INVOLVEMENT OF SUMOylation IN ALZHEIMER'S DISEASE

S. MARCELLI[1,2,*], F. IANNUZZI[1,*], E. FICULLE[3,*], S. AQUARO[4], A. CIECHANOVER[5], R. NISTICÒ[6,7] and M. FELIGIONI[1,3]

[1]*Laboratory of Neuronal Cell Signaling, EBRI Rita Levi-Montalcini Foundation, Rome, Italy*
[2]*Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy*
[3]*Department of Neurorehabilitation Sciences, Casa di Cura Policlinico, Milano, Italy*
[4]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Arcavacata di Rende, Cosenza, Italy*
[5]*Technion Integrated Cancer Center (TICC), The Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, Haifa, Israel*
[6]*Pharmacology of Synaptic Disease, EBRI Rita Levi-Montalcini Foundation, Rome, Italy*
[7]*University of Rome "Tor Vergata", Rome, Italy*

*These authors share co-first authorship

Albeit enormous progress having been achieved in both therapy and management of Alzheimer's disease, a unique etiology and an appropriately effective treatment for this neurodegenerative condition still remain elusive. Consequently, this disabling pathology is presently recognized as the most common cause of chronic dementia among the ageing population, characterized by progressive cognitive decline, including memory impairment and deficiencies in recognition, language and skilled movements. Emerging evidence concerning the alteration of post-translational modifications (PTMs) supports their implication in an attractive new area of investigation potentially implicated in AD pathophysiology. APP, Aβ, tau, BACE1, GSK3β and various other proteins related to AD generally undergo PTMs and, interestingly, impairment of such physiological processes are being more and more related to AD onset or progression. In this study the effects are discussed of PTMs on AD-related proteins and their potential contribution to the development of AD. In particular, special concern is placed on the SUMOylation of AD target proteins, underlining the association of such PTM with the impairment of intracellular trafficking, protein aggregation and degradation, neuronal development and synaptic transmission, classically known to be AD hallmarks. Since similar events have also been reported for Parkinson's disease, polyglutamine disorders such as Huntington's disease, amyotrophic lateral sclerosis and prion disease, the potential role is also briefly reviewed of SUMOylation on the etiology of these related neurological diseases.

Alzheimer disease and PTMs

Human life expectancy is nowadays largely increased in respect to the past as a result of the reduced mortality achieved by medical care improvement and general amelioration of quality of life. A consequence of life elongation is the growing frequency of pathologies related to ageing. In fact, in 2015 more than 47 million people worldwide resulted

Key words: SUMOylation, Alzheimer, tau, APP, prion disease, glycogen synthase kinase, 3β α-synuclein, Parkinson, Huntington, amyotrophic lateral sclerosis

Corresponding author:
Dr Marco Feligioni,
Laboratory of Neuronal Cell Signaling,
EBRI Rita Levi-Montalcini Foundation,
via del Fosso di Fiorano 64, 00143, Rome, Italy
Tel.: +39 06 501703112 - Fax: +39 06 50 170 3335
e-mail: m.feligioni@ebri.it

2279-5855 (2016)

affected by dementia, the most common pathology in the elderly, unfortunately expected to triple by 2050 (Mayeux and Stern, 2012).The most common type of dementia is Alzheimer's disease (AD), a severe neurodegenerative condition characterized by the progressive decline of cognitive and behavioral functions. It appears clear how this severe pathology might have a deep socio-economic impact on society, particularly as an early diagnosis or an appropriate treatment do not yet exist. It is well known that the histopathological hallmarks characterizing AD are neurofibrillary tangles (NFTs) and amyloid-β (Aβ) aggregates, mostly located in the brain sections at *post-mortem* examinations of AD specimens. Brain regions affected include temporal and fronto-temporal lobes, entorhinal cortex, amygdala and hippocampus (Holtzman et al, 2011), which are known as brain areas involved in learning processes and memory as well as in emotion formation.

Amyloid-β plaques are extracellular structures composed of a central core of $A\beta_{40}$ and $A\beta_{42}$ aggregated peptides, surrounded by dystrophic neurites (Wogulis et al, 2005). According to the "amyloid cascade hypothesis" of AD, these aggregated and toxic peptides are the result of the amyloidogenic cleavage of the amyloid precursor protein (APP), a type I integral membrane glycoprotein mostly expressed in the brain (Mattson, 2004) whose role is not clearly defined yet, although it is surely important for the development of neurites, synaptic plasticity, cellular adhesion and neurons survival (Zheng and Koo, 2006). On the other hand, neurofibrillary tangles are intracellular structures composed of abnormally phosphorylated tau protein, assembled into paired helical filaments (PHFs) (Grundke-Iqbal et al, 1986). Tau is a microtubule associated protein (MAP) mainly found in the axons of CNS neurons, having a role in both axonal development and microtubule stabilization via their polymerization (Mandelkow and Mandelkow, 2012).

When tau becomes hyper-phosphorylated it gains a particular conformation that reduces its interactions with the cytoskeleton, leading to destabilization and depolymerisation of microtubules (Kirkitadze et al, 2001).

Both NFTs and Aβ aggregates have been linked to synaptotoxicity, axonal degeneration (Nisticò et al, 2012) and impaired cellular communication, that later turn into gliosis, neurodegeneration and widespread neuronal death. Although various processes including aging, gene mutations, neuroinflammation, oxidative stress or chronic circulatory insufficiency are risk factors for the onset and progression of AD (Selkoe, 2001), many other molecular changes are involved in AD development. Post-translational modifications (PTMs), for instance, have been implicated in this emerging and interesting field of research and are recently object of several scientific studies.

To date, it has been demonstrated that most of the key players in AD are modified by PTMs during the disease progression and that these events potentially contribute to their functional alteration observed in AD.

PTMs are endowed with an utmost physiological relevance, since they can modulate the localization, interactions and functions of proteins in a quick and reversible way (Russell et al, 2014). In particular, their fine regulation is critical for the nervous system function because PTMs participate in the control of synaptic communication, homeostatic modulation and general brain functioning (Di Antonio and Hicke, 2004; Routtenberg and Rekart, 2005; Lee et al, 2014).

Consequently, PTM homeostatic dysfunction most probably contributes to the arising of pathological conditions, and, in the case of AD, possibly PTM dysfunction contributes to cognitive alteration and neurodegeneration. Abnormal protein phosphorylation, ubiquitination or SUMOylation are among the PTMs reported in literature as potentially involved in the etiopathogenesis of AD (Lee et al, 2001; Ross and Poirier, 2004; Nisticò et al, 2014).

This review particularly focuses on the relationships between altered protein SUMOylation and AD, with special concern on the proteins mostly associated with this chronic neurodegenerative disease, notably Aβ and tau.

SUMOylation pathway

SUMOylation is a reversible post-translational modification occurring through the covalent binding

of the 11 kDa *Small Ubiquitin-like MOdifier* (SUMO) peptide to specific lysine residues on target proteins. As a result, SUMOylation is implicated in the normal protein functionality, regulating transcription factor transactivation, protein-protein interactions and subcellular localization of certain proteins (Feligioni et al, 2015; Sarge and Parke-Sarge, 2009).

Three SUMO paralogues are known to be abundantly expressed in vertebrate brains, namely SUMO1, SUMO2 and SUMO3. Since the latter two subtypes share about 95% sequence homology, they are commonly referred to as SUMO2/3 (Johnson, 2004).

Similarly to the ubiquitination pathway, the SUMOylation process requires three enzymes to complete the cycle, namely SUMO-E1 activating enzyme, SUMO-E2 conjugating enzyme and SUMO-E3 ligating enzyme.

The pathway starts with the cleavage of the C-terminus of SUMO peptides by sentrin-specific proteases (SENP), in order to expose a diglycine (GG) motif that can be covalently attached, via a thioester bond, to a cysteine residue of a SUMO-E1 activating enzyme (Aos1 and Uba2 heterodimer) in an ATP-dependent manner. This complex becomes the substrate for the SUMO-E2 conjugating enzyme, (Ubc-9) which lastly transports SUMO onto the lysine residue of the target protein, generally by means of a specific SUMO-E3 ligase. The cycle is completed by the reversible removal of SUMO from its target substrates, operated by one of the SENP proteases (Droescher et al, 2013). For SUMO conjugation, a specific consensus sequence has been described, that is *ψKxE/D*, where *ψ* is a bulky hydrophobic residue and *x* can be any amino acid followed by an acidic residue.

Involvement of SUMOylation in AD

Several studies on AD have shown that various proteins are subjected to post-translational modifications such as phosphorylation, ubiquitination, and SUMOylation (Atkin and Paulson, 2014; Tenreiro et al, 2014). SUMOylation is currently largely studied in Alzheimer's disease since SUMO moieties establish covalent interactions with fundamental AD proteins such as AβPP, tau, BACE1, GSK3β and JNK (Feligioni and Nisticò, 2013).

From a clinical prospective, it was reported that AD patients present altered levels of SUMOylation and SUMO-related protein expression. Indeed, SUMO3 labelling was found to be increased in *post-mortem* brain sections from AD patients, especially in the hippocampus, which is the brain region responsible for learning and memory and the most affected in AD (Li et al, 2003). The protease SENP3, which participates in the de-SUMOylation process, was shown to be down-regulated in inferior parietal lobes of sporadic AD patients (Weeraratna et al, 2007). Furthermore, the SUMO conjugating enzyme Ubc9 has been hypothesized to be responsible for AD development. In fact, a genomic analysis of Korean patients' DNA affected by late-onset AD showed variations of the Ubc9 gene (UBE2I) that might significantly support the risk of developing AD for the Korean population (Ahn et al, 2009). In AD animal models, instead, controversial results were observed in the expression of the main components of the SUMOylation pathway. Although global levels of SUMO1 or SUMO2/3 were not altered significantly in that model, however several individual SUMO2/3 bands were reported to be considerably decreased. Ensuing studies showed increased SUMO1 and SUMO2/3 levels in APP overexpressing and aged mice (Yang et al, 2012; Yun et al, 2013). In addition to this controversy is the fact that decreased SUMO2 levels were found in old APP mice (17 months), whereas increased SUMO1, Ubc9, and SENP1 levels were observed in young APP mice (3 and 6 months) (Nisticò et al, 2014).

SUMOylation OF PROTEINS RELATED TO AD

In the last decade, an increasing number of proteins associated with AD pathophysiological mechanisms has been reported to undergo SUMOylation (Martins et al, 2016). In the following paragraph we summarize the knowledge on the SUMOylation of several proteins known to be related to the formation of AD pathological hallmarks. To date, researchers are still working to understand whether and to what extent SUMOylation is a putative mechanism

contributing to protein misfolding or aggregation (Feligioni et al, 2015).

Tau SUMOylation

AD is classically characterized by high levels of misfolded tau protein forming neurofibrillary tangles (Goedert et al, 1992). Tau has been reported to be a SUMO1 target at lysine K340, a residue located within the 4R-tau, one of the interaction sides with microtubules (Dorval and Fraser, 2006). The *in vivo* interaction between tau and SUMO1 was confirmed by immunohistochemistry experiments in which SUMO1 immunoreactivity co-localized with phosphorylated tau aggregated in neuritic plaques of APP transgenic mice but not in mutant tau transgenic mice, indicating that APP processing can take part in the SUMO-tau relationship. Conversely, NFTs from *post-mortem* AD brain were found prominently ubiquitinated but not immunoreactive for tau-SUMO1 conjugates (Takahashi et al, 2008).

Takahashi and co-workers demonstrated that proteasomal inhibition significantly decreased tau SUMOylation, but increased tau ubiquitination (Takahashi et al, 2008). Proteasome impairment is a common feature in several aging-related neurodegenerative disorders, consequently, as Dorval and Fraser suggested, down-regulation of tau sumoylation in response to proteasome failure may explain why neurofibrillary tangles are not labeled by SUMO immunostaining (Dorval and Fraser, 2006). This results suggest that tau can also be ubiquitinated and degraded by the proteasome through both ubiquitin-dependent and ubiquitin-independent pathways (David et al, 2002; Shimura et al, 2004).

Recently, it has been reported that SUMOylation of lysine K340 stimulates tau phosphorylation and inhibits ubiquitination-mediated tau degradation, thus favoring its aggregation (Luo et al, 2014).

APP and Aβ SUMOylation

SUMOylation seems to be involved in the modulation of APP processing and/or trafficking and subsequently in its proteolytic amyloidogenic processing (Hardy and Selkoe, 2002). Consequently, modulation of the cleavage of APP into Aβ could potentially reduce Aβ-induced toxicity and could be associated with cognitive deficits.

It has been found that APP can be SUMOylated *in vitro* with an efficiency of ~34% both by SUMO1 and SUMO2 at lysines 587 and 595. Covalent modifications of APP lysines K587 and K595 by both SUMO1 and SUMO2 were reported to decrease Aβ aggregation levels in HeLa cells overexpressing APP (Zhang and Sarge, 2008), probably due to the close spatial proximity and unfavorable steric congestion between the β-secretase cleavage site and the SUMOylatable lysines on APP (Martins et al, 2016). On the other hand, SUMOylation by either SUMO1 or SUMO2 is associated with decreased levels of Aβ aggregates, suggesting that APP SUMOylation could have protective effects against the amyloidogenic APP processing. However this effect was not observed in overexpressing K587R and K595R mutants (Zhang and Sarge, 2008).

Intriguingly, K595 is also site of the well-known APP mutation (K595N) associated with the autosomal dominant Swedish early-onset familial form of AD (Mullan et al, 1992). Therefore, an attractive hypothesis could already be formulated wherein impeding APP SUMOylation at this lysine residue may contribute to the increased Aβ production and higher pathogenicity characterizing the Swedish mutation (Sarge and Park-Sarge, 2009).

Controversially, Aβ peptides have been reported to directly affect the SUMO machinery in downstream signaling pathways, behaving like neurotoxins at synaptic sites when at high concentrations (Holtzman, 2011; Mucke and Selkoe, 2012).

The potential *in vivo* effects of Aβ on SUMOylation have been investigated by using APP overexpressing mice and Tg2576 mouse models. No changes were detected in global SUMO1 or SUMO2/3 conjugation levels (McMillan et al, 2011), albeit in 18-month-old APP/PS1 double transgenic mice, unconjugated SUMO1 was found elevated and SUMO1 immunoreactivity was enhanced and partially co-localized with amyloid plaques in respect to control mice (Yang et al, 2012). These data suggest that SUMO1 expression can be altered by Aβ toxicity.

A similar increase in free unconjugated SUMO2/3 levels was observed in the hippocampus

of 25-month-old C57BL/6 wild type mice, inversely correlating with their behavioral performances. Such augmented free SUMO may indicate a diminished efficacy of SUMO conjugation and therefore higher levels of free unconjugated SUMO moieties (Yang et al, 2012).

The expression levels of SUMOylated proteins during AD onset and throughout the disease progression have also been investigated in order to elucidate an age-related correlation of the SUMO/deSUMOylation balance. At early stages of the pathology (3 and 6 months) Tg2576 mice presented an increase in SUMO1 expression and conjugation compared to wild type in both cortex and hippocampus, but not at later stages (17 months). SUMO2 modification was instead decreased in old transgenic mice but not in earlier time windows (Nisticò et al, 2014).

Given the differences so far observed, a possible opposite functional role has been speculated for SUMO1 versus SUMO2 conjugations, wherein SUMO1 overexpression may impair synaptic function and spine morphology, whereas SUMO2 might be beneficial against the age-related memory decline (Matsuzaki et al, 2015).

GSK3β SUMOylation

Several other proteins, known to be involved in signaling pathways crucial to the pathophysiology of AD, have been identified as SUMO conjugation targets.

GSK3β, for instance, plays a pivotal role in the pathogenesis of both sporadic and familial forms of AD. Glycogen synthase kinase 3β (GSK3β) is a serine/threonine protein kinase involved in several physiological processes, such as glycogen metabolism and gene transcription. It is one of the main kinases associated with the hyperphosphorylation of tau and plaque-associated microglial-mediated inflammatory responses (Martins et al, 2016). GSK3β was shown to phosphorylate the majority of sites on tau including Ser396/Ser404 (PHF-1) epitope, which is directly involved in microtubule destabilization and paired helical filament formation in AD brains (Hanger et al, 1992; Hanger et al, 1998; Bramblett et al, 1993; Takashima et al, 1998). Reciprocal interactions have also been established between Aβ and GSK3β, indicating that Aβ seems to be strictly linked with GSK3β activation. The hypothesis that Aβ-mediated GSK3β activation could be achieved through Tyr216 phosphorylation has emerged and an increase of pGSK3βTyr216 was found in numerous models of Aβ treatment (Hu et al, 2009; Terwel et al, 2008; Pajak et al, 2009; Selenica et al, 2013). Pajak et al. (2009) revealed a co-localization of pGSK3βTyr216 and Aβ deposits in PC12 cells overexpressing human Amyloid Precursor Protein.

A recent *in vitro* study presented a correlation between pGSK3βTyr216 and tau phosphorylation at PHF-1 epitope, the characteristic GSK3β motif. Tyr216 phosphorylation could promote nuclear accumulation of pTau at PHF-1 epitope, an early event known to be implied in AD pathology. As a consequence, any GSK3β inhibitor reducing Tyr216 phosphorylation could be used to prevent tau hyperphosphorylation at the pathological PHF-1 site (Noel et al, 2016).

Recently, SUMOylation of GSK3β was demonstrated to induce the phosphorylation and the subsequent activation of the kinase at Tyr216 (Eun Jeoung et al, 2008), thus suggesting that SUMOylation of protein kinases or phosphatases may also contribute to the increased tau phosphorylation (Gong and Iqbal, 2008; Wang et al, 2007). Therefore, SUMOylation of GSK3β may represent another promising target for AD therapy.

IMPLICATION OF SUMOYLATION IN OTHER NEURODEGENERATIVE DISEASES

In the last decade, the deregulation of SUMOylation homeostasis has been also associated with neurodegenerative diseases other than AD. Indeed, if SUMOylation in AD contributes to alter solubility and proteolytic processing of Aβ and tau proteins, a similar activity has been recognized for various proteins implicated in the onset and progression of several diseases, including Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis (ALS) and prion disease.

We here provide an overview of the recently unveiled impact that SUMOylation has on the

etiology and progression of these AD-related neurological diseases.

SUMOylation impact on α-synuclein

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by the loss of dopaminergic neurons which leads to cardinal motor signs, i.e. rigidity, bradykinesia and resting tremor (Bose and Beal, 2016). Several genes have been associated with the hereditary forms of the disease, encoding proteins such as parkin, DJ-1 and especially α-synuclein.

Similarly to tau, α-synuclein belongs to the family of natively unfolded neuronal proteins (Schweers et al, 1994; Weinreb et al, 1996). It is commonly localized at presynaptic terminals (Iwai et al, 1995) and implicated in MAP kinase signaling, synaptic vesicle trafficking and neurotransmission (Iwata et al, 2001; Chandra et al, 2005; Chandra et al, 2004; Liu et al, 2004).

In pathological conditions, generally referred to as "*synucleinopathies*" (including Parkinson's disease, Dementia with Lewy bodies and Multiple System Atrophy), α-synuclein can undergo a conformational transition from random coil to β-pleated sheet conformation, progressively leading to fibrillization, formation of oligomers, and accumulation of insoluble aggregates and intracellular inclusion bodies that represent the hallmarks of this class of diseases (Goedert, 2001; Spillantini et al, 1997; Kahle et al, 2002; Barghorn et al, 2004; Conway et al, 2000).

α-synuclein is known to be the target of several PTMs including SUMOylation (Kim et al, 2011; Krumova et al, 2011), which may affect protein localization, structure and aggregation-prone aptitudes (Feligioni et al, 2015; Goedert, 2001). Indeed, various investigations proved the occurrence of α-synuclein SUMOylation by means of cell-free assays (Krumova et al, 2011) in *in vitro* cell-based assays (Krumova et al, 2011; Oh et al, 2011) and co-localization imaging (Kim et al, 2011; Pountney et al, 2005). In particular, SUMO1 was found to be predominantly conjugated via mono-SUMOylation, whereas SUMO2 and SUMO3 could form polymer chains of poly-SUMOylation (Matic et al, 2008).

Notably, insoluble α-synuclein aggregates were found to be SUMOylated in neuronal cell cultures from both Parkinson's disease and Dementia with Lewy bodies affected brains (Feligioni et al, 2015; Kim et al, 2011). In addition, endogenous α-synuclein was found to be efficiently conjugated in His_6-SUMO transgenic mice, interestingly confirming that this PTM does occur *in vivo* in the mouse brain under physiological circumstances (Krumova et al, 2011).

Fifteen lysine residues have been localized within α-synuclein core repeats, among which K96 is a classical SUMO consensus acceptor site (VK96KD), whereas K102 is a closely related motif (GK102NE) Feligioni et al, 2015). These two sites account for more than 50% of the α-synuclein SUMO conjugates, as confirmed by mutagenesis assays (Dorval and Fraser, 2006; Krumova et al, 2011).

Increasing evidence highlights the SUMOylation of α-synuclein as an important marker in PD pathogenesis. Indeed, this PTM seems to abolish fibril formation *in vitro*, with a 50% covalent conjugation of a single SUMO moiety being already efficient in completely blocking the α-synuclein pathognomonic fibrillation (Krumova et al, 2011; Shahpasandzadeh et al, 2014). Furthermore, overexpression of mutant K96R/K102R α-synuclein in dopaminergic *substantia nigra pars compacta* neurons of a PD rodent model substantially exacerbated the protein toxicity and its neurodegenerative effect (Krumova et al, 2011). These studies are speculative of a molecular mechanism wherein SUMOylation may act as a modulator of fibril formation, by maintaining the aggregation-prone α-synuclein in solution (Krumova et al, 2011).

Interestingly, a site- and isoform-dependent effect of SUMOylation on α-synuclein aggregation was elucidated by means of protein semi-synthesis experiments, indicating that SUMOylation at K102 is a better inhibitor of aggregation than the modification at K96 and that SUMO1 modification is a better aggregation inhibitor than SUMO3 (Abeywardana and Pratt, 2015).

On the other hand, further investigations found that a higher proportion of oligomeric α-synuclein species were SUMOylated *in vitro*, but only upon proteasome inhibition (Anderson et al, 2006;

Iwatsubo, 2003). Indeed, treatment of mammalian cells with the proteasome inhibitor MG-132 usually leads to the formation of stable and multi-ubiquitinated protein inclusions that are more easily SUMOylated and can ultimately end up in cell death (Yuan et al, 2008; Xie et al, 2010). Conclusively, SUMOylation might impinge in synucleinopathies in two ways, either by inhibiting the propensity of α-synuclein to aggregate or by affecting the cell degradative machinery and hence the clearance of α-synuclein aggregates (Vijayakumaran et al, 2015).

Last but not least, recent studies also suggested a role for SUMOylation in mitochondrial dynamics, with a clear pivotal implication in PD pathogenesis. Indeed, SUMOylation activates DJ-1, thus decreasing mitochondrial oxidative stress, while reducing the amount of parkin available for mitochondrial recruitment. In addition, SUMOylation is known to modulate mitochondrial fission via modification of the dynamin-related protein 1 (DRP1) (Feligioni et al, 2015; Guerra de Souza et al, 2016).

SUMO, Huntingtin and polyglutamine disorders

Similarly to PD, an aberrant accumulation of insoluble high-molecular-weight protein complexes has been observed in Huntington's disease (HD), another neurodegenerative disorder caused by the expansion of a CAG polyglutamine (polyQ) repeats within the gene coding for the Huntingtin (Htt) protein (MacDonald et al, 1993). As soon as the threshold of the minimal polyglutamine repetition length is exceeded (generally above 35 to 45 repeats), the resultant expanded protein undergoes conformational changes and therefore aggregates, forming typical "amyloid-like" inclusion bodies (Poirier et al, 2002; Steffan et al, 2004) which determine neuronal death in specific brain regions, including basal ganglia, cerebellum, brainstem and spinal motor nuclei (Ross, 1995).

Htt appears to have a neuroprotective function by promoting the production of neurotrophic factors such as BDNF (Cattaneo et al, 2001). Several studies have suggested a role for the proteolytic cleavage of this polyglutamine protein, since only antibodies to the N-terminus of Htt, but not at the C-terminus, are able to recognize the intranuclear inclusions characterizing HD (Li et al, 2000; Toneff et al, 2002). Specifically, the fragmented Httex1p N-terminus has been identified as an important mediator of aggregation, localization and protein stability (Thompson et al, 2009; Atwel and Truant, 2008; Sivanandam et al, 2011; Zheng et al, 2013).

Both the full-length Htt and the related pathogenic fragment can be relevantly regulated by PTMs including SUMOylation (Ehrnhoefer et al, 2011; Pennuto et al, 2009; Zheng and Diamond, 2012), with an effect in the promotion of their stabilization (Steffan et al, 2004). Proteomic analysis and cell-based assays confirmed the relevance of SUMO-conjugation of aggregated proteins, with K6 and K9 appearing as primary conjugation sites (Steffan et al, 2004; O'Rourke et al, 2013), and with SUMO2 specifically eliciting a dose-dependent increase in insoluble Htt (Tatham et al, 2011).

In parallel, ubiquitination appeared to reduce poly-Q toxicity, presumably by promoting the degradation of toxic proteins, and possibly competing with SUMOylation for the same lysine residues in the amino-terminal domain (Desterro et al, 1998; Hoege et al, 2002; Lin et al, 2003). To confirm, HeLa cells treated with proteasome inhibitors resulted to accumulate conjugated forms of all SUMO paralogs in insoluble protein inclusions as well as in the accumulation on SUMO2 substrates of lysine-63-linked polyubiquitin chains (Feligioni et al, 2015; Tatham et al, 2011).

A remarkable accumulation of SUMO2-modified Htt was also found in the insoluble fractions of *post-mortem* striata from HD patients, suggesting that this PTM could indeed be relevant in humans to regulate the age-related pathogenic accumulation of mutant Htt (O'Rourke et al, 2013). Co-localization studies implying truncated Htt fragment and His-tagged SUMO further supported this hypothesis (Sipione et al, 2002).

The consequences of Htt SUMOylation have also been investigated by mutation of the target lysines K6 and K9 of the *Drosophila* protein (MacDonald et al, 1993). SUMOylation deficient mutant substantially prevented degeneration, indicating that SUMOylation at these sites may correlate with the pathological insult (Steffan et al, 2004).

Among the SUMOylation enzymes, PIAS1, a SUMO E3 ligase, has been elected as a potential candidate SUMO ligase for Htt (O'Rourke et al, 2013) and therefore suggested as possible therapeutic target in HD treatment. PIAS1 was indeed able to regulate the accumulation of insoluble Htt polypeptides in HeLa cells (O'Rourke et al, 2013). Moreover, the genetic reduction of the homologous PIAS enzyme in *Drosophila* proved to be neuroprotective, and a reduction of Drosophila SUMO smt3 was effective in the same direction (Steffan et al, 2004).

Finally, SUMO2 activity and expression is commonly highly influenced by cellular stress (Saitoh and Hinchey, 2000), therefore several studies are now focusing on the eventual correlations between increased SUMOylation and oxidative stress, known to be implicated in the HD pathology (Browne and Beal, 2006).

SUMOylation and amyotrophic lateral sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a late-onset neurodegenerative condition characterized by the selective loss of motor neurons, ultimately leading to paralysis and death within 2–5 years from diagnosis (Zarei et al, 2015). Although most ALS cases are sporadic, approximately 20% have been linked to familial autosomal dominant mutations in the gene encoding the copper-zinc superoxide dismutase SOD1, with G93R and G85R being the most frequent (Andersen, 2006). The resulting SOD1 proteins are supposed to undergo *gain-of-function* mutations, acquiring cytotoxic properties (Cleveland, 1999; Takeuchi et al, 2002; Guégan et al, 2002).

The major pathologic hallmark of familial ALS (fALS) is the aggregation of mutated SOD1, which misfolds and forms proteinaceous intracellular inclusions (Bruijn et al, 1998; Son et al, 2003; Watanabe et al, 2001).

The exact mechanisms of aggregation still remain unclear, however PTMs of SOD1 may also be involved. Given the fact that SUMOylation is known to regulate protein stability and aggregation-prone attitudes (Feligioni et al, 2015), SOD1 SUMOylation has been investigated in order to evaluate a potential involvement in the pathological process. Notably, by means of an *in vitro* SUMOylation assay, human SOD1 was found to be a specific substrate of SUMO1 but not of SUMO2 or SUMO3 isoforms in the NSC34 motor neuron cell line (Niikura et al, 2014; Cashman et al, 1992). On the other hand, mutated G93R and G85R SOD1 proteins were found to be also modified by SUMO2 and SUMO3 (Niikura et al, 2014) and this modification might modulate the degradation process (Niikura et al, 2014; Fei et al, 2006).

K9 and K75 were identified by means of a SUMOplot prediction software (*www.abgent.com/doc/sumoplot*) as potential lysine targets for SUMO-conjugation, subsequently confirmed via mutagenesis studies. Particularly, K75R mutation abrogated the SUMO1 modification of SOD1, whereas no clear cut results were obtained concerning K9R mutations, depending upon *in vitro* versus *in vivo* assays (Niikura et al 2014; Fei et al, 2006).

Fei and coworkers (2006) found that SUMO1ylation at K75 both enhanced SOD1 stability and promoted its aggregation. Accordingly, SUMO1 overexpression was found to support SOD1 aggregation, moreover co-localizing with the aggregates and with a parallel reduction in the levels of free SUMO-1 (Dangoumau et al, 2016). These results, however, were not replicated for SUMO2 and SUMO3, possibly indicating a role for the sole SUMO1 in the pathological process of fALS (Fei et al, 2006). To confirm, another more recent study underlined that preventing the SUMOylation of mutant SOD1 via substitution of lysine 75 significantly reduced the number of motor neuronal cells with aggregates (Dangoumau et al, 2016).

On the other hand, SUMO3 was identified as the principal responsible for the promotion and acceleration of fALS-linked mutant SOD1 aggregation in NSC43 cells by Niikura et al (2014). Again, K75R but not K9R mutants showed a significant reduction in the rate of aggregation, supporting previous findings. In addition, the non-SUMOylated mutant SOD1 was not detected to co-immunoprecipitate with SUMO, while the not-SUMOylatable not-aggregating wild type SOD1 actually did. Considering that the majority of the SOD1 protein was found in its non-SUMOylated

form, the authors suggested that SUMO3 conjugation of mutant SOD1 may accelerate protein aggregation, possibly by recruiting such a large amount of non-SUMOylated SOD1 protein (Niikura et al, 2014).

Finally, further studies highlighted the connection between oxidative stress and ALS, with special concern toward the SUMO2/3 isoform, known to have a role in these responses (Tempé et al, 2008; Parakh et al, 2013; Nakamura et al, 2012). Therefore, it has been proposed that a possible stress-induced elevation of SUMO3ylation during ALS might accelerate the aggregation of mutant SOD1 in motor neurons, consequently activating the cytotoxic pathways at the basis of ALS pathophysiology (Nakamura et al, 2012). Additional data will be required to support this interesting hypothesis, further supporting a pivotal role for SUMOylation as potential therapeutic target in ALS.

Insights of SUMOylation in prionic diseases

Prion diseases, also known as Transmissible Spongiform Encephalopathies (TSEs), are a class of self-perpetuating and infectious neurodegenerative pathologies (Aguzzi and Polymenidou, 2004), including Creutzfeldt-Jakob disease, fatal familial insomnia and kuru in humans, scrapie in small ruminants (Prusiner et al, 1993), bovine spongiform encephalopathy or "*mad cow*" disease in cattle (Wells et al, 1987), chronic wasting disease in deer and elk (Williams and Young, 1980), and feline spongiform encephalopathy in both wild and domestic cats (Wyatt et al, 1991).

TSEs causative agent is supposed to be a "small cellular proteinaceous infectious particle" named *prion* (PrP^C) (Prusiner, 1982; Caughey and Baron, 2006; Chiesa and Harris, 2001), encoded by the *PRNP* gene, in the short arm of human chromosome 20. To date, all known cases of familial prion disease co-segregate with *PRNP* mutations (Sparkes et al, 1986).

PrP^C is an Asn-linked glycosylated protein supposed to play a role in signal transduction and metal homeostasis, as well as to act as antioxidant and be protective against apoptosis (McKinley et al, 1991). Indeed, $PrP^{-/-}$ animals show altered synaptic function (Richt et al, 2007), circadian rhythms (Tobler et al, 1996), motor function and copper metabolism (Nazor et al, 2007; Katamine et al, 1998), as well as impaired response to oxidative stress (Collinge, 2001).

Although the physiological function of cellular prion protein and its actual involvement in disease remain quite elusive, the central event in prion pathogenesis is ascribed to the conformational conversion of normal PrP^C into the neurotoxic and misfolded PrP^{Sc} isoform, characterized by a higher content of β-sheet structures (Pan et al, 1993; Safar et al, 1993; Meyer et al, 1986). Such isoform not only propagates itself but also imposes its abnormal conformation to the cellular PrP^C molecules of the host, often ending up in misfolding, decreased solubility and aggregation into amyloid-like plaques (Aguzzi and Polymenidou, 2004; Chiesa and Harris, 2001; Gasset et al, 1992; Meier et al, 2003). Consequently, prion disease is characterized by widespread neurodegeneration, with affected individuals exhibiting clinical symptoms of both cognitive and motor dysfunctions.

As PrP^C is synthesized as a family of four distinct isoforms, attention has been focused on the ^{Cyt}PrP form, a minor member that segregates outside the secretory route and can induce neurocytotoxicity (Hegde et al, 1998; Juanes et al, 2009). ^{Cyt}PrP is synthesized by alternative initiation of translation from a downstream AUG, coding for Met-8 in human PrP^C [$_{Hu}PrP(M8)$] and for Met-15 in hamster [$_{Ha}PrP(M15)$].

Accumulation of ^{Cyt}PrP sensitizes cells to death (Ma and Lindquist, 2002; Ma et al, 2002), since it spills into the cytosol and accesses the nucleus forming insoluble aggregates when the proteasome is inhibited (Yedida et al, 2001; Drisaldi et al, 2003).

Interestingly, access to the nucleus has been associated to a possible SUMO conjugation of this protein. Indeed, $_{Ha}PrP(M15)$ isolated from nuclear fractions of hamster brain homogenates was recognized by anti-SUMO-1 antibodies but not by anti-ubiquitin or anti-SUMO 2/3 antibodies, showing a variable level of SUMO-1 conjugation (Juanes et al, 2009).

Such PTM might underlie the SUMO-mediated on/off functional switch of PrP^c nuclear population

and suggest a high physiological relevance and an important fine-tuning for the activity of this protein.

Moreover, expression of $_{Ha}$PrP(M15) was involved in the dysregulation of cellular growth, resulting in an antiproliferative activity and G_0/G_1 phase arrest (Juanes et al, 2009). $_{Ha}$PrP(M15) might therefore be considered as a possible candidate for promotion of G_1 phase arrest during cell differentiation and its SUMO-modification may represent the switch to its alternative functional states.

Further investigation will be required to better provide novel insight into PrP^C physiological and pathological balance.

CONCLUSIONS

To date, SUMOylation has been recognized as an essential intracellular mechanism implicated in various physiological and pathological cellular pathways. By means of modification of target proteins, SUMOylation can indeed induce changes in their localization, trafficking, function, and stability. In the very complex frame of AD onset and progression, the alteration of metabolic pathways at molecular level, including PTMs, surely plays a fundamental role, possibly contributing to or exacerbating AD pathology.

The present review focuses on the SUMOylation of proteins known to be involved in AD and similar neurodegenerative diseases.

A deeper knowledge on the role of SUMOylation in Alzheimer's disease is indeed interesting and it will be important in the future to determine how SUMO modifications might contribute to the formation of the specific AD markers, including early synaptic failure, aggregation of misfolded proteins, and metabolic impairment. Moreover it will be attractive to unveil how SUMOylation might impact on neuronal proteins in normal conditions, and, eventually, how this process might become deregulated at the onset or during the progression of disease. Considerable evidence indicates that protein SUMOylation actually takes part in the process of aggregation of several proteins intimately connected with the etiology of neurodegenerative diseases, such as Aβ, Tau, α-synuclein, mutant Htt and SOD1.

Definitely, a broader understanding of these mechanisms would lead to undeniable pharmacological and clinical benefits, with the selective targeting of SUMOylated proteins potentially becoming a future therapeutic strategy for neurological disorders, including AD.

ACKNOWLEDGEMENTS

This work was partly supported by research grants from: "H. Lundbeck A/S, Denmark", Casa di Cura del Policlinico di Milano (CPPS, Milan) and from the Italian Ministry of Health to IRCCS Neurological Institute "C. Mondino" (RicercaCorrente2013–2015).

REFERENCES

Abeywardana T, Pratt MR (2015) Extent of Inhibition of α-synuclein aggregation in vitro by SUMOylation Is conjugation site- and SUMO isoform-selective. *Biochem* **54**(4): 959–61.

Aguzzi A, Polymenidou M (2004) Mammalian Prion biology: one century of evolving concepts. *Cell* **116**(2): 313–27.

Ahn K , Song JH, Kim DK, Park MH, Jo SA, Koh YH (2009) Ubc9 gene polymorphisms and late-onset Alzheimer's disease in the Korean population: a genetic association study. *Neurosci Lett* **465**(3): 272–75.

Andersen PM (2006) Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Curr Neurol Neurosci Rep* **6**(1): 37–46.

Anderson JP, Walker DE, Goldstein JM, de Laat R et al. and Chilcote TJ (2006) Phosphorylation of Ser-129 Is the dominant pathological modification of alpha-synuclein in familial and sporadic lewy body disease. *J Biol Chem* **281**(40): 29739–52.

Atkin G, Paulson H. (2014) Ubiquitin pathways in neurodegenerative disease. *Front Mol Neurosci* **7**: 63.

Atwal RS, Truant R (2008) A stress sensitive ER membrane-association domain in Huntingtin protein defines a potential role for Huntingtin in the regulation of autophagy. *Autophagy* **4**(1): 91–93.

Barghorn S, Davies P, Mandelkow E (2004) Tau paired helical filaments from Alzheimer's disease brain and assembled in vitro are based on beta-structure in the core domain. *Biochemistry* **43**(6): 1694–1703.

Bose A, Beal MF (2016) Mitochondrial dysfunction in Parkinson's disease. *J Neurochem* **139** Suppl 1:216-231.

Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VM (1993) Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron* **10**(6): 1089–99.

Browne SE, Beal MF (2006) Oxidative damage in Huntington's disease pathogenesis. *Antioxid Redox Signal* **8**(11-12): 2061–73.

Bruijn LI, Houseweart MK, Kato S, Anderson KL et al. and Cleveland DW (1998) Aggregation and motor neuron toxicity of an ALS-Linked SOD1 mutant independent from wild-type SOD1. *Science* **281**(5384): 1851–54.

Cashman NR, Durham HD, Blusztajn JK, Oda K et al. and Antel JP (1992) Neuroblastoma X Spinal Cord (NSC) Hybrid cell lines resemble developing motor neurons. *Dev Dyn* **194**(3): 209–21.

Cattaneo E, Rigamonti D, Goffredo D, Zuccato C, Squitieri F, Sipione S (2001) Loss of normal Huntingtin Function: new developments in Huntington's disease research. *Trends Neurosci* **24**(3): 182–88.

Caughey B, Baron GS (2006) Prions and their partners in crime. *Nature* **443**(7113): 803–10.

Chandra S, Fornai F, Kwon HB, Yazdani U et al. and Südhof TC (2004) Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. *Proc Natl Acad Sci U S A* **101**(41): 14966–71.

Chandra S, Gallardo G, Fernández-Chacón R, Schlüter OM, Südhof TM (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell* **123**(3): 383–96.

Chiesa R, Harris DA (2001) Prion Diseases: What Is the neurotoxic molecule? *Neurobiol Dis* **8**(5): 743–63.

Cleveland DW (1999) From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. *Neuron* **24**(3): 515–20.

Collinge J (2001) Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* **24**: 519–50.

Conway KA, Harper JD, Lansbury PT (2000) Fibrils Formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry* **39**(10): 2552–63.

Dangoumau A, Marouillat S, Burlaud Gaillard J, Uzbekov R et al. and Vourc'h P (2016) Inhibition of pathogenic mutant SOD1 aggregation in cultured motor neuronal cells by prevention of its SUMOylation on lysine 75. *Neurodegener Dis* **16**(3-4): 161–71.

David DC, Layfield R, Serpell L, Narain Y, Goedert M, Spillantini MG (2002) Proteasomal degradation of tau protein. *J Neurochem* **83**(1): 176–85.

Desterro JM, Rodriguez MS, Hay RT (1998) SUMO-1 Modification of IkappaBalphainhibits NF-kappaB activation. *Mol cell* **2**(2): 233–39.

DiAntonio A, Hicke L (2004) Ubiquitin-Dependent regulation of the synapse. *Annu Rev Neurosci* **27**: 223–46.

Dorval V, Fraser PE (2006) Small Ubiquitin-like Modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein. *J Biol Chem* **281**(15): 9919–24.

Drisaldi B, Stewart RS, Adles C, Stewart LR et al. and Harris DA (2003) Mutant PrP Is delayed in its exit from the endoplasmic reticulum, but neither wild-type nor mutant PrP undergoes retrotranslocation prior to proteasomal degradation. *J Biol Chem* **278**(24): 21732–43.

Droescher M, Chaugule VK, Pichler A (2013) SUMO rules: regulatory concepts and their implication in neurologic functions. *Neuromolecular Med* **15**(4): 639–60.

Ehrnhoefer DE, Sutton L, Hayden MR (2011) Small Changes, big impact: posttranslational modifications and function of Huntingtin in Huntington disease. *Neuroscientist* **17**(5): 475–92.

Eun Jeoung L, Sung Hee H, Jaesun C, Sung Hwa S et al. and Sang Sun K (2008) Regulation of glycogen synthase kinase 3beta functions by modification of the small ubiquitin-like modifier. *Open biochem J* **2**: 67–76.

Fei E, Jia N, Yan M, Ying Z et al. and Wang G (2006) SUMO-1 Modification increases human SOD1 stability

and aggregation. B*iochem Biophys Res Commun* **347**(2): 406–12.

Feligioni M, Marcelli S, Knock E, Nadeem U, Arancio O, Fraser PE (2015) SUMO modulation of Protein Aggregation and Degradation. *AIMS Mol Sci* **2**(4): 382–410.

Feligioni M, Nisticò R (2013) SUMO: A (oxidative) stressed protein. N*euromolecular Med* **15**(4): 707–19.

Gasset M, Baldwin MA, Lloyd DH, Gabriel JM et al. and Prusiner SB (1992) Predicted alpha-helical regions of the prion protein when synthesized as peptides form amyloid. *Proc Natl Acad Sci U S A* **89**(22): 10940–44.

Goedert M (2001) Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci* **2**(7): 492–501.

Goedert M, Spillantini MG, Cairns NJ, Crowther RA (1992) Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* **8**(1): 159–68.

Gong CX, Iqbal K (2008) Hyperphosphorylation of Microtubule-Associated Protein Tau: A Promising Therapeutic Target for Alzheimer Disease. *Curr Med Chem* **15**(23): 2321–28.

Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM (1986) Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem* **261**(13): 6084–89.

Guégan C, Vila M, Teismann P, Chen C et al. and Teissman P (2002) Instrumental activation of bid by caspase-1 in a transgenic mouse model of ALS. *Mol Cell Neurosci* **20**(4): 553–62.

Guerra de Souza AC, Prediger RD, Cimarosti H (2016) SUMO-Regulated mitochondrial function in Parkinson's disease. *J Neurochem* **137**(5): 673–86.

Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH (1992) Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett* **147**(1): 58–62.

Hanger DP, Betts JC, Loviny TL, Blackstock WP, Anderton BH (1998) New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry. *J Neurochem* **71**(6): 2465–76.

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**(5580): 353–56.

Hegde RS, Mastrianni JA, Scott MR, DeFea KA et al. and Lingappa VR (1998) A transmembrane form of the prion protein in neurodegenerative disease. *Science* **279**(5352): 827–34.

Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* **419**(6903): 135–41.

Holtzman DM (2011) CSF biomarkers for Alzheimer's disease: current utility and potential future use. *Neurobiol Aging* **32** Suppl 1: S4–9.

Holtzman DM, Morris JC, Goate AM (2011) Alzheimer's disease: the challenge of the second century. *Sci Transl Med* **3**(77): 77sr1.

Hu S, Begum AN, Jones MR, Oh MS et al. and Frautschy SA (2009) GSK3 Inhibitors show benefits in an Alzheimer's Disease (AD) Model of neurodegeneration but adverse effects in control animals. *Neurobiol Dis* **33**(2): 193–206.

Iwai A, Masliah E, Yoshimoto M, Ge N et al. and Saitoh T (1995) The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron* **14**(2): 467–75.

Iwata A, Maruyama M, Kanazawa I, Nukina N (2001) Alpha-synuclein affects the MAPK Pathway and accelerates cell death. *J Biol Chem* **276**(48): 45320–29.

Iwatsubo T (2003) Aggregation of alpha-synuclein in the pathogenesis of Parkinson's disease. *J Neurol* **250** Suppl 3:III11–14.

Johnson ES (2004) Protein modification by SUMO. *Annu Rev Biochem* **73**: 355–82.

Juanes ME, Elvira G, García-Grande A, Calero M, Gasset M (2009) Biosynthesis of prion protein nucleocytoplasmic isoforms by alternative initiation of translation. *J Biol Chem* **284**(5): 2787–94.

Kahle PJ, Neumann M, Ozmen L, Muller V et al. and Haass C (2002) Hyperphosphorylation and insolubility

of alpha-synuclein in transgenic mouse oligodendrocytes. *EMBO Rep* **3**(6): 583–88.

Katamine S, Nishida N, Sugimoto T, Noda T et al. and Miyamoto T (1998) Impaired motor coordination in mice lacking prion protein. *Cell Mol Neurobiol* **18**(6): 731–42.

Kim YM, Jang WH, Quezado MM, Oh Y, Chung KC, Junn E, Mouradian MM (2011) Proteasome inhibition induces α-synuclein SUMOylation and Aggregate formation. *J Neurol Sci* **307**(1-2): 157–61.

Kirkitadze MD, Condron MM, Teplow DB (2001) Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis. *J Mol Biol* **312**(5): 1103–19.

Krumova P, Meulmeester E, Garrido M, Tirard M et al. and Weishaupt JH (2011) Sumoylation inhibits alpha-synuclein aggregation and toxicity. *J Cell Biol* **194**(1): 49–60.

Lee L, Dale E, Staniszewski A, Zhang H et al. and Arancio O (2014) Regulation of synaptic plasticity and cognition by SUMO in normal physiology and Alzheimer's disease. *Sci Rep* **4**: 7190.

Lee VM, Goedert M, Trojanowski TQ (2001) Neurodegenerative Tauopathies. *Annu Rev Neurosci.* **24**: 1121–59.

Li H, Li SH, Johnston H, Shelbourne PF, Li XJ (2000) Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity. *Nat Genet* **25**(4): 385–89.

Li Y, Wang H, Wang S, Quon D, Liu YW, Cordell B (2003) Positive and negative regulation of APP amyloidogenesis by sumoylation. *Proc Natl Acad Sci U S A* **100**(1): 259–64.

Lin X, Liang M, Liang YY, Brunicardi FC, Feng XH (2003) SUMO-1/Ubc9 promotes nuclear accumulation and metabolic stability of tumor suppressor Smad4. *J Biol Chem* **278**(33): 31043–48.

Liu S, Ninan I, Antonova I, Battaglia F et al. and Arancio O (2004) Alpha-synuclein produces a long-lasting increase in neurotransmitter release. *EMBO J* **23**(22): 4506–16.

Luo HB, Xia YY, Shu XJ, Liu ZC et al. and Wang JZ (2014) SUMOylation at K340 inhibits tau degradation through deregulating its phosphorylation and ubiquitination. *Proc Natl Acad Sci U S A* **111**(46): 16586–91.

Ma J, Lindquist S (2002) Conversion of PrP to a self-perpetuating PrPSc-like Conformation in the Cytosol. *Science* **298**(5599): 1785–88.

Ma J, Wollmann R, Lindquist S (2002) Neurotoxicity and Neurodegeneration when PrP accumulates in the cytosol. *Science* **298**(5599): 1781–85.

MacDonald ME, Barnes G, Srinidhi J, Duyao MP et al. and Penney J (1993) Gametic but not somatic instability of CAG repeat length in Huntington's disease. *J Med Genet* **30**(12): 982–86.

Mandelkow EM, Mandelkow E (2012) Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Med* **2**(7): a006247.

Martins WC, Tasca CI, Cimarosti H (2016) Battling Alzheimer's Disease: Targeting SUMOylation-Mediated Pathways. *Neurochem Res* **41**(3): 568–78.

Matic I, Van Hagen M, Schimmel J, Macek B et al. and Vertegaal ACO (2008) In vivo identification of human small ubiquitin-like modifier polymerization sites by high accuracy mass spectrometry and an in vitro to in vivo strategy. *Mol Cell Proteomics* **7**(1): 132–44.

Matsuzaki S, Lee L, Knock E, Srikumar T et al. and Fraser PE (2015) SUMO1 Affects synaptic function, spine density and memory. *Sci Rep* **5**: 10730.

Mattson MP (2004) Pathways towards and away from Alzheimer's disease. *Nature* **430**(7000): 631–39.

Mayeux R, Stern Y (2012) Epidemiology of Alzheimer disease. *Cold Spring Harb Perspect Med* **2**(8).

McKinley MP, Meyer RK, Kenaga L, Rahbar F, Cotter R, Serban A, Prusiner SB (1991) Scrapie prion rod formation in vitro requires both detergent extraction and limited proteolysis. *J Virol* **65**(3): 1340–51.

McMillan LE, Brown JT, Henley JM, Cimarosti H. (2011) Profiles of SUMO and ubiquitin conjugation in an Alzheimer's disease model. *Neurosci Lett* **502**(3): 201–8.

Meier P, Genoud N, Prinz M, Maissen M et al. and Aguzzi A (2003) Soluble dimeric prion protein binds PrP(Sc) in vivo and antagonizes prion disease. *Cell*

113(1): 49–60.

Meyer RK, McKinley MP, Bowman KA, Braunfeld MB, Barry RA, Prusiner SB (1986) Separation and properties of cellular and scrapie prion proteins. *Proc Natl Acad Sci U S A* **83**(8): 2310–14.

Mucke L, Selkoe DJ (2012) Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med* **2**(7): a006338.

Mullan M, Crawford F, Axelman K, Houlden H, Lilius L, Winblad B, Lannfelt L (1992) A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-Terminus of Beta-amyloid. *Nat Genet.* **1**(5): 345–47.

Nakamura T, Cho DH, Lipton SA (2012) Redox regulation of protein misfolding, mitochondrial dysfunction, synaptic damage, and cell death in neurodegenerative diseases. *Exp Neurol* **238**(1): 12–21.

Nazor K, Seward T, Telling GC (2007) Motor behavioral and neuropathological deficits in mice deficient for normal prion protein expression. *Biochim Biophys Acta* **1772**(6): 645–53.

Niikura T, Kita Y, Abe Y (2014) SUMO3 Modification accelerates the aggregation of ALS-linked SOD1 mutants. *PloS One* **9**(6): e101080.

Nisticò R, Pignatelli M, Piccinin S, Mercuri NB, Collingridge G (2012) Targeting synaptic dysfunction in Alzheimer's disease therapy. *Mol Neurobiol* **46**(3): 572–87.

Nisticò R, Ferraina C, Marconi V, Blandini F, Negri L, Egebjerg J, Feligioni M (2014) Age-related changes of protein SUMOylation balance in the AβPP Tg2576 mouse model of Alzheimer's disease. *Front Pharmacol* **5**: 63.

Noel A, Barrier L, Ingrand S (2016) The Tyr216 phosphorylated form of GSK3β contributes to tau phosphorylation at PHF-1 epitope in response to Aβ in the nucleus of SH-SY5Y cells. *Life Sci* **158**: 14–21.

O'Rourke JG, Gareau JR, Ochaba J, Song W et al. and Thompson LM (2013) SUMO-2 and PIAS1 Modulate insoluble mutant huntingtin protein accumulation. *Cell Rep* **4**(2): 362–75.

Oh Y, Kim YM, Mouradian MM, Chung KC (2011) Human polycomb protein 2 promotes α-synuclein aggregate formation through covalent SUMOylation. *Brain Res* **1381**: 78–89.

Pajak B, Songin M, Strosznajderv JB, Gajkowska B (2009) Alzheimer's disease genetic mutation evokes ultrastructural alterations: correlation to an intracellular abeta deposition and the level of GSK-3beta-P(Y216) phosphorylated form. *Neurotoxicology* **30**(4): 581–88.

Pan KM, Baldwin M, Nguyen J, Gasset M et al. and Cohen FE (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci U S A* **90**(23): 10962–66.

Parakh S, Spencer DM, Halloran MA, Soo KY, Atkin JD (2013) Redox regulation in amyotrophic lateral sclerosis. *Oxid Med Cell Longev* **2013**: 408681.

Pennuto M, Palazzolo I, Poletti A (2009) Post-translational modifications of expanded polyglutamine proteins: impact on neurotoxicity. *Hum Mol Genet* **18**(R1): R40–47.

Poirier MA, Li H, Macosko J, Cai S, Amzel M, Ross CA (2002) Huntingtin spheroids and protofibrils as precursors in polyglutamine fibrilization. *J Biol Chem* **277**(43): 41032–37.

Pountney DL, Voelcker NH, Gai WP (2005) Annular Alpha-synuclein oligomers are potentially toxic agents in alpha-synucleinopathy. Hypothesis. *Neurotox Res* **7**(1-2): 59–67.

Prusiner SB (1982) Novel Proteinaceous infectious particles cause scrapie. *Science* **216**(4542): 136–44.

Prusiner SB, Groth D, Serban A, Koehler R et al. and DeArmond SJ (1993) Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc Natl Acad Sci U S A* **90**(22): 10608–12.

Richt JA, Kasinathan P, Hamir AN, Castilla J et al. and Kuroiwa Y (2007) Production of cattle lacking prion protein. *Nat Biotechnol* **25**(1): 132–38.

Ross CA (1995) When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* **15**(3): 493–96.

Ross CA, Poirier MA (2004) Protein aggregation and neurodegenerative disease. *Nat Med* **10** Suppl: S10–17.

Routtenberg A, Rekart JL (2005) Post-Translational protein modification as the substrate for long-lasting

memory. *Trends Neurosci* **28**(1): 12–19.

Russell CL, Koncarevic S, Ward MA (2014) Post-translational modifications in Alzheimer's disease and the potential for new biomarkers. *J Alzheimers Dis* **41**(2): 345–64.

Safar J, Roller PP, Gajdusek DC, Gibbs CJ (1993) Conformational Transitions, dissociation, and unfolding of scrapie amyloid (prion) protein. *J Biol Chem* **268**(27): 20276–84.

Saitoh H, Hinchey J (2000) Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. *J Biol Chem* **275**(9): 6252–58.

Sarge KD, Park-Sarge OK (2009) Sumoylation and human disease pathogenesis. *Trends Biochem Sci* **34**(4): 200–205.

Schweers O, Schönbrunn-Hanebeck E, Marx A, Mandelkow E (1994) Structural studies of tau protein and Alzheimer paired helical filaments show no evidence for beta-structure. *J Biol Chem* **269**(39): 24290–97.

Selenica MLB, Brownlow M, Jimenez JP, Lee DC et al. and Morgan D (2013) Amyloid oligomers exacerbate tau pathology in a mouse model of tauopathy. *Neurodegener Dis* **11**(4): 165–81.

Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* **81**(2): 741–66.

Shahpasandzadeh H, Popova B, Kleinknecht A, Fraser PE, Outeiro TF, Braus GH (2014) Interplay between SUMOylation and phosphorylation for protection against α-synuclein inclusions. *J Biol Chem* **289**(45): 31224–40.

Shimura H, Schwartz D, Gygi SP, Kosik KS (2004) CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. *J Biol Chem* **279**(6): 4869–76.

Sipione S, Rigamonti D, Valenza M, Zuccato C et al. and Cattaneo E (2002) Early Transcriptional Profiles in huntingtin-inducible striatal cells by microarray analyses. *Hum Mol Genet* **11**(17): 1953–65.

Sivanandam VN, Jayaraman M, Hoop CL, Kodali R, Wetzel R, van der Wel PCA (2011) The Aggregation-enhancing huntingtin N-terminus is helical in amyloid fibrils. *J Am Chem Soc* **133**(12): 4558–66.

Son M, Cloyd CD, Rothstein JD, Rajendran B, Elliott JL (2003) Aggregate formation in Cu,Zn superoxide dismutase-related proteins. *J Biol Chem* **278**(16): 14331–36.

Sparkes RS, Simon M, Cohn VH, Fournier RE et al. and Mohandas T (1986) Assignment of the human and mouse prion protein genes to homologous chromosomes. *Proc Natl Acad Sci U S A* **83**(19): 7358–62.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in lewy bodies. *Nature* **388**(6645): 839–40.

Steffan JS, Agrawal N, Pallos J, Rockabrand E et al. and Marsh JL (2004) SUMO modification of huntingtin and Huntington's disease pathology. *Science* **304**(5667): 100–104.

Takahashi K, Ishida M, Komano H, Takahashi H (2008) SUMO-1 Immunoreactivity co-localizes with phospho-tau in APP Transgenic mice but not in mutant tau transgenic mice. *Neurosci Lett* **441**(1): 90–93.

Takashima A, Honda T, Yasutake K, Michel G et al. and Yamaguchi H (1998) Activation of tau protein kinase I/glycogen synthase kinase-3beta by amyloid beta peptide (25-35) enhances phosphorylation of tau in hippocampal neurons. *Neurosci Res* **31**(4): 317–23.

Takeuchi H, Kobayashi Y, Ishigaki S, Doyu M, Sobue G (2002) Mitochondrial localization of mutant superoxide dismutase 1 triggers caspase-dependent cell death in a cellular model of familial amyotrophic lateral sclerosis. *J Biol Chem* **277**(52): 50966–72.

Tatham MH, Matic I, Mann M, Hay RT (2011) Comparative Proteomic analysis identifies a role for SUMO in protein quality control. *Sci Signal* **4**(178): rs4.

Tempé D, Piechaczyk M, Bossis G (2008) SUMO under stress. *Biochem Soc Trans* **36**(Pt 5): 874–78.

Tenreiro S, Eckermann K, Outeiro TF (2014) Protein Phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci* **7**: 42.

Terwel D, Muyllaert D, Dewachter I, Borghgraef P, Croes S, Devijver H, Van Leuven F (2008) Amyloid activates GSK-3beta to aggravate neuronal tauopathy in bigenic mice. *Am J Pathol* **172**(3): 786–98.

Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N et al. and Steffan JS (2009) IKK Phosphorylates huntingtin and targets it for degradation by the proteasome and

lysosome. *J Cell Biol* **187**(7): 1083–99.

Tobler I, Gaus SE, Deboer T, Achermann P et al. and Manson JC (1996) Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* **380**(6575): 639–42.

Toneff T, Mende-Mueller L, Wu Y, Hwang SR et al. and Hook V (2002) Comparison of huntingtin proteolytic fragments in human lymphoblast cell lines and human brain. *J Neurochem* **82**(1): 84–92.

Vijayakumaran S, Wong MB, Antony H, Pountney DL (2015) Direct And/or Indirect Roles for SUMO in Modulating Alpha-Synuclein Toxicity. *Biomolecules* **5**(3): 1697–1716.

Wang JZ, Grundke-Iqbal I, Iqbal K (2007) Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur J Neurosci* **25**(1): 59–68.

Watanabe M, Dykes-Hoberg M, Culotta VC, Price DL, Wong PC, Rothstein JD (2001) Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. *Neurobiol Dis* **8**(6): 933–41.

Weeraratna AT, Kalehua A, Deleon I, Bertak D et al. and Taub DD (2007) Alterations in immunological and neurological gene expression patterns in Alzheimer's disease tissues. *Exp Cell Res* **313**(3): 450–61.

Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT (1996) NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* **35**(43): 13709–15.

Wells GA, Scott AC, Johnson CT, Gunning RF et al. and Bradley R (1987) A novel progressive spongiform encephalopathy in cattle. *Vet Rec* **121**(18): 419–20.

Williams ES, Young S (1980) Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *J Wildl Dis* **16**(1): 89–98.

Wogulis M, Wright S, Cunningham D, Chilcote T, Powell K, Rydel RE (2005) Nucleation-dependent polymerization is an essential component of amyloid-mediated neuronal cell death. *J Neurosci* **25**(5): 1071–80.

Wyatt J, Pearson G, Smerdon T, Gruffydd-Jones T, Wells G, Wilesmith J. (1991) Naturally Occurring scrapie-like spongiform encephalopathy in five domestic cats. *Vet Rec* **129**(11): 233–36.

Xie W, Li X, Li C, Zhu W, Jankovic J, Le W (2010) Proteasome Inhibition modeling nigral neuron degeneration in Parkinson's disease. *J Neurochem* **115**(1): 188–99.

Yang QG, Wang F, Zhang Q, Xu WR, Chen YP, Chen GH (2012) Correlation of increased hippocampal sumo3 with spatial learning ability in old C57BL/6 mice. *Neurosci Lett* **518**(2): 75–79.

Yedidia Y, Horonchik L, Tzaban S, Yanai A, Taraboulos A (2001) Proteasomes and ubiquitin are involved in the turnover of the wild-type prion protein. *EMBO J* **20**(19): 5383–91.

Yuan BZ, Chapman JA, Reynolds SH (2008) Proteasome inhibitor MG132 induces apoptosis and inhibits invasion of human malignant pleural mesothelioma cells. *Transl Oncol* **1**(3): 129–40.

Yun SM, Cho SJ, Song JC, Song SY et al. and Koh YH (2013) SUMO1 modulates Aβ generation via BACE1 accumulation. *Neurobiol Aging* **34**(3): 650–62.

Zarei S, Carr K, Reiley L, Diaz K et al. and Chinea A (2015) A comprehensive review of amyotrophic lateral sclerosis. *Surg Neurol Int* **6**: 171.

Zhang YQ, Sarge KD (2008) Sumoylation of amyloid precursor protein negatively regulates abeta aggregate levels. B*iochem Biophys Res Commun* **374**(4): 673–78.

Zheng H, Koo EH (2006) The amyloid precursor protein: beyond amyloid. *Mol Neurodegener* **1**: 5.

Zheng Z, Li A, Holmes BB, Marasa JC, Diamond MI (2013) An N-terminal nuclear export signal regulates trafficking and aggregation of huntingtin (Htt) protein exon 1. *J Biol Chem* **288**(9): 6063–71.

Zheng Z, Diamond MI (2012) Huntington disease and the huntingtin protein. *Prog Mol Biol Transl Sci* **107**: 189–214.

ALZHEIMER'S DISEASE AND DEPRESSION: TWO SIDES OF THE SAME COIN?

M.G. MORGESE[1], L. TRABACE[1] and V. CUOMO[2]

[1]*Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy*
[2]*Department of Physiology and Pharmacology, "Sapienza", University of Rome, Rome, Italy*

Recent evidence indicates that the prevalence of depression, as well as Alzheimer's disease (AD), has reached epidemic proportions in the last decades. Chronic stress is considered a widely accepted risk factor for the development of both depressive symptoms and AD pathology. Indeed, high cortisol levels, and thus hypothalamic pituitary adrenal (HPA)-axis hyperactivity, have been indicated as the most frequent alteration in patients affected by depression and AD. Furthermore, depressive state has been pointed as an early manifestation of AD, advocating an overlap between these neuropathological events. In this regard, we have demonstrated that central soluble beta amyloid 1-42 (Aβ) peptide induces a depressive-like behavior in rats, with altered HPA axis activation, reduced cortical serotonin and neurotrophin levels. Alterations in monoamine content is a common feature of these neuropathologies. In addition, neuroinflammation and microglial activation, as well as altered oxidative homeostasis, appear as other potential causal factors to explain the AD-depression link. Thus, the main aim of the present review is to summarize biological substrates and pathological pathways common to depressive state and AD, widely accepted as co-morbid diseases.

Increasing evidence has linked depressive state to the development of neurological disorders such as Alzheimer's disease (AD). In this regard, depression has been indicated as a risk factor for AD (Ownby et al, 2006; Sun et al, 2008). Indeed, depression is common in pre-clinical AD, and may represent an early manifestation of this disease before the appearance of cognitive impairments (Geerlings et al, 2000; Visser et al, 2000). In particular, subjects with mild cognitive impairment and depression have more than twice the risk of developing Alzheimer-type dementia than non-depressed patients (Modrego and Ferrandez, 2004). It has been reported that neuropsychiatric symptoms may represent prodromal symptoms of dementia deriving from neurobiological changes in specific cerebral regions, thus not simply an emotional reaction to the awareness of the dementing diseases (Andersen et al, 2005). Soluble forms of beta amyloid peptide (Aβ) have been implicated in the development of early memory deficits and neuropsychiatric symptoms (Rowan et al, 2005). Indeed, it has been hypothesized that the insoluble amyloid plaques may represent an early mechanism responsible for the sequestering of small, diffusible conglomerate of Aβ peptide reported to cause impairment in synaptic structure and function (Walsh and Selkoe, 2007). Accordingly, in the early stages of AD, significant cognitive deficits have been directly attributed to soluble Aβ fragments (Cleary et al, 2005; Mattson, 2004) and increased levels of soluble Aβ oligomers caused synaptic dysfunction (Hardy and Selkoe, 2002; Selkoe and Schenk,

Key words: Alzheimer's disease, depression, noradrenaline, serotonin, beta amyloid

Corresponding author:
Maria Grazia Morgese, Ph.D.
Dept. of Clinical and Experimental Medicine
University of Foggia
Via Napoli, 20
71121 Foggia, Italy
Tel.: +39 0881 588052
e-mail: mariagrazia.morgese@unifg.it

2279-5855 (2016)

2003), endorsing the hypothesis that increased central soluble Aβ levels may also be implicated in the development of non-cognitive AD symptoms. In line with these findings, we previously demonstrated that soluble Aβ, intracerebrally injected in rats, is able to generate a depressive-like state accompanied by reduction in serotonin (5-HT) and neurotrophin levels (Colaianna et al, 2010) and altered hypothalamic pituitary adrenal (HPA) axis response and noradrenaline (NA) levels (Morgese et al, 2014). It has been hypothesized that cognitive decline and mood alterations share common neuropathological mechanisms leading to increased vulnerability of depressed patients versus AD pathology (Aznar and Knudsen, 2011). Alterations in monoamine content and increased susceptibility to stress are common features of both AD and depression, as well as alteration in neuroinflammatory and pro-oxidative states. The aim of the present review was to summarize the main knowledge in regard to biological and pathological substrates commonly shared between AD and depression with the scope of shedding light on new therapeutic approaches.

AMYLOIDOGENIC AND NON-AMYLOIDOGENIC PATHWAYS

Amyloid β is the main component of the amyloid plaques, one of the neuropathological hallmarks of AD (Di Carlo et al, 2012). However, mounting evidence suggests that Aβ, besides its well-accepted neurotoxic activity, may hold physiological roles (Hardy and Selkoe, 2002). This peptide, in its soluble form, is produced and secreted during normal cellular activity and has been shown to modulate synaptic activity without overt signs of neurotoxicity (Grilli et al, 2010; Mura et al, 2010; Preda et al, 2008). Physiologically, the neuromodulatory role of Aβ would be important for the correct functioning of neurotransmitter systems; however, in pathological conditions, Aβ-modulating synaptic activity could trigger functional alterations on neurotransmission (Mura et al, 2012). The Aβ peptide derives from the sequential proteolytic cleavage of amyloid precursor protein (APP) by β-site APP cleaving enzyme 1 (BACE1), known as β-secretase, and γ−secretase, a multi-subunit protease complex comprised of proteins such as presenilin1 or 2 (Cole and Vassar, 2007; Hardy and Selkoe, 2002). The action of β-secretase on APP leads to the formation of a shorter fragment β-secretase-generated C-terminal APP, known as β-CTF, that is a substrate for γ−secretase complex, which activity results in the production of Aβ peptide and APP intracellular domain (known as AICD). This pathway is recognized as the amyloidogenic pathway associated to synaptic loss (Grabrucker et al, 2011). APP can also be metabolized by α-secretase, thus producing α-CTF that γ−secretase transforms into AICD and p3 peptides (Chow et al, 2010). This pathway is the non- amyloidogenic pathway since α-secretase cuts APP in the Aβ region, thus impeding its production (Chow et al, 2010). This pathway has been reported to be neurotrophic and neuroprotective (Chow et al, 2010), thus many investigations have been steered at individuating the mechanism that can move APP processing towards α-secretase-mediated derivatives, as discussed below.

MONOAMINES IN DEPRESSION AND AB PRODUCTION

The cathecolaminergic theory of affective disorders was first presented in 1965 by Schildkraut (Schildkraut, 1995), who stated the crucial role of lower central NA availability in the insurgence of depression. Later, in 1995 the contribution of 5-HT became evident, with the discovery that deficiency of brain serotonergic activity leads to increased vulnerability to stress (Maes and Meltzer, 1995; Mann, 1999). Although such initial monoaminergic theory has been revised over time, available pharmacological treatment options are still mainly based on central NA and 5-HT enhancement. Alterations in these neurotransmitter systems have also been reported in AD and, in particular, Aβ has been shown to modulate and to be modulated by these systems. In this regard, we previously found that soluble Aβ is able to reduce 5-HT in the prefrontal cortex, but not in hippocampus or nucleus accumbens, of rats receiving a single intracerebroventricular (icv) injection 7 days earlier (Colaianna et al, 2010). In good agreement, impairment of the serotonergic

system has been reported in the very early stages of AD (Egashira et al, 2005; Kepe et al, 2006; Versijpt et al, 2003). Moreover, it is well accepted that impaired 5-HT neurotransmission in the prefrontal area is central to both depressive disorders (Krishnan and Nestler, 2008) and several neurodegenerative diseases (Egashira et al, 2005; Mattson, 2004). Indeed, post-mortem and clinical evidence indicated substantial disruption of the serotonergic system in AD (Lanctot et al, 2001; Morgan et al, 1987). Furthermore, the risk of developing AD is higher in individuals with a history of depression (Kessing and Andersen, 2004). On the other hand, post-mortem studies performed on AD patients revealed low 5-HT and relative receptors content (Reynolds et al, 1995). It has been shown that the overexpression of the APP gene containing the Swedish mutations causing familial AD, an *in vitro* model of familiar AD, can affect the sensitivity of the 5-HT_{1B} receptor subtype and related systems (Tajeddinn et al, 2016). Moreover, clinical evidence revealed that serotonin-selective re-uptake inhibitors (SSRI) significantly improve depressant symptoms and daily activities in AD patients (Werner and Covenas, 2015). Interestingly, it has been shown that serotonergic system activation is able to negatively modulate interstitial Aβ content. Indeed, it has been reported in transgenic animal models of AD that enhancing of 5-HT signalling, including after administration of SSRI antidepressants, rapidly reduced Aβ production *in vivo* via activation of extracellular regulated kinase (ERK) (Cirrito et al, 2011; Fisher et al, 2016). Furthermore, data originating from PET imaging study performed in cognitively normal individuals and data from retrospective analysis on antidepressant use indicated that increased 5-HT signalling correspond to lower Aβ accumulation (Sheline et al, 2014; Vlassenko et al, 2011). In further agreement, 5-HT_4, 5-HT_6, and 5-HT_7 receptor activation leads to reduced interstitial Aβ and simultaneous pharmacological blockade of the two receptor subtypes, 5-HT_4 and 5-HT_7, prevented such an effect (Cho and Hu, 2007; Fisher et al, 2016). Interestingly, the expression of these two receptor subtypes has been shown to be reduced with aging (Rodriguez et al, 2012). Molecules acting as agonists on 5-HT_4 receptor subtype hold antidepressant properties, as demonstrated in different depression animal models and paradigms, while reduced serotonergic tone results in a depressive-like profile (Lucas et al, 2007; Zotti et al, 2013). In further agreement, genetic depletion of these receptors in mice leads to reduced serotonergic spontaneous electrical activity and lower content of 5-HT and relative metabolite, such as 5-hydroxyindol acetic acid (Conductier et al, 2006). Moreover, pharmacological activation of 5-HT_4 receptors resulted in enhanced short and long-term memory function (Meneses, 2007) and increased population amplitude spike in the CA1 region of hippocampus also in transgenic AD model (Spencer et al, 2004), suggesting that molecules acting on this target may result very useful either in depression or AD. On the other hand, 5-HT stimulates APP release by activating 5-HT_{2A} and 5-HT_{2C} receptor subtypes. Furthermore, polymorphisms in gene encoding for 5-HT_{2A} receptors, whose binding reduction has been reported in AD brains (Versijpt et al, 2003), have been described in AD patients affected by major depression (Holmes et al, 2003). Antagonists of 5-HT_{2A} receptors have been shown to induce antidepressant effects (Patel et al, 2004), and co-administration of these compounds with SSRI has been proposed in order to obtain a synergistic effect (Patel et al, 2004). In addition, transgenic mice deleted of 5-HT_{2A} receptors display an altered phenotype with symptoms correlated to depression (Popa et al, 2005). In regard to 5-HT_{2C} receptors, both agonists and antagonists have been proposed for their antidepressant activity (Cryan and Lucki, 2000; Cryan et al, 2005; Steardo et al, 2000). In addition, targeting 5-HT_6 receptors is a novel therapeutic strategy that is being pursued in AD, and a randomized, double-blind, placebo-controlled study aimed at investigating the efficacy and tolerability of the 5-HT_6 receptor antagonist, SB-742457, in subjects with mild-to-moderate and probable AD revealed a safe profile and possible utility in improving cognitive symptoms of AD (Maher-Edwards et al, 2010). However, antagonists of these receptor subtypes have been indicated as useful also in the treatment of non-cognitive symptoms associated to AD (Garcia-Alloza et al,

2004). Polymorphism of 5-HT_6 receptors has been associated to altered response to antidepressant treatment in major depressive disorder (Lee et al, 2005), although contrasting results have been reported (Wu et al, 2001).

In order to highlight the mechanism through which increased 5-HT signalling may reduce Aβ production, it has been shown that blockade of PKA, a downstream signalling arising also after 5-HT receptor activation, can prevent the Aβ lowering effect of SSRI and 5-HT4, 5-HT 6, 5-HT7 agonists, indicating the crucial role of this kinase (Fisher et al, 2016). Moreover, the same Authors found that the reduction in interstitial Aβ induced by SSRI was mediated via α secretase-related mechanisms (Fisher et al, 2016). The activity of this enzyme has been shown to be modulated either by PKA (Efthimiopoulos et al, 1996; Robert et al, 2001; Xu et al, 1996), or by other kinases such as PKC (Buxbaum et al, 1990; Caporaso et al, 1992), ERK or phosphatidyl inositol-3 kinase (Mills et al, 1997; Solano et al, 2000). This enzymatic machinery is shared by other neurotransmitters, such as NA, reported to be interconnected to 5-HT and to be implicated either in depression or AD. In fact, serotonergic system functioning is strictly linked to the noradrenergic system. It has been proposed that these systems control each other via heteroceptors. In particular, inhibitory α_2 receptors (α_2AR) are present at 5-HT terminal levels, while 5-HT_3 receptors are localized at NA terminals. Thus, a negative feedback has been hypothesized, considering that increased 5-HT levels correspond to NA release, which in turn inhibits further 5-HT release via α_2AR activation (Mongeau et al, 1997). Increased α_2AR have been found in post-mortem brains of depressed patients (Meana et al, 1992; Ordway et al, 1994) and a theory of α_2AR supersensitivity in depression was early postulated (Charney et al, 1981). Upregulation of α_2AR density was demonstrated by using radiolabeled agonists in patients with depressive disorders, indicating a selective increase in the density of high-affinity conformational state α_2AR, which can suggest enhanced G protein coupling to the receptor (Cottingham and Wang, 2012). The antidepressant effect of tricyclic compounds has been linked to their ability to bind α_2AR and to function as arrestin-based ligands (Cottingham et al, 2015). B-arrestins, a small family of regulators of G protein- coupled receptors, are responsible for desensitization or internalization of such receptors along with initiation of their own signalling (Jiang et al, 2013). Such α_2AR/arrestin recruitment leads to receptor endocytosis and downregulation of this receptor expression after long–term use (Cottingham et al, 2015). These effects have been reported for several antidepressant compounds of this class (Cottingham et al, 2014), with some differences. In particular, for amitriptyline, an interaction around 14-fold stronger with the receptor has been reported, and it is has been described as a weaker driver of arrestin recruitment, and it preferentially recruits a different arrestin subtype, while imipramine uniquely holds a slight ability to drive α2AR endocytosis in arrestin-null cells (Cottingham et al, 2014). This molecular arrestin-related mechanism has been evidenced also for desipramine (Cottingham et al, 2011). Profound alterations in the NA system have also been reported in early AD (Haglund et al, 2006) and it has been found that α2AR activation promotes amyloidogenesis by disrupting the cellular mechanism that prevents deleterious APP processing (Chen et al, 2014). Furthermore, targeting β-arrestin is a novel field of research for early treatment of AD. High levels of β-arrestin 1 were found in post-mortem AD brains, as well as in transgenic animal model of AD (Liu et al, 2013). β-arrestin signalling complex has also been suggested as a target for antidepressants and as a depression marker (Schreiber et al, 2009). In addition, an overexpression of β-arrestin 2 in AD patients has also been reported. Such overexpression leads to increased Aβ production while silencing the gene encoding for β-arrestin 2, either in transgenic animal model or cell cultures, reduces Aβ generation by regulating ɣ- secretase activity (Thathiah et al, 2013). In further agreement, deletion of β-arrestin-2 was reported of being protective toward loss of dendritic spine mediated by Aβ in hippocampal neurons (Pontrello et al, 2012). Many reports have indicated that Aβ directly interacts with the noradrenergic system and, in particular, it has been found that Aβ directly binds to β-adrenergic

receptors (Igbavboa et al, 2006; Wang et al, 2011). Wang and Colleagues (2011) demonstrated, *in vitro*, that Aβ may cause desensitization and subsequently internalization of β2 adrenergic receptors in prefrontal cortical neurons (Wang et al, 2011) and the internalization process was dependent on adaptor protein arrestin 3 and GTPase dynamin, but not arrestin 2. Therefore, these findings indicate that many mechanisms are possibly occurring in the interaction between Aβ and noradrenergic receptors. Furthermore, β2 adrenergic receptor activation has been shown to mediate Aβ-induced phosphorylation of tau both through *in vivo* and *in vitro* studies (Wang et al, 2013). Ultimately, polymorphisms in the gene encoding for β2 adrenergic receptor have been associated with increased risk of developing sporadic late onset AD (Yu et al, 2008).

Moreover, still confirming the dual role of noradrenergic system in depression and AD, alterations in β adrenergic receptors have also been reported in depressed patients (Mann et al, 1986). Accordingly, it has been reported that activation of central β adrenergic receptors, either type 1, 2 or 3, leads to antidepressant effects (Gu et al, 2012; Overstreet et al, 2008; Zhang et al, 2003).

With regard to the dopaminergic system, impairment of its neurotransmission has been implicated in many diseases, including depression (Schmidt et al, 2001), and several preclinical studies have indicated the involvement of dopaminergic receptors, either D1, D2 or D3, in antidepressant effects (Pytka et al, 2016). In particular, it was shown that prefrontal cortical and hippocampal areas of AD patients were characterized by lower DA receptor expression, either D1-like or D2-like subtypes (Kemppainen et al, 2003; Kumar and Patel, 2007). The nucleus accumbens was reported to be highly affected in AD, since the expression of D2-like receptors, dopaminergic transporter and tyrosine hydroxylase enzyme were found altered in this brain region (Allard et al, 1990; Joyce et al, 1997; Murray et al, 1995; Rinne et al, 1986). In this regard, atrophy of this nucleus was evidenced in imaging studies in a cohort of late, but not early, onset AD patients (Pievani et al, 2013). In further agreement, we have previously shown that an acute icv injection of Aβ blunted dopamine (DA) release in the prefrontal cortical area (Trabace et al, 2007), and it was found that acute Aβ administration disrupted the cholinergic control of DA release particularly in the nucleus accumbens (Preda et al, 2008). In addition, in AD patients, drugs able to increase dopaminergic function resulted in improved cortical neurotransmission and synaptic plasticity, as revealed by electrophysiological findings, as well as in improved cognitive performances (Koch et al, 2014; Martorana et al, 2013; Martorana et al, 2009). Thus, even if drugs acting on the dopaminergic system play a marginal role in the treatment of AD and depression, this still remains a field open for future investigations.

CHRONIC STRESS AND NEUROINFLAMMATION: A BRIDGE BETWEEN DEPRESSION AND AD

Chronic stress is considered a widely accepted risk factor for the development of depressive symptoms. Indeed, high cortisol levels, and thus HPA-axis hyperactivity, have been indicated as the most frequent alteration occurring in patients affected by major depressive disorder (Stetler and Miller, 2011). However, in atypical depression, also HPA axis hypo-activity has been reported (Gold et al, 2002). Among molecular mechanisms proposed to explain HPA-dysregulation and depression insurgence, alteration in neurotrophin levels has been proposed. In particular, low brain derived neurotrophic factor (BDNF) levels have been described after prenatal stress (Boersma et al, 2014). Glucocorticoids have been related to such an effect, since corticosterone is able to down-regulate both mRNA and protein BDNF content (Schaaf et al, 1998). In this light, mice over-expressing glucocorticoids showed an increased anxiety-like behavior (Sarrazin et al, 2009). It has been proposed that 5-HT depletion in depression can be related to shifting the conversion of tryptophan (TRP), the 5-HT precursor, into kynurenine (KYN) instead of to 5-HT (Oxenkrug, 2013; Oxenkrug et al, 2013). On the other hand, peripherally released glucocorticoids have been shown to induce tryptophan

2,3-dioxygenase (TDO) responsible for converting TRP into KYN later converted into kynurenic acid (KYNA). In addition, stress can lead to KYN production at central level by increasing pro-inflammatory cytokines, such as interleukin (IL)1-β and IL-6, as well as tumor necrosis factor (TNF)-α, that have been shown to induce another enzyme, indolamine 2,3-dioxygenase (IDO) (O'Farrell and Harkin, 2015). In addition, stress-induced corticotrophin releasing factor (CRF) release seems strictly implicated in enhancing cytokine circulating levels derived from activated macrophages either centrally or peripherally (Leonard and Song, 2002). Moreover, 5-HT has an inhibitory effect on glucocorticoids production by acting at amygdaloid complex, such deficiency would result in a lack of HPA axis inhibition, thus prompting a further 5-HT reduction (Oxenkrug, 2013). In this light, antidepressant treatment with fluoxetine has been shown to attenuate neuroinflammation (Shan et al, 2016; Valera et al, 2014; Zhang et al, 2012). Interestingly, activation of immune response by Poly I:C administration in rats was reported to induce depressive behavior accompanied by decreased BDNF levels in frontal cortex and activated KYN pathway (Gibney et al, 2013). Moreover, TRP metabolism at microglial level leads to KYN metabolites with reactive oxidative and toxic properties (Guillemin et al, 2005; Guillemin et al, 2003a). Microglial dysfunction has been proposed as a link between depression and AD (Santos et al, 2015). In this regard, depression is a highly comorbid condition in AD and mild cognitive impairment (Apostolova and Cummings, 2008; Steinberg et al, 2008). However, some Authors have proposed that depression is not a risk factor for AD, but rather an early symptom (Panza et al, 2010; Richard et al, 2013). Accordingly, we demonstrated that the Aβ-induced depressive-like behavior was associated with reduced cortical 5-HT and BDNF levels and with altered HPA axis activation (Colaianna et al, 2010; Morgese et al, 2014). We have found that Aβ inhibits HPA axis and increases NA release in prefrontal cortex and hippocampus of rats, while at amigdaloid level NA content was reduced (Morgese et al, 2014). Accordingly, blunted acute HPA axis response was also evidenced in a transgenic AD model after immobilization stress, as well as chronically enhanced negative feedback on the axis (Justice et al, 2015). The central role of Aβ in response to stress is emerging. In particular, it has been reported that modern life-like stress and psychosocial-stress exacerbate Aβ-pathology (Baglietto-Vargas et al, 2015; Srivareerat et al, 2009). Moreover, *in vivo* microdialysis experiments in a transgenic animal model of AD subjected to chronic stress revealed an increase in interstitial Aβ levels (Kang et al, 2007). The effect of acute restraint stress on Aβ seems mediated by higher neuronal/synaptic activity in the hippocampus and via CRF. Indeed, CRF seems to increase Aβ production by regulating ɣ-secretase activity, an enzyme involved in APP cleaving (Park et al, 2015). Consistent with this hypothesis, dysregulation of the HPA occurs early in AD, resulting in increased cortisol levels (Hoogendijk et al, 2006; Swaab et al, 2005; Swaab et al, 1994), and it has been shown that glucocorticoid administration (at concentrations mimicking stress response) enhances Aβ formation by increasing steady-state levels of APP and β-APP cleaving enzyme (Green et al, 2006). In addition, it has been reported that in a transgenic AD animal model, chronic isolation stress exacerbates Aβ plaque deposition (Dong et al, 2004) via enhanced corticosterone levels and increased expression of glucocorticoid and CRF1 receptors (CRFR1) (Dong et al, 2008). Accordingly, genetic increase of CRF release accelerates neuropathology and cognitive decline in an AD mouse model (Dong et al, 2012). Furthermore, chronic CRFR1 antagonists and blocking of PKA were able to revert the previously reported deleterious effects of chronic isolation stress, inducing lower corticosterone and Aβ levels in the same animal model (Dong et al, 2014). Chronic stress was shown to drive APP processing and to induce the amyloidogenic pathway and exogenous central Aβ administration triggers APP misprocessing, indicating that the actions of Aβ mimic those of stress and glucorticoids (Catania et al, 2009). In this regard, we recently reported that pre-, peri- and post-natal dietary conditions that lead to higher vulnerability to stress correspond to

increased plasma Aβ levels (Morgese et al, 2016). In our experience, activation of HPA axis, and thus increased NA levels, occurs very early after exogenous Aβ injection, as early as 2 hours after central administration, and is mediated through inducible nitric oxide synthase (iNOS) and central IL-1 receptors (Morgese et al, 2015). Astrocytic iNOS activation was shown to potentiate N-methyl D-aspartate (NMDA)-induced neurotoxicity (Hewett et al, 1994), and NOS modulation has been linked to antidepressant properties of drugs (Wegener et al, 2004). It has also been reported that NA can regulate glial activation (Braun et al, 2014). Furthermore, *in vitro* studies have evidenced a protective effect of NA toward toxicity Aβ-induced by increasing neurotrophic factor expression via activation of β adrenergic receptor signalling cascade (Counts and Mufson, 2010; Liu et al, 2015). Thus, we can hypothesize that the NA release, in our experimental conditions, may represent an early acute protective phenomenon occurring after Aβ induction of pro-inflammatory pathway that in condition of repetitive stressor stimuli, such as chronic stress, leads to HPA dysregulation. Furthermore, reduced NA concentration in locus coeruleus projecting areas facilitates the inflammatory reaction of microglial cells after Aβ exposure, thus impairing microglial migration and phagocytosis, thereby decreasing Aβ clearance (Heneka et al, 2010a; Heneka et al, 2010b). Interestingly, it has been reported that NA inhibits iNOS induction after inflammatory stimuli in astrocytes (Feinstein et al, 1993) and microglia (Dello Russo et al, 2004). In our preliminary data, we found that single icv injection of Aβ increased IL-1β at prefrontal level, and this increase is linked to a high rate of activated microglia. On the other hand, the KYN pathway could be involved taking into account the role of TRP metabolites on microglia. Indeed, it has been reported that a TRP metabolite, quinolinic acid, produced in microglial cells, can interact with NMDA receptors, ultimately resulting in excitotoxicity and neurotoxicity (Guillemin et al, 2005; Guillemin et al, 2003b) and Aβ was shown to increase IDO mRNA and quinolinic acid production in human microglia (Guillemin et al, 2003a). Accordingly, our data have recently demonstrated that Aβ injection leads to both increased cortical glutamate levels, as revealed by microdialysis technique, and memory impairment. Moreover, modulation of NMDA receptors with memantine prevented such behavioral outcome (Tucci et al, 2014). Intriguingly, pharmacological modulation of NMDA with memantine was suggested as therapeutic tool for treatment of depression either linked or not to AD (Berman et al, 2000; Reisberg et al, 2003).

OXIDATIVE STRESS IN DEPRESSION AND AB

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly produced in the processes of respiration and energy production. When the neural antioxidant defenses of the organism are inadequate, an imbalance between pro-oxidant and antioxidant processes occurs; free radicals accumulate, disturbing the physiological oxidative homeostasis and then leading to the so-called oxidative stress. The brain is the most vulnerable organ to oxidative damage due to its peculiar functions and structures (Li et al, 2013). Elevated ROS and RNS levels, such as oxygen and hydrogen peroxide-derived or nitric oxide-derived peroxynitrites, activate a series of neurobiological toxic events that ultimately lead to DNA, protein, carbohydrate and fatty acid damage with the production of species like 8-hydroxy-2'deoxyguanosine or 4-hydroxy-nonenal (Li et al, 2013). It has been proposed that oxidative stress can be considered as a dynamic continuum of brain response that, depending on internal and external conditions, can move toward aging, aging cognitive decline, depression, mild cognitive impairment, and ultimately to AD (Rodrigues et al, 2014). Concerning depression, reduced antioxidant capacity was found in a rat model of depression, since low levels of reduced glutathione, glutathione peroxidase and vitamin C were observed (Eren et al, 2007a). Further confirming these correlations, treatment with the NA and 5-HT re-uptake inhibitor venlafaxine was reported to revert the increased lipid peroxidation induced by experimental depressive-like behavior in rats (Eren et al, 2007b). Accordingly, duloxetine was recently shown to reduce oxidative stress in

the hippocampus and dorsal root ganglion of rats as well as fluoxetine (Demirdas et al, 2016). In a mice model of stress, reduced catalase and glutathione reductase activity in the hippocampus and in the cerebral cortex were demonstrated, indicating a change in the central antioxidant defense system (Moretti et al, 2012). Furthermore, in the same work, the Authors showed that antioxidant treatment and fluoxetine treatment both reverse depressive-like behavior and brain oxidative damage (Moretti et al, 2012). Likewise, consistent proof of the antioxidant effects of antidepressants support the hypothesis that their antioxidant properties can contribute to the improvement in clinical aspects (Bilici et al, 2001; Herken et al, 2007; Khanzode et al, 2003).

On the other hand, oxidative stress has been shown as an early feature of AD pathology (Aluise et al, 2010; Pallas et al, 2008; Singh et al, 2010) and researchers have extensively worked in this way in order to possibly elucidate pathogenetic mechanisms for AD (Ansari and Scheff, 2010; Gella and Durany, 2009; Gibson et al, 1999; Srikanth et al, 2011). Protein oxidation is a frequently hypothesized potential factor in the progression of AD (Bonet-Costa et al, 2016), along with mitochondrial impairment and oxidative stress, considering the role of mitochondria in regulating energy and oxygen metabolism (Adiele and Adiele, 2016). Mitochondrial defect hypothesis in the development of this neurological disorder has been supported by clinical findings (Reddy, 2007; Reddy, 2006). In fact, mitochondrial dysfunction has been linked to synaptic and neuronal stress in AD in either post-mortem studies or animal and cell models (Du et al, 2010; Moreira et al, 2010; Swerdlow and Khan, 2009). In this regard, a recent *in vitro* study showed the beneficial effect of mitochondria-targeted antioxidant treatment on Aβ-induced neuronal mitochondrial depolarization, cytochrome c oxidase deactivation, and adenosine triphosphate (ATP) deficiency acting by suppressing superoxide elevation and intracellular lipid oxidation (Hu and Li, 2016).

Several lines of evidence indicate that Aβ induces oxidative stress (Butterfield et al, 2013). It has been proposed that Aβ can be inserted as oligomers into the bilayer and serves as a source of ROS. This process in turn is considered the triggering factor of lipid peroxidation initiation (Butterfield et al, 2002).

As stated in the previous section, neuroinflammation plays a prominent role in the progression of AD (Rosi et al, 2004) and it should be underlined that oxidative stress may trigger an active, self-perpetuating cycle of chronic neuro-inflammation, further promoting oxidative stress and in turn leading to irreversible neuronal dysfunction and cell death (Maccioni et al, 2009; Mhatre et al, 2004). In further agreement, several evidences indicated that interaction between oxidative stress and neuroinflammatory processes drives Aβ generation (Agostinho et al, 2010; Candore et al, 2010; Yao et al, 2004). Furthermore, it is worth noting that increased ROS production is also associated with chronic stress. In particular, we previously described in a rodent paradigm of chronic psychosocial stress a hyperactivation of HPA axis that corresponds to a significant increase in ROS production either at central or peripheral level (Colaianna et al, 2013). This animal paradigm was also shown to induce depressive–like behavior (Ieraci et al, 2016). Hence, all the reviewed mechanisms linking AD and depression seem to be closely correlated.

CONCLUSIONS

In conclusion, in the present review we have summarized the main available knowledge that indicates possible common neurobiological/ neuropthological bases shared between depression and AD. The data retrieved are confluent and their merging can help in improving interpretation of these comorbid conditions. It is worthy of note that, either for AD or depression, despite frenetic research having led to densely populated literature, the exiguity of pharmacological tools available makes mandatory a further effort in order to identify novel therapeutic approaches. Therefore, a deeper understanding of the molecular basis for co-existence of aberrations in the reviewed pathways may result in identifying pharmaceutical targets useful as adjuvant or combination therapies to improve efficacy outcomes, as well as side effect burden, of conventional treatment schemes.

ACKNOWLEDGEMENTS

This study was supported by FIR 2015-2018 program from Apulia Region to MGM.

REFERENCES

Adiele RC, Adiele CA (2016). Mitochondrial Regulatory Pathways in the Pathogenesis of Alzheimer's Disease. *J Alzheimers Dis* 2016 Jul 6; 53(4):1257-70. doi: 10.3233/JAD-150967.

Agostinho P, Cunha RA, Oliveira C (2010). Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Curr Pharm Des* **16:** 2766-2778.

Allard P, Alafuzoff I, Carlsson A, Eriksson K, Ericson E, Gottfries CG, Marcusson JO (1990). Loss of dopamine uptake sites labeled with [3H]GBR-12935 in Alzheimer's disease. *Eur Neurol* **30:** 181-185.

Aluise CD, Robinson RA, Beckett TL, Murphy MP et al and Butterfield DA (2010). Preclinical Alzheimer disease: brain oxidative stress, Abeta peptide and proteomics. *Neurobiol Dis* **39:** 221-228.

Andersen K, Lolk A, Kragh-Sorensen P, Petersen NE, Green A (2005). Depression and the risk of Alzheimer disease. *Epidemiology* **16:** 233-238.

Ansari MA, Scheff SW (2010). Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *J Neuropathol Exp Neurol* **69:** 155-167.

Apostolova LG, Cummings JL (2008). Neuropsychiatric manifestations in mild cognitive impairment: a systematic review of the literature. *Dement Geriatr Cogn Disord* **25:** 115-126.

Aznar S, Knudsen GM (2011). Depression and Alzheimer's disease: is stress the initiating factor in a common neuropathological cascade? *J Alzheimers Dis* **23:** 177-193.

Baglietto-Vargas D, Chen Y, Suh D, Ager RR et al and LaFerla FM (2015). Short-term modern life-like stress exacerbates Abeta-pathology and synapse loss in 3xTg-AD mice. *J Neurochem* **134:** 915-926.

Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47:** 351-354.

Bilici M, Efe H, Koroglu MA, Uydu HA, Bekaroglu M, Deger O (2001). Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J Affect Disord* **64:** 43-51.

Boersma GJ, Lee RS, Cordner ZA, Ewald ER, Purcell RH, Moghadam AA, Tamashiro KL (2014). Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. *Epigenetics* **9:** 437-447.

Bonet-Costa V, Pomatto LC, Davies KJ (2016). The Proteasome and Oxidative Stress in Alzheimer's Disease. *Antioxid Redox Signal 2016 Aug 25. [Epub ahead of print]*

Braun D, Madrigal JL, Feinstein DL (2014). Noradrenergic regulation of glial activation: molecular mechanisms and therapeutic implications. *Curr Neuropharmacol* **12:** 342-352.

Butterfield DA, Castegna A, Lauderback CM, Drake J (2002). Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. *Neurobiol Aging* **23:** 655-664.

Butterfield DA, Swomley AM, Sultana R (2013). Amyloid beta-peptide (1-42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. *Antioxid Redox Signal* **19:** 823-835.

Buxbaum JD, Gandy SE, Cicchetti P, Ehrlich ME et al and Greengard P (1990). Processing of Alzheimer beta/A4 amyloid precursor protein: modulation by agents that regulate protein phosphorylation. *Proc Natl Acad Sci U S A* **87:** 6003-6006.

Candore G, Bulati M, Caruso C, Castiglia L et al and Vasto S (2010). Inflammation, cytokines, immune response, apolipoprotein E, cholesterol, and oxidative stress in Alzheimer disease: therapeutic implications. *Rejuvenation Res* **13:** 301-313.

Caporaso GL, Gandy SE, Buxbaum JD, Ramabhadran TV, Greengard P (1992). Protein phosphorylation regulates secretion of Alzheimer beta/A4 amyloid precursor protein. *Proc Natl Acad Sci U S A* **89:** 3055-3059.

Catania C, Sotiropoulos I, Silva R, Onofri C, Breen KC, Sousa N, Almeida OF (2009). The amyloidogenic potential and behavioral correlates of stress. *Mol Psychiatry* **14:** 95-105.

Charney DS, Menkes DB, Heninger GR (1981). Receptor sensitivity and the mechanism of action of antidepressant treatment. Implications for the etiology and therapy of depression. *Arch Gen Psychiatry* **38:** 1160-1180.

Chen Y, Peng Y, Che P, Gannon M et al and Wang Q (2014). alpha(2A) adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction. *Proc Natl Acad Sci U S A* **111:** 17296-17301.

Cho S, Hu Y (2007). Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. *Exp Neurol* **203:** 274-278.

Chow VW, Mattson MP, Wong PC, Gleichmann M (2010). An overview of APP processing enzymes and products. *Neuromolecular Med* **12:** 1-12.

Cirrito JR, Disabato BM, Restivo JL, Verges DK et al and Sheline YI (2011). Serotonin signaling is associated with lower amyloid-beta levels and plaques in transgenic mice and humans. *Proc Natl Acad Sci U S A* **108:** 14968-14973.

Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH (2005). Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci* **8:** 79-84.

Colaianna M, Schiavone S, Zotti M, Tucci P et al and Trabace L (2013). Neuroendocrine profile in a rat model of psychosocial stress: relation to oxidative stress. *Antioxid Redox Signal* **18:** 1385-1399.

Colaianna M, Tucci P, Zotti M, Morgese MG et al and Trabace L (2010). Soluble beta amyloid(1-42): a critical player in producing behavioural and biochemical changes evoking depressive-related state? *Br J Pharmacol* **159:** 1704-1715.

Cole SL, Vassar R (2007). The Alzheimer's disease beta-secretase enzyme, BACE1. *Mol Neurodegener* **2:** 22.

Conductier G, Dusticier N, Lucas G, Cote F et al and Compan V (2006). Adaptive changes in serotonin neurons of the raphe nuclei in 5-HT(4) receptor knock-out mouse. *Eur J Neurosci* **24:** 1053-1062.

Cottingham C, Chen Y, Jiao K, Wang Q (2011). The antidepressant desipramine is an arrestin-biased ligand at the alpha(2A)-adrenergic receptor driving receptor down-regulation in vitro and in vivo. *J Biol Chem* **286:** 36063-36075.

Cottingham C, Ferryman CJ, Wang Q (2015). alpha2 Adrenergic Receptor Trafficking as a Therapeutic Target in Antidepressant Drug Action. *Prog Mol Biol Transl Sci* **132:** 207-225.

Cottingham C, Percival S, Birky T, Wang Q (2014). Tricyclic antidepressants exhibit variable pharmacological profiles at the alpha(2A) adrenergic receptor. *Biochem Biophys Res Commun* **451:** 461-466.

Cottingham C, Wang Q (2012). alpha2 adrenergic receptor dysregulation in depressive disorders: implications for the neurobiology of depression and antidepressant therapy. *Neurosci Biobehav Rev* **36:** 2214-2225.

Counts SE, Mufson EJ (2010). Noradrenaline activation of neurotrophic pathways protects against neuronal amyloid toxicity. *J Neurochem* **113:** 649-660.

Cryan JF, Lucki I (2000). Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine(2C) receptors. *J Pharmacol Exp Ther* **295:** 1120-1126.

Cryan JF, Valentino RJ, Lucki I (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev* **29:** 547-569.

Dello Russo C, Boullerne AI, Gavrilyuk V, Feinstein DL (2004). Inhibition of microglial inflammatory responses by norepinephrine: effects on nitric oxide and interleukin-1beta production. *J Neuroinflammation* **1:** 9.

Demirdas A, Naziroglu M, Ovey IS (2016). Duloxetine Reduces Oxidative Stress, Apoptosis, and Ca2+ Entry Through Modulation of TRPM2 and TRPV1 Channels in the Hippocampus and Dorsal Root Ganglion of Rats. *Mol Neurobiol*. 2016 Jul 21. [Epub ahead of print]

Di Carlo M, Giacomazza D, San Biagio PL (2012). Alzheimer's disease: biological aspects, therapeutic perspectives and diagnostic tools. *J Phys Condens Matter* **24:** 244102.

Dong H, Goico B, Martin M, Csernansky CA, Bertchume A, Csernansky JG (2004). Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience* **127:** 601-609.

Dong H, Murphy KM, Meng L, Montalvo-Ortiz J, Zeng Z, Kolber BJ et al and Csernansky JG (2012). Corticotrophin releasing factor accelerates neuropathology

and cognitive decline in a mouse model of Alzheimer's disease. *J Alzheimers Dis* **28:** 579-592.

Dong H, Wang S, Zeng Z, Li F, Montalvo-Ortiz J, Tucker C et al and Csernansky JG (2014). Effects of corticotrophin-releasing factor receptor 1 antagonists on amyloid-beta and behavior in Tg2576 mice. *Psychopharmacology (Berl)* **231:** 4711-4722.

Du H, Guo L, Yan S, Sosunov AA, McKhann GM, Yan SS (2010). Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. *Proc Natl Acad Sci U S A* **107:** 18670-18675.

Efthimiopoulos S, Punj S, Manolopoulos V, Pangalos M, Wang GP, Refolo LM, Robakis NK (1996). Intracellular cyclic AMP inhibits constitutive and phorbol ester-stimulated secretory cleavage of amyloid precursor protein. *J Neurochem* **67:** 872-875.

Egashira N, Iwasaki K, Takashima A, Watanabe T et al and Fujiwara M (2005). Altered depression-related behavior and neurochemical changes in serotonergic neurons in mutant R406W human tau transgenic mice. *Brain Res* **1059:** 7-12.

Eren I, Naziroglu M, Demirdas A (2007a). Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. *Neurochem Res* **32:** 1188-1195.

Eren I, Naziroglu M, Demirdas A, Celik O, Uguz AC, Altunbasak A et al and Uz E (2007b). Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat. *Neurochem Res* **32:** 497-505.

Feinstein DL, Galea E, Reis DJ (1993). Norepinephrine suppresses inducible nitric oxide synthase activity in rat astroglial cultures. *J Neurochem* **60:** 1945-1948.

Fisher JR, Wallace CE, Tripoli DL, Sheline YI, Cirrito JR (2016). Redundant Gs-coupled serotonin receptors regulate amyloid-beta metabolism in vivo. *Mol Neurodegener* **11:** 45.

Garcia-Alloza M, Hirst WD, Chen CP, Lasheras B, Francis PT, Ramirez MJ (2004). Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. *Neuropsychopharmacology* **29:** 410-416.

Geerlings MI, Schoevers RA, Beekman AT, Jonker C, Deeg DJ, Schmand B et al and Van Tilburg W (2000). Depression and risk of cognitive decline and Alzheimer's disease. Results of two prospective community-based studies in The Netherlands. *Br J Psychiatry* **176:** 568-575.

Gella A, Durany N (2009). Oxidative stress in Alzheimer disease. *Cell Adh Migr* **3:** 88-93.

Gibney SM, McGuinness B, Prendergast C, Harkin A, Connor TJ (2013). Poly I:C-induced activation of the immune response is accompanied by depression and anxiety-like behaviours, kynurenine pathway activation and reduced BDNF expression. *Brain Behav Immun* **28:** 170-181.

Gibson GE, Park LC, Zhang H, Sorbi S, Calingasan NY (1999). Oxidative stress and a key metabolic enzyme in Alzheimer brains, cultured cells, and an animal model of chronic oxidative deficits. *Ann N Y Acad Sci* **893:** 79-94.

Gold PW, Gabry KE, Yasuda MR, Chrousos GP (2002). Divergent endocrine abnormalities in melancholic and atypical depression: clinical and pathophysiologic implications. *Endocrinol Metab Clin North Am* **31:** 37-62, vi.

Grabrucker AM, Schmeisser MJ, Udvardi PT, Arons M, Schoen M, Woodling NS et al and Boeckers TM (2011). Amyloid beta protein-induced zinc sequestration leads to synaptic loss via dysregulation of the ProSAP2/Shank3 scaffold. *Mol Neurodegener* **6:** 65.

Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM (2006). Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci* **26:** 9047-9056.

Grilli M, Lagomarsino F, Zappettini S, Preda S, Mura E, Govoni S, Marchi M (2010). Specific inhibitory effect of amyloid-beta on presynaptic muscarinic receptor subtypes modulating neurotransmitter release in the rat nucleus accumbens. *Neuroscience* **167:** 482-489.

Gu L, Liu YJ, Wang YB, Yi LT (2012). Role for monoaminergic systems in the antidepressant-like effect of ethanol extracts from Hemerocallis citrina. *J Ethnopharmacol* **139:** 780-787.

Guillemin GJ, Meininger V, Brew BJ (2005). Implications for the kynurenine pathway and quinolinic acid in amyotrophic lateral sclerosis. *Neurodegener Dis* **2:** 166-176.

Guillemin GJ, Smith DG, Smythe GA, Armati PJ, Brew BJ (2003a). Expression of the kynurenine pathway enzymes

in human microglia and macrophages. *Adv Exp Med Biol* **527:** 105-112.

Guillemin GJ, Smythe GA, Veas LA, Takikawa O, Brew BJ (2003b). A beta 1-42 induces production of quinolinic acid by human macrophages and microglia. *Neuroreport* **14:** 2311-2315.

Haglund M, Sjobeck M, Englund E (2006). Locus ceruleus degeneration is ubiquitous in Alzheimer's disease: possible implications for diagnosis and treatment. *Neuropathology* **26:** 528-532.

Hardy J, Selkoe DJ (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297:** 353-356.

Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A et al and Kummer MP (2010a). Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proc Natl Acad Sci U S A* **107:** 6058-6063.

Heneka MT, O'Banion MK, Terwel D, Kummer MP (2010b). Neuroinflammatory processes in Alzheimer's disease. *J Neural Transm* **117:** 919-947.

Herken H, Gurel A, Selek S, Armutcu F, Ozen ME, Bulut M et al and Akyol O (2007). Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. *Arch Med Res* **38:** 247-252.

Hewett SJ, Csernansky CA, Choi DW (1994). Selective potentiation of NMDA-induced neuronal injury following induction of astrocytic iNOS. *Neuron* **13:** 487-494.

Holmes C, Arranz M, Collier D, Powell J, Lovestone S (2003). Depression in Alzheimer's disease: the effect of serotonin receptor gene variation. *Am J Med Genet B Neuropsychiatr Genet* **119B:** 40-43.

Hoogendijk WJ, Meynen G, Endert E, Hofman MA, Swaab DF (2006). Increased cerebrospinal fluid cortisol level in Alzheimer's disease is not related to depression. *Neurobiol Aging* **27:** 780 e781-780 e782.

Hu H, Li M (2016). Mitochondria-targeted antioxidant mitotempo protects mitochondrial function against amyloid beta toxicity in primary cultured mouse neurons. *Biochem Biophys Res Commun*.

Ieraci A, Mallei A, Popoli M (2016). Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice. *Neural Plasticity* **2016:** 13.

Igbavboa U, Johnson-Anuna LN, Rossello X, Butterick TA, Sun GY, Wood WG (2006). Amyloid beta-protein1-42 increases cAMP and apolipoprotein E levels which are inhibited by beta1 and beta2-adrenergic receptor antagonists in mouse primary astrocytes. *Neuroscience* **142:** 655-660.

Jiang T, Yu JT, Tan MS, Zhu XC, Tan L (2013). beta-Arrestins as potential therapeutic targets for Alzheimer's disease. *Mol Neurobiol* **48:** 812-818.

Joyce JN, Smutzer G, Whitty CJ, Myers A, Bannon MJ (1997). Differential modification of dopamine transporter and tyrosine hydroxylase mRNAs in midbrain of subjects with Parkinson's, Alzheimer's with parkinsonism, and Alzheimer's disease. *Mov Disord* **12:** 885-897.

Justice NJ, Huang L, Tian JB, Cole A et al and Zheng H (2015). Posttraumatic stress disorder-like induction elevates beta-amyloid levels, which directly activates corticotropin-releasing factor neurons to exacerbate stress responses. *J Neurosci* **35:** 2612-2623.

Kang JE, Cirrito JR, Dong H, Csernansky JG, Holtzman DM (2007). Acute stress increases interstitial fluid amyloid-beta via corticotropin-releasing factor and neuronal activity. *Proc Natl Acad Sci U S A* **104:** 10673-10678.

Kemppainen N, Laine M, Laakso MP, Kaasinen V et al and Rinne JO (2003). Hippocampal dopamine D2 receptors correlate with memory functions in Alzheimer's disease. *Eur J Neurosci* **18:** 149-154.

Kepe V, Barrio JR, Huang SC, Ercoli L et al and Phelps ME (2006). Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proc Natl Acad Sci U S A* **103:** 702-707.

Kessing LV, Andersen PK (2004). Does the risk of developing dementia increase with the number of episodes in patients with depressive disorder and in patients with bipolar disorder? *J Neurol Neurosurg Psychiatry* **75:** 1662-1666.

Khanzode SD, Dakhale GN, Khanzode SS, Saoji A, Palasodkar R (2003). Oxidative damage and major depression: the potential antioxidant action of selective serotonin re-uptake inhibitors. *Redox Rep* **8:** 365-370.

Koch G, Di Lorenzo F, Bonni S, Giacobbe V, Bozzali

M, Caltagirone C, Martorana A (2014). Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. *Neuropsychopharmacology* **39:** 2654-2661.

Krishnan V, Nestler EJ (2008). The molecular neurobiology of depression. *Nature***455:** 894-902.

Kumar U, Patel SC (2007). Immunohistochemical localization of dopamine receptor subtypes (D1R-D5R) in Alzheimer's disease brain. *Brain Res* **1131:** 187-196.

Lanctot KL, Herrmann N, Mazzotta P (2001). Role of serotonin in the behavioral and psychological symptoms of dementia. *J Neuropsychiatry Clin Neurosci* **13:** 5-21.

Lee SH, Lee KJ, Lee HJ, Ham BJ, Ryu SH, Lee MS (2005). Association between the 5-HT6 receptor C267T polymorphism and response to antidepressant treatment in major depressive disorder. *Psychiatry Clin Neurosci* **59:** 140-145.

Leonard BE, Song C (2002). Changes in the immune system in rodent models of depression. *Int J Neuropsychopharmacol* **5:** 345-356.

Li J, O W, Li W, Jiang ZG, Ghanbari HA (2013). Oxidative stress and neurodegenerative disorders. *Int J Mol Sci* **14:** 24438-24475.

Liu X, Ye K, Weinshenker D (2015). Norepinephrine Protects against Amyloid-beta Toxicity via TrkB. *J Alzheimers Dis* **44:** 251-260.

Liu X, Zhao X, Zeng X, Bossers K, Swaab DF, Zhao J, Pei G (2013). beta-arrestin1 regulates gamma-secretase complex assembly and modulates amyloid-beta pathology. *Cell Res* **23:** 351-365.

Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S et al and Debonnel G (2007). Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* **55:** 712-725.

Maccioni RB, Rojo LE, Fernandez JA, Kuljis RO (2009). The role of neuroimmunomodulation in Alzheimer's disease. *Ann N Y Acad Sci* **1153:** 240-246.

Maes M, Meltzer HY (1995). The Serotonin Hypothesis of Major Depression. In: (ed)^(eds). *Neuropsychopharmacology: The Forth Generation of Progress*, edn. New York: Raven Press. p^pp.

Maher-Edwards G, Zvartau-Hind M, Hunter AJ, Gold M, Hopton G, Jacobs G et al and Williams P (2010). Double-blind, controlled phase II study of a 5-HT6 receptor antagonist, SB-742457, in Alzheimer's disease. *Curr Alzheimer Res* **7:** 374-385.

Mann JJ (1999). Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology* **21:** 99S-105S.

Mann JJ, McBride PA, Stanley M (1986). Postmortem serotonergic and adrenergic receptor binding to frontal cortex: correlations with suicide. *Psychopharmacol Bull* **22:** 647-649.

Martorana A, Di Lorenzo F, Esposito Z, Lo Giudice T, Bernardi G, Caltagirone C, Koch G (2013). Dopamine D(2)-agonist rotigotine effects on cortical excitability and central cholinergic transmission in Alzheimer's disease patients. *Neuropharmacology* **64:** 108-113.

Martorana A, Mori F, Esposito Z, Kusayanagi H et al and Koch G (2009). Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. *Neuropsychopharmacology* **34:** 2323-2328.

Mattson MP (2004). Pathways towards and away from Alzheimer's disease. *Nature* **430:** 631-639.

Meana JJ, Barturen F, Garcia-Sevilla JA (1992). Alpha 2-adrenoceptors in the brain of suicide victims: increased receptor density associated with major depression. *Biol Psychiatry* **31:** 471-490.

Meneses A (2007). Stimulation of 5-HT1A, 5-HT1B, 5-HT2A/2C, 5-HT3 and 5-HT4 receptors or 5-HT uptake inhibition: short- and long-term memory. *Behav Brain Res* **184:** 81-90.

Mhatre M, Floyd RA, Hensley K (2004). Oxidative stress and neuroinflammation in Alzheimer's disease and amyotrophic lateral sclerosis: common links and potential therapeutic targets. *J Alzheimers Dis* **6:** 147-157.

Mills J, Laurent Charest D, Lam F, Beyreuther K, Ida N, Pelech SL, Reiner PB (1997). Regulation of amyloid precursor protein catabolism involves the mitogen-activated protein kinase signal transduction pathway. *J Neurosci* **17:** 9415-9422.

Modrego PJ, Ferrandez J (2004). Depression in patients with mild cognitive impairment increases the risk of developing dementia of Alzheimer type: a prospective cohort study. *Arch Neurol* **61:** 1290-1293.

Mongeau R, Blier P, de Montigny C (1997). The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. *Brain Res Brain Res Rev* **23:** 145-195.

Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G (2010). Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochim Biophys Acta* **1802:** 2-10.

Moretti M, Colla A, de Oliveira Balen G, dos Santos DB et al and Severo Rodrigues AL (2012). Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. *J Psychiatr Res* **46:** 331-340.

Morgan DG, May PC, Finch CE (1987). Dopamine and serotonin systems in human and rodent brain: effects of age and neurodegenerative disease. *J Am Geriatr Soc* **35:** 334-345.

Morgese MG, Colaianna M, Mhillaj E, Zotti M et al and Trabace L (2015). Soluble beta amyloid evokes alteration in brain norepinephrine levels: role of nitric oxide and interleukin-1. *Front Neurosci* **9:** 428.

Morgese MG, Tucci P, Colaianna M, Zotti M, Cuomo V, Schiavone S, Trabace L (2014). Modulatory activity of soluble beta amyloid on HPA axis function in rats. *Curr Pharm Des* **20:** 2539-2546.

Morgese MG, Tucci P, Mhillaj E, Bove M, Schiavone S, Trabace L, Cuomo V (2016). Lifelong Nutritional Omega-3 Deficiency Evokes Depressive-Like State Through Soluble Beta Amyloid. *Mol Neurobiol*.

Mura E, Preda S, Govoni S, Lanni C et al and Marchi M (2010). Specific neuromodulatory actions of amyloid-beta on dopamine release in rat nucleus accumbens and caudate putamen. *J Alzheimers Dis* **19:** 1041-1053.

Mura E, Zappettini S, Preda S, Biundo F et al and Marchi M (2012). Dual effect of beta-amyloid on alpha7 and alpha4beta2 nicotinic receptors controlling the release of glutamate, aspartate and GABA in rat hippocampus. *PLoS One* **7:** e29661.

Murray AM, Weihmueller FB, Marshall JF, Hurtig HI, Gottleib GL, Joyce JN (1995). Damage to dopamine systems differs between Parkinson's disease and Alzheimer's disease with parkinsonism. *Ann Neurol* **37:** 300-312.

O'Farrell K, Harkin A (2015). Stress-related regulation of the kynurenine pathway: Relevance to neuropsychiatric and degenerative disorders. *Neuropharmacology*.

Ordway GA, Widdowson PS, Smith KS, Halaris A (1994). Agonist binding to alpha 2-adrenoceptors is elevated in the locus coeruleus from victims of suicide. *J Neurochem* **63:** 617-624.

Overstreet DH, Stemmelin J, Griebel G (2008). Confirmation of antidepressant potential of the selective beta3 adrenoceptor agonist amibegron in an animal model of depression. *Pharmacol Biochem Behav* **89:** 623-626.

Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D (2006). Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. *Arch Gen Psychiatry* **63:** 530-538.

Oxenkrug G (2013). Serotonin-kynurenine hypothesis of depression: historical overview and recent developments. *Curr Drug Targets* **14:** 514-521.

Oxenkrug G, Ratner R, Summergrad P (2013). Kynurenines and vitamin B6: link between diabetes and depression. *J Bioinform Diabetes* **1**.

Pallas M, Camins A, Smith MA, Perry G, Lee HG, Casadesus G (2008). From aging to Alzheimer's disease: unveiling "the switch" with the senescence-accelerated mouse model (SAMP8). *J Alzheimers Dis* **15:** 615-624.

Panza F, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Imbimbo BP et al and Solfrizzi V (2010). Late-life depression, mild cognitive impairment, and dementia: possible continuum? *Am J Geriatr Psychiatry* **18:** 98-116.

Park HJ, Ran Y, Jung JI, Holmes O, Price AR, Smithson L et al and Felsenstein KM (2015). The stress response neuropeptide CRF increases amyloid-beta production by regulating gamma-secretase activity. *EMBO J* **34:** 1674-1686.

Patel JG, Bartoszyk GD, Edwards E, Ashby CR, Jr. (2004). The highly selective 5-hydroxytryptamine (5-HT)2A receptor antagonist, EMD 281014, significantly increases swimming and decreases immobility in male congenital learned helpless rats in the forced swim test. *Synapse* **52:** 73-75.

Pievani M, Bocchetta M, Boccardi M, Cavedo E, Bonetti M, Thompson PM, Frisoni GB (2013). Striatal morphology in early-onset and late-onset Alzheimer's disease: a preliminary study. *Neurobiol Aging* **34:** 1728-1739.

Pontrello CG, Sun MY, Lin A, Fiacco TA, DeFea KA, Ethell IM (2012). Cofilin under control of beta-arrestin-2 in NMDA-dependent dendritic spine plasticity, long-term depression (LTD), and learning. *Proc Natl Acad Sci U S A* **109:** E442-451.

Popa D, Lena C, Fabre V, Prenat C, Gingrich J, Escourrou P et al and Adrien J (2005). Contribution of 5-HT2 receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT2A receptors. *J Neurosci* **25:** 11231-11238.

Preda S, Govoni S, Lanni C, Racchi M, Mura E, Grilli M, Marchi M (2008). Acute beta-amyloid administration disrupts the cholinergic control of dopamine release in the nucleus accumbens. *Neuropsychopharmacology* **33:** 1062-1070.

Pytka K, Podkowa K, Rapacz A, Podkowa A, Zmudzka E, Olczyk A et al and Filipek B (2016). The role of serotonergic, adrenergic and dopaminergic receptors in antidepressant-like effect. *Pharmacol Rep* **68:** 263-274.

Reddy PH (2007). Mitochondrial dysfunction in aging and Alzheimer's disease: strategies to protect neurons. *Antioxid Redox Signal* **9:** 1647-1658.

Reddy PH (2006). Mitochondrial oxidative damage in aging and Alzheimer's disease: implications for mitochondrially targeted antioxidant therapeutics. *J Biomed Biotechnol* **2006:** 31372.

Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ, Memantine Study G (2003). Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med* **348:** 1333-1341.

Reynolds GP, Mason SL, Meldrum A, De Keczer S, Parnes H, Eglen RM, Wong EH (1995). 5-Hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br J Pharmacol* **114:** 993-998.

Richard E, Reitz C, Honig LH, Schupf N et al and Luchsinger JA (2013). Late-life depression, mild cognitive impairment, and dementia. *JAMA Neurol* **70:** 374-382.

Rinne JO, Sako E, Paljarvi L, Molsa PK, Rinne UK (1986). Brain dopamine D-2 receptors in senile dementia. *J Neural Transm* **65:** 51-62.

Robert SJ, Zugaza JL, Fischmeister R, Gardier AM, Lezoualc'h F (2001). The human serotonin 5-HT4 receptor regulates secretion of non-amyloidgenic precursor protein. *J Biol Chem* **276:** 44881-44888.

Rodrigues R, Petersen RB, Perry G (2014). Parallels between major depressive disorder and Alzheimer's disease: role of oxidative stress and genetic vulnerability. *Cell Mol Neurobiol* **34:** 925-949.

Rodriguez JJ, Noristani HN, Verkhratsky A (2012). The serotonergic system in ageing and Alzheimer's disease. *Prog Neurobiol* **99:** 15-41.

Rosi S, Ramirez-Amaya V, Hauss-Wegrzyniak B, Wenk GL (2004). Chronic brain inflammation leads to a decline in hippocampal NMDA-R1 receptors. *J Neuroinflammation* **1:** 12.

Rowan MJ, Klyubin I, Wang Q, Anwyl R (2005). Synaptic plasticity disruption by amyloid beta protein: modulation by potential Alzheimer's disease modifying therapies. *Biochem Soc Trans* **33:** 563-567.

Santos LE, Beckman D, Ferreira ST (2015). Microglial dysfunction connects depression and Alzheimer's disease. *Brain Behav Immun*.

Sarrazin N, Di Blasi F, Roullot-Lacarriere V, Rouge-Pont F et al and Piazza PV (2009). Transcriptional effects of glucocorticoid receptors in the dentate gyrus increase anxiety-related behaviors. *PLoS One* **4:** e7704.

Schaaf MJ, de Jong J, de Kloet ER, Vreugdenhil E (1998). Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. *Brain Res* **813:** 112-120.

Schildkraut JJ (1995). The catecholamine hypothesis of affective disorders: a review of supporting evidence. 1965. *J Neuropsychiatry Clin Neurosci* **7:** 524-533; discussion 523-524.

Schmidt K, Nolte-Zenker B, Patzer J, Bauer M, Schmidt LG, Heinz A (2001). Psychopathological correlates of reduced dopamine receptor sensitivity in depression, schizophrenia, and opiate and alcohol dependence. *Pharmacopsychiatry* **34:** 66-72.

Schreiber G, Golan M, Avissar S (2009). Beta-arrestin signaling complex as a target for antidepressants and as a depression marker. *Drug News Perspect* **22:** 467-480.

Selkoe DJ, Schenk D (2003). Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol* **43:** 545-584.

Shan H, Bian Y, Shu Z, Zhang L, Zhu J, Ding J et al and Hu G (2016). Fluoxetine protects against IL-1beta-induced neuronal apoptosis via downregulation of p53. *Neuropharmacology* **107:** 68-78.

Sheline YI, West T, Yarasheski K, Swarm R et al and Cirrito JR (2014). An antidepressant decreases CSF Abeta production in healthy individuals and in transgenic AD mice. *Sci Transl Med* **6:** 236re234.

Singh M, Dang TN, Arseneault M, Ramassamy C (2010). Role of by-products of lipid oxidation in Alzheimer's disease brain: a focus on acrolein. *J Alzheimers Dis* **21:** 741-756.

Solano DC, Sironi M, Bonfini C, Solerte SB, Govoni S, Racchi M (2000). Insulin regulates soluble amyloid precursor protein release via phosphatidyl inositol 3 kinase-dependent pathway. *FASEB J* **14:** 1015-1022.

Spencer JP, Brown JT, Richardson JC, Medhurst AD, Sehmi SS, Calver AR, Randall AD (2004). Modulation of hippocampal excitability by 5-HT4 receptor agonists persists in a transgenic model of Alzheimer's disease. *Neuroscience* **129:** 49-54.

Srikanth V, Maczurek A, Phan T, Steele M, Westcott B, Juskiw D, Munch G (2011). Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. *Neurobiol Aging* **32:** 763-777.

Srivareerat M, Tran TT, Alzoubi KH, Alkadhi KA (2009). Chronic psychosocial stress exacerbates impairment of cognition and long-term potentiation in beta-amyloid rat model of Alzheimer's disease. *Biol Psychiatry* **65:** 918-926.

Steinberg M, Shao H, Zandi P, Lyketsos CG et al and Cache County I (2008). Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: the Cache County Study. *Int J Geriatr Psychiatry* **23:** 170-177.

Steardo L, Monteleone P, Trabace L, Cannizzaro C, Maj M, Cuomo V (2000). Serotonergic modulation of rat pineal gland activity: in vivo evidence for a 5-Hydroxytryptamine(2C) receptor involvement. *J Pharmacol Exp Ther* **295:**266-273.

Stetler C, Miller GE (2011). Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom Med* **73:** 114-126.

Sun X, Steffens DC, Au R, Folstein M et al and Qiu WQ (2008). Amyloid-associated depression: a prodromal depression of Alzheimer disease? *Arch Gen Psychiatry* **65:** 542-550.

Swaab DF, Bao AM, Lucassen PJ (2005). The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev* **4:** 141-194.

Swaab DF, Raadsheer FC, Endert E, Hofman MA, Kamphorst W, Ravid R (1994). Increased cortisol levels in aging and Alzheimer's disease in postmortem cerebrospinal fluid. *J Neuroendocrinol* **6:** 681-687.

Swerdlow RH, Khan SM (2009). The Alzheimer's disease mitochondrial cascade hypothesis: an update. *Exp Neurol* **218:** 308-315.

Tajeddinn W, Persson T, Calvo-Garrido J, Seed Ahmed M et al and Aarsland D (2016). Pharmacological Modulations of the Serotonergic System in a Cell-Model of Familial Alzheimer's Disease. *J Alzheimers Dis* **53:** 349-361.

Thathiah A, Horre K, Snellinx A, Vandewyer E et al and De Strooper B (2013). beta-arrestin 2 regulates Abeta generation and gamma-secretase activity in Alzheimer's disease. *Nat Med* **19:** 43-49.

Trabace L, Kendrick KM, Castrignano S, Colaianna M et al and Govoni S (2007). Soluble amyloid beta1-42 reduces dopamine levels in rat prefrontal cortex: relationship to nitric oxide. *Neuroscience* **147:** 652-663.

Tucci P, Mhillaj E, Morgese MG, Colaianna M et al and Trabace L (2014). Memantine prevents memory consolidation failure induced by soluble beta amyloid in rats. *Front Behav Neurosci* **8:** 332.

Valera E, Ubhi K, Mante M, Rockenstein E, Masliah E (2014). Antidepressants reduce neuroinflammatory responses and astroglial alpha-synuclein accumulation in a transgenic mouse model of multiple system atrophy. *Glia* **62:** 317-337.

Versijpt J, Van Laere KJ, Dumont F, Decoo D et al and Korf J (2003). Imaging of the 5-HT2A system: age-, gender-, and Alzheimer's disease-related findings. *Neurobiol Aging* **24:** 553-561.

Visser PJ, Verhey FR, Ponds RW, Kester A, Jolles J (2000). Distinction between preclinical Alzheimer's disease and depression. *J Am Geriatr Soc* **48:** 479-484.

Vlassenko AG, Mintun MA, Xiong C, Sheline YI, Goate AM, Benzinger TL, Morris JC (2011). Amyloid-beta plaque growth in cognitively normal adults: longitudinal [11C] Pittsburgh compound B data. *Ann Neurol* **70:** 857-861.

Walsh DM, Selkoe DJ (2007). A beta oligomers - a decade of discovery. *J Neurochem***101:** 1172-1184.

Wang D, Fu Q, Zhou Y, Xu B, Shi Q, Igwe B et al and Xiang YK (2013). beta2 adrenergic receptor, protein kinase A (PKA) and c-Jun N-terminal kinase (JNK) signaling pathways mediate tau pathology in Alzheimer disease models. *J Biol Chem* **288:** 10298-10307.

Wang D, Yuen EY, Zhou Y, Yan Z, Xiang YK (2011). Amyloid beta peptide-(1-42) induces internalization and degradation of beta2 adrenergic receptors in prefrontal cortical neurons. *J Biol Chem* **286:** 31852-31863.

Wegener G, Bandpey Z, Heiberg IL, Volke V, Trabace L, Rosenberg R, Harvey BH (2004). Combined chronic treatment with citalopram and lithium does not modify the regional neurochemistry of nitric oxide in rat brain. *J Physiol Pharmacol* **55:**575-586.

Werner FM, Covenas R (2015). Review: Classical neurotransmitters and neuropeptides involved in generalized epilepsy in a multi-neurotransmitter system: How to improve the antiepileptic effect? *Epilepsy Behav*.

Wu WH, Huo SJ, Cheng CY, Hong CJ, Tsai SJ (2001). Association study of the 5-HT(6) receptor polymorphism (C267T) and symptomatology and antidepressant response in major depressive disorders. *Neuropsychobiology* **44:** 172-175.

Xu H, Sweeney D, Greengard P, Gandy S (1996). Metabolism of Alzheimer beta-amyloid precursor protein: regulation by protein kinase A in intact cells and in a cell-free system. *Proc Natl Acad Sci U S A* **93:** 4081-4084.

Yao Y, Chinnici C, Tang H, Trojanowski JQ, Lee VM, Pratico D (2004). Brain inflammation and oxidative stress in a transgenic mouse model of Alzheimer-like brain amyloidosis. *J Neuroinflammation* **1:** 21.

Yu JT, Tan L, Ou JR, Zhu JX, Liu K, Song JH, Sun YP (2008). Polymorphisms at the beta2-adrenergic receptor gene influence Alzheimer's disease susceptibility. *Brain Res* **1210:** 216-222.

Zhang F, Zhou H, Wilson BC, Shi JS, Hong JS, Gao HM (2012). Fluoxetine protects neurons against microglial activation-mediated neurotoxicity. *Parkinsonism Relat Disord* **18 Suppl 1:** S213-217.

Zhang HT, Huang Y, O'Donnell JM (2003). Antagonism of the antidepressant-like effects of clenbuterol by central administration of beta-adrenergic antagonists in rats. *Psychopharmacology (Berl)* **170:** 102-107.

Zotti M, Colaianna M, Morgese MG, Tucci P, Schiavone S, Avato P, Trabace L (2013). Carvacrol: from ancient flavoring to neuromodulatory agent. *Molecules* **18:**6161-6172.

OLD AND NEW EVIDENCE FOR NEUROPEPTIDE INVOLVEMENT IN ALZHEIMER'S DISEASE

C. SEVERINI[1,2], C. PETRELLA[1,2], P. PETROCCHI PASSERI[1] and R. POSSENTI[3]

[1]*Institute of Cell Biology and Neurobiology, CNR, Rome, Italy*
[2]*IRCCS Fondazione Santa Lucia, Rome, Italy*
[3]*Department of Medicine of Systems, University of Rome Tor Vergata, Rome, Italy*

Alzheimer's disease (AD) is an irreversible degenerative disorder characterized by degeneration of neurons in different brain areas and by progressive cognitive and functional decline. Various deranged mechanisms play a role in the disease process all inducing neuronal death, the inevitable event occurring in AD. Novel therapeutic approaches using disease-modifying treatment are being investigated with the intention of influencing multiple pathways involved in AD. Because of their putative roles as neurotransmitters, neuromodulators, and neuroregulators in the central nervous system, neuropeptides have been the object of considerable research. Postmortem studies have provided evidence that several neuropeptide-containing neurons are pathologically altered in brain areas of AD patients, as well as in the brain of animal models of AD. In addition, altered levels of neuropeptides have been found in cerebrospinal fluid (CSF) of AD patients, getting insights into the potential role of neuropeptides in the pathophysiology of AD and offering the possibility to identify novel biomarkers of this pathology. The role exerted by neuropeptides seems particularly interesting since they are generally neuroprotective and widely distributed in brain areas responsible for learning and memory processes. The present review summarizes the recent findings on neuropeptide involvement in AD, with a focus on the contribution of thyrotrophin-releasing hormone, cholecystokinin, bradykinin and chromogranin/secretogranin family, describing brain distribution and the role played in AD and in cognitive functions, as well as their neuroprotective properties. Convincing evidence has been provided for the protective role of these neuropeptides against neurodegeneration observed in AD, both *in vitro* and *in vivo*, identifying neuropeptide receptors as potential therapeutic targets.

Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting more than 20 million individuals worldwide. It is clinically characterized by memory defeat and cognitive impairment, accompanied by neuronal loss in the cerebral cortex, hippocampus, basal forebrain, locus coeruleus and dorsal raphe and by a significant damage of basal forebrain cholinergic neurons in brain (Pákáski M, Kálmán J, 2008).

Histopathologically, the hallmarks of AD are the ubiquitous presence of intra-neuronal fibrillary tangles and extracellular deposits of beta amyloid (Aβ) fibrils in senile plaques. While in physiological conditions the phosphorylation-modified tau protein stabilizes the axonal microtubules in the central nervous system (CNS), in AD tau protein may undergo abnormal phoshorylation, hyperphosphorylation and some other modifications (nitration, ubiquitination,

Key words: Alzheimer's disease, TRH, CCK, bradykinin, chromogranin/secretogranins

Corresponding author:
Cinzia Severini,
CNR, Institute of Cell Biology and Neurobiology, CNR,
Via Fosso di Fiorano, 64,
00143, Rome, Italy
Tel.: +39 06 501703234 - Fax: +39 06 501703311
e-mail: cinzia.severini@cnr.it

2279-5855 (2016)

truncation, shift, prolyl isomerization), leading to intraneuronal amassing of tau protein bringing about a disruption of neuronal cell communication (Zhao et al, 2014).

The amyloid (or Aβ) hypothesis (Hardy and Higgins, 1992) has become the dominant model of AD pathogenesis, indicating a crucial role for the production of Aβ peptides which aggregate into oligomers and further deposit as plaques, and guiding the development of potential treatments. The increased Aβ production leads to an amyloid core formation around which neurites, astrocytes, and glial cells accumulate, thus forming senile plaques and activating an immune system response (Mrak and Griffin, 2001; Tuppo and Arias, 2005).

According to this hypothesis, soluble Aβ oligomers are identified as the principal neurotoxic agent in AD pathology (Haass & Selkoe, 2007). This so-called "amyloid hypothesis" was partially modified by Selkoe and Hardy (2016), indicating the crucial role exerted by neuritic alteration in the immediate vicinity of AD plaques, raising the possibility of decreased efficiency of neurotransmission along them.

The pathophysiology of AD involves disturbances and imbalances occurring in a variety of mechanisms. Besides Aβ production, neurofibrillary tangle accumulation and inflammation, various deranged mechanisms, such as chronic oxidative stress, mitochondrial dysfunction, hormone imbalance, mitotic dysfunction, calcium mishandling, and genetic components play a role in the disease process, all inducing neuronal death, the inevitable event occurring in AD (Anand et al, 2014). The disease has been extensively studied looking for a therapy, however acetylcholinesterase inhibitors and memantine are the only drugs currently approved for treatment, nevertheless providing symptomatic treatment without altering the course of the disease. As a consequence, it is of great importance to develop disease modifying substances that might counteract or slow down neurodegeneration.

The role exerted by neuropeptides seems particularly interesting since they are generally neuroprotective and are involved in learning and memory processes.

Neuropeptides are small proteic molecules (from 3 to 100 amino acids) which mediate or modulate the neuronal communication by binding to specific cell surface receptors: they can act as true neurotransmitters or as neuromodulators (Hallberg, 2015).

Radioimmunoassay and immunohistochemistry have allowed to draw exact distribution maps of individual neuropeptides and their receptors in the central (CNS) and peripheral (PNS) nervous systems. Generally, neuropeptides originate in the body of the nerve cell from precursors with high molecular weight (pre-pro-peptides), usually biologically inactive, whose processing leads to the formation of one or more neuropeptides endowed with biological effects (Hallberg and Nyberg, 2003). Once released, neuropeptides can function as neuromodulators reaching their receptors at a considerable distance from the site of release. For this special form of endocrine transmission the concept has been proposed of volume transmission (VT) and extra-synaptic neurotransmission (Fuxe et al, 2012), further confirmed by the frequent discrepancy observed between neuropeptides and their cognate receptor distribution in many brain areas.

This transmission mode expands the classical concept of synaptic transmission based on a communication point by point and emphasizes the capacity of the transmitter molecules, especially peptides, to influence large target areas.

Unlike classical neurotransmitters, inactivated by specific synaptic reuptake mechanisms, neuropeptide activity is disabled by enzymatic proteolysis mediated by various extracellular peptidases, or is induced by the decrease of their concentration due to dilution. This neuropeptide degradation can lead to the formation of fragments that have similar or very different biological activities in respect to parent peptides (Nyberg and Hallberg, 2007).

Considering the large distribution of neuropeptides in the CNS and their ability to modulate cognitive functions, it is not surprising that numerous neuropeptides are differentially affected in AD (Table I).

The role exerted by neuropeptides in AD has been recently reviewed. Van Dam et al, (2013) summarized pathophysiological mechanisms and

Table I. *Changes in neuropeptide levels in AD patients and AD animal models, compared to the corresponding controls (healthy patients or wild type animals).*

	AD human brain		AD human CSF		AD animal models	
Pep tide	**Levels**	**References**	**Levels**	**References**		**References**
TRH	↓	Biggins *at al.*, 1983 (amigdala) Luo *at al.*, 2002 (hippocampus) Yong-Hong *at al.*, 2013 (blood)	↑	Pekary *at al.*, 1991 (TRHGly)		
CCK	↓	Perry *at al.*, 1981 (cortex) Sagar *at al.*, 1984 (hippocampus) Mazurek and Beal, 1991 (cortex) Lofberg *at al.*, 1996 (cortex)			↓	Diez *at al.*, 2003 (hippocampus of APP23 mice)
	↑	Struble *at al.*, 1987 (amyloid plaques) Perry *at al.*, 1981 (amyloid plaques)			↑	Diez *at al.*, 2003 (hippocampus, cortex and amyloid plaques of V717F mice)
BK					↑	Iores-Marcal *at al.*, 2006 (BK fragment in CSF of Aβ infused rats)
CgA	↑	(amyloid plaques): Marksteiner *at al.*, 2002, Rangon *at al.*, 2003, Lechner *at al.*, 2004, Willis et al 2011	↓	Blennow et al 1995; Eder et al 1998; Simonsen et al 2007; Perrin et al 2011; Jahn et al 2011; Mattson et al 2012; Paterson et al 2014; Wildsmith *at al.* 2014		
CgB	↓	Marksteiner et al 2002 (hippocampus) Lechner *at al.* 2004 (cortex)	↓	Eder et al 1998; Mattson et al 2010, 2012, 2013		
	↑	(amyloid plaques): Marksteiner et al 2002; Lechner *at al.* 2004; Willis *at al.* 2008, 2011				
	AD human brain		**AD human CSF**		**AD animal models**	
Peptide	**Levels**		**References**		**Levels**	
SgII	↓	Marksteiner et al 2002 (hippocampus) Lechner *at al.* 2004 (cortex)	↓	Eder et al 1998; Matsson et al 2010; Spellman *at al.* 2015	↑	Willis *at al.*, 2008 (amyloid plaques of V717I and K670M/ N671L mice)
	↑	Kaufmann 1998 (amyloid plaques, hippocampus, cortex) Marksteiner et al 2002 (amyloid plaques) Lechner *at al.* 2004 (amyloid plaques)				

SgIII	↑	Plà *at al.* 2013 (cortex)				
7B2	↑	Winsky-Sommerer *at al.*, 2003 (cortex) Helwig et al 2013 (amyloid plaques)			↑	Helwig et al 2013 (amyloid plaques of APP/PSEN1 mice)
ProSAAS	↑	Wada et al 2004 Hoshino *at al.* 2014 (amyloid plaques)	↓	Jahn *at al.* 2011	↑	Hoshino *at al.* 2014 (brain of APdE9 mice)
VGF		Cocco *at al.* 2010 (cortex)	↓	Carrette el al 2003; Jahn *at al.* 2011; Wujte *at al.* 2012; Spellman *at al.* 2015; Hottla *at al.* 2015; Hendricson *at al.* 2015		

therapeutic opportunities of vasopressin and oxytocin, somatostatin, neuropeptide Y (NPY), corticotropin releasing hormone (CRH), urocortin, galanin, vasoactive intestinal peptide (VIP), neurotensin, opioid peptides, angiotensin and substance P (SP). Willis et al, (2011) and Severini et al, (2016) reviewed the involvement of chromogranins and SP in AD, respectively.

The aim of the present review is to update information on the activity of old and new neuropeptides involved in AD, i.e. thyrotrophin-releasing hormone, cholecystokinin, bradykinin and chromogranin/secretogranin family.

THYROTROPIN-RELEASING HORMONE

Thyrotrophin-Releasing Hormone (TRH) was the first hypothalamic releasing hormone to be isolated and characterized. The name TRH derives from its action on the anterior pituitary, where it stimulates, *in vivo* and *in vitro*, not only the synthesis and release of thyrotrophin (TSH), but also of prolactin (PRL), and in some species also of growth hormone (GH) (Joseph-Bravo et al, 2015). TRH is a tripeptide, identified in 1969, derived from a 242-amino precursor acid protein (ProTRH). In addition to its neuroendocrine function, stimulating the thyroid gland, TRH has functions of neurotransmitter and neuromodulator in both the CNS and PNS. It was demonstrated to co-localize and to be co-secreted with other neurotransmitters in different nerve cell types in both CNS and peripheral tissues (Hrabovszky and Lipositis, 2008). ProTRH mRNA and TRH itself are widely distributed throughout the brain in extra-hypothalamic regions, including the olfactory system, reticular thalamic nucleus, amigdala, the hippocampus, piriform cortex, and striatum, where it plays a neuromodulatory role (Jackson and Reichlin, 1974; Pekary, 1998) (Fig. 1).

TRH has been shown to produce a variety of behavioral changes and neuropharmacological effects independent of its thyrotrophin-releasing properties, by acting via interaction with two different membrane receptors. TRH receptor 1 (TRH-R1) and receptor 2 (TRH-R2) are typical G-protein-coupled receptors, coupling to Gq ang G11, pertussin-toxin-insensitive G proteins that activate PLC-β (Gershengorn and Osman, 1996). Consistent with the endocrine functions, THR-R1 predominates in hypothalamic nuclei, however, is present in brainstem regions and spinal cord motoneurons where it is involved in autonomic and somatomotor control. THR-R2 mRNA is widely distributed throughout the brain with highest levels in the thalamus, cerebral and cerebellar cortex, medial habenulae, medial geniculate nucleus, pontine nuclei, and the entire reticular formation (Heuer et al, 2000). TRH has been proposed to play a role in AD. Significant differences in hypothalamic or pituitary functions were observed by Albert et al,

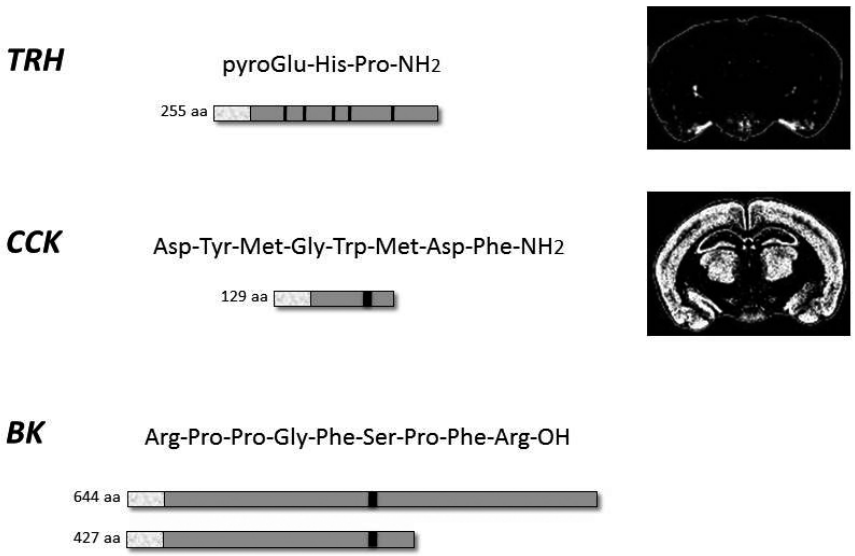

Fig. 1. *TRH, CCK and BK precursor sequence and brain distribution. The scheme represents the approximate length and the number of amino acids of the precursor of TRH, CCK and BK and their amino acid sequence. The leader signal for secretory protein is indicated as light shadowed block. The black blocks indicate the biological active fragments identified. In the right panels are shown images of in situ hybridization for the corresponding gene expression in mouse CNS (Source from Allen Brain Atlas, mouse.brain-map.org).*

(1993) between patients with senile dementia of AD type and control subjects undergoing the TRH test, while other studies failed to demonstrate a significant impairment under TRH stimulation (Gomez et al, 2000). Other works, focusing on the TRH-TSH-thyroid axis, confirmed an abnormal function of hypothalamic or pituitary functions in AD patients showing that, compared to healthy controls, the AD patients had significantly lower levels of TRH in the blood (Yong-Hong et al, 2013).

During the last decades, the relationship between TRH and AD in human subjects has been extensively studied. Increased levels of TRH were found in CSF (Pekary et al, 1991), and decreased levels in hippocampal regions (Luo et al, 2002) and amigdala (Biggins et al, 1983), despite other studies not describing significant differences (Yates et al, 1983; Bouras et al, 1986; Nemeroff et al, 1989; Banky et al, 1992). TRH has been shown to have neuroprotective functions in primary neuronal cultures (Koenig et al, 1996) due, at least in part, to its potent inhibition of GSK-3 via PKC-mediated phosphorylation (Luo and Stopa, 2004). The neuroprotective activity of TRH, together with the known facilitation of cholinergic functions, suggested a therapeutic potential of TRH in AD. It was, in fact, demonstrated that TRH administration can improve memory function in AD patients (Yarbrough and Pomara, 1985; Mellow et al, 1989) and also in the fimbria-fornix lesioned rat model (Bennet et al, 1997).

Possible mechanisms for TRH neuroprotection have been proposed. Among others, it has been suggested that a signal transduction pathway linking TRH with GSK3β activity and tau phosphorylation could be responsible for the formation of neurofibrillary tangles associated with dementia of AD. By binding to its receptor, mostly the TRH-R2 subunit receptor, TRH should be able to activate the G-protein coupled receptor (GPCR), finally triggering the MAPK signal pathway to inhibit GSK3β activity and prevent tau phosphorylation (Luo and Stopa, 2004).

CHOLECYSTOKININ

Cholecystokinin (CCK), a peptide originally discovered as a gastrin-like molecule of the gastrointestinal tract, plays an important role in

the release of pancreatic enzymes, gall bladder contraction, and gastric motility. As recently reviewed (Beinfeld handbook 2013 ,738-743), CCK is one of the most abundant and widely expressed neuropeptides in the brain, essentially as CCK8 amide, in the sulphated form. CCK shares the same five carboxyl-terminal amino acids with gastrin, which is believed to have evolved from CCK. CCK has been shown to be present in microgram quantities in the brain, distributed in high levels in the hippocampus, amygdala, septum, olfactory tubercles, caudate nucleus and the hypothalamus, ventral tegmental area and substantia nigra (Schiffmann et al, 1991) (Fig. 1).

CCK co-localizes with several key neurotransmitters and neuromodulators, prevalently γ-aminobutyric acid (GABA), as well as endocannabinoids, dopamine, serotonin and vasoactive intestinal peptide (Hokfelt et al, 1980; Somogyi et al, 1984; Kosaka et al, 1985; Katona et al, 1999), providing additional evidence for its wide-ranging role in physiological functions in neuronal networks.

CCK interacts with two G-protein (G_q and G_{11}) receptors, activating a variety of intracellular signal transduction pathways (Williams et al, 2002). Protein kinase C activation is the main signaling for CCKA receptor (CCKA-R), while adenylyl cyclase is for CCKB receptor (CCKB-R). CCKA-R is relatively specific for sulphated CCK8, while CCKB-R (identical to the gastrin receptor) interacts also with un-sulphated CCK8, CCK4 and gastrin and represents the main CCK receptor in the brain. CCK acts as an excitatory neurotransmitter modulating the release and function of other neurotransmitters and is involved in diverse normal behaviors, such as learning and memory, feeding, nociception and satiety. The central importance of CCK in neuronal networks is also reflected by its involvement in a variety of neurological and neuropsychiatric disorders including anxiety, panic attacks, schizophrenia and epilepsy (Lee and Soltesz, 2011). Several reports suggest a modulatory role of CCK in memory processing (Flood et al, 1987, Kovács and De Wied, 1994), an aspect of crucial relevance in AD, in which memory and other cognitive functions are impaired. It was demonstrated that CCK enhances memory retention and protects cholinergic neurons against basal forebrain lesions (Sugaya et al, 1992).

Alterations in the CCK system have also been correlated with AD. Indeed, despite the content of CCK in the brain of AD patients being generally relatively unchanged, in the most severe cases it was found to be reduced (Perry et al, 1981; Sagar et al, 1984; Mazurek and Beal, 1991; Löfberg et al, 1996). In addition to reduction in CCK content in brain tissues, neuritic plaques in the AD brain have been demonstrated to contain CCK (Struble et al, 1987; Perry et al, 1981).

Moreover, the CCK receptors are also affected, as a significant down-regulation was demonstrated of both CCKA and CCKB receptors in the brain of patients with AD and with mild cognitive impairment, suggesting that these receptors could play a role in AD development (Hokama et al, 2014; Lin et al, 2014).

Alterations in CCK content have been found also in AD animal models. Unlike the decrease in peptide levels reported in studies on AD human brain, increased levels of CCK were demonstrated in hippocampus and cortex, as well as in neuritic plaques of 18- and 26-month-old transgenic mice overexpressing V717F human beta-amyloid precursor protein (Diez et al, 2000). By contrast, in APP23 mice a decrease in CCK immunoreactivity was shown in hippocampal mossy fibers (Diez et al, 2003).

BRADYKININ

The kinins bradykinin (BK) and kallidin, also called Lys^{10}-BK, are oligopeptides released in the plasma or interstitial fluid after the cleavage of kininogens by kallikreins, a family of serine proteases. Plasma kallikrein and tissue kallikrein 1 (KK1) are the main enzymes involved in kinin source in blood and tissue, respectively. Kininogen-1 gene (KNG1) is a glycoprotein that contains the BK sequence in its mid portion (Fig. 1).

Kinins are potent vasodilators, promote natriuresis and diuresis, and have beneficial cardiovascular effects, however, they also promote pain and inflammation (Bhoola et al, 1992). Components of the kallikrein-kinin system are present in blood,

heart, aorta, brown adipose tissue, adrenal and lung. In the brain, they are localized in the cerebral cortex, brain stem, cerebellum, hypothalamus, hippocampus, and pineal gland, among others. They are found surrounding blood vessels, in neurons and glial cells (Raidoo and Bhoola, 1998).

In human plasma, BK is rapidly metabolized by kininases, among which aminopeptidase P and carboxypeptidase N. However, the major degradation pathway of BK involves also kininase II and angiotensin-converting enzyme (ACE), which is also responsible for the conversion of inactive angiotensin I into the vasopressor angiotensin II (Boola et al, 1992).

Biological effects of kinins are produced by activation of two transmembrane receptors coupled to G proteins (Gα and Gq), namely B1 and B2 receptor (Regoli and Barabe, 1980). Most actions of kinins are mediated by B2 receptor, which has high affinity for BK and is considered a constitutive receptor (Regoli et al, 1998). On the other hand, B1 receptor possesses higher affinity to des-Arg^{9}-BK and Lys-des-Arg^{9}-BK and has limited distribution in tissues under physiological conditions. However, it is mainly expressed in pathological conditions such as chronic inflammation, infection or injury (Regoli and Barabe, 1980). Activation of kinin receptors induces phospholipase C stimulation and promotes intracellular calcium mobilization, as well as release of nitric oxide (NO), especially on neurons and blood vessels (Marceau, Regoli, 2004).

The role exerted by kallikrein-kinin system in AD and other neurological disorders was recently reviewed (Viel and Buck, 2011; Naffah-Mazzacoratti et al, 2014). It was demonstrated that proteolytic enzymes levels are altered in AD (Ladror et al, 1994). Among these, intracerebral kallikrein seems to play an important role in the pathogenesis of AD, as demonstrated by a reduced kallikrein-like enzyme activity, due to a reduction in the gene expression in cerebral tissue of AD patients (Aoyagi et al, 1990). Likewise, increased expression of kallikrein 10 and kallikrein 6 were observed in CSF, plasma and whole blood of patients with AD, showing a strong relationship between the kallikrein-kinin system and brain degeneration (Diamandis et al, 2000; 2004).

Additionally, an activation of the contact/kinin system in CSF of patients with AD has been reported as the result of an anionic interaction of residues within the region 1–11 of $A\beta_{1-42}$ with factor XII, inducing kallikrein generation. This finding seems to be characteristic for brain of AD patients, since in the CSF of patients with neuroimmune inflammatory disease (multiple sclerosis, chronic inflammatory demyelinating polyneuropathy) there was no evidence of increased cleavage of high molecular weight kininogen (Bergamaschini et al, 2001).

According to Farrall et al, (2009), the frontal cortex of patients with AD shows high levels of plasma kallikrein as well as its mRNA. This finding and the high enzyme activity suggest that kinin production could influence cerebral blood flow and vascular permeability altered in AD.

A more direct evidence for BK involvement in AD pathology was demonstrated analyzing BK release and its processing in brain and cerebrospinal fluid (CSF) of rats infused chronically with Aβ (Iores-Marçal et al, 2006). In CSF of animals infused with Aβ, BK concentration was significantly increased, however, in the brain of Aβ group, only a BK fragment was detected. These results suggest that the kallikrein-kinin system is activated in this AD animal model, and that BK is efficiently inactivated by kininases in brain. Since it was reported that in cell cultures BK can increase alpha-secretase processing of APP, inducing decreased $A\beta_{1-40}$, a major constituent of amyloid plaques, BK inactivation could ultimately contribute to the increased senile plaque deposits in the rat brain (Nitsch et al, 1998).

In addition to the variations in kallikrein-kinin enzymatic system, also BK receptors expression appeared to be modified in AD, mainly related to neuroinflammation (Marceau and Bachvarov, 1998, Viel and Buck, 2011). Indeed, in cultured skin fibroblasts from AD patients an increase in the number of BK receptors was reported (Huang et al, 1995), as well as biochemical abnormalities in B2 receptor functions (Jong et al, 2002).

Moreover, increased expression of B1 receptor was found in hippocampal astrocytes of AD mice. In the same work, the ability of B1R antagonists

to abrogate amyloidosis and cerebrovascular and memory deficits was demonstrated, providing evidence for a harmful role for B1R in AD pathogenesis (Lacoste et al, 2013), despite other experimental data indicating that in Tg-SwDI mice B1R activation plays an important role in limiting the accumulation of Aβ in AD-like brain (Passos et al, 2013).

It was also shown that chronic i.c.v. injection of $A\beta_{1-40}$ promotes significant increase in densities of kinin B1 and B2 receptors, mainly in brain regions related to cognitive behavior (Viel et al, 2008). Nevertheless, a single i.c.v. injection of aggregated $A\beta_{1-40}$ induced an increase in B1 receptor expression in hippocampus, but did not modify B2 expression in the same area (Prediger et al, 2008). These variable results suggest that the involvement of the kinin system in Aβ toxicity could be a function of the quantity of Aβ, as well as a function of the exposure time of tissue to the peptide.

On the whole, while B1R is certainly involved in neuroinflammation related to AD, B2R preferentially seems to mediate neuroprotective effects. Activation of B2R by BK was demonstrated to reduce inflammation and neuronal death (Noda et al, 2007) and to promote neurogenesis (Trujillo et al, 2012). Furthermore, it was shown that activation of B2 receptors, but not B1 receptors, up-regulates mRNA for nerve growth factor (NGF) in glial cells, establishing a neuroprotective condition (Noda et al, 2007a).

New advance in the role exerted by BK receptors in AD animal models is represented by the availability of B1 and B2 knockout mice. Following Aβ infusion, B1 knockout mice did not show any difference in memory behavior compared to control animals with the same treatment, while B2 knockout mice resulted in a significant reduction in memory consolidation (Amaral et al, 2010). These data demonstrate that, following chronic infusion with Aβ, B1 receptor could play an important role in the neurodegenerative process, while B2 receptor could have a neuroprotective role. This is further confirmed by the increased number of Aβ plaques found in B2 knockout mice infused with Aβ, pointing to B2 receptor as a potential therapeutic target in AD (Caetano et al, 2015).

Another point to be considered is the potential activity of ACE inhibitors in AD (Zou and Michikawa, 2008). ACE inhibitors (such as enalapril, ramipril and many others) are well-established as important antihypertensive drugs, liable to block conversion of inactive angiotensin I into the vasopressor angiotensin II. However, they also increase half-life of BK, enhancing levels of circulating BK and potentiating BK receptors. At least *in vivo*, ACE inhibitor activity is mainly mediated by B2 receptors, as demonstrated by the inhibitory effect exerted by B2 receptor antagonists (Marceau and Regoli, 2004). The ability of ACE inhibitors to modulate the kinin system could be responsible, at least in part, for the observed neuroprotective activity.

CHROMOGRANIN/SECRETOGRANIN FAMILY

The chromogranin/secretogranin family represents an extended but functionally conserved family of proteins, including chromogranins (chromogranin A and chromogranin B), secretogranins (secretogranin II and secretogranin III), and related proteins (7B2, NESP55, proSAAS, and VGF). They are localized in secretory vesicles and are variously distributed in endocrine, neuronal and neuroendrocrine cells, as well as in the immune system and occasionally in other tissues, subserving essential roles in the regulated secretory pathway that is responsible for controlled delivery of peptides, hormones, neurotransmitters, and growth factors. In the brain, they are widely localized in different areas (Fig. 2).

The first granins identified were chromogranin A (CgA) and chromogranin B (CgB), purified from adrenal medulla, but other proteins were successively added to this family, all sharing some commune features. They are large acidic proteins, sometimes glycosylated or sulphated, having the ability to bind calcium and, although very soluble, to aggregate in the acidic compartment, inducing the formation of dense core granules in the presynaptic structures. Granins regulate different functions, acting as chaperons for protein sorting, modulating prohormone convertase activity and regulating secretory vesicle content release. Moreover, through the secretory pathway, most of them are proteolitically

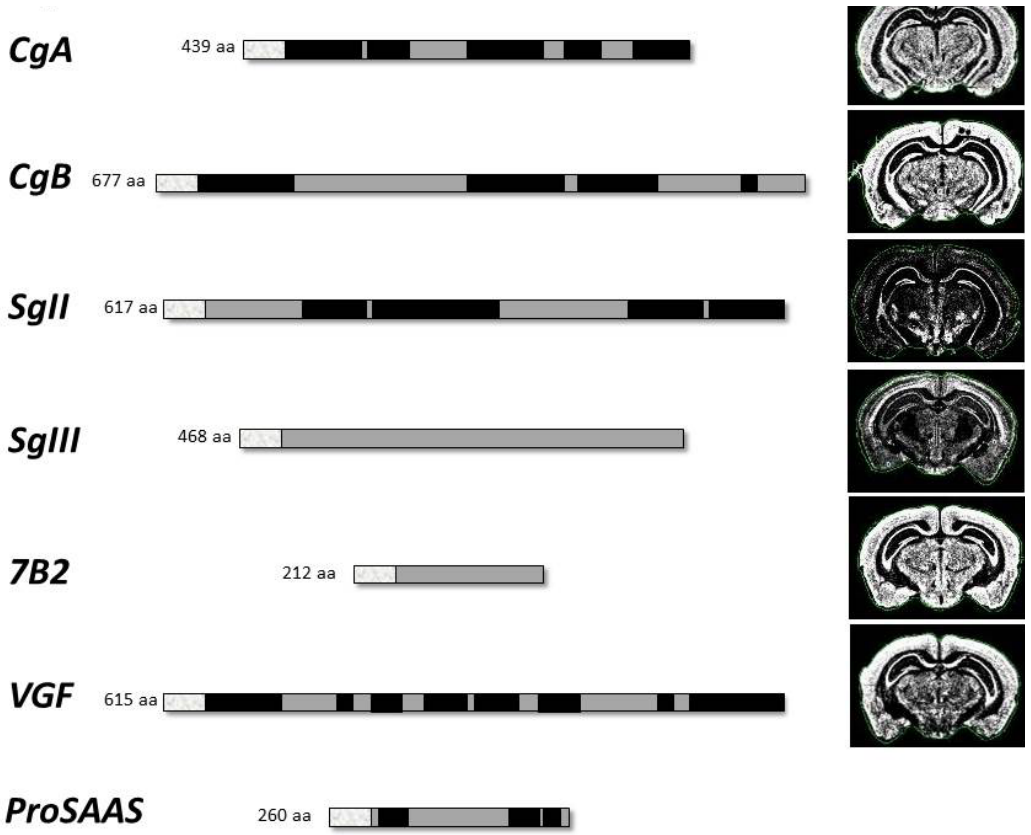

Fig. 2. *Granin precursors sequence and brain distribution. The scheme represents the approximate length and the number of amino acids of the precursor of CgA, CgB, SgII, SgIII, 7B2, VGF and ProSAAS. The leader signal for secretory protein is indicated as light shadowed block. The black blocks indicate the biological active fragments identified. In the right panels are shown images of in situ hybridization for the corresponding gene expression in mouse CNS (Source from Allen Brain Atlas, mouse.brain-map.org).*

processed in different biological active peptides that are stored in large dense core granules and released upon secretory stimulation (for extensive review see Bartolomucci et al, 2011). Despite repeated attempts to demonstrate the existence of cognate receptors, no evidence for specific granin receptors, or the precise mechanisms of action has been produced to date. Since granins are largely distributed in the CNS and are involved in synaptic functions, several studies investigated the potential utility of granins as diagnostic biomarkers for AD and other neurodegenerative diseases (Bartolomucci et al, 2011, Willis et al, 2011). However, due to the processing of these large precursor molecules in many different neuropeptides, it is difficult to recognize the precise identity of these fragments because often Authors refer to the whole precursor.

a) Chromogranin A

CgA, a 439-amino-acid protein, was the first identified granin and the most extensively studied (Winkler and Fischer-Colbrie, 1992). CgA was postulated to be an immunostimulator in AD, contributing to neuroinflammation started by Aβ peptides (Heneka et al, 2010). Indeed, intense staining for CgA was demonstrated in about 30%

of Aβ plaques in AD cortical samples, frequently surrounded by hyperactivated microglia (Marksteiner et al, 2002; Rangon et al, 2003; Lechner et al, 2004; Willis et al, 2011). CgA increase in neuritic plaques correlates with an increase in Catestatin (CST, an internal fragment of CgA), able to activate pro–caspase-1 (Wu et al, 2013).

There is evidence that CgA activates microglia to a reactive phenotype and stimulates the release of microglial cytotoxins, suggesting that this peptide may contribute to the continued and neurotoxic activation of microglia in AD (Lechner et al, 2004).

CgA staining increase in the amyloid plaques inversely correlates with CgA levels found in the CSF, significantly reduced in patients with AD or taupathies (Blennow et al, 1995; Eder et al, 1998; Simonsen et al, 2007; Perrin et al, 2011; Jahn et al, 2011; Mattson et al, 2012; Paterson et al, 2014; Wildsmith et al, 2014). Among CgA derived peptides, only Serpin (26 aa at C-terminal) showed a neuroprotective activity, as demonstrated *in vitro*, in AtT20 neuronal cell line (Koshimizu et al, 2011). To date, no neuroprotective functions have been identified for Vasostatin, Pancrestatin and Chromacin, the other major forms of the CgA-derived neuropeptides, endowed with neuroendocrine activities.

b) Chromogranin B

CgB, a pre-pro-protein of 677 amino acids, shares several features with CgA, including wide expression throughout the endocrine and nervous systems, acidic protein backbone, random-coil structure, and heat stability. CgB is abundantly expressed in many neurons and peptidergic endocrine cells (Bartolomucci et al, 2011).

Immunostaining of post-mortem brains from AD patients showed positive CgB reactivity in amyloid plaques, more prominent in hippocampal regions (Marksteiner et al, 2002; Lechner et al, 2004; Willis et al, 2008, 2011), while levels of CgB peptides in CSF of AD patients were significantly decreased, compared to control subjects (Eder et al, 1998; Mattson et al, 2010; 2012; 2013).

c) Secretogranin II

SgII is a 617 amino acid pre-pro-protein highly conserved across evolution and, together with CgB is the major soluble constituent of the large dense core vesicles of presynaptic structures (Fischer-Colbrie et al, 1995). Several studies showed an increased SgII immunostaining in amyloid plaques, while a significant reduction in immunoreactivity was described in different brain areas of AD patients (Kaufmann, 1998; Marksteiner et al, 2002; Lechner et al, 2004). Alterations in SgII content have been reported also in animal models of AD. Indeed, in brain of transgenic mice overexpressing human APP751 with the London (V717I) and Swedish (K670M/N671L) mutations, about 40% of amyloid-beta plaques were associated with SgII, however no immunostaining reduction was observed in specific brain areas, compared to controls, as otherwise reported in AD patients (Willis et al, 2008).

From SgII processing three major products are obtained: Secretoneurin (SN, 33 aa sequence, near the N-terminal portion), EM-66 (central portion) and Manserin (40 aa sequence, near the C-terminal region). Consistent with the decreased level of CgA and CgB found in in CSF of AD patients, also SN or other SgII fragments were found to be reduced (Eder et al, 1998; Matsson et al, 2010; Spellman et al, 2015). In addition, SN neuropeptide was demonstrated to promote *in vitro* and *in vivo* neuroprotection after oxygen/glucose deprivation, suggesting an anti-apoptotic activity through Jak2/Stat3 signaling pathway (Shyu et al, 2008).

d) Secretogranin III

SgIII is an acidic 468 amino acid secretory protein, well conserved during evolution, from mammals to fish. It has been identified as a specific binding protein for CgA, suggesting that it can play a central role in secretory granule biogenesis (Hosaka and Watanabe, 2010).

Although different fragments of SgIII have been detected in several neuroendocrine cell types from various species (Holtious et al, 1996), no biologically active peptides derived from SgIII have been described. In the cerebral cortex of AD patients increased levels of SgIII were observed in dystrophic neurites surrounding amyloid plaques (Plà et al, 2013). Additionally, SgIII was detected in CSF but

no significant differences between AD patients and control subjects were reported (Perrin et al, 2011).

e) 7B2

The smaller granin 7B2 (212 amino acids) is perhaps the most evolutionarily conserved member of the granin family, containing a C-terminal peptide acting as inhibitor of the prohormone convertase 2 (PC2), responsible for the proteolytic processing of many precursor proteins, among which neuropeptides precursors (Mbikay et al, 2001). Alterations, often conflicting, in PC2 and 7B2 levels have been shown in AD brain, related to a dysregulation in the level of different neuropeptides. A marked decrease in the ratio of the PC2 precursor to the total enzymatic pool was observed in the frontal cortex of AD patients, corresponding to an increase in the binding protein 7B2 (Winsky-Sommerer et al, 2003). On the contrary, other studies reported increased levels of PC2 in AD brain, while no differences were detected in the levels of 7B2 (Yakovlera et al, 2007). Likewise, no significant differences were found in the 7B2 immunoreactivity in various brain regions obtained from patients with AD and from control subjects (Iguchi et al, 1987).

More recently, in the hippocampus and substantia nigra of human AD-affected brains, as well as in the brains of APP/PSEN1 mice, 7B2 was found highly co-localized with Aβ plaques and α-synuclein deposits (Helwig et al, 2013). In the same work it was demonstrated that 7B2 efficiently prevents *in vitro* fibrillation and formation of Aβ aggregates, establishing this neural protein as an anti-aggregation chaperon associated with neurodegenerative diseases. In addition, recombinant 7B2 efficiently blocked the neurocytotoxic effect of $A\beta_{1-42}$, significantly increasing cell viability of Neuro-2A cells (Helwig et al, 2013).

f) ProSAAS

ProSAAS granin is a 260 amino acid precursor protein suggested to function as peptide precursor only in higher vertebrates (Bartolomucci et al, 2011). Like 7B2, it has been suggested to regulate the activity of the pro-hormone convertase PC1 (Lee et al, 2004). The potential role exerted by proSAAS in the pathogenesis of AD was suggested by the evidence that N-proSAAS or proSAAS-like molecules are trapped within the tau fibrils and accumulated in tau inclusions in the AD patients brains (Wada et al, 2004). In addition, in the brain of 12-month-old APdE9 mice, and in the cortex of human AD affected brain, proSAAS immunoreactivity co-localizes with amyloid plaques deposits (Hoshino et al, 2014).

Like other granins, ProSAAS fragments were found decreased in CSF of AD patients in respect to healthy subjects, suggesting that they could represent, together with other neuropeptides, biomarkers for AD (Jahn et al, 2011). As previously reported for 7B2, additional evidence for the role of ProSAAS in AD was demonstrated by the amyloid anti-aggregant activity of an internal ProSAAS fragment (ProSAAS 97-180), shown to efficiently prevent the fibrillation of $A\beta_{1-42,}$ *in vitro* (Hoshino et al, 2014). In the same study, the Authors reported that the recombinant, as well as the endogenously synthesized proSAAS, was able to prevent the neurotoxic effect of $A\beta_{1-42}$ in Neuro2a cells.

g) VGF

The large secretogranin VII, early named VGF, is a 615 amino acid protein precursor of several biological active peptides. A dozen of them have been detected in CSF of patients with neurodegenerative diseases. In CSF of AD patients, both an N-terminal fragment and an internal peptide (VGF 365-375) were found to be decreased (Carrette el al, 2003; Jahn et al, 2011; Wujte et al, 2012; Spellman et al, 2015; Hottla et al, 2015; Hendricson et al, 2015), suggesting that VGF could be a potential biomarker for this disorder. Moreover, in parietal cortex from patients with AD, a reduction in different VGF peptides was shown (Cocco et al, 2010), whereas an increase of VGF expression was reported in peripheral T cells in patients with AD, compared to aged healthy controls (Busse et al, 2015).

Interestingly, it was demonstrated that VGF-derived peptides exert important neuronal stimulatory activity. TLQP-62 (C-terminal VGF peptide) has an antidepressant activity (Lin et al, 2014), increases neuronal electrical excitability in hippocampus neurons (Takker-Varia et al, 2007), while TLQP-21 (a smaller C-terminal VGF peptide)

protects from apoptosis cerebellar granule cells after potassium deprivation (Severini et al, 2008). Moreover, in primary cortical and hippocampal cell cultures, TLQP-21 showed a neuroprotective activity against Aβ toxicity, while other fragments had minor or no neuroprotective effect (Possenti R., unpublished data).

So far, only for TLQP-21 peptide a receptor has been recently discovered, identifying the variously distributed complement C3a receptor-1 (C3AR1) as a target for TLQP-21 (Hannedouche et al, 2013; Cero et al, 2014).

CONCLUSIONS

AD has become a great clinical problem in our society and the prevalence of AD is likely to increase among the aging population worldwide. To date, no treatment has been found that could slow down the progression of the disease or that could prevent cholinergic cell death, as the current therapeutic approach to AD is of a symptomatic type. Since the pathology of AD is very complex and different pathomechanisms are involved, the ultimate goal of a sustainable disease-modifying treatment in AD is to slow down disease progression by addressing the neurodegenerative processes, acting at multiple pathways. The role exerted by neuropeptides seems particularly interesting since they are generally neuroprotective, widely distributed in brain areas responsible for learning and memory processes, and their levels are altered in both human disease and in animal experimental models. Since neuropeptides could represent biomarkers of disease progression, this seems of great potential utility for AD because of inherent difficulties assessing brain function and finding a diagnosis in this pathology. In addition, convincing evidence has been provided for the protective role of several neuropeptides against neurodegeneration both *in vitro* and *in vivo*, identifying neuropeptide receptors as potential therapeutic targets.

REFERENCES

Albert M, Jenike M, Nixon R, Nobel K. (1993) Thyrotropin response to thyrotropin-releasing hormone in patients with dementia of the Alzheimer type. *Biol Psychiatry* **33**(4):267-71.

Amaral FA, Lemos MT, Dong KE, Bittencourt MF, Caetano AL, Pesquero JB, Viel TA, Buck HS(2010) Participation of kinin receptors on memory impairment after chronic infusion of human amyloid-beta 1-40 peptide in mice. *Neuropeptides* **44**(2):93-7.

Anand R, Gill KD, Mahdi AA (2014) Therapeutics of Alzheimer's disease: Past, present and future. *Neuropharmacology* **76** pt A: 27-50.

Aoyagi T, Wada T, Nagai M, Kojima F, Harada S, Takeuchi T, Takahashi H, Hirokawa K, Tsumita T (1990) Deficiency of kallikrein-like enzyme activities in cerebral tissue of patients with Alzheimer's disease. *Experientia* **46**(1): 94-7.

Bartolomucci A, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SR (2011) The extended granin family: structure, function, and biomedical implications. *Endocr Rev* **32**(6): 755-97.

Bennett GW, Ballard TM, Watson CD, Fone KC (1997) Effect of neuropeptides on cognitive function. *Exp Gerontol* **32**(4-5): 451-69.

Beinfeld MC (2013) CCK. *Handbook of Biologically Active Peptides, second edition, edited by Abba J. Kastin,* ***chapter 99****: 738-743.*

Bergamaschini L, Donarini C, Gobbo G, Parnetti L, Gallai V (2001) Activation of complement and contact system in Alzheimer's disease. *Mech Ageing Dev* **122**: 1971–1983.

Bhoola KD, Figueroa CD and Worthy K (1992) Bioregulation of kinins: kallikreins, kininogens and kininases. *Pharmacol Rev* **44**: 1-80.

Biggins JA, Perry EK, McDermott JR, Smith AI, Perry RH, Edwardson JA (1983) Post mortem levels of thyrotropin-releasing hormone and neurotensin in the amygdala in Alzheimer's disease, schizophrenia and depression. *J Neurol Sci* **58**: 117-22.

Blennow K, Davidsson P, Wallin A, Ekman R (1995) Chromogranin A in cerebrospinal fluid: a biochemical marker for synaptic degeneration in Alzheimer's disease? *Dementia* **6**(6):306-11.

Bouras C,de St Hilaire-Kafi S, Constantinidis J (1986)

Neuropeptides in Alzheimer's disease: a review and morphological results . *Prog Neuropsychopharmacol Biol Psychiatry* **10**: 271-86.

Busse S, Steiner J, Glorius S, Dobrowolny H, Greiner-Bohl S, Mawrin C et al. and Busse M (2015) VGF expression by T lymphocytes in patients with Alzheimer's disease. *Oncotarget* **6**(17):14843-51.

Caetano AL, Dong-Creste KE, Amaral FA, Monteiro-Silva KC, Pesquero JB, Araujo MS et al. and Buck HS (2015) Kinin B2 receptor can play a neuroprotective role in Alzheimer's disease. *Neuropeptides* **53**:51-62.

Carrette O, Demalte I, Scherl A, Yalkinoglu O, Corthals G et al. and Sanchez JC (2003) A panel of cerebrospinal fluid potential biomarkers for the diagnosis of Alzheimer's disease. *Proteomics* **3**(8): 1486-94.

Cero C, Vostrikov VV, Verardi R, Severini C, Gopinath T et al. and Bartolomucci A (2014) The TLQP-21 peptide activates the G-protein-coupled receptor C3aR1 via a folding-upon-binding mechanism. *Structure* **22**(12): 1744-53.

Cocco C, D'Amato F, Noli B, Ledda A, Brancia C, Bongioanni P, Ferri GL (2010) Distribution of VGF peptides in the human cortex and their selective changes in Parkinson's and Alzheimer's diseases. *J Anat* **217**(6): 683-93.

Diamandis EP, Yousef GM, Petraki C, Soosaipillai AR (2000) Human kallikrein 6 as a biomarker of alzheimer's disease. *Clin Biochem* **33**:663–667.

Diamandis EP, Scorilas A, Kishi T, Blennow K, Luo LY et al. and Sjogren M (2004) Altered kallikrein 7 and 10 concentrations in cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. *Clin Biochem* **37**:230–237.

Diez M, Koistinaho J, Kahn K, Games D, Hökfelt T (2000) Neuropeptides in hippocampus and cortex in transgenic mice overexpressing V717F beta-amyloid precursor protein--initial observations. *Neuroscience* **100**(2): 259-86.

Diez M, Danner S, Frey P, Sommer B, Staufenbiel M, Wiederhold KH, Hökfelt T (2003) Neuropeptide alterations in the hippocampal formation and cortex of transgenic mice overexpressing beta-amyloid precursor protein (APP) with the Swedish double mutation (APP23). *Neurobiol Dis* **14**(3): 579-94.

Eder U, Leitner B, Kirchmair R, Pohl P, Jobst KA et al. and Winkler H (1998) Levels and proteolytic processing of chromogranin A and B and secretogranin II in cerebrospinal fluid in neurological diseases. *J Neural Transm* (Vienna) **105**(1): 39-51.

Farrall AJ, Wardlaw JM (2009) Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiol Aging* **30**(3): 337-52.

Fischer-Colbrie R, Laslop A, Kirchmair R (1995) Secretogranin II: molecular properties, regulation of biosynthesis and processing to the neuropeptide secretoneurin. *Prog Neurobiol* **46**:49–70.

Flood JF, Smith GE, Morley JE (1987) Modulation of memory processing by cholecystokinin: dependence on the vagus nerve. *Science* **236**(4803): 832-4.

Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Diaz-Cabiale Z, Rivera A et al. and Agnati LF(2012) Extrasynaptic neurotransmission in the modulation of brain function. Focus on the striatal neuronal-glial networks. *Front Physiol* **3**:136.

Gershengorn MC, Osman R (1996) Molecular and cellular biology of thyrotropin-releasing hormone receptors. *Physiol Rev* **76**(1): 175-91.

Gomez JM, Aguilar M, Soler J (2000) Growth hormone and thyrotropin hormone secretion in Alzheimer'disease. *J Nutr Health Aging* **4**:229-32.

Hallberg M, Nyberg F (2003) Neuropeptide conversion to bioactive fragments--an important pathway in neuromodulation. *Curr Protein Pept Sci* **4**(1): 31-44.

Hallberg M (2015) Neuropeptides: metabolism to bioactive fragments and the pharmacology of their receptors. *Med Res Rev* **35**(3): 464-519.

Hannedouche S, Beck V, Leighton-Davies J, Beibel M, Roma G et al. and Bassilana F (2013) Identification of the C3a receptor (C3AR1) as the target of the VGF-derived peptide TLQP-21 in rodent cells. *J Biol Chem* **288**(38): 27434-43.

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**(5054): 184-5.

Haass C, Selkoe DJ (2007) Soluble protein oligomers

in neurodegeneration: lessons from Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol **8**(2): 101-12.

Helwig M, Hoshino A, Berridge C, Lee SN et al. and Lindberg I (2013) The neuroendocrine protein 7B2 suppresses the aggregation of neurodegenerative disease-related proteins. *J Biol Chem* **288**(2): 1114-24.

Hendrickson RC, Lee AY, Song Q, Liaw A, Wiener M, Paweletz CPet al. and Yates NA (2015) High Resolution Discovery Proteomics Reveals Candidate Disease Progression Markers of Alzheimer's Disease in Human Cerebrospinal Fluid. *PLoS One* **10**(8): e0135365.

Heneka MT, O'Banion MK, Terwel D, Kummer MP (2010) Neuroinflammatory processes in Alzheimer's disease. *J Neural Transm* (Vienna) **117**(8): 919-47.

Heuer H, Schäfer MK, O'Donnell D, Walker P, Bauer K (2000) Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. *J Comp Neurol* **428**(2): 319-36.

Hokama M, Oka S, Leon J, Ninomiya T, Honda H et al. and Nakabeppu Y (2014) Altered expression of diabetes-related genes in Alzheimer's disease brains: the Hisayama study. *Cereb Cortex* **24**(9): 2476-88.

Hökfelt T, Rehfeld JF, Skirboll L, Ivemark B, Goldstein M, Markey K (1980) Evidence for coexistence of dopamine and CCK in meso-limbic neurones. *Nature* **285**(5765): 476-8.

Hölttä M1, Minthon L, Hansson O, Holmén-Larsson J, Pike I et al. and Gobom J (2015) An integrated workflow for multiplex CSF proteomics and peptidomics-identification of candidate cerebrospinal fluid biomarkers of Alzheimer's disease. *J Proteome Res* **14**(2): 654-63.

Hosaka M, Watanabe T (2010) Secretogranin III: a bridge between core hormone aggregates and the secretory granule membrane. *Endocr J* **57**(4):275-86.

Hoshino A, Helwig M, Rezaei S, Berridge C, Eriksen JL, Lindberg I (2014) A novel function for proSAAS as an amyloid anti-aggregant in Alzheimer's disease. *J Neurochem* **128**(3): 419-30.

Hrabovszky E, Liposits Z (2008) Novel aspects of glutamatergic signalling in the neuroendocrine system. J Neuroendocrinol 20(6): 743-51.

Huang HM, Lin TA, Sun GY, Gibson GE (1995) Increased inositol 1,4,5-trisphosphate accumulation correlates with an up-regulation of bradykinin receptors in Alzheimer's disease. *J Neurochem* **64**(2): 761-6.

Iguchi H, Chan JS, Seidah NG, Chrétien M (1987) Evidence for a novel pituitary protein (7B2) in human brain, cerebrospinal fluid and plasma: brain concentrations in controls and patients with Alzheimer's disease. *Peptides* **8**(4): 593-8.

Iores-Marçal LM, Viel TA, Buck HS, Nunes VA, Gozzo AJ et al. and Araujo MS(2006) Bradykinin release and inactivation in brain of rats submitted to an experimental model of Alzheimer's disease. *Peptides* **27**(12): 3363-9.

Jahn H, Wittke S, Zürbig P, Raedler TJ, Arlt S et al. and Wiedemann K (2011) Peptide fingerprinting of Alzheimer's disease in cerebrospinal fluid: identification and prospective evaluation of new synaptic biomarkers. *PLoS One* **6**(10): e26540.

Jackson IM and S. Reichlin (1974) Thyrotropin-releasing hormone (TRH): distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. *Endocrinology* **95**: 854–862.

Jong YJ, Dalemar LR, Seehra K, Baenziger NL (2002) Bradykinin receptor modulation in cellular models of aging and Alzheimer's disease. *Int Immunopharmacol* **2**(13-14): 1833-40.

Joseph-Bravo P, Jaimes-Hoy L, Uribe RM, Charli JL (2015) 60 YEARS OF NEUROENDOCRINOLOGY: TRH, the first hypophysiotropic releasing hormone isolated: control of the pituitary-thyroid axis. *J Endocrinol* **226**(2): T85-T100.

Katona I, Sperlágh B, Sík A, Käfalvi A, Vizi ES, Mackie K, Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* **19**(11): 4544-58.

Kaufmann WA, Barnas U, Humpel C, Nowakowski K, DeCol C et al. and Marksteiner J (1998) Synaptic loss reflected by secretoneurin-like immunoreactivity in the human hippocampus in Alzheimer's disease. *Eur J Neurosci* **10**(3): 1084-94.

Koenig ML, Yourick DL, Meyerhoff JL (1996) Thyrotropin-releasing hormone (TRH) attenuates glutamate-stimulated increases in calcium in primary

neuronal cultures. *Brain Res* **730**: 143–9.

Kosaka T, Kosaka K, Tateishi K, Hamaoka Y, Yanaihara N, Wu JY, Hama K (1985) GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. *J Comp Neurol* **239**(4): 420-30.

Koshimizu H, Cawley NX, Yergy AL, Loh YP (2011) Role of pGlu-serpinin, a novel chromogranin A-derived peptide in inhibition of cell death. *J Mol Neurosci* **45**(2): 294-303.

Kovács GL, De Wied D (1994) Peptidergic modulation of learning and memory processes. *Pharmacol Rev* **46**(3): 269-91.

Lacoste B, Tong XK, Lahjouji K, Couture R, Hamel E (2013) Cognitive and cerebrovascular improvements following kinin B1 receptor blockade in Alzheimer's disease mice. *J Neuroinflammation* **10**:57.

Ladror US, Wang GT, Klein WL, Holzman TF, Krafft GA (1994) Potential beta PP-processing proteinase activities from Alzheimer's and control brain tissues. *J Protein Chem*. **13**(4): 357-66.

Lechner T, Adlassnig C, Humpel C, Kaufmann WA, Maier H et al. and Marksteiner J (2004) Chromogranin peptides in Alzheimer's disease. *Exp Gerontol* **39**(1): 101-13.

Lee SN, Prodhomme E, Lindberg I (2004) Prohormone convertase 1 (PC1) processing and sorting: effect of PC1 propeptide and proSAAS. *J Endocrinol* **182**(2): 353-64.

Lee SY, Soltesz I (2011) Cholecystokinin: a multi-functional molecular switch of neuronal circuits. *Dev Neurobiol* **71**(1): 83-91.

Lin H, Zhang T, Wu Y, Wang Y, Wang W, Wang Q (2014) Related genes and potential biomarkers for early diagnosis of Alzheimer's disease: a preliminary study based on DNA microarray. *Am J Alzheimers Dis Other Demen* **29**(1): 90-5.

Lin P, Wang C, Xu B, Gao S, Guo J et al. and Zhou W (2014) The VGF-derived peptide TLQP62 produces antidepressant-like effects in mice via the BDNF/TrkB/CREB signaling pathway. *Pharmacol Biochem Behav* **120**:140-8.

Löfberg C, Harro J, Gottfries CG, Oreland L (1996) Cholecystokinin peptides and receptor binding in Alzheimer's disease. *J Neural Transm* (Vienna) **103**(7): 851-60.

Luo L, Yano N, Mao Q, Jackson IM, Stopa EG (2002) Thyrotropin releasing hormone (TRH) in the hippocampus of Alzheimer patients. *J Alzheimers Dis* **4**(2): 97-103.

Luo L, Stopa EG (2004) Thyrotropin releasing hormone inhibits tau phosphorylation by dual signaling pathways in hippocampal neurons. *J Alzheimers Dis* **6**(5): 527-36.

Marceau F, Hess JF and Bachvarov DR (1998) The B1 receptors for kinins. *Pharmacol Rev* **50**(3): 357-386.

Marceau F, Regoli D (2004) Bradykinin receptor ligands: therapeutic perspectives. *Nat Rev Drug Discov* **3**(10): 845-52.

Marksteiner J, Kaufmann WA, Gurka P, Humpel C (2002) Synaptic proteins in Alzheimer's disease. *J Mol Neurosci* **18**(1-2): 53-63.

Mattsson N, Johansson P, Hansson O, Wallin A, Johansson JO et al. and Zetterberg H (2010) Converging pathways of chromogranin and amyloid metabolism in the brain. *J Alzheimers Dis* **20**(4): 1039-49.

Mattsson N, Portelius E, Rolstad S, Gustavsson M, Andreasson U et al. and Zetterberg H (2012) Longitudinal cerebrospinal fluid biomarkers over four years in mild cognitive impairment. *J Alzheimers Dis* **30**(4): 767-78.

Mattsson N, Insel P, Nosheny R, Zetterberg H et al. and Weiner M (2013) CSF protein biomarkers predicting longitudinal reduction of CSF β-amyloid42 in cognitively healthy elders. *Transl Psychiatry* **3**:e293.

Mazurek MF, Beal MF (1991) Cholecystokinin and somatostatin in Alzheimer's disease postmortem cerebral cortex. *Neurology* **41**(5): 716-9.

Mbikay M, Seidah NG, Chrétien M (2001) Neuroendocrine secretory protein 7B2: structure, expression and functions. *Biochem J* **357**:329–342.

Mellow AM, Sunderland T, Cohen RM, Lawlor BA et al. and Murphy DL (1989) Acute effects of high-dose thyrotropin releasing hormone infusions in Alzheimer's disease. *Psychopharmacology* (Berl) **98**(3): 403-7.

Mrak RE, Griffin WS (2001) Interleukin-1, neuroinflammation, and Alzheimer's disease Neurobiol Aging **22**(6): 903-8.

Naffah-Mazzacoratti Mda G, Gouveia TL, Simões PS, Perosa SR (2014) What have we learned about the kallikrein-kinin and renin-angiotensin systems in neurological disorders? *World J Biol Chem* **5**(2): 130-40.

Nemeroff CB, Kizer JS, Reynolds GP, Bissette G (1989) Neuropeptides in Alzheimer's disease: a postmortem study. *Regul Pept* **25**(1): 123-30.

Nitsch RM, Kim C, Growdon JH (1998) Vasopressin and bradykinin regulate secretory processing of the amyloid protein precursor of Alzheimer's disease. *Neurochem Res* **23**(5): 807-14.

Noda M, Kariura Y, Pannasch U, Nishikawa K, Wang L et al. and Wada K (2007b) Neuroprotective role of bradykinin because of the attenuation of pro-inflammatory cytokine release from activated microglia. *J Neurochem* **101**(2): 397-410.

Noda M, Sasaki K, Ifuku M, Wada K (2007a) Multifunctional effects of bradykinin on glial cells in relation to potential anti-inflammatory effects. *Neurochem Int* **51**(2-4): 185-91.

Nyberg F, Hallberg M (2007) Peptide conversion--a potential pathway modulating G-protein signaling. *Curr Drug Targets* **8**(1): 147-54.

Pákáski M, Kálmán J (2008) Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. *Neurochem Int* **53**(5): 103-11.

Passos GF, Medeiros R, Cheng D, Vasilevko V, Laferla FM, Cribbs DH (2013) The bradykinin B1 receptor regulates Aβ deposition and neuroinflammation in Tg-SwDI mice. *Am J Pathol* **182**(5):1740-9.

Paterson RW, Bartlett JW, Blennow K et al. and Schott JM (2014) Cerebrospinal fluid markers including trefoil factor 3 are associated with neurodegeneration in amyloid-positive individuals. *Transl Psychiatry* **4**:e419.

Pekary AE, Gwirtsman H, Tourtellotte WW, Smith VP, Hershman JM (1991) High levels of thyrotropin-releasing hormone precursor peptide immunoreactivity and binding substance occur in human cerebrospinal fluid. *Neuroendocrinology* **53**(3): 246-52.

Pekary AE (1998) Is Ps4 (prepro-TRH [160-169]) more than an enhancer for thyrotropin-releasing hormone? *Thyroid* **8**(10): 963-8.

Perrin RJ, Craig-Schapiro R, Malone JP, Shah AR, Gilmore P et al. and Fagan AM (2011) Identification and validation of novel cerebrospinal fluid biomarkers for staging early Alzheimer's disease. *PLoS One* **6**(1): e16032.

Perry EK, Blessed G, Tomlinson BE, Perry RH, Crow TJ et al. and Arregui A (1981) Neurochemical activities in human temporal lobe related to aging and Alzheimer-type changes. *Neurobiol Aging* **2**(4): 251-6(1981).

Perry RH, Dockray GJ, Dimaline R, Perry EK, Blessed G, Tomlinson BE (1981) Neuropeptides in Alzheimer's disease, depression and schizophrenia. A post mortem analysis of vasoactive intestinal peptide and cholecystokinin in cerebral cortex. *J Neurol Sci* **51**(3): 465-72.

Plá V, Paco S, Ghezali G, Ciria V, Pozas E, Ferrer I, Aguado F (2013) Secretory sorting receptors carboxypeptidase E and secretogranin III in amyloid β-associated neural degeneration in Alzheimer's disease. *Brain Pathol* **23**(3): 274-84.

Prediger RD, Medeiros R, Pandolfo P, Duarte FS et al. and Takahashi RN (2008) Genetic deletion or antagonism of kinin B(1) and B(2) receptors improves cognitive deficits in a mouse model of Alzheimer's disease. *Neuroscience* **151**(3): 631-43.

Raidoo DM, Bhoola KD (1998) Pathophysiology of the kallikrein-kinin system in mammalian nervous tissue. *Pharmacol Ther* **79**(2): 105-27.

Rangon CM, Haïk S, Faucheux BA, Metz-Boutigue MH et al. and Aunis D (2003) Different chromogranin immunoreactivity between prion and a-beta amyloid plaque. *Neuroreport* **14**(5): 755-8.

Regoli D and Barabe J (1980) Pharmacology of bradykinin and related kinins. *Pharmacol Rev* **32**: 1-46.

Regoli D, Nsa Allogho S, Rizzi A and Gobeil FJ (1998) Bradykinin receptors and their antagonists. *Eur J Pharmacol* **348**(1): 1-10.

Sagar SM, Beal MF, Marshall PE, Landis DM, Martin JB (1984) Implications of neuropeptides in neurological diseases. *Peptides* **5 Suppl 1**:255-62.

Schiffmann SN, Vanderhaeghen JJ (1991) Distribution of cells containing mRNA encoding cholecystokinin in the rat central nervous system. *J Comp Neurol* **304**(2):

219-33.

Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* **8**(6): 595-608.

Severini C, Ciotti MT, Biondini L, Quaresima S, Rinaldi AM, Levi A, Frank C, Possenti R (2008) TLQP-21, a neuroendocrine VGF-derived peptide, prevents cerebellar granule cells death induced by serum and potassium deprivation. *J Neurochem* **104**(2): 534-44.

Severini C, Petrella C, Calissano P (2016) Substance P and Alzheimer's disease: emerging novel roles. *Curr Alzheimer Res* [Epub ahead of print].

Shyu WC, Lin SZ, Chiang MF, Chen DC, Su CY et al. and Li H (2008) Secretoneurin promotes neuroprotection and neuronal plasticity via Jak2/Stat3 pathway in murine models of stroke. *J Clin Invest* **118**(1): 133-48.

Simonsen AH, McGuire J, Podust VN, Hagnelius NO et al. and Waldemar G (2007) A novel panel of cerebrospinal fluid biomarkers for the differential diagnosis of Alzheimer's disease versus normal aging and frontotemporal dementia. *Dement Geriatr Cogn Disord* **24**(6): 434-40.

Somogyi P, Hodgson AJ, Smith AD, Nunzi MG, Gorio A, Wu JY (1984 Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material *J Neurosci* **4**(10): 2590-603.

Spellman DS, Wildsmith KR, Honigberg LA, Tuefferd M, Baker D et al. and Potter WZ (2015) Development and evaluation of a multiplexed mass spectrometry based assay for measuring candidate peptide biomarkers in Alzheimer's Disease Neuroimaging Initiative (ADNI) CSF. *Proteomics Clin Appl* **9**(7-8): 715-31.

Struble RG, Powers RE, Casanova MF, Kitt CA, Brown EC, Price DL (1987) Neuropeptidergic systems in plaques of Alzheimer's disease. *J Neuropathol Exp Neurol* **46**(5): 567-84.

Sugaya K, Takahashi M, Kubota K (1992) Cholecystokinin protects cholinergic neurons against basal forebrain lesion. *Jpn J Pharmacol* **59**(1): 125-8.

Thakker-Varia S, Krol JJ, Nettleton J, Bilimoria PM et al. and Alder J (2007) The neuropeptide VGF produces antidepressant-like behavioral effects and enhances proliferation in the hippocampus. *J Neurosci* **27**(45): 12156-67.

Trujillo CA, Negraes PD, Schwindt TT, Lameu C, Carromeu C et al. and Ulrich H (2012) Kinin-B2 receptor activity determines the differentiation fate of neural stem cells. *J Biol Chem* **287**(53): 44046-61.

Tuppo EE, Arias HR (2005) The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol* **37**(2): 289-305.

Van Dam D, Van Dijck A, Janssen L, De Deyn PP (2013) Neuropeptides in Alzheimer's disease: from pathophysiological mechanisms to therapeutic opportunities. *Curr Alzheimer Res* **10**(5): 449-68.

Viel TA, Lima Caetano A, Nasello AG, Lancelotti CL, Nunes VA, Araujo MS, Buck HS (2008) Increases of kinin B1 and B2 receptors binding sites after brain infusion of amyloid-beta 1-40 peptide in rats. *Neurobiol Aging* **29**(12):1805-14.

Viel TA, Buck HS (2011) Kallikrein-kinin system mediated inflammation in Alzheimer's disease in vivo. *Curr Alzheimer Res* **8**(1): 59-66.

Wada M, Ren CH, Koyama S, Arawaka S, Kawakatsu S et al. and Kato T (2004) A human granin-like neuroendocrine peptide precursor (proSAAS) immunoreactivity in tau inclusions of Alzheimer's disease and parkinsonism-dementia complex on Guam. *Neurosci Lett* **356**(1): 49-52.

Wiedermann CJ (2000) Secretoneurin: a functional neuropeptide in health and disease. *Peptides* **21**(8): 1289-98.

Wijte D, McDonnell LA, Balog CI, Bossers K et al. and Mayboroda OA (2012) A novel peptidomics approach to detect markers of Alzheimer's disease in cerebrospinal fluid. *Methods* **56**(4): 500-7.

Wildsmith KR, Schauer SP, Smith AM, Arnott D et al. and Honigberg LA (2014) Identification of longitudinally dynamic biomarkers in Alzheimer's disease cerebrospinal fluid by targeted proteomics. *Mol Neurodegener* **9**:22.

Williams JA, Sans MD, Tashiro M, Schäfer C, Bragado MJ, Dabrowski A (2002) Cholecystokinin activates a variety of intracellular signal transduction mechanisms in rodent pancreatic acinar cells. *Pharmacol Toxicol* **91**(6): 297-303.

Willis M, Prokesch M, Hutter-Paier B, Windisch M, Stridsberg M et al. and Marksteiner J (2008) Chromogranin B and Secretogranin II in transgenic mice overexpressing human APP751 with the London (V717I) and Swedish (K670M/N671L) mutations and in Alzheimer patients. *J Alzheimers Dis* **13**(2): 123-35.

Willis M, Leitner I, Jellinger KA, Marksteiner J (2011) Chromogranin peptides in brain diseases. *J Neural Transm* (Vienna) **118**(5): 727-35.

Winkler H, Fischer-Colbrie R (1992) The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* **49**:497–528.

Winsky-Sommerer R, Grouselle D, Rougeot C, Laurent V, David JP et al. and Epelbaum J (2003) The proprotein convertase PC2 is involved in the maturation of prosomatostatin to somatostatin-14 but not in the somatostatin deficit in Alzheimer's disease. *Mol Neurodegener* **9**:22.

Wu Z, Sun L, Hashioka S, Yu S, Schwab C et al. and Nakanishi H(2013) Differential pathways for interleukin-1β production activated by chromogranin A and amyloid β in microglia. *Neurobiol Aging* **34**(12): 2715-25.

Yakovleva T, Marinova Z, Kuzmin A, Seidah NG, Haroutunian V, Terenius L, Bakalkin (2007) Dysregulation of dynorphins in Alzheimer disease. *G.Neurobiol Aging* **28**(11): 1700-8.

Yarbrough GG, Pomara N (1985) The therapeutic potential of thyrotropin releasing hormone (TRH) in Alzheimer's disease (AD). *Prog Neuropsychopharmacol Biol Psychiatry* **9**(3): 285-9.

Yates CM, Harmar AJ, Rosie R, Sheward J, Gordon A et al. and Fink G (1983) Thyrotropin-releasing hormone, luteinizing hormone-releasing hormone and substance P immuno-reactivity in post-mortem brain from cases of Alzheimer-type dementia and Down's syndrome. *Brain Res* **258**(1): 45-52.

Yong-Hong L, Xiao-Dong P, Chang-Quan H, Bo Y, Qing-Xiu L (2013) Hypothalamic-pituitary-thyroid axis in patients with Alzheimer disease (AD). *J Investig Med* **61**(3): 578-81.

Zhao LN, Lu L, Chew LY, Mu Y (2014) Alzheimer's disease--a panorama glimpse. *Int J Mol Sci* **15**(7): 12631-50.

Zou K, Michikawa M (2008) Angiotensin-converting enzyme as a potential target for treatment of Alzheimer's disease: inhibition or activation? *Rev Neurosci* **19**(4-5): 203-12.

CENTRAL NEURODEGENERATION AND DIABETES

M. SOLIGO[1], V. PROTTO[1], S. PICCININ[2], R. NISTICÒ[2,3] and L. MANNI[1]

[1]*Institute of Translational Pharmacology, Consiglio Nazionale Delle Ricerche (CNR), Rome, Italy*
[2]*European Brain Research Institute (EBRI), Rita Levi-Montalcini Foundation, Rome, Italy*
[3]*Department of Biology, University of Rome Tor Vergata, Rome, Italy*

Hundreds of millions of people currently suffer from diabetes in the world and the epidemiological perspective regarding diabetes and its complications in the medium period is somewhat frightening. Among other end-organ damage, diabetes induces brain cell sufferance and neurodegeneration, associated with cognitive decline, a disease state referred to as "diabetic encephalopathy" (DE). In Type-1 diabetes, always emerging during childhood and adolescence, when the brain is in a crucial development stage, the diabetes-induced brain damage leads to lower IQ and poor cognitive performances. Type-2 diabetes, associated with the cluster of risk factors of the metabolic syndrome and interacting with the physiological aging process, participates in the development of major forms of senile dementia, including Alzheimer's disease. Therapies aimed at controlling hyperglycemia and the insulin metabolism do not guarantee the reversal of established DE symptomatology, making diabetes-associated cognitive decline a disease still lacking effective therapeutic approaches. In this review, the clinical studies characterizing cognitive and structural abnormalities induced by diabetes are briefly reviewed. Then we study the mechanisms of central neurodegeneration induced by the disruption of glucose and insulin homeostasis, investigated by using animal models of both Type-1 and Type-2 diabetes.

The metabolism of the brain is strictly dependent on glucose as the energy source and the brain itself is the greatest glucose consumer among all body organs. On average, the adult brain is 2% of total body weight but his oxygen and glucose consumptions in the resting awake state are respectively 20% and 25% of their total body disposal (Mergenthaler et al, 2013). The fact that the energy metabolism of the brain is almost purely based on glucose oxidation is also indicated by the respiratory quotient, that in the brain is nearly 1 (Sokoloff, 1999). Glucose is transported from plasma into the brain by facilitated diffusion across the blood-brain barrier. Different types of glucose transporters (GLUTs) participate in this process and, among them, GLUT-1 and GLUT-3 are the major subtypes involved in glucose transportation across the blood-brain barrier (Duelli et al, 1994; McKinney, 2005; Rao et al, 2006; Chen and Zhong, 2013; Mergenthaler et al, 2013). Within the brain, glucose is either oxidized to produce ATP, essential as energy supply for neurons and non-neuronal cells, and for neurotransmitter synthesis (Mergenthaler et al, 2013) or used to synthesize glycogen, that is stored in astrocytes (Phelps, 1972; Mergenthaler et al, 2013). Substrates alternative to glucose could be also used as energy supplier in particular conditions, such as ketone bodies in infants (Brekke et al, 2015) or glycogen and amino acids after extended fasting

Key words: *diabetes mellitus, diabetic encephalopathy, Alzheimer's disease, neurodegeneration, dementia, hyperglycemia, insulin, neurotrophins*

Corresponding author:
Luigi Manni, Ph.D.
Institute of Translational Pharmacology,
Consiglio Nazionale Delle Ricerche (CNR),
via del Fosso del Cavaliere 100, 00133 Rome, Italy
Tel.: +39 0649934248
e-mail: luigi.manni@ift.cnr.it

2279-5855 (2016)

in adults (Chen and Zhong, 2013).

Thus, the brain's high-energy consumption and its dependence on glucose metabolism, makes it particularly vulnerable to disturbances in glucose homeostasis, such as those observed in pre-diabetic states and during diabetes. In this review, we will briefly summarize the relevant clinical data on the characteristic of mild to severe cognitive impairments observed in diabetic patients. For a more comprehensive and detailed insight into the clinical features of the so-called "diabetic encephalopathy" (DE) we refer the readers to the huge amount of literature published in the last 25 years (reviewed, among others, in: Dejgaard et al, 1991; Gispen and Biessels, 2000; Desrocher and Rovet, 2004; Biessels and Gispen, 2005; Brands et al, 2005; Mijnhout et al, 2006; Biessels et al, 2008; Chen and Zhong, 2013; Cameron, 2015; Moheet et al, 2015). We will then go into the research aimed at elucidating the distinctive behavioral, functional, structural and molecular features of diabetic brain, as revealed by using animal models of diabetes. We will attempt to touch the main aspect of a multifaceted and heterogeneous topic, indicating both the seminal as well as the most updated bibliographic references, to be used by the reader interested in further in-depth analysis over part or the whole general argument.

COGNITIVE IMPAIRMENT IN DIABETES

Diabetes

Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia. In 2013 about 380 million people worldwide suffered for diabetes (Shi and Hu, 2014), with an estimates of 642 million people affected by 2040 (http://www.diabetesatlas.org/) and an increase in the proportion of diabetic people >65 years of age. Two main types of diabetes mainly account for these epidemiological data: Type-1 and Type-2 diabetes mellitus (T1DM and T2DM respectively).

T1DM generally develops at a young age (children, early adulthood) and accounts for 5% to 10% of all cases of diabetes. T1DM results from autoimmune beta-cell destruction in the pancreas and is characterized by a complete lack of insulin production. Thus, patients with T1DM require treatment with insulin to achieve normoglycemia and to survive (Daneman, 2006). Most of T1DM patients develop microvascular complication before midlife, associated with complications such as retinopathy, nephropathy and peripheral neuropathy (Daneman, 2006).

In T2DM the principal defect is insulin resistance, lately associated with a relative insulin deficiency (Stumvoll et al, 2005). Beside an important genetic component, lifestyle and obesity seem to be main pathogenic factors for T2DM. Type 2 diabetes accounts for 90% to 95% of all diagnosed diabetes cases and the incidence of T2DM strongly increases with age. Insulin resistance is often associated with a pattern of metabolic and vascular abnormalities, namely obesity, dyslipidemia, hypertension and chronic pro-inflammatory state, which form a cluster of risk factors called metabolic syndrome (Eckel et al, 2005). All of these abnormalities represent a separate risk factor for the development of cerebral complications associated with cognitive decline. In this review we will only focus on the proper "diabetic" abnormalities (i.e. hyper- and hypoglycemia, hypo- and hyperinsulinemia, insulin resistance) but it should be taken into account that the association of proper "diabetic" risk factors with one or more of the previously cited "metabolic" factors could worsen the outcome of an eventual cerebral complication in diabetic patients.

Pre-diabetes is a condition in which a person has elevated blood glucose levels but does not meet diagnostic criteria for diabetes. People with prediabetes can have impaired fasting glucose or impaired glucose tolerance, or both and are likely to develop a frank diabetes in a relatively short time. Since hyperglycemia is an important risk factor for the development of central neurodegeneration (discussed in a further section of the text), prediabetes represent a clinical condition in which a mild cognitive impairment associated with brain cell damage can develop (S Roriz-Filho et al, 2009; Yau et al, 2010).

Diabetic encephalopathy

The progressive alterations in cerebral function

and structure that occur in association with diabetes are mainly referred to as "diabetic encephalopathy" (DE) (Dejgaard et al, 1991; Mijnhout et al, 2006). The diabetes-associated cognitive dysfunctions have been first described in the early 1920's (Miles and Root, 1922) and the term "diabetic encephalopathy" introduced in the 1950 (De Jong, 1950). The first structural evidence for DE was produced in 1966, when a peculiar pattern of diffuse degenerative abnormalities of the cerebral tissue were found in a post-mortem study on the brains of young long-term T1DM patients, likely dependent upon angiopathy and a primary diabetic factor (Reske-Nielsen et al, 1966). The term diabetic encephalopathy has been subject to some controversy, and a decade ago Mijnhout and colleagues (Mijnhout et al, 2006) proposed the term "diabetes-associated cognitive decline" (DACD) to describe the state of mild to moderate cognitive impairment solely attributable to diabetes. They also put forward the correct observation that pathogenic research on DACD (or DE) should focus on T1DM, since in T2DM several biases are introduced by the co-morbidity with metabolic syndrome-associated risk factors (Mijnhout et al, 2006).

Manifestations of diabetic encephalopathy include changes in several cognitive domains. While it has been recognized that both T1DM and T2DM lead to a significant acceleration of the "physiologic" brain aging process (Biessels et al, 2002; Biessels et al, 2008; S Roriz-Filho et al, 2009; Sims-Robinson et al, 2010; Wang et al, 2014), the pattern of cognitive impairment differs between the two diabetes types, and so is also for some structural abnormalities revealed by neuroimaging studies.

Different types of diabetes affect different cognitive processes

Human studies on T1DM should be categorized at least according to the age of patients enrolled. Indeed, T1DM insurgence is prevalent in young people and infants and the outcome influenced by the age of onset, the duration of diabetes, the efficacy of glycemic control and the presence of microvascular complication. Cross sectional analysis on children evidenced that the early the onset, the worse the cognitive impairment (recently reviewed in: Moheet et al, 2015). This reflects the selective vulnerability of developing brain to dysregulation in glucose homeostasis (Ryan et al, 1993; Brands et al, 2005; Moheet et al, 2015). The main cognitive domains affected in children and adolescents with T1DM are spatial intelligence, information-processing speed, psychomotor efficiency, memory, attention and executive function (Biessels et al, 1994; Desrocher and Rovet, 2004; Brands et al, 2005; Ho et al, 2008; Moheet et al, 2015). Interestingly, longitudinal studies, evaluating the progression of cognitive decline in adult T1DM patients, revealed that an efficient glycemic control always block it or slow it down, impeding its progression toward more severe forms of cognitive deficit and dementia (Ott et al, 1999; Moheet et al, 2015). From a structural standpoint, imaging studies indicated, as main neuropathological correlates of T1DM in adults, a global cerebral atrophy, a significant decrease of grey matter volume and density in the cortex and hippocampus and increased occurrence of white matter hyper-intensities (WMH), probably related to ischemic lesions (Ho et al, 2008; Moheet et al, 2015; Nunley et al, 2015). Moreover, de-organization of cortical networks was also found in T1DM patients, positively associated with the occurrence of microvascular complications (Lyoo et al, 2013; Ryan et al, 2015; van Duinkerken et al, 2016).

Studies on T2DM patients revealed that the main cognitive processes affected by diabetes are verbal fluency and mental speed, executive function, processing speed, memory (Biessels et al, 2008; Umegaki, 2014; Moheet et al, 2015). In elderly T2DM patients the cognitive impairments may be more pronounced, and can interfere with day-to-day functioning. In addition, the incidence of dementia appears to be doubled in elderly subjects with diabetes (Ott et al, 1999; Cole et al, 2007; Umegaki, 2014). Reduced efficiency of brain networks and longer white matter path length were also described in middle-aged T2DM patients (Kim et al, 2016). It is worth noticing that several, if not all, of the clinical studies on aged T2DM patients are more probably biased by the concomitant presence of one or more risk factors belonging to the above-cited metabolic syndrome. This makes difficult the direct association of cognitive

impairments with the peculiar features of T2DM (i.e. insulin resistance and hyperglycemia). However, studies on pre-diabetic patients indicated that, even in absence of co-morbid factors, insulin resistance *per-se* is sufficient to generate significant impairment in intellectual functioning, verbal memory and psychomotor efficiency (S Roriz-Filho et al, 2009; Yau et al, 2010). As for structural abnormalities evidenced by neuroimaging, the WMH also characterize the brain of T2DM patients, indicating microvascular sufferance in the brain (van Harten et al, 2007; de Bresser et al, 2010; Brundel et al, 2014). Brain atrophy with reduced gray and white matter volume was also reported, with the temporal lobe and hippocampal region being the more susceptible to damage (den Heijer et al, 2003; Brundel et al, 2014). Again, structural analysis of the brain of pre-diabetic adolescents, also revealed reduced white matter volume and reduced white and grey matter microstructural integrity (S Roriz-Filho et al, 2009; Yau et al, 2010).

ANIMAL STUDIES

The effects of diabetes on the brain encompass cognitive impairment, structural pathological changes, functional defects in signal conduction and neurotransmission, dysfunction of synaptic plasticity, alteration in the neurogenesis, dysregulation of general homeostatic balance in brain cells and tissue, abnormal functionality of molecular systems (i.e. neurotrophins) supporting and regulating the maintenance of brain cell phenotypes and functionality. The use of animal models allowed characterizing almost all of these features of DE.

Animal models of DE

Several animal models have been developed and used extensively in diabetes research (Rees and Alcolado, 2005) (Table 1). The study of the effects of perturbed or disrupted glucose homeostasis, associated with insulin deficit (or reduced insulin signaling) on the development of cognitive deficits and on the underlying mechanisms of DE, has made main use of the streptozotocin (STZ) and the BB rat models of T1DM (Rees and Alcolado, 2005; Sima et al, 2009).

STZ is a pancreatic beta-cells cytotoxic agent, which could be injected systemically to deplete

Table I. *Animal models of diabetes used to study brain and neurobehavioral dysfunctions.*

	Model	Glucose	Insulin
Type 1 Diabetes	i.p. STZ	+	-
	NOD mouse	+	-
	BB/Wor rat	+	-
	Akita mouse	+	-
Type 2 Diabetes	Zucker fa/fa rat	+	+
	Diabetic Zucker rat	+	+
	db/db mouse	+	+
	OLETF rat	+	+
	GK rat	+	+
Brain insulin resistance	i.c.v. STZ	+	+

The presence of hyperglycemia is common in all of the diabetes animal models, independently of the genetic profile. i.p.: intra-peritoneum. i.c.v.: intra-cerebroventricular.(+): increased plasma and/or brain levels. (–): decreased plasma and brain levels.

insulin-producing cells in a dosage-dependent way. The nitrosurea moiety of STZ is responsible for its toxicity on pancreas insulin-producing cells, which is probably mediated through a decrease in NAD levels and the formation of intracellular free radicals. The GLUT-2 glucose transporter is responsible for STZ transport across the cell membrane, thus, since the GLUT-2 is not expressed at the blood-brain barrier, direct effects of STZ on the brain after systemic administration are unlikely (Biessels and Gispen, 2005). Like diabetic patients, STZ-diabetic rats develop end-organ damage affecting the eyes, kidneys, heart, blood vessels, and peripheral nervous system. STZ model has been used as a model of DE, since it induces behavioral dysfunction similar to human DE (You et al, 2009) and, among others, alterations in the brain cholinergic system (Welsh and Wecker, 1991), deregulations of brain neurotrophins (Sposato et al, 2007; Soligo et al, 2015), alterations of Tau metabolism (Clodfelder-Miller et al, 2006).

Biobreeding rat also known as the BB rat is an inbred laboratory rat strain that spontaneously develops autoimmune Type 1 Diabetes (Rees and Alcolado, 2005). The strain was derived from a colony of outbred Wistar rats that spontaneously develop hyperglycemia and ketoacidosis. They have been used to study the effects of cerebral ischemia on diabetic brain (Sutherland et al, 1992), some mechanisms of the impaired central insulin/insulin growth factor (IGF) signaling (Li et al, 2005) and the therapeutic role of insulin C-peptide replacement in DE (Sima et al, 2008; Sima et al, 2009).

Animal models of spontaneous T2DM have been also used, although the genetic defects that underlies the development of diabetes-like pathology is not the primary defect encountered in human T2DM. Most of these animal models of T2DM are characterized by dysregulations in lipid metabolism and often by insulin resistance. Among them mouse and rats defective for leptin receptor (db/db mouse and Zucker rats) and rats characterized by obesity and impaired glucose tolerance secondary to the reduced expression of cholecystokinin-2 receptor gene, the Otsuka Long-Evans Tokushima fatty (OLEFT) rats (Biessels and Gispen, 2005; Rees and Alcolado, 2005).

Behavioral studies on animal models of DE

The cognitive performance of diabetic rats have been extensively tested in the Morris Water Maze (MWM). MWM is a test for spatial learning that involve several cognitive components such as selective attention, building of internal representation of external environment, storage and retrieval of information, problem solving (Bannerman et al, 1995). STZ rats showed impaired performances in the MWM starting ten weeks after STZ treatment (Biessels et al, 1996; Kamal et al, 2000). Continuous delivery of insulin by subcutaneous insulin pellets prevented the learning deficits if insulin treatment was started at the onset of diabetes, but not if insulin delivery was started after the learning ability was just impaired (Biessels et al, 1998). Moreover, an interaction between diabetic cognitive impairment and aging was also reported, since aged rats performed worse than young-adult rats after diabetes of the same duration (Kamal et al, 2000). Prolonged latencies in the MWM were also found in 8 months old BB rats (Li et al, 2002), associated with hippocampal neuron apoptosis and deregulations in IGF and insulin signaling.

Other behavioral paradigms have been also tested in STZ and BB rats. STZ rats displayed decrease in social interaction and increased fear-related and anxiety-related behavior (reviewed in: Reagan, 2012)., while BB rats exhibited impaired working and reference memories when tested in the radial arm maze (Sima et al, 2009).

Learning and memory deficits have also been reported in some, but not all, the studies examining hippocampal-dependent behavior in T2DM models (Reagan, 2012), but a correlation between cognitive impairment and hyperglycemia or insulin resistance is rather difficult, if not impossible. In general, the outcome of behavioral test in diabetic animal seems to be strictly dependent on the patho-physiological features of the animal model, and the particular behavioral test, those related to hippocampal function and plasticity being the more explored and more consistent in results.

Synaptic plasticity and neurotransmission

Since hippocampus plays a crucial role in spatial

learning and memory (Eichenbaum et al, 2007), the hippocampal synaptic plasticity has been studied by several groups as a neuro-functional correlate of the impaired cognitive performance in behavioral tasks. In particular, long-term potentiation (LTP) and long-term depression (LTD) has been studied in hippocampal slices from STZ-treated rodents. Both LTP and LTD are generated by increased postsynaptic calcium concentration (Yang et al, 1999), and represent respectively an activity-dependent strengthening or reduction in the efficacy of neuronal synapses. A deficit in the N-methyl-D-aspartate (NMDA) glutamate receptor-dependent LTP in the dentate gyrus, CA1 and CA3 fields of the hippocampus has been reported, with a gradual increase over time and a maximum expression 12 weeks after diabetes induction (Biessels et al, 1999; Kamal et al, 1999). As for behavioral tests, insulin treatment prevents the development but is less effective against existing LTP deficits (Biessels et al, 1998). On the other hand, expression of LTD is enhanced in the CA1 field of hippocampi from diabetic rats (Kamal et al, 1999; Artola et al, 2005).

The mechanisms underlying the alterations in hippocampal synaptic plasticity in STZ-diabetic rats seem to be mostly related to glutamatergic neurotransmission and postsynaptic deficits in membrane excitability and/or in the intracellular signaling generated by LTP and LTD induction (Biessels et al, 2002). Reduced impulse conduction velocity was found in presynaptic fibers (Candy and Szatkowski, 2000), while paired-pulse facilitation in the CA1 field is unaffected (Biessels et al, 1996). LTP/LTD deficits in diabetic rodents are associated with both reductions in glutamate receptors NMDA- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-mediated currents in the hippocampus (Gardoni et al, 2002; Kamal et al, 2006). AMPA receptor binding activity and functional activities is reduced in the hippocampus of STZ rats (Chabot et al, 1997; Kamal et al, 2006). Additionally, NR2B NMDA subunit is decreased in the hippocampus of STZ rats 12 weeks after diabetes induction and Ca+/CaM-stimulated phosphorylation of hippocampal NR2A and NR2B subunits expressed in the post-synaptic density is reduced (Gardoni et al, 1999). Additionally, the basal level of glutamate in the dentate gyrus of STZ rats after 12 weeks of diabetes is reduced (Reisi et al, 2009) suggesting another mechanism through which glutamatergic tone may be modulated in the hippocampus of STZ rats.

Other neurotransmitter systems, i.e. GABA and acetylcholine (Ach), could be involved in the diabetes-induced alteration in synaptic plasticity. Both GABA and Ach modulates glutamatergic neurons excitability and plastic response after glutamate-based neurotransmission in the hippocampus (Teles-Grilo Ruivo and Mellor, 2013), and represent the neurochemical connection between the septal nuclei and the hippocampus. A deficit in Ach metabolism in the brain of STZ rats (Welsh and Wecker, 1991) and a cholinergic receptor dysfunction and decreased GABAergic neuroprotective inhibitory function in the hippocampus of STZ rats have been reported (Sherin et al, 2012). Thus, the deficit in synaptic plasticity in STZ rodents is attributable to dysregulations in the whole circuitry generating and modulating the LTP and LTD.

Electrophysiological studies in experimental models of T2DM failed to demonstrate consistent alterations in synaptic plasticity. Some of them indicated that T2DM animals exhibit deficits in LTP (Gerges et al, 2003; Alzoubi et al, 2005; Stranahan et al, 2008), others have failed to observe electrophysiological changes (Belanger et al, 2004). The differences in the physiological characteristics of the T2DM animals used in these studies most probably account for these apparently incongruent findings.

Structural deficits

Diabetes-induced neuroanatomical deficits, because of increased neuronal vulnerability, play an important role in the development of altered neuroplasticity, and in the electrophysiological properties of neurons. The diabetes-induced increase in neuronal apoptosis, especially in the hippocampus, has been described in a great number of studies in STZ-treated rodents and in the BB model (extensively reviewed in: Sadeghi et al, 2016). STZ-treated rodents also exhibit structural deficits in the neuronal processes, directly correlated with the deficit in plasticity and electrophysiological properties. STZ-induced diabetes provokes dendritic atrophy of hippocampal CA3 pyramidal neurons

as well as dendritic atrophy and decreases in spine density in CA1 pyramidal neurons (Reagan, 2012), and in the entorhinal and sensory cortices (Zhou et al, 2013). STZ-induced dendritic changes include synaptic reorganization in the dentate gyrus and the CA1 region of the hippocampus (Grillo et al, 2005). Decreases in spine density have also been observed in the dentate gyrus of db/db mice (Stranahan et al, 2009). Diabetes-induced deficits in morphological plasticity also include decreased dendritic length and spine density in the pre-frontal cortex of STZ rats (Martinez-Tellez et al, 2005).

Other measures of increased neuronal vulnerability have been identified in the hippocampus of diabetic rodents including reductions in cell proliferation and neurogenesis (Jackson-Guilford et al, 2000; Revsin et al, 2005; Beauquis et al, 2006; Beauquis et al, 2008). The process of neurogenesis consists of proliferation of new cells from progenitors, differentiation into astrocytes, oligodendrocytes or neurons, and survival and incorporation of newborn cells into target regions. Neurogenesis in the dentate gyrus supports the acquisition and consolidation of memories in the hippocampus and guarantees hippocampus structural plasticity across the lifespan (Leuner and Gould, 2010). STZ-induced diabetes consistently decreases hippocampal cell proliferation and neurogenesis (recently reviewed in: Ho et al, 2013). Cell proliferation is impaired as early as 4 weeks after STZ with an almost immediate reduction in immature neuronal differentiation. Newly generated cells in STZ rats also shown a reduced rate of survival, (Zhang et al, 2008). Thus, STZ-induced diabetes consistently decreases hippocampal cell proliferation and survival and also affects neuronal differentiation, probably contributing to impaired learning and memory based on the results from cognitive tests (Ho et al, 2013). Interestingly, results consistent with those obtained on STZ-treated rodents were also obtained using a genetic model of T1DM, the nonobese diabetic (NOD) mice (Beauquis et al, 2008). Beside the impaired cell proliferation and neuronal differentiation, NOD mice also display a pre-existing disposition towards decreased hippocampal neurogenesis long before conversion into a diabetic state (Beauquis et al, 2008).

Hippocampal neurogenesis has been studied in a number of animal models of T2D (Ho et al, 2013). Zucker rats and their db/db mice analogous T2DM model, have decreased hippocampal cell proliferation and lower levels of neuronal differentiation compared to their non-diabetic counterparts (Stranahan et al, 2008; Yi et al, 2009). Studies on other models of T2DM did not produce results consistent with those presented above, and this could be ascribed to the heterogeneity of risk factors active in inducing the neuroplasticity defect (Ho et al, 2013).

Neurotrophic deficit

Increasing evidences have been produced about a generalized neurotrophic deficit produced by diabetes in the brain (Sima and Li, 2005; Sima, 2010) (Fig. 1). The decreased trophic support to brain cells is due to a deficit in neurotrophic insulin signaling (discussed below) and to a dysregulated production and signaling of a class of factors called neurotrophins (NTs). NTs are structurally related molecules derived from a common ancestor gene (Hallbook, 1999). Two NTs, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) have been mainly investigated in the diabetic brain, since they are heavily involved in the generation and maintenance of hippocampal synaptic plasticity, in the trophic support to the central cholinergic, dopaminergic and glutamatergic systems and in general as neurotrophic correlates for learning and memory related behavior (Chaldakov et al, 2009; Ho et al, 2013; Hempstead, 2014). NTs and their precursors, proNTs, are both biologically active (Hempstead, 2014). The binding of BDNF or NGF to their tyrosine kinase (Trk) cognate receptors generates an intracellular signaling cascade supporting cell survival and phenotypic maintenance, neurogenesis as well as the generation of synaptic plasticity and of its neurobehavioral correlates (Lim et al, 2003; Allen and Dawbarn, 2006; Soule et al, 2006; Frielingsdorf et al, 2007; Gottmann et al, 2009) (Fig. 1). The challenge of proNTs to the p75NTR-sortilin receptor complex, on the other hand, induces neuronal sufferance and apoptosis and impaired neurogenesis and synaptic plasticity (Lu, 2003; Guo et al, 2013; Hempstead, 2014; Yang et al, 2014; Chen et al, 2016). Thus impaired proNTs production, maturation and signaling could play an important role in generating

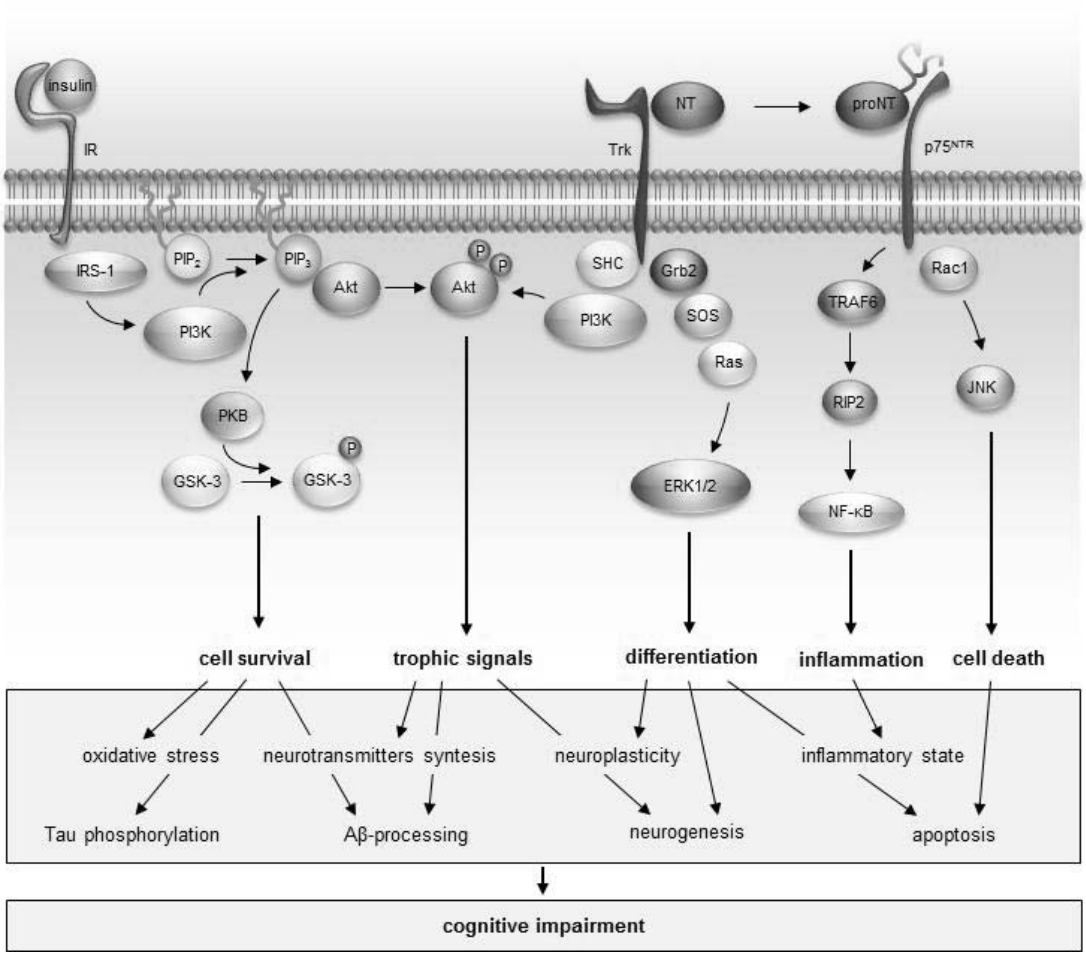

Fig. 1. *Neurotrophic signaling produced by insulin and neurotrophins in the brain. The signaling cascades generated by tyrosyne kinase receptors share common downstream mediators (i.e. Akt) and regulate brain cell proliferation, survival, differentiation, and functions. All these physiologic cellular and tissue functions are dysregulated by diabetes, and contribute to the cognitive impairment and the diabetic encephalopathy.*

the diabetic brain phenotype.

Plasma BDNF levels decrease in humans with T2DM and was inversely associated with fasting plasma glucose, but not with insulin (Krabbe et al, 2007). BDNF produced by cerebrovascular tissue decreases early after induction of diabetes in STZ-treated rats (Navaratna et al, 2011) and is less expressed in hippocampal neurons, compared with healthy rats (Liu et al, 2013). There are clues that the trophic BDNF signaling through its receptor TrkB diminished in STZ-treated rats (Navaratna et al, 2013). NGF and its receptor levels were found decreased in both STZ (Sposato et al, 2007; Rocco et al, 2013; Soligo et al, 2015) and BB (Sima et al, 2008; Sima et al, 2009) T1DM models. Moreover, the proNGF content increased after STZ treatment and the ratio between NGF and proNGF decreased in the brain of diabetic rats (Soligo et al, 2015), suggesting an impaired maturation of the NT and a signaling shifted toward neuronal sufferance.

PATHOGENESIS OF DIABETIC ENCEPHALOPATHY AND ITS LINK TO DEMENTIA

Patients with diabetes have nearly twofold higher risk to develop vascular dementia or Alzheimer's disease (AD) (for a review see: Sims-Robinson et al, 2010).

Impaired metabolic parameters, such as hyperglycemia, hyperinsulinemia and insulin resistance, positively correlate with the development of dementia. Elevated blood glucose levels increase the risk of dementia in both diabetic and nondiabetic individuals by 40% and 18%, respectively (Crane et al, 2013). These findings indicate that fluctuations in blood glucose levels could negatively influence brain function, even in the absence of T2D or impaired glucose tolerance. As stated in the first chapters of this review, only pure "diabetic" factors will be discussed here. Nevertheless, beside the direct effects on nerve cells of deregulation in glucose and insulin function (Cole et al, 2007; Sims-Robinson et al, 2010; Mergenthaler et al, 2013), several risk factors associated with diabetes have been investigated as players in central neurodegeneration occurring in diabetic patients. Among others, dysfunction of calcium metabolism and mitochondrial activity, dyslipidemia, hypercholesterolemia and genetic alterations of the lipid transporter apolipoprotein E (APOE) seems to play major roles in the development of neurodegeneration and dementia, especially in T2DM, and in its link with AD (Cole et al, 2007; Sims-Robinson et al, 2010; Dar et al, 2014; de la Monte and Tong, 2014).

Hyperglycemia-mediated cell damage

Hyperglycemia causes patho-physiological processes, including oxidative stress, advanced glycation end-products (AGE) formation and microvascular pathology (Biessels et al, 2002) (Fig. 2). Excessive glucose availability in the brain results in an increased flux of glucose through the polyol and hexosamine pathways, disturbances of intracellular second messenger pathways and an imbalance in the generation and scavenging of reactive oxygen species (ROS), and the formation of AGEs (Brownlee, 2001). These latter are a group of molecules formed by irreversible, non-enzymatic reactions between sugars and the free amino groups of proteins, lipids and nucleic acids. Auto-oxidation of glucose leads to the formation of oxygen radicals, which are intermediates in the AGE pathway (Hunt et al, 1988). The formation and accumulation of AGEs are normal during aging, but are exacerbated in patients with diabetes (Brownlee, 1995). The link of AGE to its receptor (RAGE) induces a series of biological processes that cause further diabetic complications (Singh et al, 2001). RAGEs, galectin-3 (a proatherogenic molecule), and the polyol pathway activation are all increased in diabetic rat brains, with concomitant increase in the activity of the glyceradehyde-3-phosphate dehydrogenase, indicating elevated oxidative stress (Aragno et al, 2005). Proinflammatory and brain injury gene markers modulated by AGEs, were also up-regulated in the hippocampus of diabetic rats (Sims-Robinson et al, 2010).

These phenomena also contributes to microvascular changes, which leads to microinfarcts and WMH, which in turn result in cognitive decline and dementia (S Roriz-Filho et al, 2009). Indeed, diabetes is associated with a basal chronic systemic inflammatory state that in turn induces endothelial dysfunction, platelet hyperactivity and the microvascular complications responsible for the development of neuropathy and retinopathy (S Roriz-Filho et al, 2009). Thickening of capillary basement membrane has also been demonstrated in the brain of diabetic patients, together with increased clinical sign of brain ischemia (S Roriz-Filho et al, 2009).

Overall these hyperglycemia-induced effects might be responsible for the "accelerated brain aging" that occurs in subjects and animals with diabetes (Kamal et al, 2000; Biessels et al, 2002; Biessels and Gispen, 2005). Indeed, hyperglycemia, causing accumulation of AGEs and ROS, may trigger a cascade of events that leads to neural aging and brain atrophy.

Insulin as a neurotrophic factor

Beside its action in regulating glucose homeostasis, insulin, through the challenge of the tyrosine kinase insulin receptor (IR) exert a proper neurotrophic action on brain neurons (Fig. 1). It is worth noticing that the insulin structurally-related insulin-growth factor (IGF) binds the insulin receptor triggering the same downstream intracellular signaling cascade. Thus, this "neurotrophic" system could be addressed as the insulin/IGF system. Insulin is either transported across the BBB and produced by brain cells (Schwartz et al, 1992), targeting IR-expressing cells, especially in the hippocampus, cerebral cortex, hypothalamus, cerebellum and olfactory bulbs (Cole et al, 2007). By challenging

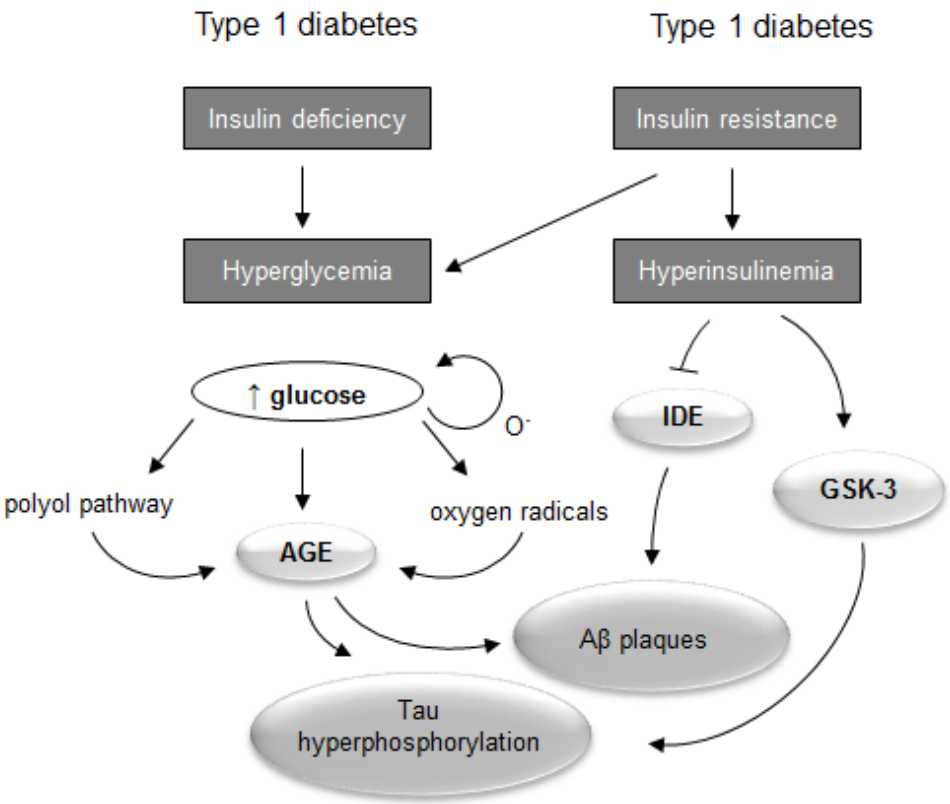

Fig. 2. *Pathogenetic mechanism activated by diabetes, leading to brain cell sufferance and diabetic encephalopaty. Hyperglycemia and abnormally high glucose levels characterize both T1DM and T2DM, which deleteriously affect cellular metabolism. T2DM is also characterized by reduced insulin signaling and increased (at least during its development) insulin levels, generating a neurotrophic deficit (see Fig. 1) and the production of hyperphosphorylated tau and of Aβ oligomers and plaques.*

IR, in the brain insulin produces marked effects on food intake behavior, is implicated in the central regulation of autonomic function and modulates synaptic transmission, learning and memory (Schwartz et al, 1992; Zhao et al, 1999). Most of these action are exerted by stimulating the auto-phosphorylation of the IR, which initiates several intracellular signaling cascades that involve phosphatidylinositol 3-kinase (PI3K) and the activation of Akt and de-activation of glycogen synthase 3 (GSK3) (Cole et al, 2007) (Fig. 1). These downstream signaling pathways are common to other neurotrophic factors, such as BDNF and NGF (Fig. 1), and their de-regulation in diabetes accounts for a generalized neurotrophic deficit (see above). Insulin signaling in neuronal cells is able to affect the metabolism of two major players in the development and progression of AD (namely amyloid-β and tau, see below), regulates neurotransmitter receptors intracellular and plasma membrane traffic and is able to promote neurite outgrowth and axonal regeneration in vitro (S Roriz-Filho et al, 2009). It is thus conceivable that both hypo-insulinemia (T1DM) and insulin resistance (T2DM) could affect neuronal metabolism and be of main pathogenic importance in the development of DE. However, as will be explained in more details in a subsequent chapter, also hyperinsulinemia could result in brain toxicity, linked to the lack of amyloid-β degradation (Farris et al, 2003) (Fig. 2).

Alzheimer's disease: a type of "diabetes"?

Numerous epidemiological studies have linked diabetes, especially T2DM, with an increased risk

of developing Alzheimer's disease (AD) (Ott et al, 1999; Sims-Robinson et al, 2010; Dar et al, 2014). In animal model of DE, several biochemical and molecular alterations have been demonstrated, that are undoubtedly very similar or equal to those observed in humans or in animal models of AD (Freude et al, 2005; Clodfelder-Miller et al, 2006; Planel et al, 2007; Malone et al, 2008; Kim et al, 2009; Kroner, 2009; Qu et al, 2011; Rocco et al, 2013; Zhou et al, 2013; de la Monte and Tong, 2014). Impairment in cerebral glucose utilization and energy metabolism occurs very early in AD patients and may precede or accompanying the initial signs and symptoms of dementia (Gispen and Biessels, 2000; Biessels, van der Heide et al, 2002). Impairments in the insulin signaling pathway in the brain have been implicated in AD and aging (Rivera et al, 2005). AD is characterized by both insulin deficiency and insulin resistance within the brain, leading to the definition of AD as an insulin-resistant brain state (Hoyer, 1998). In recent years AD has been also referred to as "type 3 diabetes" (Rivera et al, 2005; Steen et al, 2005) and an animal model specifically developed to mimic the development of an AD-like pathology by inducing a brain insulin resistance through intra-cerebroventricular injection of STZ (Mayer et al, 1990).

The mechanisms underlying the connection between diabetic brain state and the development of AD have been in part exposed in this chapter and concern with the toxicity of high glucose levels and the possible deleterious effects of abnormal insulin signaling, the increased oxidative and pro-inflammatory status of diabetic brain. Moreover, some direct connection exist between these diabetic features and some of the specific molecular hallmarks of AD. Several animal studies demonstrated an AD-like metabolism of tau protein and the amyloid-β in diabetic rodents. Tau is hyperphosphorylated in the brain of diabetic rodents (Freude et al, 2005; Clodfelder-Miller et al, 2006; Planel et al, 2007; Kim et al, 2009; Rocco et al, 2013). Furthermore, experimental diabetes in animal models of AD exacerbates the hyperphosphorylation of tau (Ke et al, 2009; Jolivalt et al, 2010; Qu et al, 2011), that is the prodromal of its aggregation in the neurofibrillary tangles (NFT), one of the main structural abnormalities found in AD brain. Deregulated amyloid-precursor protein (APP) metabolism and increased production of amyloid-β, the main component of the senile plaques and of neurotoxic fibrils in AD, has been described in animal models of diabetes and, similarly to what has been found for tau protein, diabetes exacerbates the AD-like symptoms in APP transgenic rodents (Liu et al, 2008; Jolivalt et al, 2010; Jung et al, 2010; Li et al, 2010; Wang et al, 2010; Cai et al, 2011; Liu et al, 2011; Currais et al, 2012; Devi et al, 2012; Subramanian and John, 2012). Tau gene expression and phosphorylation are regulated by the insulin/IGF signaling (Schubert et al, 2004; de la Monte et al, 2011). Tau hyper-phosphorylation in diabetic animals is due to deactivation of the PI3K and Akt pathways and overactivation of kinases like the GSK3, related, among other neurotrophic signaling deficits, to a defect in the IR signaling (de la Monte and Tong, 2014). Moreover, insulin inhibits intracellular degradation of amyloid-β by competing as a substrate for their common clearance mechanism, the insulin-degrading enzyme (IDE) (Gasparini et al, 2002), which has also been found decreased in the brain of AD patients (Cook et al, 2003).

CONCLUSIONS

Clinical and preclinical models studies, starting in the early 1920's and with great intensity and effort since the 1980's, have accumulated evidences about the distinctive features and the pathogenesis of the damage that disrupted glucose and/or insulin homeostasis causes to the brain (Moheet et al, 2015). The need for an intensive research effort on this topic arise from the terrific data about the current incidence and future perspectives of the diabetes epidemics and the social costs that diabetes and its complication actually produces and will generate in the near future (Shi and Hu, 2014). Both T1DM and T2DM induced hyperglycemia and led to brain damage, neurobehavioral and cognitive deficits (Biessels et al, 2008; Reagan, 2012; Crane et al, 2013). Differences in insulin availability and in compensatory mechanisms between the two main types of diabetes accounts for the differences in how brain cells and functions are damaged. Since T1DM lacks insulin while T2DM is characterized by hypofunctional IRs, overproduction

of insulin, and the comorbidity with the many factors of the metabolic syndrome, different treatment approaches are most probably needed to avoid or control the central neurodegenerative damage associated with the two types of diabetes. Potential treatments actually falls in two main categories: the restoration of glycemic control and the specific support to neurodegeneration and its causes (oxidative stress, neurotrophic deficit, vascular dysfunction, etc.). The re-establishment of euglycemia, with different pharmacological approaches, depending on the type of diabetes, has been proven useful in counteracting the development of cognitive and neurodegenerative alterations, in humans and even more in diabetes animal models (Biessels and Gispen, 2005; Biessels et al, 2008). However, diabetes in humans often remains undiagnosed or uncontrolled for time sufficient to generate irreversible brain damage, generating the need for treatment specifically addressed at neurodegenerative changes.

The link between T2DM and the development of AD is gaining more and more ground (Yang and Song, 2013; Dar et al, 2014). Both clinical and experimental studies indicated that impaired cerebral glucose/insulin metabolism is a pathological feature in AD patients and precedes the clinical symptoms and pathological alterations found in the AD brain. In this respect, the need for the development of animal models more effectively reproducing human T2DM should also be mentioned. The vast majority of the preclinical data on DE, up until now, have been produced using the very simple, reliable and effective STZ animal model (Biessels and Gispen, 2005), but the validation of the obtained data on models of T2DM is often inconsistent, though strongly needed to translate the potential treatments screened on STZ-treated animals into the clinical research.

REFERENCES

Allen SJ, Dawbarn D (2006) Clinical relevance of the neurotrophins and their receptors. *Clin Sci (Lond)* **110**: 175-191.

Alzoubi KH, Aleisa AM, Alkadhi KA (2005) Impairment of long-term potentiation in the CA1, but not dentate gyrus, of the hippocampus in Obese Zucker rats: role of calcineurin and phosphorylated CaMKII. *J Mol Neurosci* **27**: 337-346.

Aragno M, Mastrocola R, Medana C, Restivo F et al and Boccuzzi G (2005) Up-regulation of advanced glycated products receptors in the brain of diabetic rats is prevented by antioxidant treatment. *Endocrinology* **146**: 5561-5567.

Artola A, Kamal A, Ramakers GM, Biessels GJ, Gispen WH (2005) Diabetes mellitus concomitantly facilitates the induction of long-term depression and inhibits that of long-term potentiation in hippocampus. *Eur J Neurosci* **22**: 169-178.

Bannerman DM, Good MA, Butcher SP, Ramsay M, Morris RG (1995) Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. *Nature* **378**: 182-186.

Beauquis J, Roig P, Homo-Delarche F, De Nicola A, Saravia F (2006) Reduced hippocampal neurogenesis and number of hilar neurones in streptozotocin-induced diabetic mice: reversion by antidepressant treatment. *Eur J Neurosci* **23**: 1539-1546.

Beauquis J, Saravia F, Coulaud J, Roig P, Dardenne M, Homo-Delarche F, De Nicola A (2008) Prominently decreased hippocampal neurogenesis in a spontaneous model of type 1 diabetes, the nonobese diabetic mouse. *Exp Neurol* **210**: 359-367.

Belanger A, Lavoie N, Trudeau F, Massicotte G, Gagnon S (2004) Preserved LTP and water maze learning in hyperglycaemic-hyperinsulinemic ZDF rats. *Physiol Behav* **83**: 483-494.

Biessels GJ, Cristino NA, Rutten GJ, Hamers FP, Erkelens DW, Gispen WH (1999) Neurophysiological changes in the central and peripheral nervous system of streptozotocin-diabetic rats. Course of development and effects of insulin treatment. *Brain* **122 (Pt 4)**: 757-768.

Biessels GJ, Deary IJ, Ryan CM (2008) Cognition and diabetes: a lifespan perspective. *Lancet Neurol* **7**: 184-190.

Biessels GJ, Gispen WH (2005) The impact of diabetes on cognition: what can be learned from rodent models? *Neurobiol Aging* **26 Suppl 1**: 36-41.

Biessels GJ, Kamal A, Ramakers GM, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH (1996) Place learning and

hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. *Diabetes* **45**: 1259-1266.

Biessels GJ, Kamal A, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH (1998) Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res* **800**: 125-135.

Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH (1994) Cerebral function in diabetes mellitus. *Diabetologia* **37**: 643-650.

Biessels GJ, van der Heide LP, Kamal A, Bleys RL, Gispen WH (2002) Ageing and diabetes: implications for brain function. *Eur J Pharmacol* **441**: 1-14.

Brands AM, Biessels GJ, de Haan EH, Kappelle LJ, Kessels RP (2005) The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes Care* **28**: 726-735.

Brekke E, Morken TS, Sonnewald U (2015) Glucose metabolism and astrocyte-neuron interactions in the neonatal brain. *Neurochem Int* **82**: 33-41.

Brownlee M (1995) Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* **46**: 223-234.

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**: 813-820.

Brundel M, Kappelle LJ, Biessels GJ (2014) Brain imaging in type 2 diabetes. *Eur Neuropsychopharmacol* **24**: 1967-1981.

Cai Z, Zhao Y, Yao S, Bin Zhao B (2011) Increases in beta-amyloid protein in the hippocampus caused by diabetic metabolic disorder are blocked by minocycline through inhibition of NF-kappaB pathway activation. *Pharmacol Rep* **63**: 381-391.

Cameron FJ (2015) The Impact of Diabetes on Brain Function in Childhood and Adolescence. *Pediatr Clin North Am* **62**: 911-927.

Candy SM, Szatkowski MS (2000) Neuronal excitability and conduction velocity changes in hippocampal slices from streptozotocin-treated diabetic rats. *Brain Res* **863**: 298-301.

Chabot C, Massicotte G, Milot M, Trudeau F, Gagne J (1997) Impaired modulation of AMPA receptors by calcium-dependent processes in streptozotocin-induced diabetic rats. *Brain Res* **768**: 249-256.

Chaldakov GN, Tonchev AB, Aloe L (2009) NGF and BDNF: from nerves to adipose tissue, from neurokines to metabokines. *Riv Psichiatr* **44**: 79-87.

Chen J, Li CR, Yang H, Liu J et al and Xu ZQ (2016) proBDNF Attenuates Hippocampal Neurogenesis and Induces Learning and Memory Deficits in Aged Mice. *Neurotox Res* **29**: 47-53.

Chen Z, Zhong C (2013) Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. *Prog Neurobiol* **108**: 21-43.

Clodfelder-Miller BJ, Zmijewska AA, Johnson GV, Jope RS (2006) Tau is hyperphosphorylated at multiple sites in mouse brain in vivo after streptozotocin-induced insulin deficiency. *Diabetes* **55**: 3320-3325.

Cole AR, Astell A, Green C, Sutherland C (2007) Molecular connexions between dementia and diabetes. *Neurosci Biobehav Rev* **31**: 1046-1063.

Cook DG, Leverenz JB, McMillan PJ, Kulstad JJ et al and Craft S (2003) Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. *Am J Pathol* **162**: 313-319.

Crane PK, Walker R, Larson EB (2013) Glucose levels and risk of dementia. *N Engl J Med* **369**: 1863-1864.

Currais A, Prior M, Lo D, Jolivalt C, Schubert D, Maher P (2012) Diabetes exacerbates amyloid and neurovascular pathology in aging-accelerated mice. *Aging Cell* **11**: 1017-1026.

Daneman D (2006) Type 1 diabetes. *Lancet* **367**: 847-858.

Dar TA, Sheikh IA, Ganie SA, Ali R et al and Zargar MA (2014) Molecular linkages between diabetes and Alzheimer's disease: current scenario and future prospects. *CNS Neurol Disord Drug Targets* **13**: 290-298.

de Bresser J, Tiehuis AM, van den Berg E, Reijmer YD et al and Utrecht Diabetic Encephalopathy Study G (2010) Progression of cerebral atrophy and white matter hyperintensities in patients with type 2 diabetes. *Diabetes Care* **33**: 1309-1314.

De Jong RN (1950) The nervous system complications in diabetes mellitus with special reference

to cerebrovascular changes. *The Journal of nervous and mental disease* **111**: 181-206.

de la Monte SM, Tong M (2014) Brain metabolic dysfunction at the core of Alzheimer's disease. *Biochem Pharmacol* **88**: 548-559.

de la Monte SM, Tong M, Bowling N, Moskal P (2011) si-RNA inhibition of brain insulin or insulin-like growth factor receptors causes developmental cerebellar abnormalities: relevance to fetal alcohol spectrum disorder. *Molecular brain* **4**: 13.

Dejgaard A, Gade A, Larsson H, Balle V, Parving A, Parving HH (1991) Evidence for diabetic encephalopathy. *Diabet Med* **8**: 162-167.

den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM (2003) Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* **46**: 1604-1610.

Desrocher M, Rovet J (2004) Neurocognitive correlates of type 1 diabetes mellitus in childhood. *Child Neuropsychol.***10**: 36-52.

Devi L, Alldred MJ, Ginsberg SD, Ohno M (2012) Mechanisms underlying insulin deficiency-induced acceleration of beta-amyloidosis in a mouse model of Alzheimer's disease. *PloS one* **7**: e32792.

Duelli R, Schrock H, Kuschinsky W, Hoyer S (1994) Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. *Int J Dev Neurosci* **12**: 737-743.

Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. *Lancet* **365**: 1415-1428.

Eichenbaum H, Yonelinas AP, Ranganath C (2007) The medial temporal lobe and recognition memory. *Annu Rev Neurosci* **30**: 123-152.

Farris W, Mansourian S, Chang Y, Lindsley L et al and Guenette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci U S A* **100**: 4162-4167.

Freude S, Plum L, Schnitker J, Leeser U et al and Schubert M (2005) Peripheral hyperinsulinemia promotes tau phosphorylation in vivo. *Diabetes* **54**: 3343-3348.

Frielingsdorf H, Simpson DR, Thal LJ, Pizzo DP (2007) Nerve growth factor promotes survival of new neurons in the adult hippocampus. *Neurobiol Dis* **26**: 47-55.

Gardoni F, Kamal A, Bellone C, Biessels GJ, et al and Di Luca M (2002) Effects of streptozotocin-diabetes on the hippocampal NMDA receptor complex in rats. *J Neurochem* **80**: 438-447.

Gardoni F, Schrama LH, van Dalen JJ, Gispen WH, Cattabeni F, Di Luca M (1999) AlphaCaMKII binding to the C-terminal tail of NMDA receptor subunit NR2A and its modulation by autophosphorylation. *FEBS Lett* **456**: 394-398.

Gasparini L, Netzer WJ, Greengard P, Xu H (2002) Does insulin dysfunction play a role in Alzheimer's disease? *Trends Pharmacol Sci* **23**: 288-293.

Gerges NZ, Aleisa AM, Alkadhi KA (2003) Impaired long-term potentiation in obese zucker rats: possible involvement of presynaptic mechanism. *Neuroscience* **120**: 535-539.

Gispen WH, Biessels GJ (2000) Cognition and synaptic plasticity in diabetes mellitus. *Trends Neurosci* **23**: 542-549.

Gottmann K, Mittmann T, Lessmann V (2009) BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Exp Brain Res* **199**: 203-234.

Grillo CA, Piroli GG, Wood GE, Reznikov LR, McEwen BS, Reagan LP (2005) Immunocytochemical analysis of synaptic proteins provides new insights into diabetes-mediated plasticity in the rat hippocampus. *Neuroscience* **136**: 477-486.

Guo J, Wang J, Zhang Z, Yan J et al and Liao H (2013) proNGF inhibits neurogenesis and induces glial activation in adult mouse dentate gyrus. *Neurochem Res* **38**: 1695-1703.

Hallbook F (1999) Evolution of the vertebrate neurotrophin and Trk receptor gene families. *Curr Opin Neurobiol* **9**: 616-621.

Hempstead BL (2014) Deciphering proneurotrophin actions. *Handb Exp Pharmacol* **220**: 17-32.

Ho MS, Weller NJ, Ives FJ, Carne CL et al and Jones TW (2008) Prevalence of structural central nervous

system abnormalities in early-onset type 1 diabetes mellitus. *J Pediatr.* **153**: 385-390.

Ho N, Sommers MS, Lucki I (2013) Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. *Neurosci Biobehav Rev* **37**: 1346-1362.

Hoyer S (1998) Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. *J Neural Transm (Vienna).* **105**: 415-422.

Hunt JV, Dean RT, Wolff SP (1988) Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *Biochem J* **256**: 205-212.

Jackson-Guilford J, Leander JD, Nisenbaum LK (2000 Oct 27) The effect of streptozotocin-induced diabetes on cell proliferation in the rat dentate gyrus. *Neurosci Lett* **293**: 91-94.

Jolivalt CG, Hurford R, Lee CA, Dumaop W, Rockenstein E, Masliah E (2010) Type 1 diabetes exaggerates features of Alzheimer's disease in APP transgenic mice. *Exp Neurol* **223**: 422-431.

Jung SW, Han OK, Kim SJ (2010) Increased expression of beta amyloid precursor gene in the hippocampus of streptozotocin-induced diabetic mice with memory deficit and anxiety induction. *J Neural Transm* **117**: 1411-1418.

Kamal A, Biessels GJ, Duis SE, Gispen WH (2000) Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. *Diabetologia* **43**: 500-506.

Kamal A, Biessels GJ, Gispen WH, Ramakers GM (2006) Synaptic transmission changes in the pyramidal cells of the hippocampus in streptozotocin-induced diabetes mellitus in rats. *Brain Res* **1073-1074**: 276-280.

Kamal A, Biessels GJ, Urban IJ, Gispen WH (1999) Hippocampal synaptic plasticity in streptozotocin-diabetic rats: impairment of long-term potentiation and facilitation of long-term depression. *Neuroscience* **90**: 737-745.

Ke YD, Delerue F, Gladbach A, Gotz J, Ittner LM (2009) Experimental diabetes mellitus exacerbates tau pathology in a transgenic mouse model of Alzheimer's disease. *PloS one* **4**: e7917.

Kim B, Backus C, Oh S, Hayes JM, Feldman EL (2009) Increased tau phosphorylation and cleavage in mouse models of type 1 and type 2 diabetes. *Endocrinology* **150**: 5294-5301.

Kim DJ, Yu JH, Shin MS, Shin YW, Kim MS (2016) Hyperglycemia Reduces Efficiency of Brain Networks in Subjects with Type 2 Diabetes. *PloS one* **11**: e0157268.

Krabbe KS, Nielsen AR, Krogh-Madsen R, Plomgaard P et al and Pedersen BK (2007) Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia* **50**: 431-438.

Kroner Z (2009) The relationship between Alzheimer's disease and diabetes: Type 3 diabetes? *Altern Med Rev* **14**: 373-379.

Leuner B, Gould E (2010) Structural plasticity and hippocampal function. *Annu Rev Psychol* **61**: 111-140, C111-113.

Li Y, Duffy KB, Ottinger MA, Ray Bet al and Greig NH (2010) GLP-1 receptor stimulation reduces amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease. *J Alzheimers Dis* **19**: 1205-1219.

Li ZG, Zhang W, Grunberger G, Sima AA (2002) Hippocampal neuronal apoptosis in type 1 diabetes. *Brain Res* **946**: 221-231.

Li ZG, Zhang W, Sima AA (2005) The role of impaired insulin/IGF action in primary diabetic encephalopathy. *Brain Res* **1037**: 12-24.

Lim KC, Lim ST, Federoff HJ (2003) Neurotrophin secretory pathways and synaptic plasticity. *Neurobiol Aging* **24**: 1135-1145.

Liu J, Feng L, Ma D, Zhang M et al and Ma S (2013) Neuroprotective effect of paeonol on cognition deficits of diabetic encephalopathy in streptozotocin-induced diabetic rat. *Neurosci Lett* **549**: 63-68.

Liu Y, Liu H, Yang J, Liu X et al and Wang G (2008) Increased amyloid beta-peptide (1-40) level in brain of streptozotocin-induced diabetic rats. *Neuroscience* **153**: 796-802.

Liu Y, Liu L, Lu S, Wang D, Liu X, Xie L, Wang G (2011) Impaired amyloid beta-degrading enzymes in brain of streptozotocin-induced diabetic rats. *J Endocrinol*

Invest **34**: 26-31.

Lu B (2003) Pro-region of neurotrophins: role in synaptic modulation. *Neuron* **39**: 735-738.

Lyoo IK, Yoon S, Renshaw PF, Hwang J et al and Jacobson AM (2013) Network-level structural abnormalities of cerebral cortex in type 1 diabetes mellitus. *PloS one* **8**: e71304.

Malone JI, Hanna S, Saporta S, Mervis RF, Park CR, Chong L, Diamond DM (2008) Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory. *Pediatr Diabetes* **9**: 531-539.

Martinez-Tellez R, Gomez-Villalobos Mde J, Flores G (2005) Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. *Brain Res* **1048**: 108-115.

Mayer G, Nitsch R, Hoyer S (1990) Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. *Brain Res* **532**: 95-100.

McKinney RA (2005) Physiological roles of spine motility: development, plasticity and disorders. *Biochem Soc Trans.* **33**: 1299-1302.

Mergenthaler P, Lindauer U, Dienel GA, Meisel A (2013) Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci* **36**: 587-597.

Mijnhout GS, Scheltens P, Diamant M, Biessels GJ et al and Heine RJ (2006) Diabetic encephalopathy: A concept in need of a definition. *Diabetologia* **49**: 1447-1448.

Miles WR, Root HF (1922) Psychologic tests applied in diabetic patients. *Arch Intern Med* **30**: 767-770.

Moheet A, Mangia S, Seaquist ER (2015) Impact of diabetes on cognitive function and brain structure. *Ann N Y Acad Sci* **1353**: 60-71.

Navaratna D, Fan X, Leung W, Lok J et al and Lo EH (2013) Cerebrovascular degradation of TRKB by MMP9 in the diabetic brain. *J Clin Invest* **123**: 3373-3377.

Navaratna D, Guo SZ, Hayakawa K, Wang X, Gerhardinger C, Lo EH (2011) Decreased cerebrovascular brain-derived neurotrophic factor-mediated neuroprotection in the diabetic brain. *Diabetes* **60**: 1789-1796.

Nunley KA, Ryan CM, Orchard TJ, Aizenstein HJ et al and Rosano C (2015) White matter hyperintensities in middle-aged adults with childhood-onset type 1 diabetes. *Neurology* **84**: 2062-2069.

Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM (1999) Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* **53**: 1937-1942.

Phelps CH (1972) Barbiturate-induced glycogen accumulation in brain. An electron microscopic study. *Brain Res* **39**: 225-234.

Planel E, Tatebayashi Y, Miyasaka T, Liu Let al and Takashima A (2007) Insulin dysfunction induces in vivo tau hyperphosphorylation through distinct mechanisms. *J Neurosci* **27**: 13635-13648.

Qu Z, Jiao Z, Sun X, Zhao Y, Ren J, Xu G (2011) Effects of streptozotocin-induced diabetes on tau phosphorylation in the rat brain. *Brain Res* **1383**: 300-306.

Rao J, Oz G, Seaquist ER (2006) Regulation of cerebral glucose metabolism. *Minerva Endocrinol* **31**: 149-158.

Reagan LP (2012) Diabetes as a chronic metabolic stressor: causes, consequences and clinical complications. *Exp Neurol* **233**: 68-78.

Rees DA, Alcolado JC (2005) Animal models of diabetes mellitus. *Diabet Med* **22**: 359-370.

Reisi P, Alaei H, Babri S, Sharifi MR, Mohaddes G, Soleimannejad E (2009) Determination of the extracellular basal levels of glutamate and GABA at dentate gyrus of streptozotocin-induced diabetic rats. *Pathophysiology* **16**: 63-66.

Reske-Nielsen E, Lundbaek K, Rafaelsen OJ (1966) Pathological changes in the central and peripheral nervous system of young long-term diabetics : I. Diabetic encephalopathy. *Diabetologia* **1**: 233-241.

Revsin Y, Saravia F, Roig P, Lima A, de Kloet ER, Homo-Delarche F, De Nicola AF (2005) Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes. *Brain Res* **1038**: 22-31.

Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM (2005) Insulin and insulin-like growth

factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *J Alzheimers Dis* **8**: 247-268.

Rocco ML, Pristera A, Pistillo L, Aloe L, Canu N, Manni L (2013) Brain Cholinergic Markers and Tau Phosphorylation are Altered in Experimental Type 1 Diabetes: Normalization by Electroacupuncture. *J Alzheimers Dis* **33**: 767-773.

Ryan CM, Williams TM, Finegold DN, Orchard TJ (1993) Cognitive dysfunction in adults with type 1 (insulin-dependent) diabetes mellitus of long duration: effects of recurrent hypoglycaemia and other chronic complications. *Diabetologia* **36**: 329-334.

Ryan JP, Aizenstein HJ, Orchard TJ, Ryan CM et al and Rosano C (2015) Age of Childhood Onset in Type 1 Diabetes and Functional Brain Connectivity in Midlife. *Psychosom Med* **77**: 622-630.

Sadeghi A, Hami J, Razavi S, Esfandiary E, Hejazi Z (2016) The Effect of Diabetes Mellitus on Apoptosis in Hippocampus: Cellular and Molecular Aspects. *Int J Prev Med.* **7**: 57.

Schubert M, Gautam D, Surjo D, Ueki K et al and Bruning JC (2004) Role for neuronal insulin resistance in neurodegenerative diseases. *Proc Natl Acad Sci U S A* **101**: 3100-3105.

Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D, Jr. (1992) Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev* **13**: 387-414.

Sherin A, Anu J, Peeyush KT, Smijin S, Anitha M, Roshni BT, Paulose CS (2012) Cholinergic and GABAergic receptor functional deficit in the hippocampus of insulin-induced hypoglycemic and streptozotocin-induced diabetic rats. *Neuroscience* **202**: 69-76.

Shi Y, Hu FB (2014) The global implications of diabetes and cancer. *Lancet* **383**: 1947-1948.

Sima AA (2010) Encephalopathies: the emerging diabetic complications. *Acta Diabetol* **47**: 279-293.

Sima AA, Li ZG (2005) The effect of C-peptide on cognitive dysfunction and hippocampal apoptosis in type 1 diabetic rats. *Diabetes* **54**: 1497-1505.

Sima AA, Zhang W, Li ZG, Kamiya H (2008) The effects of C-peptide on type 1 diabetic polyneuropathies and encephalopathy in the BB/Wor-rat. *Exp Diabetes Res* **2008**: 230458.

Sima AA, Zhang W, Muzik O, Kreipke CW, Rafols JA, Hoffman WH (2009) Sequential abnormalities in type 1 diabetic encephalopathy and the effects of C-Peptide. *Rev Diabet Stud* **6**: 211-222.

Sims-Robinson C, Kim B, Rosko A, Feldman EL (2010) How does diabetes accelerate Alzheimer disease pathology? *Nat Rev Neurol* **6**: 551-559.

Singh R, Barden A, Mori T, Beilin L (2001) Advanced glycation end-products: a review. *Diabetologia* **44**: 129-146.

Sokoloff L (1999) Energetics of functional activation in neural tissues. *Neurochem Res* **24**: 321-329.

Soligo M, Protto V, Florenzano F, Bracci-Laudiero L, De Benedetti F, Chiaretti A, Manni L (2015) The mature/pro nerve growth factor ratio is decreased in the brain of diabetic rats: Analysis by ELISA methods. *Brain Res* **1624**: 455-468.

Soule J, Messaoudi E, Bramham CR (2006) Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. *Biochemical Society transactions* **34**: 600-604.

Sposato V, Manni L, Chaldakov GN, Aloe L (2007) Streptozotocin-induced diabetes is associated with changes in NGF levels in pancreas and brain. *Arch Ital Biol* **145**: 87-97.

S Roriz-Filho J1, Sá-Roriz TM, Rosset I, Camozzato AL et al and Roriz-Cruz M (2009) (Pre)diabetes, brain aging, and cognition. *Biochim Biophys Acta* **1792**: 432-443.

Steen E, Terry BM, Rivera EJ, Cannon JL et al and de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *J Alzheimers Dis* **7**: 63-80.

Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP (2008) Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nat Neurosci* **11**: 309-317.

Stranahan AM, Lee K, Martin B, Maudsley S, Golden E, Cutler RG, Mattson MP (2009) Voluntary exercise and

caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus* **19**: 951-961.

Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP (2008) Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus* **18**: 1085-1088.

Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* **365**: 1333-1346.

Subramanian S, John M (2012) Intranasal administration of insulin lowers amyloid-beta levels in rat model of diabetes. *Indian J Exp Biol* **50**: 41-44.

Sutherland GR, Peeling J, Sutherland E, Tyson R, Dai F, Kozlowski P, Saunders JK (1992) Forebrain ischemia in diabetic and nondiabetic BB rats studied with 31P magnetic resonance spectroscopy. *Diabetes* **41**: 1328-1334.

Teles-Grilo Ruivo LM, Mellor JR (2013) Cholinergic modulation of hippocampal network function. *Front Synaptic Neurosci.* **5**: 2.

Umegaki H (2014) Type 2 diabetes as a risk factor for cognitive impairment: current insights. *Clin Interv Aging.* **9**: 1011-1019.

van Duinkerken E, Ijzerman RG, Klein M, Moll AC et al and Tijms BM (2016) Disrupted subject-specific gray matter network properties and cognitive dysfunction in type 1 diabetes patients with and without proliferative retinopathy. *Hum Brain Mapp* **37**: 1194-1208.

van Harten B, Oosterman J, Muslimovic D, van Loon BJ, Scheltens P, Weinstein HC (2007) Cognitive impairment and MRI correlates in the elderly patients with type 2 diabetes mellitus. *Age Ageing* **36**: 164-170.

Wang JQ, Yin J, Song YF, Zhang L et al and Jing YH (2014) Brain aging and AD-like pathology in streptozotocin-induced diabetic rats. *J Diabetes Res* **2014**: 796840.

Wang X, Zheng W, Xie JW, Wang T, Wang SL, Teng WP, Wang ZY (2010) Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model. *Mol Neurodegener* **5**: 46.

Welsh B, Wecker L (1991) Effects of streptozotocin-induced diabetes on acetylcholine metabolism in rat brain. *Neurochem Res* **16**: 453-460.

Yang J, Harte-Hargrove LC, Siao CJ, Marinic T et al and Hempstead BL (2014) proBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. *Cell reports* **7**: 796-806.

Yang SN, Tang YG, Zucker RS (1999) Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation. *J Neurophysiol* **81**: 781-787.

Yang Y, Song W (2013) Molecular links between Alzheimer's disease and diabetes mellitus. *Neuroscience* **250**: 140-150.

Yau PL, Javier DC, Ryan CM, Tsui WH, Ardekani BA, Ten S, Convit A (2010) Preliminary evidence for brain complications in obese adolescents with type 2 diabetes mellitus. *Diabetologia* **53**: 2298-2306.

Yi SS, Hwang IK, Yoo KY, Park OK et al and Yoon YS (2009) Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. *Neurochem Res* **34**: 1039-1046.

You JS, Kim CJ, Kim MY, Byun YG, Ha SY, Han BS, Yoon BC (2009) Long-term treadmill exercise-induced neuroplasticity and associated memory recovery of streptozotocin-induced diabetic rats: an experimenter blind, randomized controlled study. *NeuroRehabilitation* **24**: 291-297.

Zhang WJ, Tan YF, Yue JT, Vranic M, Wojtowicz JM (2008) Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. *Acta Neurol Scand* **117**: 205-210.

Zhao W, Chen H, Xu H, Moore E, Meiri N, Quon MJ, Alkon DL (1999) Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. *J Biol Chem* **274**: 34893-34902.

Zhou Y, Luo Y, Dai J (2013) Axonal and dendritic changes are associated with diabetic encephalopathy in rats: an important risk factor for Alzheimer's disease. *J Alzheimers Dis* **34**: 937-947.

GLIAL CELLS MEDIATE THE BROAD NEUROPROTECTIVE AND ANTI-AMYLOIDOGENIC ACTIONS OF PAINLESS NGF IN THE BRAIN VIA THE CXCL12 CHEMOKINE

S. CAPSONI[1] and A. CATTANEO[1,2]

[1]*Bio@SNS Laboratory of Biology, Scuola Normale Superiore, Pisa, Italy*
[2]*Rita Levi-Montalcini European Brain Research Institute (EBRI), Roma, Italy*

Nerve Growth Factor (NGF) is a therapeutic candidate for Alzheimer's disease. Due to its pain-inducing activity, in current clinical trials NGF is delivered locally into the brain by neurosurgery, but data on the efficacy of local NGF delivery in decreasing β-amyloid deposition are not available. To reduce the NGF pain-inducing side effects, thus avoiding the need for local brain injection, we developed painless NGF (hNGFp), inspired by the human genetic disease HSAN V. hNGFp has identical neurotrophic potency as wild type human NGF, but a ten-fold lower pain sensitizing activity. The results of a recent new study (Capsoni et al 2016) shed new light on the neuroprotective mechanism by hNGFp and are highly relevant for the design of NGF-based clinical trials. In that study we mimicked, in the 5xFAD mouse model, the intraparenchymal delivery of hNGFp used in clinical trials and found it to be ineffective in decreasing Aβ plaque load. On the contrary, the same dose of hNGFp delivered intranasally, which was instead widely biodistributed in the brain and did not induce pain, showed a potent anti-amyloidogenic action and rescued synaptic plasticity and memory deficits in this aggressive neurodegeneration model. This result has significant implications for the interpretation of the results of NGF clinical trials in AD and for their design. As for the mechanism of the potent and broad neuroprotection by hNGFp, it was found that hNGFp acts on glial cells, modulating inflammatory proteins such as the soluble TNFα receptor II and the chemokine CXCL12. The rescuing effects by hNGFp are mediated by CXCL12, since pharmacological inhibition of CXCL12 receptor CXCR4 occludes most hNGFp effects. These findings have significant therapeutic implications which are discussed in this article: i) they establish that a widespread exposure of the brain is required for NGF to fully exert its neuroprotective actions; ii) they identify a new anti-neurodegenerative pathway, linking hNGFp to inflammatory chemokines and cytokines, as a broad target for new therapeutic opportunities for neurodegenerative diseases.

NGF and AD

A selective vulnerability of basal forebrain cholinergic neurons (BFCNs) contributes to cognitive decline in Alzheimer's Disease (AD) patients (Bartus et al, 1982; Whitehouse et al, 1982). BFCNs depend on the neurotrophin Nerve Growth Factor (NGF) (Levi-Montalcini, 1952; Levi-Montalcini, 1987) for their maintenance and survival (Mufson et al, 1999). For this reason, the clinical application of NGF as a therapeutic agent for AD is being pursued, with a strong rationale, that goes well beyond its well-established actions on BFCNs, and is based on a broad anti-amyloidogenic action of NGF (Mufson et al, 1999; Capsoni and Cattaneo, 2006; Capsoni et

Key words: painless NGF, neuroprotection, Alzheimer's disease, CXCL12 chemokine, microglia

Corresponding author:
Dr. Simona Capsoni,
Department of Medical and Specialty Surgical Sciences,
University of Ferrara, Italy
e-mail: simona.capsoni@unife.it

2279-5855 (2016)

al, 2011; Cattaneo and Calissano, 2012). However, due to the poor biodistribution of NGF to the brain after systemic delivery (Poduslo and Curran, 1996) and, most importantly, to its potent pro-nociceptive activity after systemic (Petty et al, 1994) and intrathecal (Malcangio et al, 2000) administration, current NGF clinical trials on AD are based on the neurosurgical injection of NGF-secreting cells or of viral vectors carrying the NGF gene (Tuszynski et al, 2005; Mandel, 2010; Eriksdotter-Jonhagen et al, 2012), close to the nucleus basalis of Meynert (NBM). These trials report a positive effect on BFCNs and on cognition, which warrant further consideration of the NGF therapeutic approach, but the outcome of the NGF treatment on AD hallmarks, such as amyloid pathology, were not described in treated patients (Tuszynski et al, 2005; Mandel, 2010; Eriksdotter-Jonhagen et al, 2012; Ferreira et al, 2015; Karami et al, 2015). In preclinical testing, after a local infusion for three months of NGF in non-human primate brains, Aβ deposition was found to be identical to that in age-matched controls (Tuszynski et al, 1998). Moreover, the highly invasive neurosurgical procedure involved in this form of NGF therapy, even if promising, is unlikely to become generally applicable to the large numbers of patients affected by dementia.

Painless NGF

Thus, to fully exploit the therapeutic potential of NGF, we sought to reduce the pain-inducing side effects of NGF. To this aim, we developed painless NGF (hNGFp), a double mutant form of NGF (hNGFP61S/R100E) inspired by the human genetic disease Hereditary Sensory Autonomic Neuropathy type V (Einarsdottir et al, 2004). hNGFp harbors the mutation R100E, to reduce the pro-nociceptive activity of NGF (Covaceuszach et al, 2009; Capsoni et al, 2012; Malerba et al, 2015) and a second mutation (P61S), allowing its specific immunodetection against endogenous NGF. hNGFp is a TrkA biased agonist, in that it effectively binds and activates TrkA, although with a different signaling profile, while its binding affinity to p75NTR is strongly reduced (Covaceuszach et al, 2010; Capsoni et al, 2012). hNGFp has identical neurotrophic potency as wild type (WT) human NGF (hNGF), but a ten-fold lower pain sensitizing activity (Capsoni et al, 2012; Malerba et al, 2015). hNGFp has therefore a broader therapeutic window than hNGF and is currently undergoing preclinical IND enabling studies, including manufacturing under GMP conditions, towards its clinical testing on man.

A recent study (Capsoni et al 2016) addresses two important questions that have significant implications towards the development of therapeutic strategies for clinical applications of NGF and of painless hNGFp. In the present paper, we i) summarize the main findings of that study and ii) discuss the implications of those results for the current ongoing clinical trials with human NGF as well as for the forthcoming clinical trials with painless hNGFp.

Capsoni et al (2016) l studied the efficacy of hNGFp in counteracting neurodegeneration and behavioral deficits in the aggressive mouse model 5xFAD (Oakley et al, 2006), starting the treatment at an age when the pathology is already evident, a situation that would be similar to a human clinical trial on AD patients. Previous studies had shown that intranasal delivery of hNGFp to AppxPS1 mice induced a dramatic reduction of the amyloid plaque load, accompanied by a rescue of the learning and memory deficits, in the Morris water maize (Capsoni et al 2012).

LACK OF EFFICACY OF INTRAPARENCHYMAL hNGFp

In this paper Capsoni et al addressed the question whether the local intraparenchymal delivery of NGF to the NBM decreases Aβ plaque load, by locally delivering hNGFp close to the NBM of 5xFAD mice, mimicking the intraparenchymal delivery route current tested in NGF clinical trials. Very surprisingly, it was found that hNGFp, despite inducing cholinergic sprouting in the ipsilateral NBM, determined no decrease of the Aβ plaque load in the cerebral cortex. Thus the local delivery close to NBM is not sufficient for hNGFp to exert an anti-amyloidogenic effect, despite a clear action on cholinergic neurons, suggesting that a more widespread distribution of hNGFp in the brain might be required to reduce Aβ deposition.

INTRANASAL DELIVERY OF hNGFp IS WIDELY DISTRIBUTED THROUGHOUT THE BRAIN AND DETERMINES A STRONG NEUROPROTECTION

To investigate whether a broader exposure of the brain to hNGFp might be more effective, hNGFp was delivered intranasally, a non-invasive way to introduce neurotrophic factors to the brain at pharmacologically and therapeutically relevant doses (Capsoni et al, 2002b; De Rosa et al, 2005; Capsoni et al, 2009; Capsoni et al, 2012; Thorne and Frey, 2001; Lochhead and Thorne, 2012; Capsoni et al 2002a; Malerba et al, 2011). A biodistribution study of hNGFp after intranasal delivery, exploiting an ELISA protocol which selectively detects the P61S tagging mutation (Covaceuszach et al, 2009; Malerba et al, 2015) showed that after intranasal administration, hNGFp widely distributes to brain areas relevant to the neuropathology, such as the cerebral cortex and hippocampus, while its systemic levels are below the detection threshold. Thus, the intranasal delivery route, together with the use of hNGFp, allows delivering higher concentrations of the neurotrophin to maximize a broad exposure of the brain, while minimizing the potentially painful consequences of systemic build-up. Indeed, after intranasal delivery, hNGFp determines a robust decrease of the amyloid plaque load in different brain areas, as well as a general decrease of soluble and insoluble Aβ42 and Aβ40, and of Aβ oligomers (AβOs). This reduced plaque load after intranasal hNGFp administration is caused, at least in part, by a reduction of the pro-amyloidogenic APP processing. To test the specificity of hNGFp action, increasing doses of brain-derived neurotrophic factor (BDNF) were intranasally administered to 5xFAD mice, but even much higher doses of BDNF were found not to determine rescue of Aβ plaque load nor of behavioural memory deficits, despite a robust sprouting of cholinergic fibers in the NBM, indicating that BDNF reached the brain targets. It was therefore concluded that hNGFp has potent anti-amyloidogenic actions that are not shared by another neuroprotective neurotrophin and that the intranasal delivery of hNGFp is much more effective than its local administration in determining a robust decrease of Aβ deposition. Thus, a more widespread action of hNGFp in the brain is required to achieve a pharmacological effect. The robust decrease of Aβ production after intranasal delivery of hNGFp to 5xFAD mice correlates functionally with a full rescue of synaptic plasticity deficits in the entorhinal cortex as well as of memory deficits in behavioural paradigms.

MODULATION OF MICROGLIA AND ASTROCYTE MORPHOLOGY AND ACTIVITY BY hNGFp

What is the cellular basis of the anti-amyloidogenic actions of hNGFp in 5xFAD mice? In these mice, Aβ is produced mainly by cortical neurons, mainly pyramidal neurons, which do not express TrkA receptors. Hence, these neurons cannot be the first targets of the potent anti- amyloidogenic action of hNGFp. We considered unlikely that activation of BFCNs by hNGFp is sufficient to induce the potent anti-amyloidogenic response observed, also because the 5xFAD mice do not have a cholinergic deficit. Thus, we postulated that microglia and/or astrocytes might be the cells mediating hNGFp actions. These cells are potential target cells for NGF (De Simone et al, 2007; Cragnolini et al, 2009; Cragnolini et al, 2012). In cortical microglia of WT mice, both p75NTR and total TrkA were undetectable *in vivo*, while they were readily detectable in cultured WT primary microglia. Both receptors were instead well expressed in the cortex of PBS-treated 5xFAD mice. Interestingly, a similar increase in p75NTR and TrkA microglia expression could be observed in brain sections from Alzheimer's disease patients.

After hNGFp treatment, p75NTR immunoreactivity in 5xFAD cortical microglia was decreased, while TrkA immunoreactivity remained high. 5xFAD mice showed a higher number of large microglial cells, characterized by a lower number of ramifications, in respect to WT mice. hNGFp decreased the number and the size of microglia cells and partially restored their morphological complexity.

5xFAD microglia cells are engulfed by different

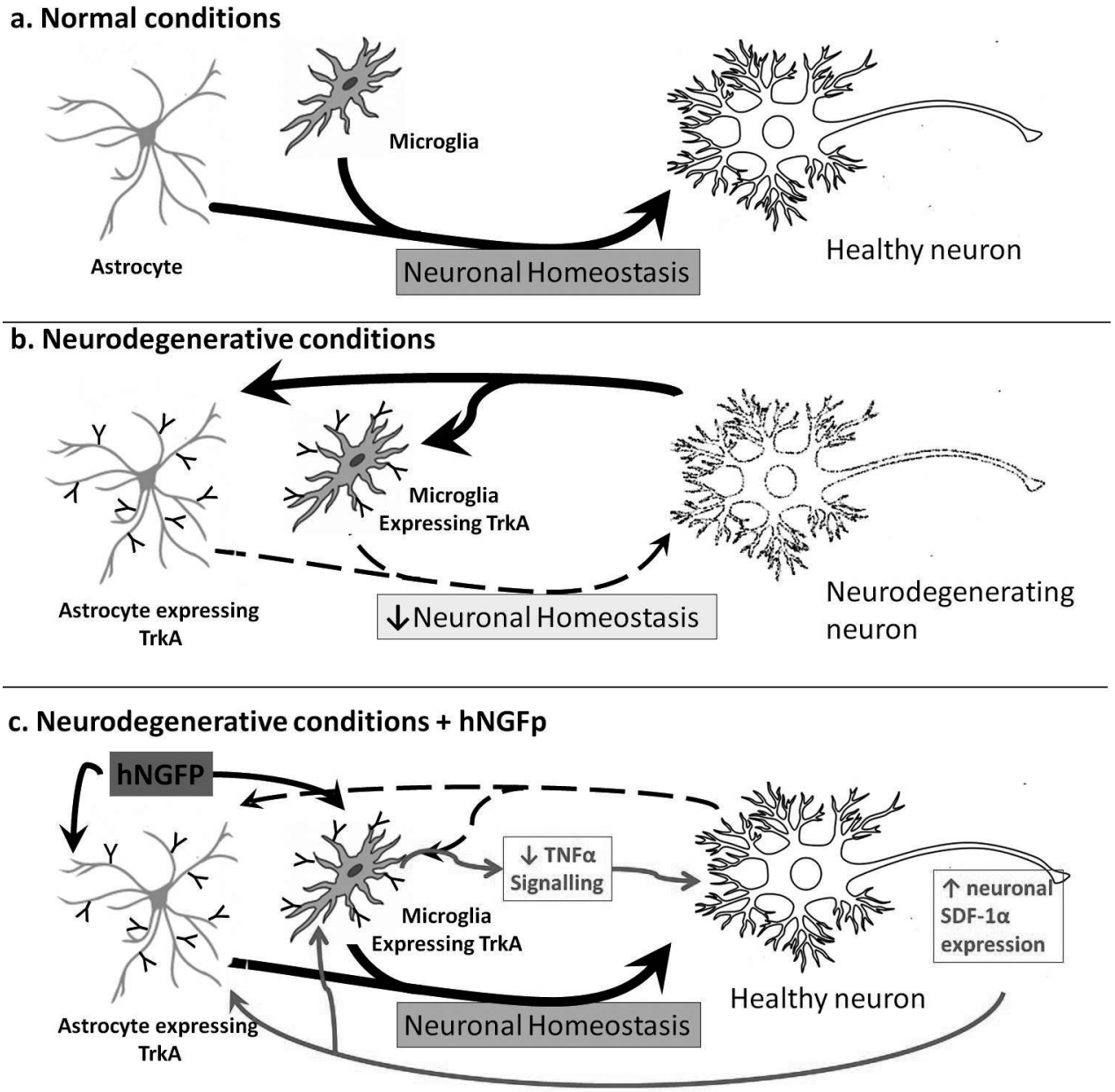

Fig. 1. *Scheme of the proposed neuroprotective mechanism by hNGFp. Under normal conditions (**a**), astrocytes and microglia do not express TrkA receptors and contribute to maintain neuronal homeostasis and welfare. In the neurodegenerating brain (**b**), astrocytes and microglia start expreressing TrkA and changing the p75NTR/TrkA ratio in favor of p75NTR, possibly as a protective reaction against neurodegeneration. After intranasal administration, hNGFp acts primarily on microglia and astrocytes (**c**), leading to a decreased availability of TN α to neurons, that in turn, possibly together with other undefined factors, mediate an increase of neuronal CXCL12 levels. This results, by as yet undefined mechanisms, in the downstream reduction of APP processing and broad neuroprotection and restoration of neuron-to-glia homeostasis.*

Aβ species. hNGFp induced a robust decrease of both Aβ and AβO immunoreactivity in cortical microglia of 5XFAD mice.

Cortical astrocytes from WT mice, similarly to microglia, do not express p75NTR and TrkA at detectable levels *in vivo*, but do so in culture. A significantly increased astrocytic p75NTR immunoreactivity was found in PBS-treated 5xFAD mice, which was decreased after hNGFp treatment. TrkA immunoreactivity in astrocytes was undetectable both in WT and PBS-treated 5xFAD mice, but a robust increase in astrocytic TrkA was observed after hNGFp administration. Most importantly, an increased expression of both p75NTR and TrkA in astrocytes could also be detected in Alzheimer's disease brain sections.

As for astrocyte morphology, 5xFAD mice showed extensive astrogliosis, characterized by an increase in the volume of individual astrocytes, which was reduced after hNGFp treatment. In addition, similarly to microglia, the treatment with hNGFp decreased Aβ and AβO immunoreactivity in 5xFAD astrocytes.

hNGFp is a preferential TrkA binder, in respect to p75NTR (Covaceuszach et al, 2010). hNGFp shifts the expression of NGF receptors towards TrkA on both cell types. Since, in PBS-treated 5xFAD mice, TrkA is expressed primarily on microglia and not in astrocytes, microglia cells might represent the primary target of hNGFp action and astrocytes may represent a secondary target. In any event, an increased degradation of cellular Aβ forms taken up by microglia and astrocytes might significantly contribute to the overall reduction of Aβ deposition after hNGFp treatment.

THE CHEMOKINE CXCL12 IS A MEDIATOR OF hNGFp NEUROPROTECTIVE AND ANTI-AMYLOIDOGENIC ACTIONS IN 5XFAD MICE

To investigate the mechanism whereby hNGFp determines the rescue of Aβ deposition and memory deficits, Capsoni et al postulated that hNGFp might modulate the expression of microglia-derived cytokines and chemokines. A cytokine and chemokine profiling revealed that hNGFp treatment increases the levels of the soluble TNFα receptor II (sTNFRII) (an extracellular decoy of TNFα), of macrophage inflammatory protein 1α and 1γ (MIP-1α and MIP-1γ) and of CXCL12 (also known as SDF-1α).

The focus was put on the CXCL12 chemokine, which has been shown to modulate Aβ expression (Parachikova and Cotman, 2007; Laske et al, 2008a; Laske et al, 2008b). Surprisingly, it was found that CXCL12 expression was not up-regulated by hNGFp treatment in 5XFAD microglia, but in neurons. Which factor could modulate CXCL12 expression in neurons? A possible microglia-derived candidate was identified in TNFα, since its levels are known to be regulated by NGF (Marshall et al, 1999; Prencipe et al, 2014). It is known that CXCL12 can be upregulated by reduced levels of TNFα (Zhang et al, 2008; Bockstal et al, 2011) in non-neuronal cells and, consistently, Capsoni et al showed that the incubation of WT and 5xFAD cultured cortical neurons with TNFα decreases CXCL12 expression, providing a link between TNF signaling and neuronal CXCL12. Also, hNGFp treatment reduced the expression of TNFα in 5xFAD microglia. Likewise, hNGFp reduced TNFα expression in WT microglia cultures, treated with AβOs. Altogether, data presented point to the reduction of microglia-derived TNFα signaling as the mechanism whereby hNGFp treatment increases neuronal CXCL12 expression.

Does CXCL12 mediate the rescuing effects of hNGFp on memory deficits and on amyloidogenic APP processing in 5xFAD mice? 5xFAD mice were therefore co-treated with hNGFp and AMD3100, an inhibitor of CXCL12 receptor CXCR4, which is expressed on neurons, astrocytes and microglia (Guyon, 2014). The treatment of 5xFAD mice with AMD3100 completely occluded and counteracted the rescuing effects of hNGFp on memory deficits, on Aβ plaque load and on the levels of AβO. AMD3100 was also found to block the hNGFp-induced modulation of presenilin 1 and BACE1 levels and the formation of APP processing products, as well as the reduction of intracellular Aβ induced by hNGFp in microglia cells.

These data demonstrate that CXCL12 mediates most of the actions of intranasally delivered hNGFp

in 5xFAD mice. In keeping with this conclusion, CXCL12 treatment of 5xFAD cultured cortical neurons also decreased AβO immunoreactivity in neurons, while it protected WT neurons from cell death induced by incubation with 7PA2 AβOs (Walsh et al, 2002).

In conclusion, the paper by Capsoni et al (2016), summarized above, demonstrates that hNGFp exerts its neuroprotective actions in 5xFAD mice via microglia and astrocytes, which under neurodegenerative conditions express TrkA. More in detail, hNGFp would act on microglia as its primary cellular target in the brain, lowering the overall TNFα signaling by microglia, thereby inducing neuronal CXCL12. This chemokine would then be an obligatory mediator of the neuroprotective and anti-amyloidogenic actions of hNGFp in 5xFAD mice.

Overall, these new data support the emerging concept that the neuroprotective actions of NGF in the brain go well beyond BFCNs (Capsoni and Cattaneo, 2006; Cattaneo et al, 2008; Capsoni et al, 2011; Cattaneo and Calissano, 2012) and demonstrate that the potent anti-amyloidogenic actions of hNGFp are exerted also in the absence of an overt cholinergic deficit of BFCNs. Most important, the study establishes glial cells (both astrocytes and microglia) as a broad target for hNGFp actions in the brain, and identifies the chemokine SDF1 as a key mediator of the neuroprotective and anti-amyloidogenic actions of hNGFp with significant clinical and therapeutic implications.

DISCUSSION

The clinical use of NGF as a therapeutic agent for Alzheimer's disease has a strong mechanistic rationale that goes beyond its well established actions on BFCNs and involves a direct anti-amyloidogenic action (Mufson et al, 1999; Capsoni and Cattaneo, 2006; Capsoni et al 2011; Cattaneo and Calissano, 2012). Thus, NGF homeostasis in the brain is crucially regulated and a disruption or alteration of this homeostasis is an upstream driver of Alzheimer's neurodegeneration. However, the clinical uses of NGF are hampered by NGF potent pain sensitizing activity, forcing current clinical trials in Alzheimer's disease patients to an invasive intraparenchymal delivery (Tuszinski et al, 2005; Eriksdotter-Jonhagen et al, 2012). To facilitate the therapeutic uses of NGF, we designed the mutant hNGFp, that has identical neurotrophic properties to hNGF, but a significantly lower pain-sensitizing activity (Capsoni et al, 2012; Malerba et al, 2015). The Capsoni et al (2016) study demonstrated the remarkable efficacy of intranasally-delivered hNGFp in counteracting neurodegeneration and behavioral deficits in the aggressive mouse model 5xFAD (Oakley et al, 2006), starting the treatment at an age when the pathology is already well evident, similar to a prospective clinical situation. On the other hand, a continuous local intraparenchymal delivery of hNGFp, close to the NBM, mimicking current NGF clinical trials in AD patients, failed to rescue neurodegeneration, despite a clear sprouting of BFCNs. This suggests that the neuroprotective actions of hNGFp cannot be mediated by the cholinergic system, also because, at this age, 5xFAD mice have no cholinergic deficit. This also shows that the known pro-cholinergic activity of hNGFp (in common with that of NGF) is an additional bonus of this neurotrophic and neuroprotective treatment. The anti-neurodegenerative action of hNGFp is highly specific for this neurotrophin, since it is not exerted by the related neurotrophin BDNF (not even at a 100-fold higher dose than hNGFp), strengthening the conclusion that the broad neuroprotective action of hNGFp is not mediated by BFCNs, which respond to both NGF and BDNF (Hefti et al, 1993; Mufson et al, 1999). The superior properties of hNGFp over those of BDNF are highly significant, since the latter neurotrophin is being considered as a therapeutic candidate for the treatment of AD and dementias (Nagahara and Tuszynski, 2011).

Moreover, the results of the Capsoni 2016 study show that the potent neuroprotective and anti-amyloidogenic actions of hNGFp require a widespread and global biodistribution of hNGFp in relevant brain regions, such as that obtained by intranasal delivery. This has the important implication that the clinical outcome of the NGF-based clinical trials, which are currently forcedly being performed

by the local injection of NGF or of NGF secreting cells. Despite the fact that the encouraging results of those trials have motivated the authorization by regulatory authorities for their further continuation and for further patient recruitment, the results obtained by the Capsoni 2016 study suggest that the outcome of those trials could have been much more positive if a more global exposure of the brain to NGF could have been achieved. Of course, the "dark side of the moon" of NGF, namely its pain sensitizing activity, does not allow for this more global exposure.

These results provide a very important motivation for the use and further development of painless NGF, thanks to its increased therapeutic window and to the cellular basis for its actions in the brain. The Capsoni 2016 study demonstrates that microglia and astrocytes mediate hNGFp actions. In 5xFAD brain, both microglia and astrocytes express TrkA and p75NTR receptors with a p75NTR/TrkA ratio in favor of p75NTR. This imbalance is changed by hNGFp, which shifts the expression of NGF receptors towards TrkA on both cell types. Previous studies have shown that microglia take up both soluble and insoluble forms of Aβ *in vitro* and *in vivo* (Bolmont et al, 2008; Lee and Landreth, 2010) but also that phagocytosis may not be effective in compensating the continuous deposition of Aβ (Bolmont et al, 2008; Sierra et al, 2013). Similarly, astrocytes have been shown to take up Aβ *in vitro* and *in vivo* (Wyss-Coray et al, 2003) with a feed-forward mechanism driven by cytokines and Aβ42, eventually leading to Aβ production also in these cells (Zhao et al, 2011). The dramatic overall reduction of Aβ deposition after hNGFp is carried out, at least partially, by a reduced APP amyloidogenic processing, and also by an increased degradation of cellular Aβ forms taken up by microglia and astrocytes.

The observed increase in the microglia-derived TNFα decoy sTNFαRII, leads to a reduced TNFα signaling by microglia, which upregulates the neuronal expression of the chemokine CXCL12, which has well-established neuroprotective (Guyon and Nahon, 2007; Li et al, 2012) and anti-amyloidogenic actions (Parachikova and Cotman, 2007; Laske et al, 2008a; Laske et al, 2008b). Most importantly, CXCL12 is downregulated in Alzheimer's disease brains (Parachikova and Cotman, 2007; Laske et al, 2008a; Laske et al, 2008b). Capsoni et al (2016) tested the hypothesis that CXCL12 might mediate the neuroprotective actions of hNGFp and, by exploiting the AMD3100 inhibitor of the CXCL12 receptor CXCR4, provided compelling evidence that CXCL12 mediates the pharmacological effects of hNGFp in 5xFAD mice.

Based on these data, we can draw a picture whereby hNGFp would act primarily on microglia and astrocytes in the neurodegenerating brain, leading to a decreased availability of TNFα to neurons that, possibly with other undefined factors, in turn mediates an increase of neuronal CXCL12 levels and the downstream reduction of APP processing, increased clearance of Aβ and broad neuroprotection and restoration of neuron-to-glia homeostasis (Fig. 1).

How exactly CXCL12 exerts these downstream neuroprotective actions remains to be seen. One could envisage that this chemokine, produced by neurons, acts on microglia and astrocytes to re-establish a correct neuron-glia homeostasis. In any event, this chemokine should mimic many of the actions of hNGFp in the 5XFAD as well as in other models. Thus, besides being a neurotrophic factor, NGF can be considered a neurokine, a concept put forward two decades ago by Levi-Montalcini (Levi-Montalcini et al 1996).

Altogether, the new Capsoni 2016 study adds a strong mechanistic rationale to the clinical uses of hNGFp in neurodegenerative diseases, and demonstrates microglia and astrocytes as cellular targets mediating its strong neuroprotective actions. Notably, glial cells mediate the rescuing effect by hNGFp, via the CXCL12 chemokine, identifying a new anti-neurodegenerative pathway that might become a target for new therapeutic opportunities for Alzheimer's and other neurodegenerative diseases. Thus, we demonstrate a direct causal reciprocal link between neurotrophins and neuroinflammatory molecules, mediated by glial cells, under clinically very relevant conditions. The broad neuroprotective actions of hNGFp, via glial cells, open the therapeutic potential of painless hNGFp to a large number of neurodegenerative conditions beyond, but including,

Alzheimer's disease, where an action through astrocytes and microglia cells could exert beneficial actions to restore a correct neuronal homeostasis.

REFERENCES

Bartus RT, Dean RL, 3rd, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**(4558): 408-14.

Bockstal V, Geurts N, Magez S (2011) Acute Disruption of Bone Marrow B Lymphopoiesis and Apoptosis of Transitional and Marginal Zone B Cells in the Spleen following a Blood-Stage Plasmodium chabaudi Infection in Mice. *J Parasitol Res* **2011**: 534697.

Bolmont T, Haiss F, Eicke D, Radde R et al. and Calhoun ME (2008) Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. *J Neurosci* **28**(16): 4283-92.

Capsoni S, Brandi R, Arisi I, D'Onofrio M, Cattaneo A (2011) A dual mechanism linking NGF/proNGF imbalance and early inflammation to Alzheimer's disease neurodegeneration in the AD11 anti-NGF mouse model. *CNS Neurol Disord Drug Targets* **10**(5): 635-47.

Capsoni S, Cattaneo A (2006) On the molecular basis linking Nerve Growth Factor (NGF) to Alzheimer's disease. *Cell Mol Neurobiol* **26**(4-6): 619-33.

Capsoni S, Covaceuszach S, Ugolini G, Spirito F et al and Cattaneo A. (2009) Delivery of NGF to the Brain: Intranasal versus Ocular Administration in Anti-NGF Transgenic Mice. *J Alzheimers Dis* **16**(2): 371-88.

Capsoni S, Giannotta S, Cattaneo A (2002) Beta-amyloid plaques in a model for sporadic Alzheimer's disease based on transgenic anti-nerve growth factor antibodies. *Mol Cell Neurosci* **21**(1): 15-28.

Capsoni S, Giannotta S, Cattaneo A (2002) Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice. *Proc Nat Acad Sci USA* **99**(19): 12432-7.

Capsoni S, Marinelli S, Ceci M, Vignone D et al and Cattaneo A (2012) Intranasal "painless" Human Nerve Growth Factor Slows Amyloid Neurodegeneration and Prevents Memory Deficits in App X PS1 Mice. *PLoS One* **7**(5): e37555.

Capsoni S, Ugolini G, Comparini A, Ruberti F, Berardi N, Cattaneo A (2000) Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. *Proc Nat Acad Sci USA* **97**(12): 6826-31.

Capsoni S, Malerba F, Carucci N, Rizzi C et al and Cattaneo A (2016) The chemokine CXCL12 mediates the anti-amyloidogenic actin of painless human nerve Growth factor. BRAIN, *in press*.

Cattaneo A, Calissano P (2012) Nerve growth factor and Alzheimer's disease: new facts for an old hypothesis. *Mol Neurobiol* **46**(3): 588-604.

Cattaneo A, Capsoni S, Paoletti F (2008) Towards non invasive Nerve Growth Factor therapies for Alzheimer's disease. *J Alzheimer's Disease*; **15**: 255-83.

Cha M, Kohan KJ, Zuo X, Ling JX, Gu JG. (2012) Assessment of chronic trigeminal neuropathic pain by the orofacial operant test in rats. *Behavioural Brain Res* **234**(1): 82-90.

Covaceuszach S, Capsoni S, Marinelli S, Pavone F et al and Cattaneo A (2010) In vitro receptor binding properties of a "painless" NGF mutein, linked to hereditary sensory autonomic neuropathy type V. *Biochem Biophys Res Commun* **391**: 824-9.

Covaceuszach S, Capsoni S, Ugolini G, Spirito F, Vignone D, Cattaneo A (2009) Development of a non invasive NGF-based therapy for Alzheimer's disease. *Curr Alzheimer Res* **6**(2): 158-70.

Cragnolini AB, Huang Y, Gokina P, Friedman WJ (2009) Nerve growth factor attenuates proliferation of astrocytes via the p75 neurotrophin receptor. *Glia* **57**(13): 1386-92.

Cragnolini AB, Volosin M, Huang Y, Friedman WJ (2012) Nerve growth factor induces cell cycle arrest of astrocytes. *Dev Neurobiol* **72**(6): 766-76.

De Rosa R, Garcia AA, Braschi C, Capsoni S, Maffei L, Berardi N, Cattaneo A (2005) Intranasal administration of nerve growth factor (NGF) rescues recognition memory deficits in AD11 anti-NGF transgenic mice. *Proc Nat Acad Sci USA* **102**(10): 3811-6.

De Simone R, Ambrosini E, Carnevale D, Ajmone-Cat MA, Minghetti L (2007) NGF promotes microglial migration through the activation of its high affinity

receptor: modulation by TGF-beta. *J Neuroimmunol* **190**(1-2): 53-60.

Einarsdottir E, Carlsson A, Minde J, Toolanen G et al and Holmberg M (2004) A mutation in the nerve growth factor beta gene (NGFB) causes loss of pain perception. *Hum Mol Genet* **13**(8): 799-805.

Eriksdotter-Jonhagen M, Linderoth B, Lind G, Aladellie L et al and Wahlberg L (2012) Encapsulated cell biodelivery of nerve growth factor to the Basal forebrain in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord* **33**(1): 18-28.

Ferreira D, Westman E, Eyjolfsdottir H, Almqvist P et al and Eriksdotter M (2015) Brain changes in Alzheimer's disease patients with implanted encapsulated cells releasing nerve growth factor. *J Alzheimers Dis* **43**(3): 1059-72.

Frey IW, Liu J, Chen XQ, Thorne RG, Fawcett JR, Ala TA, et al. (1997) Delivery of ^{125}I-NGF to the brain via the olfactory route. *Drug Delivery* 4: 87-92.

Guyon A. (2014) CXCL12 chemokine and its receptors as major players in the interactions between immune and nervous systems. *Front Cell Neurosci* 8: 65.

Guyon A, Nahon JL (2007) Multiple actions of the chemokine stromal cell-derived factor-1alpha on neuronal activity. *J Mol Endocrinol* 2007; **38**(3): 365-76.

Hefti F, Knusel B, Lapchak PA (1993) Protective effects of nerve growth factor and brain-derived neurotrophic factor on basal forebrain cholinergic neurons in adult rats with partial fimbrial transections. *Progress in Brain Res* **98**: 257-63.

Karami A, Eyjolfsdottir H, Vijayaraghavan S, Lind G et al. and Eriksdotter M (2015) Changes in CSF cholinergic biomarkers in response to cell therapy with NGF in patients with Alzheimer's disease. *Alzheimers Dement* **11**(11):1316-28. doi: 10.1016/j.jalz.2014.11.008. Epub 2015 Feb 9.

Kimura R, Devi L, Ohno M (2010) Partial reduction of BACE1 improves synaptic plasticity, recent and remote memories in Alzheimer's disease transgenic mice. J *Neurochem* **113**(1): 248- 61.

Laske C, Stellos K, Eschweiler GW, Leyhe T, Gawaz M (2008) Decreased CXCL12 (SDF-1) plasma levels in early Alzheimer's disease: a contribution to a deficient hematopoietic brain support? *J Alzheimers Dis* **15**(1): 83-95.

Laske C, Stellos K, Stransky E, Seizer P et al. and Gawaz M (2008) Decreased plasma and cerebrospinal fluid levels of stem cell factor in patients with early Alzheimer's disease. *J Alzheimers Dis* **15**(3): 451-60.

Lee CY, Landreth GE (2010) The role of microglia in amyloid clearance from the AD brain. *J Neural Transm* **117**(8): 949-60.

Levi-Montalcini R (1952) Effects of mouse tumor transplantation on the nervous system. *Ann N Y Acad Sci* **55**(2): 330-44.

Levi-Montalcini R (1987) The Nerve Growth Factor 35 years later. *Science* **237**: 1154-62

Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A (1996) Nerve growth factor: from neurotrophin to neurokine. *Trends Neurosci* **19**(11): 514-20.

Li M, Hale JS, Rich JN, Ransohoff RM, Lathia JD (2012) Chemokine CXCL12 in neurodegenerative diseases: an SOS signal for stem cell-based repair. *Trends Neurosci* **35**(10): 619-28.

Lochhead JJ, Thorne RG (2012) Intranasal delivery of biologics to the central nervous system. *Adv Drug Deliv Rev* 64(7): 614-28.

Malcangio M, Ramer MS, Boucher TJ, McMahon SB (2000) Intrathecally injected neurotrophins and the release of substance P from the rat isolated spinal cord. *Eur J Neurosci* **12**(1): 139-44.

Malerba F, Paoletti F, Bruni Ercole B, Materazzi S et al. and Cattaneo A. (2015) Functional characterization of human ProNGF and NGF mutants: identification of NGF P61SR100E as a "painless" lead investigational candidate for therapeutic applications. *PLoS ONE* **10**(9): e0136425.

Malerba F, Paoletti F, Capsoni S, Cattaneo A (2011) Intranasal delivery of therapeutic proteins for neurological diseases. *Expert Opin Drug Deliv* **8**(9): 1-20.

Mandel RJ (2010) CERE-110, an adeno-associated virus-based gene delivery vector expressing human nerve growth factor for the treatment of Alzheimer's disease. *Curr Opin Mol Ther* **12**(2): 240-7.

Marshall JS, Gomi K, Blennerhassett MG, Bienenstock

J (1999) Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism. *J Immunol* **162**(7): 4271-6.

Mufson EJ, Kroin JS, Sendera TJ, Sobreviela T (1999) Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. *Prog Neurobiol* **57**(4): 451-84.

Nagahara AH, Tuszynski MH (2011) Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nat Rev Drug Discov* 10(3): 209-19.

Oakley H, Cole SL, Logan S, Maus E et al and Vassar R (2006) Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* **26**(40): 10129-40.

Parachikova A, Cotman CW (2007) Reduced CXCL12/CXCR4 results in impaired learning and is downregulated in a mouse model of Alzheimer disease. *Neurobiol Dis* **28**(2): 143-53.

Petty BG, Cornblath DR, Adornato BT, Chaudhry V et al and Peroutka SJ (1994) The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. *Ann Neurol* **36**(2): 244-6.

Poduslo JF, Curran GL (1996) Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. *Brain Res Mol Brain Res* **36**(2): 280-6.

Prencipe G, Minnone G, Strippoli R, De Pasquale L et al and Bracci-Laudiero L (2014) Nerve growth factor downregulates inflammatory response in human monocytes through TrkA. *J Immunol* **192**(7): 3345-54.

Sierra A, Abiega O, Shahraz A, Neumann H (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. *Frontiers Cell Neurosci* **7**: 6.

Thorne RG, Frey WH 2nd (2001) Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet* **40**(12): 907-46.

Tuszynski MH, Smith DE, Roberts J, McKay H, Mufson E (1998) Targeted intraparenchymal delivery of human NGF by gene transfer to the primate basal forebrain for 3 months does not accelerate beta- amyloid plaque deposition. *Exper Neurol* **154**(2): 573-82.

Tuszynski MH, Thal L, Pay M, Salmon DP et al. and Conner J (2005) A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med* **11**(5): 551-5.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK et al. and Selkoe DJ. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**(6880): 535-9.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**(4537): 1237-9.

Wyss-Coray T, Loike JD, Brionne TC, Lu E et al. and Husemann J (2003) Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med* 2003; **9**(4): 453-7.

Zhang Q, Guo R, Schwarz EM, Boyce BF, Xing L (2008) TNF inhibits production of stromal cell-derived factor 1 by bone stromal cells and increases osteoclast precursor mobilization from bone marrow to peripheral blood. *Arthritis Res Ther* **10**(2): R37.

Zhao J, O'Connor T, Vassar R (2011) The contribution of activated astrocytes to Abeta production: implications for Alzheimer's disease pathogenesis. *J Neuroinflamm* **8**: 150.

POTENTIAL PREVENTION AND TREATMENT OF NEURODEGERNERATIVE DISEASES: OLIVE POLYPHENOLS AND HYDROXYTYROSOL

V. CALABRESE[1] and R. CREA[2]

[1]Department of Biomedical and Biotechnological Sciences, School of Medicine, University of Catania, Catania, Italy
[2]CreAgri Incorporated, Hayward, CA, USA

The Authors contributed equally to this manuscript

Emerging interest has recently focused on markers of oxidative stress and neuroinflammation in neurodegenerative disorders as abnormal redox homeostasis, oxidative stress and altered antioxidant systems have been considered important factors underlying the pathogenesis of major degenerative disorders, such as Parkinson's disease (PD) and Alzheimer's disease (AD). Basal levels of oxidants are indispensible for redox signaling to produce adaptive cellular responses such as vitagenes linked to cell survival, but at higher levels are detrimental to cells, contributing to aging and to the pathogenesis of numerous age-related diseases. Altered expression of genes related to oxidative stress, oxidative damage to DNA, protein and lipids, as well as alterations in the redox state act synergistically, and contribute to the course of major age-related diseases. However, the concept that low levels of stress can induce responses that may be protective against the pathogenic processes is a frontier area of neurobiological research focal to understanding and developing therapeutic approaches to neurodegenerative pathologies. This paper introduces the concept of cellular stress response and hormesis and its applications to the field of neuroprotection. Here hormetic mechanisms are reviewed as possibility of targeted therapeutic manipulation in a cell-, tissue- and/or pathway-specific manner at appropriate points in the AD and PD disease processes. We address and propose the potential therapeutic utility of olive polyphenols and hydroxytyrosol as potential pharmacological modulators of neuroinflammation and activators of the keap1/nfr2/are pathway as a rationale for treating neurodegenerative disorders.

The lengthening life span of the world's population is causing a dramatic increase in the prevalence of neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's (AD) (Kim et al, 2016; Li and Le, 2013).

A major complication of normal "healthy" ageing is the increasing risk for age-related diseases like cardiovascular diseases, diabetes mellitus, and dementia that can adversely affect the quality of life in general, increasing the risk of co-morbidities and mortality. The burden caused by ageing-associated pathologies is therefore obvious (Kenion et al, 1993; Judy et al, 2013). At a cellular and molecular level, "ageing" can be defined as a progressive deterioration of physiological functions ultimately leading to systemic dysfunction and death (Nkuipou-Kenfack et al, 2014). This might include the accumulation of senescent cells thereby limiting regenerative abilities (Nkuipou-Kenfack et al, 2014b). Ageing is a complex systemic process and the major

Key words: Parkinson's disease, Alzheimer's disease, hormesis, cellular stress response, inflammation, heme oxygenase, olive polyphenols, hydroxytyrosol

Corresponding author:
Prof. Vittorio Calabrese,
Department of Biomedical and Biotechnological Sciences,
University of Catania,
Via Andrea Doria, 95100 Catania, Italy
Tel.: +39 095 4781120 - Fax: +39 095 580138
e-mail: calabres@unict.it

2279-5855 (2016)

gap in ageing research remains the insufficient knowledge about pathways shifting from normal "healthy" ageing to disease-associated pathological ones (Campisi, 2013; Lopez-Otin et al, 2013). Oxidants, at a basal level, are essential for redox signaling and modulation of stress responses, such as vitagenes, which are associated to an increase in cell survival, but at higher levels, reactive molecular species are detrimental contributing to aging and the pathogenesis of numerous aging-related diseases (Dattilo et al, 2015; Calabrese et al, 2015; Calabrese et al, 2015). Mitochondrial dysfunction is characteristic of several neurodegenerative disorders, and evidence for mitochondria being a site of damage in neurodegenerative disorders is partially based on decreases in respiratory chain complex activities as in PD, AD, and Huntington's disease (Calabrese et al, 2001). Such defects in respiratory complex activities, possibly associated with oxidant/antioxidant balance perturbation, are thought to underlie defects in energy metabolism and induce cellular degeneration (Cornelius et al, 2013; Calabrese et al, 2010; Mancuso et al, 2007). PD is a progressive neurodegenerative disorder with onset typically occurring in the sixth or seventh decade of life. The main clinical features of PD, namely bradykinesia, rigidity and tremor, result from dopamine depletion in the striatum, due to nigral neuronal loss. Although a number of hypotheses for the pathogenesis of PD have been proposed, including defective DNA repair mechanisms, specific genetic defects, viral disorders, lack of a neurotrophic hormone, or toxic compounds present in the environment, none, however, completely explains the cascade of events responsible for the cause and the course of the disease (Vaubel et al, 2016). A loss in nigral dopaminergic neurons is normally observed during the course of normal aging, and when a pathological process adds to this and neurons are lost beyond the threshold of 80% of the neurons present at birth, PD can develop. However, increasingly, both genetic and epidemiological evidence now point to a maternal mitochondrial origin of PD (Goldstein et al, 2015, Goldstein et al, 2016). AD is also a progressive neurodegenerative disorder leading to cognitive decline, neuropsychiatric symptoms, disability, caregiver burden, and premature death. It represents the most prevalent cause of dementia and its incidence rates exponentially increase with increasing age (Ziegler-Graham et al, 2008). The number of Americans living with AD is rapidly increasing. An estimated 5.4 million Americans of all ages have AD in 2016. One in nine people aged 65 years and older has AD and by mid-century, someone in the United States will develop the disease every 33 seconds (Brookmeyer et al, 2007).

It is now accepted that "neuroinflammation", is a common feature of neurological and neurodegenerative diseases and that controlling the degree of neuroinflammation may delay or prevent the onset of neurodegenerative disorders (Schwartz and Deczkowska, 2016). Efficient functioning of maintenance and repair processes seems to be crucial for both survival and physical quality of life. This is accomplished by a complex network of the so-called longevity assurance processes, which are composed of several genes, termed vitagenes (Calabrese et al, 2010b). Modulation of the "neuroinflammation" process and enhancing the endogenous cellular defence mechanisms via the sirtuin, Nrf2 and related pathways that integrate adaptive stress responses may represent an innovative approach to therapeutic intervention in diseases causing chronic tissue damage, such as in neurodegeneration (Calabrese et al, 2012). Dietary olive polyphenols, especially hydroxytyrosol (HT), have been demonstrated *in vitro* and *in vivo* to be potent anti-inflammatory (Bitler et al, 2005), partially via the modulation of the NF-kb cellular pathway (Richard et al, 2011) as well as neuroprotective through the activation of hormetic pathways, including sirtuins and vitagenes. The present paper introduces the potential use of olive polyphenols (hydroxytyrosol) as neuroprotective agents and their potential effect on the hormetic dose-response concept including its scientific foundations, toxicological and pharmacological implications, and its applications in the field of neuroprotection and their mechanistic foundations.

PARKINSON'S DISEASE

Parkinson's disease (PD), the second most

common late-age onset neurodegenerative disease, is a degenerative disorder affecting 1-2% of the population after the age of about 50 years (Beitz, 2014; Eriksen et al, 2003; Campolo et al, 2016). Currently, no disease modifying therapy is available except for the symptomatic alleviating treatment. Two cardinal pathological features of PD are the presence of Lewy body and Lewy neurites in remaining neurons and the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Bellucci et al, 2016; Rodriguez-Oroz et al, 2009). The latter is believed to be the cause for major motor symptoms in PD patients, including tremors, rigidity, bradykinesia, and impaired balance. L-DOPA therapy, as major currently available PD therapy, is able to alleviate motor symptoms (Olanow, 2015), however no disease modifying therapy able to prevent dopaminergic neuron loss or halt the neurodegenerative progression in PD currently exists. Among all PD cases, over 90% of all PD is sporadic, while approximately 10% represent familial cases associated with mutations in various genes (Zimprich et al, 2004; Jawed et al, 2016). Although a number of hypotheses, including defective DNA repair mechanisms, specific genetic defects, viral disorder, lack of a neurothrophic hormone or toxic compounds present in the environment, have been proposed, none completely explains the cascade of events responsible for the cause and the course of the disease. Oxidative stress is commonly believed to be the underlying cause of cellular dysfunction and cell death in both sporadic and familial PD (Blesa et al, 2015), although the precise etiology of PD remains elusive. The nigral dopaminergic neurons appear to be highly vulnerable to oxidative stress due to high production of reactive oxygen species (ROS), as by-products of dopamine (DA) metabolism and to high iron concentration (Zucca et al, 2015). DA is easily oxidized to dopamine quinone (DAQ) (Sulzer and Zecca, 2000; Jawed et al, 2016), which reacts with sulfhydryl groups in cysteinyl proteins to form Michael adducts and generate ROS through redox cycling (Cornwell and Ma, 2007; Hastings, 2009; Segura-Aguilar et al, 2014; Wang et al, 2006). In addition to DA metabolism, mitochondrial dysfunction and neuroinflammation in PD are also believed to contribute to the increased ROS levels in the substantia nigra (Dias et al, 2013). Consistent with the notion that oxidative stress contributes to PD pathology, high levels of lipid peroxidation, increased protein oxidation, and depletion of glutathione are common in the substantia nigra of PD brains (Danielson and Andersen, 2008; Zhang et al, 2016). *In vivo*, ROS is continuously produced and can be eliminated by cellular antioxidant mechanisms such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH). When the balance between ROS production and elimination is disrupted, extra ROS causes oxidative stress that impairs normal cellular function. In patients or animal models of PD, SOD and CAT are increased, whereas GPx and GSH are decreased (Rajasankar et al, 2009), indicating ROS imbalance and oxidative stress. Based on these observations, it has been proposed that antioxidants may be a possible approach against PD. However, none of the antioxidants, including vitamin E, vitamin C, Coenzyme Q10, green tea polyphenols, and docosahexaenoic acid (Chen et al, 2007; Fahn, 1992; Goldstein et al, 2015) have provided convincing data of significant therapeutic efficacy. The failure of the antioxidant therapeutic trials indicates that oxidative stress might be just one of the contributing factors to PD pathogenesis, and antioxidants alone are not sufficient to halt the pathological progression in PD. Equally important, antioxidant bioavailabilty and capacity to cross the blood brain barrier may be important factors in supporting their therapeutic activity. A large body of evidence supports the role of free radicals in the pathogenesis of the disease (Hyun et al, 2002). Levels of lipid hydroperoxides are increased 10-fold in the SN in PD (Hyun et al, 2002). Decreased glutathione peroxidase and catalase activities associated to increased SOD activity lead to increased levels of hydrogen peroxide (Dexter et al, 1991; Zhang et al, 2016). This, in dopaminergic cells is primarily produced by MAO via deamination of dopamine and also non-enzimatically by autoxidation of dopamine. Hydrogen peroxide, by reacting with

reduced forms of transition metals, e.g., iron (II) or copper (I), gives rise to the powerful oxidant hydroxyl radical and oxidative damage to nigral membrane lipids, proteins and DNA ensues. The role of iron in brain oxidative injury, has been extensively considered (Mattson et al, 2002). Dexter et al. (Dexter et al, 1991) reported a 31-35% increase in the total iron content in parkinsonian SN compared to control tissue, which was associated to decreased levels of the iron storage protein ferritin, contrasting with a significant decrease of iron binding protein levels in the CSF. A shift of iron II/iron III ratio in the SN from almost 2:1 in the normal brain to 1:2 in the parkinsonian brain (Li and Dryhurst, 1997). Hence, a distinct possibility exists that excessive free radical generation occurs in this region, leading to the death of nigral neurons. In addition, substantia nigra is a dopamine-rich brain area, and catechols, including DOPA and dopamine, have been demonstrated to be cytotoxic *in vitro*, presumably by formation of covalent bonds between their quinone forms and macromolecules of vital importance, primarily represented by thiol groups (Spencer et al, 2002; Zhang et al, 2016). In fact, an intermediate in the autoxidation of catechols to quinone is the free radical semiquinone. Both autoxidation steps generate reduced forms of molecular oxygen, such as superoxide anion and hydrogen peroxide, which in addition to hydrogen peroxide produced by the MAO-dependent catabolism of dopamine, contribute to maintain considerably levels of the highly reactive hydroxyl radical which, reacting with free thiol groups, may contribute to the decreased levels of GSH and correspondent increase in GSSG found in the SN (Calabrese et al, 2002; Calabrese et al, 2004). This is of special importance considering that nigral cells also contain neuromelanin, a pigmented substance related to lipofuscin and derived from dopamine. Neuromelanin has been demonstrated to have high affinity for iron III, and this iron-melanin interaction might have pathogenetic implications. In fact, the synthesis of neuromelanins from dopamine is known to produce more oxidative damage than the synthesis from other catecholamines (Spencer et al, 1994; Goldstein et al, 2016) and, in addition, neuromelanins polymerize from pheomelanin in a process that requires cysteine for synthesis, thus competing with γ-glutamyl cysteine synthetase which utilizes cysteine for GSH synthesis. Under these circumstances the GSH system in the SN could result in a position of increased demand and decreased synthetic capability, hence contributing to the high vulnerability of this region to peroxidative injury (Calabrese et al, 2001; Calabrese et al, 2002; Calabrese et al, 2004). This is confirmed by the study of Perry et al, which showed that GSH levels in the SN were significantly lower than other brain regions (Perry et al, 1982). Moreover, a 40% decrease has been also reported in GSH in the SN of PD, associated to significant increase in oxidized glutathione (Sian et al, 1994; Goldstein et al, 2015). Recently, it has been demonstrated in PD patients that the proportion of dopaminergic neurons with immunoreactive NF-kB (nuclear factor -kB) in their nuclei was more than 70-fold than in control subjects (Hunot et al, 1997). A possible relationship between the nuclear localization of NFkB in mesencephalic neurons of PD patients and oxidative stress in such neurons has been shown *in vitro* with primary cultures of rat mesencephalon, where translocation of NF-kB is preceded by a transient production of free radicals during apoptosis induced by activation of the sphingomyelin-dependent signalling pathway with C2-ceramide (France-Lanord et al, 1997). The data suggest that this oxidant-mediated apoptogenic transduction pathway may play a role in the mechanism of neuronal death in PD (Moore et al, 2003; Dawson and Dawson, 2002; Mc Naught et al, 2001; Schapira et al, 1990). Moreover, a potential role for exocytotoxic processes in PD has been strengthened by the observations that there appears to be a mitochondrial encoded defect in complex I activity of the electron transport chain (Schapira et al, 1990). An impairment of oxidative phosphorylation will enhance vulnerability to excitotoxicity (Xin et al, 2000; Goldstein et al, 2016). Substantia nigra neurons possess N-methyl-D-aspartate receptors and there are glutamatergic inputs into the substantia nigra from both the cerebral cortex and the subthalamic nucleus. After activation of excitatory amino acid receptors, it has been suggested that there is an influx of calcium followed

by activation of neuronal nitric oxide (NO) synthase, which can then lead to the generation of peroxynitrite (Bechtold and Brown, 2003). Consistent with such a mechanism, studies of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in both mice and primates have shown that inhibition of neuronal NO synthase exerts neuroprotective effects, raising the prospect that excitatory amino acid antagonists for neuronal NO synthase inhibitors might be useful in the treatment of PD (Dawson and Dawson, 2002; Dawson and Dawson, 1995).

Mitochondria and Parkinson's disease

Mitochondrial involvement in PD has long been established with the identification of complex I deficiency in the *substantia nigra* (SN) (Calabrese et al, 2007b). In respect to this, it has been proposed that PD patients may have inherited higher proportions of mutant mitochondria leading to specific ETC defect and increased pathogenic susceptibility. The presence of complex I deficiency in PD raises the possibility to determine whether this is caused by an mtDNA defect. Platelets are derived from bone marrow megakaryocytes and thus have respiratory chain complexes with both nuclear and mtDNA encoded subunits. Circulating platelets can be used to generate cells without mtDNA (by exposure, as mentioned above, over several passages to ethidium bromide). The cells can survive in a medium supplemented with pyruvate and uridine. These cells can serve as recipient for a donor of mtDNA, for instance enucleate cells or platelets containing mitochondria but not nuclei, thus replicating in a cellular system (called a *cybrid*) mtDNA and mtDNA encoded ETC subunits from the donor and nuclear DNA encoded subunits from the host cells. If a respiratory defect is present in the donor mitochondria then it will be perpetuated in the cybrids following fusion, thereby providing evidence for a direct mitochondrial causality. Studies performed applying this model to investigate the origin of complex I deficiency in PD platelets have demonstrated a significant complex I deficiency in the mixed cybrids from PD patients compared to cybrid controls. These studies have also indicated that in PD a mtDNA defect caused the mitochondrial deficiency and, in addition, that mtDNA defect was most likely heteroplasmic and associated with parallel changes in mitochondrial membrane potential (Calabrese et al, 2007b).

Furthermore, a large body of evidence supports the role of free radicals in the pathogenesis of PD (Mancuso et al, 2007). The role of iron in brain oxidative injury has been extensively considered (Zecca et al, 2004; Sun YM et al. 2016). Dexter et al. (Dexter et al, 1991) reported a 31-35% increase in the total iron content in parkinsonian SN compared to control tissue, which was associated with decreased levels of the iron storage protein ferritin, contrasting with a significant decrease of iron binding protein levels in the CSF, a shift of iron II/iron III ratio in the SN from almost 2:1 in the normal brain to 1:2 in the parkinsonian brain (Li and Dryhurst, 1997). Hence, a distinct possibility exists that excessive free radical generation occurs in this region, leading to the death of nigral neurons. In addition, substantia nigra is a dopamine-rich brain area, and catechols, including DOPA and dopamine, have been demonstrated to be cytotoxic *in vitro*, presumably by formation of covalent bonds between their quinone forms and thiol groups on important macromolecules. In fact, an intermediate in the autoxidation of catechols to quinone is the free radical semiquinone. Both autoxidation steps generate reduced forms of molecular oxygen, such as superoxide anion and hydrogen peroxide, which in addition to hydrogen peroxide produced by the MAO-dependent catabolism of dopamine, contribute to the production of increased levels of the highly reactive hydroxyl radical, which reacting with free thiol groups may contribute to the decreased levels of GSH and corresponding increase in GSSG found in the SN. This is of special importance considering that nigral cells also contain neuromelanin, a pigmented substance related to lipofuscin and derived from dopamine. Neuromelanin has been demonstrated to have high affinity for iron III, and this iron-melanin interaction might have pathogenic implications. In fact, the synthesis of neuromelanins from dopamine is known to produce more oxidative damage than the synthesis from other catecholamines (Zucca et al, 2015) and, in addition, neuromelanins polymerize from pheomelanin in a process that requires cysteine

for its synthesis, thus competing with γ-glutamyl cysteine synthetase which utilizes cysteine for GSH synthesis. Under these circumstances, the GSH system in the SN could find itself in a position of increased demand and decreased synthetic capability, and hence contribute to the high vulnerability of this region to peroxidative injury. This is supported by the study of Perry et al, which showed that GSH levels in the SN were significantly lower than in other brain regions (Perry et al, 1982). Moreover, a 40% decrease in GSH content in the SN of PD has been also reported to be associated with a significant increase in oxidized glutathione (Mischley et al, 2013). Recently, it has been demonstrated in PD patients that the proportion of dopaminergic neurons with immunoreactive NF-kB in their nuclei was more than 70-fold than in control subjects (Hunot et al, 1997). A possible relationship between the nuclear localization of NF-kB in mesencephalic neurons of PD patients and oxidative stress in such neurons has been shown *in vitro* with primary cultures of rat mesencephalon, where translocation of NF-kB is preceded by a transient production of free radicals during apoptosis induced by activation of the sphingomyelin-dependent signalling pathway with C2-ceramide (Hunot et al, 1997). These data suggest that this oxidant-mediated apoptogenic transduction pathway may play a role in the mechanism of neuronal death in PD (Hunot et al, 1997). PD can also be produced experimentally after exposure to the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) which causes a parkinsonian syndrome in humans and non-human primates after concentration of the active metabolite of MPTP (MPP^+) into dopamine neurons. Mitochondrial sequestration of MPP^+ occurs via electrochemical gradient where it then selectively inhibits complex I. This inhibition leads in turn to loss of mitochondrial membrane potential and ATP depletion. Inhibition of complex I by MPP+ is associated also with diversion of electrons through complex II, which is known to produce 7 times more free radicals than the normal flux through complex I (Meyer et al, 2016). In addition, MPP^+ neurotoxicity in isolated cells is dependent on p53 and nitric oxide synthase (NOS) which causes significant rises in the formation of NO-derived oxidants peroxynitrite and hydroxyl radical (Hare et al, 2013). However, further studies are warranted to elucidate the intracellular cascade of events leading to cell death in disorders showing slow progression over many years, such as PD.

ALZHEIMER'S DISEASE

Alzheimer's disease is also a progressive neurodegenerative disorder leading to cognitive decline, neuropsychiatric symptoms, disability, caregiver burden, and premature death. It represents the most prevalent cause of dementia and its incidence rates exponentially increase with increasing age (Ziegler-Graham et al, 2008). One in nine people aged 65 and over has Alzheimer's disease and by mid-century, someone in the United States will develop the disease every 33 seconds. These numbers will escalate rapidly in coming years, as the baby boom generation has begun to reach age 65 and beyond, the age range of greatest risk of Alzheimer's. By 2050, the number of people aged 65 and older with Alzheimer's disease may nearly triple, from 5.2 million to a projected 13.8 million, barring the development of medical breakthroughs to prevent or cure the disease. Previous estimates based on high range projections of population growth provided by the U.S. Census suggest that this number may be as high as 16 million. The disease is predicted to affect 1 in 85 people globally worldwide by 2050 (Brookmeyer et al, 2007). The neuropathological hallmarks of AD, in mouse models and postmortem patient brains, are diffuse amyloid plaques – which are frequently surrounded by dystrophic neurites - and intracellular neurofibrillary tangles, respectively constituted by amyloid β (Aβ) and hyperphosphorylated microtubule-associated protein tau (Lalla et al, 2013). In addition, dominant mutations were found in the Amyloid Precursor Protein (APP) gene and in the presenilin 1 and 2 genes (PSEN1 and PSEN2), which encode for components of gamma-secretase, leading to EOAD (Giri et al, 2016). APP is cleaved by beta-secretase and gamma-secretase sequentially to generate Abeta 1-40 and Abeta 1-42 amyloid peptides, respectively, which accumulate forming the amyloid plaques

(Mendiola-Precoma et al, 2016; Huang et al. 2016). Research since the discoveries of amyloid β (Aβ) and tau has provided detailed information on molecular pathogenetic events, yet little is known about the cause of Alzheimer's disease and no cure is available (Scheltens et al, 2016). Emerging evidence suggests that inflammation has a pivotal role in the pathogenesis of various neurological disorders, and understanding of interactions between the immune system and the nervous system might be key to the prevention or delay of most late-onset CNS diseases. Neuroinflammation, a specialized immune response of the nervous system, has been related to the onset of some chronic degenerative diseases of the central nervous system (CNS) characterized by a progressive neuronal death in specific regions of the CNS. This neuronal loss seems to be the cause of motor and cognitive deficits that characterize neurodegenerative diseases. Brain inflammation has been linked to many of these diseases, including amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), PD and, particularly, AD (Calabrese et al, 2010; Trovato et al, 2016). An increasing number of studies have proposed a strong correlation between reactive oxygen species (ROS)-induced oxidative stress dysfunction of protein metabolism and the pathogenesis of AD. Indeed, the brain has a large potential oxidative capacity but a limited ability to counteract oxidative stress (Cobb et al, 2015; Tramutola et al, 2016, Chiurchiù et al, 2016). One possible vehicle for deposition and accumulation of Aβ in AD is oxidative stress, mediated by the production of reactive oxygen species. In biological systems, ROS are a category of important free radicals, such as superoxide and hydroxyl radicals produced as a by-product of oxidative phosphorylation in the mitochondria's electron transport chain, with smaller amounts originating from cellular membrane, endoplasmic reticulum and peroxisomes (Federico et al, 2012; Murphy, 2009, Santos et al, 2009). ROS are physiologically present at minimal concentration within the cell, and in normal conditions there is a steady-state balance between pro-oxidants and antioxidants, which is necessary to ensure optimal efficiency of antioxidant defense. The body can be exposed to ROS directly from exogenous and endogenous sources. The first include drugs and toxic chemicals that change the balance of oxidants/antioxidants in favor of the oxidation (Valavanidis et al, 2009; Yamamori et al, 2012); the latter includes overproduction of reactive oxygen intermediates by the mitochondrial electron transport chain. ROS can also act as necessary signaling molecules (Dickinson et al, 2011, Finkel et al, 2011; Brieger et al, 2012). However, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative stress ensues with consequential severe damage to cellular components, such as DNA, proteins and lipids, and plays a pivotal role in leading an irreversible cellular damage (Tramutola et al, 2016a; Calabrese et al, 2012). In the process of aging and neurodegenerative diseases, the decline of normal antioxidant defense mechanisms increases the brain's vulnerability to oxidative stress. ROS have the ability to modify crucial molecules in the cell, including proteins shown to be involved in neurodegenerative diseases. Misregulation of ROS, due to either mitochondrial dysfunction, age, or both, has been implicated in numerous neurodegenerative diseases (Li Zuo et al, 2015). AD brain has been reported to be under oxidative stress that may play an important role in the pathogenesis and progression of AD (Swomley et al, 2015; Chakrabarti, 2013). Several lines of evidence support a fundamental role for oxidative stress-mediated event in the pathogenesis of the disease. Amyloid-β-peptide (1-42) has been shown to induce protein oxidation in both *in vitro* and *in vivo* studies (Tramutola, 2016; Levault, 2015; Di Domenico, 2016). The production of ROS and its involvement in AD pathogenesis are also supported by the high level of lipid peroxidation found in the brain of AD patients, as well as by the increased levels of HNE found postmortem in their cerebrospinal fluid (CSF) (Di Bona et al, 2010). In addition, β-amyloid-induced damage promotes the generation of ROS, causing cell death and neurodegeneration, and induces also glial activation, thus causing local inflammation. Furthermore, oxidative stress promotes apoptosis of neurons of the brain and gene duplication without cell division, leading to aneuploidy and DNA damage (Querfurth et al, 2010). Oxidative stress can damage DNA, also causing breakage of the filaments and large deletions, and can affect different enzymatic and

mitogenic pathways (Von Bernhardi et al, 2012; Butterfield et al, 2006). Furthermore, brain mitochondrial-derived oxidative stress occurs at the early stage of AD, contributing to AD pathogenesis. With the progression of the disease, amyloid (Aβ) enters mitochondria promoting superoxide formation and consequently inducing oxidative stress, which then triggers redox-mediated changes in cell signaling, such as c-Jun NH2-terminal kinase (JNK)–Mitogen-activated protein kinases (MAPKs) pathway, associated to activation of nuclear factor-kB (NF-kB) and release of pro-inflammatory IL and cytokines (Peng et al, 2016). Currently, treatment of Alzheimer's disease's patients involves drugs that are only able to reduce symptoms or delay the progression of the disease, such as cholinesterase inhibitors and drugs that target the glutamatergic system. It also known that unhealthy dietary structure and nutritional status affect the progression of AD. Therefore, a nutritional intervention should be considered as an important part of a therapeutic approach aimed at decreasing the complications of AD as well as slowing down AD progression. Extra virgin olive oil, an integral ingredient of the "Mediterranean diet," has been shown to exert benefits against the risk of mild cognitive impairment. Consistent with this notion, different antioxidant strategies are being studied and are intended not only to reduce the deleterious ROS activity, but also to promote the regenerative capacity of the adult brain (Lee et al, 2010). Oxidative stress has been extensively studied in neurologic diseases including AD, PD, MS, ALS and dementia (Andersen, 2004). This is not surprising since neuronal cells are especially susceptible to oxidative stress and subsequent cellular damage (including cell death). In a disease such as Alzheimer's, oxidative stress/ oxidative damage is felt to play a key role in the loss of neurons and the progression to dementia. Recent studies have shown increased glutathionylation of specific proteins in AD patients compared with control subjects (Newman et al, 2007). The exact function of this reversible oxidative modification is unknown. Further studies investigating the specific *in vivo* effects of S-glutathionylation in oxidative stress are important to determining the role of S-glutathionylation in the AD brain and neurodegenerative disorders.

GSH levels in human tissues normally range from 0.1 to 10 millimolar, most concentrated in the liver (up to 10 mM) while plasma concentration is in the micromolar range. Oxidative stressors that can deplete GSH include ultraviolet and other radiations, viral infections, environmental toxins, heavy metals, inflammation and dietary deficiencies of GSH precursors and enzyme cofactors. The sulfhydryl (-SH) group on the cysteinyl portion confers a strong electron-donating character. As electrons are lost the molecule becomes oxidized, and two such molecules become dimerized by a disulfide bridge to form glutathione disulfide or oxidized glutathione (GSSG). This linkage is reversible upon re-reduction. GSH is under tight homeostatic control both intracellularly and extracellularly. A dynamic balance is maintained between GSH synthesis, its recycling from GSSG, and its utilization. GSH synthesis involves two closely linked, enzymatically controlled reactions that utilize ATP. First, cysteine and glutamate are combined, by gamma-glutamyl cysteinyl synthetase. Second, GSH synthetase combines gamma-glutamylcysteine with glycine to generate GSH. As GSH levels rise, they self-limit further GSH synthesis; otherwise, cysteine availability is usually rate-limiting. GSH recycling is catalyzed by glutathione disulfide reductase, which uses reducing equivalents from NADPH to reconvert GSSG to 2GSH. The reducing power of ascorbate helps to conserve systemic GSH. GSH is used as a cofactor by multiple peroxidase enzymes, to detoxify peroxides generated from oxygen radical attack on biological molecules, to reduce oxidized centers on DNA, proteins, and other biomolecules, and glutathione S-transferases (GST) to conjugate GSH with endogenous substances and to exogenous electrophiles and diverse xenobiotics. Direct attack by free radical and other oxidative agents can also deplete GSH, despite the homeostatic glutathione redox cycle attempts to keep GSH restored (Anathy et al, 2012). The liver is the largest GSH reservoir. GSH equivalents circulate in the blood predominantly as cystine, the oxidized and more stable form of cysteine. Cells import cystine from the blood, reconvert it to cysteine and from it synthesize GSH. Conversely, inside the cell GSH helps to re-reduce oxidized forms of other antioxidants such as ascorbate and alpha-tocopherol

(Meister, 1994). GSH is an extremely important cell protectant by directly quenching reactive free radicals and GSH/GSSG balance is crucial to maintain cellular homeostasis and survival. GSH is the essential cofactor for many enzymes which require thiol-reducing equivalents, and helps to keep redox-sensitive active sites on enzymes in the necessary reduced state. Other thiol cell systems - the metallothioneins, thioredoxins, and other redox regulator proteins - are ultimately regulated by GSH levels and the GSH/GSSG redox ratio. Glutathione status is a highly sensitive indicator of cell functionality and viability. In humans, GSH depletion is linked to a number of disease states: as intracellular GSH becomes reduced, the cell functionality is progressively reduced (Fisher-Wellman et al, 2013).

GSH is present in the brain in millimolar concentrations (Dringen, 2000). Although there are some reports favoring the transport of GSH across the blood brain barrier and their uptake into brain cells, it still needs to be established whether these play a role in GSH homeostasis in the brain (Peterson et al, 2013). Nevertheless, the constituent amino acids of GSH may cross the blood brain barrier and be utilized for GSH synthesis in the brain following the same pathway as described above. Several reports employing different techniques have shown the presence of GSH both in neurons and glial cells (Maybodi et al, 1999), but the concentration of GSH appears to be higher in brain astrocytes compared to neurons. Apart from the antioxidant functions of GSH in the brain, extracellular GSH has been hypothesized to have additional functions as a neurotransmitter (Janaky et al, 2008), neurohormone, in the detoxification of glutamate and in leukotriene metabolism (Dringen, 2000). It has been observed that there is an age-dependent depletion in intracellular GSH in the cerebrospinal fluid during aging in humans (Samuelsson et al, 2011). Studies from others have shown that aged mice have a 30% decrease in levels of GSH compared with younger animals. Since the brain requires extensive ROS detoxification, it is evident that a decrease in GSH content could increase oxidative damage, making the brain more susceptible to neurological disorders (Bharath et al, 2005). Decreased GSH availability in the brain is believed to promote mitochondrial damage by likely increasing the levels of oxidative stress in this organelle. Depletion of brain GSH has been shown to result in decreases in mitochondrial enzyme activities in as well as losses in ATP production in the aging murine brain. Based on these notions, one of the ways to counteract oxidative stress-mediated disruption of GSH homeostasis - a central feature of neurodegeneration - is to replenish the GSH pool either by increasing the synthesis of the tripeptide or by slowing its degradation. There are several studies where administration of precursors of GSH metabolism, such as γ-glutamyl cysteine, have been used to increase the levels of GSH in rat brains (Schulz et al, 2000). Similarly, precursors of cysteine synthesis have been administered in various animal models to accelerate cysteine production in the brain thus increasing GSH levels. GSH replacement can also be achieved by administering thiol reagents such as GSH itself or GSH analogs. GSH cannot easily penetrate the blood brain barrier due to the presence of the cysteine SH group and is not efficiently absorbed into neuronal cells in the brain (Kannan et al, 1999). Hence, rather than GSH, modified forms of GSH such as GSH analogs have generally been used *in vivo*. Recently, Butterfield et al. demonstrated that the xanthate D609 – acting as a GSH mimetic – protected against amyloid beta (1-42)-induced oxidative damage in primary neurons and isolated synaptosomes (Perluigi et al, 2006-Sultana et al, 2006). Further studies are in progress to test the use of GSH or related molecules in GSH metabolism as therapeutics in the treatment of neurodegenerative diseases to elicit an increase in brain GSH levels.

HORMESIS AND CELLULAR STRESS RESPONSE

The brain is a complex network consisting of a massive number of interacting components, whose functional organization ultimately derives from integration of plasticity systems and environmental factors. Within this context, where more than 86 billion neurons, 1 quadrillion synaptic connections, and 1 trillion bit/second equivalent plasticity processes take place, hormesis defines thresholds of adaptive responses for a given as potentially harmful stimulus

which at low dose has the capacity of promoting beneficial changes associated to adaptive plasticity mechanisms (Calabrese et al, 2010; Du et al, 2016). Signaling by reactive oxygen species, produced during aerobic metabolism, follow the mechanistic determinism of hormetic laws, whereby at low levels free radical generation regulate a wide array of physiological responses, whereas when exceeding generation occurs, such as in abnormal cell metabolism conditions, cell function and viability are under threat (Calabrese et al, 2011). Thus, an increase in ROS formation with the associated disruption of redox homeostasis results in the alteration of biologically relevant mechanisms involved in neuronal plasticity (Calabrese et al, 2012; Dattilo et al, 2015). As a basic principle of a hormetic phenomenon, restoration of disrupted homeostasis can induce adaptive conditions associated with higher level of stress tolerance than the prior stage. Due to the ability of cells to respond, counteracting both external and internal sources of stress, cellular hormesis defines a general phenomenon whereby a mild stress-induced stimulation results in biologically beneficial effects, whereas cell death represents a final process where failure in adaptation or unsuccessful adaptation occur (Calabrese et al, 2015a; Calabrese et al, 2015b).

The dose-response concept is often ignored in the assessment of disease processes, inclusive of neurovascular disorders. However, dose-response dynamics and effects are inherently involved with normal physiological processes in development and aging, and enable cellular adaption(s) and function in response to various types and levels of stress (Segev-Amzaleg et al, 2013). Of particular note for this review is that analysis of many of these processes reveals a hormetic, biphasic dose-response pattern. Hormesis defines a biphasic dose or concentration response relationship that may be induced via the actions of endogenous agonists or in response to numerous types of stress agents or conditions such as thermal change, hypoxia, traumatic injury, psychological challenges as well as stress induced by exercise, specific dietary regimes and other lifestyle activities **(**Calabrese, 2007a; Calabrese, 2008a; Calabrese and Baldwin, 2002). The concept of hormesis has emerged as a significant dose-response model in toxicology and pharmacology (Calabrese et al, 2010; Calabrese et al, 2007b; Calabrese et al, 2008b, 2008c). The enhanced recognition of hormesis has occurred principally because traditional dose-response models, such as the threshold model, have not been able to account for the occurrence of non-random biological activity below well-established thresholds of response (Calabrese et al, 2013; Calabrese et al, 2016a; Calabrese et al, 2016b). These biphasic dose-response relationships, referred to here as hormesis from the Greek meaning to excite, display a low-dose stimulation with very specific quantitative features for the amplitude and width of the enhanced response and a high-dose inhibition (Calabrese and Blain, 2011). Despite the current interest in hormesis by the toxicology community, quantitatively similar hormetic-U-shaped dose responses have long been recognized by researchers to be involved with factors affecting memory, learning, and performance, as well as nutritional antioxidants and oxidative stress-mediated neurodegenerative responses in cellular models for various diseases such as Alzeheimer's and Parkinson's (Calabrese, 2008a). Several lines of *in vitro* evidence have implicated ROS in the pathogenesis of neuronal death (Calabrese et al, 2010) and different markers of oxidative stress are found in postmortem examination of brains from patients with many neurodegenerative disorders. DNA oxidation, protein oxidation, and lipid peroxidation have been reported in brain regions containing neurofibrillary tangles and senile plaques from Alzheimer's disease patients (Calabrese et al, 2009). However, also dopaminergic neurons in the substantia nigra of brains of patients suffering from Parkinson's disease also exhibit hallmarks of oxidative stress (Calabrese et al, 2010). The oxidative stress hypothesis for neurodegeneration postulates that cumulative oxidative damage over time explain the late life onset and the slow progressive nature of neurodegenerative disorders (Calabrese et al, 2007a). The CNS is especially vulnerable to free radical damage as a result of its high oxygen consumption rate, its abundant lipid content, and the low levels of antioxidant enzymes compared with other tissues (Calabrese et al, 2009; Calabrese et al, 2011; Calabrese et al, 2012). These age-related effects, associated with genetic and environmental risk factors, may provide

an emerging mechanism for the high incidence of neurodegenerative disorders in the elderly.

Consistent with this evidence, particular attention has been paid to study the neuroprotective properties of endogenous agonists such as beta-amyloid, antioxidants, iron chelating and anti-inflammatory agents with particular regard to polyphenols (Puzzo et al, 2012). According to the principles of hormesis, appropriate endogenous agonists such a beta amyloid, as well as stressful agents, including drugs, toxins and natural substances, when given at low doses may elicit a positive response. This protection may occur as part of normal metabolism as in the case of endogenous agents or via the induction of an adaptation to or protection from the stressor, whereas at higher concentration the toxic effect prevails (Calabrese et al, 2015; Calabrese et al, 2016c).

HEME OXYGENASE SYSTEM AND NEURODEGENERATIVE DISORDERS

The role of ROS and RNS in the pathogenesis of neurodegenerative disorders has been clearly demonstrated (Islam et al, 2016; Bhat et al, 2015, Jomova et al, 2010; Uttara et al, 2009). With regard to the contribution of HO-1 in neurodegeneration, there is no consensus in literature. In fact, it is not to be questioned that HO-1 is neuroprotective, but there is evidence of a detrimental effect of this enzyme in neural tissues, probably due to the possible toxic effects of CO and iron (Motterlini and Otterbein, 2010). Due to its strong antioxidant properties and wide distribution within the CNS, HO-1 has been proposed as a key enzyme in the prevention of brain damage (Calabrese et al, 2009). In transgenic mice over-expressing HO-1, the neuroprotective effect of this enzyme in a model of ischemic brain damage has been demonstrated in neurons and attributed the HO-1 beneficial effects, due to an increase of pro-survival molecules such as cGMP, bcl-2, bilirubin-biliverdin redox cycle and the iron-sequestering protein, as well as to a reduction of pro-apoptotic p53 (Beschorner et al, 2000; Ren et al, 2011; Alfieri et al, 2013). Up regulation of HO-1 in the *substantia nigra* of PD patients has been demonstrated. In these patients, nigral neurons containing cytoplasmic Lewy bodies exhibited in their proximity maximum HO-1 immunoreactivity (Yu et al, 2013). As with AD (Schipper, 2000), upregulation of HO-1 in the nigral dopaminergic neurons by oxidative stress was shown (Shipper et al, 1998). Hemin, an inducer of HO-1, effectively inhibited experimental autoimmune encephalomyelitis (EAE, an animal model of the human disease MS) (Yingru et al, 2001). In contrast, tin-mesoporphyrin-IX, an inhibitor of HO activity, markedly exacerbated EAE (Yingru et al, 2001). These results suggest that endogenous HO-1 plays an important protective role in EAE and MS. Particularly interesting is the role played by HO-1 in AD, a neurodegenerative disorder which involves a chronic inflammatory response associated with both oxidative brain injury and beta-amyloid-associated pathology. Significant increases in the levels of HO-1 have been observed in AD brains in association with neurofibrillary tangles, and also HO-1 mRNA was found increased in AD neocortex and cerebral vessels (Smith et al, 1994). HO-1 increase was not only in association with neurofibrillary tangles, but also co-localized with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains (Schipper et al, 2006). In addition, Takeda et al. explored the relationship between HO-1 and *tau* protein, this latter being the major component of intraneuronal neurofibrillary tangles in AD. In transfected neuroblastoma cells overexpressing HO-1, the activity of this enzyme was increased, and conversely, the level of *tau* protein was significantly decreased when compared with antisense HO-1 or vector transfected cells (Takeda et al, 2000). The suppression of *tau* protein expression was almost completely counteracted by zinc-deuteroporphyrin, a specific inhibitor of HO activity (Takeda et al, 2000). The activated forms of extracellular signal-regulated kinases (ERKs) were also decreased in cells overexpressing HO-1 although no changes in the expression of total ERKs were observed (Takeda et al, 2000). Taken together, all these findings do not allow to single out a product of HO activity as the main neuroprotective factor; rather a complex puzzle of regulatory interactions between heme degradation products and cellular pathways involved in cell death/survival is hypothesized.

In human trials, higher adherence to the Mediterranean diet was associated with the reduced cognitive decline and the risk of AD (Safouris et al, 2015). The beneficial effects of extra virgin olive oil in preventing and delaying the onset of AD and declining the severity of its symptoms had been found in transgenic AD mice (Psaltopoulou et al, 2013). HT administrated to 3-month-old female transgenic APP/PS1 mice at 5 mg/kg/day for 6 months resulted in electroencephalography (EEG) activity and cognitive function improvement, reduction of mitochondrial oxidative stress and neuroinflammation (Peng et al, 2016). In the same study HT not only successfully rescued altered mitochondrial complex IV expression, but also normalized mitochondrial complex I-IV activities compromised in this transgenic AD brain. APP/PS1 mice showed higher levels of protein carbonyls and 4-HNE, markers of peroxidative damage to proteins and lipids, associated to lower expression of SOD2 than in control animals, which were normalized by HT administration. Additionally, GSH depletion and HO-1 and NQO-1 up-regulation observed in AD mice in response to Aβ-induced oxidative stress, were reversed by HT. Remarkably, APP/PS1 mice showed NFkB/MAPK-dependent activation of NLRP3 inflammasome with increased IL-6 and COX-2 expression, which were significantly decreased by HT administration (Peng et al, 2016).

Moreover, in the general metabolism of dopamine by monoamine oxidase (MAO), oxidative-deamination of dopamine generates DOPAL (3,4-dihydroxyphenylacetaldehyde), which is further oxidized to the carboxylic acid (3,4-dihydroxyphenylacetic acid, DOPAC) by aldehyde dehydrogenase. Although DOPAC is the major metabolite of dopamine in brain, a small portion of DOPAL is reduced to DOPET by aldehyde or aldose reductase (Rodríguez-Morató et al, 2015). DOPAL is a highly reactive neurotoxic metabolite, suggested to operate *in vivo* in the pathogenesis of PD (Goldstein et al, 2015). In AD, disruption of

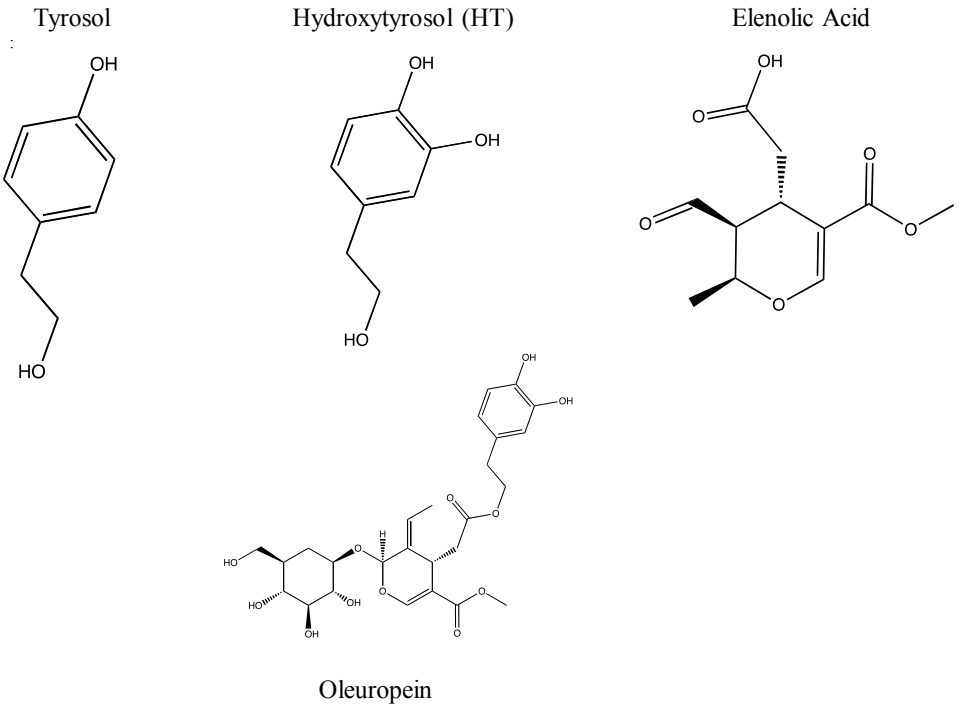

Fig. 1. *Chemical structures of inducers of the Keap1/Nrf2/ARE pathway.*

dopamine metabolism is associated with increasing oxidative stress conditions, in which highly reactive aldehydes, such as 4-hydroxynonenal and malondialdehyde, are formed and which can inhibit aldehyde dehydrogenase 2 (ALDH2). Consistent with this notion, as the soluble forms of oligomeric amyloid beta are involved in the loss of synaptic plasticity and memory, especially in early phases of AD, interestingly, stimulation of dopamine D1/D5 receptors (D1R/D5R) increases surface expression of synaptic α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate subtype glutamate and N-methyl-D-aspartate subtype glutamate receptors, thus facilitating the induction of the late phase of long-term potentiation (LTP), probably via a related mechanism (Xiang et al, 2016). In addition, aberrant promoter methylation of multiple genes has been associated with increased AD risk in males. (Ji et al, 2016). Given these findings, viable DOPET can be of value to reduce levels of DOPAL in AD patients. We posit that this putative link between oxidative stress and the generation of endogenous will be important to further research focusing on mechanisms involved in neurotoxicity and neurodegeneration which are particularly relevant in the study of AD, as well as to PD and more in general in neuropsychiatric spectrum disorders.

OLIVE POLYPHENOLS AND HYDROXYTYROSOL AS POTENTIAL PHARMACOLOGICAL MODULATORS OF NEUROINFLAMMATION AND ACTIVATORS OF THE KEAP1/NFR2/ARE PATHWAY: A RATIONALE FOR TREATING NEURODEGENERATIVE DISORDERS

Recent epidemiological studies support the efficacy of the Mediterranean diet, not only against cardiovascular and cancer diseases but also against the cognitive decline associated with ageing (Scarmeas et al, 2006; Feart et al, 2009; Valls-Pedret et al, 2012; Martinez-Lapiscina et al, 2013). In this respect, several studies highlight the role played by phenolic components of olives and olive oil in counteracting both neuroinflammation, neurotoxicity and amyloid aggregation. We herein discuss the olive polyphenol modulatory effects on the cellular pro-inflammation NF-kb pathway, their involvement in the activation of the Nfr2/ Phase II genes and ability to interfere with the amyloid aggregation in the context of their potential benefits in the onset and progression of PD and AD. Particular attention is paid to the effects of hydroxytyrosol, the most biologically active and "intriguing" compound of the olive polyphenols' family, proven to have an ideal pharmacological profile, i.e. a combination of antioxidant and anti-inflammatory activity with an excellent safety profile, high bioavailability, tissue distribution and multiple mechanism.

Hydroxytyrosol (HT) was originally found in small amounts in olive oil, at concentrations less than 1 mg/kg (Montedoro et al, 1992; Petroni et al, 1994). It is a product of the hydrolysis of its natural olive ester precursors, oleuropein (OLE), verbascoside and lingstroside, and is formed during the maturation of olives, storage and preparation of table olives. Its large scale extraction from olive oil for use in clinical applications was shown to be both expensive and unmanageable. More conveniently, large quantities of HT were produced by harvesting it from an olive oil production by-product, the olive vegetation water (OVW or olive juice), which represents 50% of the weight of the olive fruit and normally discarded as wastewater. The process developed by CreAgri is both solvent-free and environmentally friendly, employs citric acid (1%) for the mild acidic hydrolysis of the naturally occurring hydroxytyrosol esters and produces HT concentration ranging from 20 g/kg to 50 g/ kg. olive dry matter (Crea, 2002). Hydroxytyrosol has a relatively simple molecular structure (Fig. 1), amazingly related to the structure of Dopamine (DA) and also known as DOPET. It is a derivative of tyrosol, a phenethyl alcohol (also referred to as (p-hydroxyphenyl) ethanol, or *p*-HPEA). In hydroxytyrosol, the central benzene ring is functionalized with an additional hydroxyl group *ortho* to the ring hydroxyl group present in tyrosol. Hydroxytyrosol is also referred to as 2-(3,4-dihydroxyphenyl) ethanol or 3,4-DHPEA. The chemical structures of these compounds are reported in Fig. 1.

In addition to the phenolic alcohols hydroxytyrosol and tyrosol, the polyphenols and their precursors found in olive products include also (3,4-dihydroxyphenyl)

ethanol glucoside and 2-(4-hydroxyphenyl)ethyl acetate); the benzoic acid derivatives gallic acid, gentisic acid, benzoic acid, vanillic acid, protocatecuic acid, *p*-hydroxybenzoic acid, and syringic acid; the cinnamic acid derivatives caffeic acid, *p*-coumaric acid, *o*-coumaric acid, ferulic acid, cinnamic acid, and sinapinic acid; other phenolic acids and derivatives including 4-(acetoxyethyl)-1,2-dihydroxybenzene, 3,4-dihydroxyphenylacetic acid ("DOPAC"), and 4-hydroxyphenylacetic acid; the secoiridoids, characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure, including oleuropein, demethyloleuropein, 10-hydroxyoleuropein, oleuropein aglycone, oleuropein aglycone dialdehyde, ligstroside, 10-hydroxyligstroside, ligstroside aglycone, ligstroside aglycone dialdehyde, the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, and the dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol; flavonoids including the flavones apigenin and luteolin, and the flavanonol (+)-taxifolin; the lignans (+)-1-acetoxypinoresinol, (+)-pinoresinol, and (+)-1-hydroxypinoresinol. The molecular structures of these compounds have been confirmed (Carrasco-Pancorbo et al, 2005). Hydroxytyrosol is a compound endowed with multiple biochemical and pharmacological activities including a strong antioxidant and anti-inflammatory power. Initially, HT activity *in vitro* and *in vivo* has been associated with its strong anti-oxidant and radical scavenging activities (Visioli et al, 1998; Rietjens et al, 2007).

The first observation of an *in vivo* anti-inflammatory activity of olive polyphenols was made in 2005 in our laboratory (Bitler et al, 2005) with a joint-inflammation, LPS-treated BALB/c mouse model. In this experiment, we evaluated the anti-inflammatory activity of olive vegetation water (OVW) obtained during olive oil milling, and containing 25 mg HT/g solid, (HT is > 45% of the total polyphenols). HT was administered in powder form (freeze dried OVW) to LSP treated BALB/c mice by oral gavage. Because some simple phenols and polyphenols with antioxidant activity had shown varying anti-inflammatory activities *in vitro*, we tested OVW and HT at two concentrations for their ability to inhibit the production of tumor necrosis factor-alpha (TNF-alpha), a pivotal cytokine in inflammation. OVW at a dose of 125 mg/mouse (500 mg/kg) reduced serum TNF-alpha levels by 95%. In the human monocyte cell line, THP-1, OVW reduced LPS-induced TNF-alpha production by 50% at a concentration of 0.5 g/L (equivalent to approximately 0.03 g/L simple and polyphenols). OVW had no toxic effects *in vitro* or *in vivo*. When OVW was combined with glucosamine, a component of proteoglycans and glycoproteins, it was shown to decrease inducible nitric oxide synthase production in cultured macrophage cells and the two compounds acted synergistically to reduce serum TNF-alpha levels in the LPS-treated mice. Interestingly, purified HT, in comparison, did not produce any reduction of TNF-alpha in any of these experiments. Since 2005, numerous studies on the HT/polyphenols anti-inflammatory activity have been published (Lockyer et al, 2016; Rodriguez-Morato et al, 2015).

Hydroxytorosl has been shown that it can cross the blood brain barrier, although in small concentrations (D'Angelo et al, 2001), has high bioavailability and tissue penetration (Tuck et al, 2001, Mateos R.et al. 2016) and an excellent safety profile (Christian et al, 2004; Soni et al, 2006; CreAgri, GRAS certification, 2004). Its use as a research tool has produced an enormous quantity of publications worldwide to more than 900 papers during the period 2000 to date. Collectively, HT and its derivatives, natural or not, could potentially find a prevention and therapeutic use in cardiovascular, cancer, chronic inflammation and neurodegenerative diseases.

An *in vitro* demonstration that HT can act on brain cell was obtained with microglia and activated astrocytes in a cellular model of Parkinson's (Crea et al, 2012). In this case, cultured brain cells challenged with LPS were exposed to increasing quantities of HT (OVW) to monitor its effect on cytokine release. Microglia and astrocytes are sources of potential cytotoxic compounds that can aggravate neuronal injury during degenerative processes such as in PD. HT was effective at concentration of 25 ng/gr in inhibiting production of TNF, IL-6 and IL-1 in microglia and in astrocytes (at higher concentration of 250ng/gr). Oleuropein (OLE) was much less effective in both cells even at higher concentration. Additional evidence that HT may act as a neuroprotective agent *in vivo*, was

provided by Schaffer (Schaffer et al, 2007 and 2010). These results pointed towards a potential impact by HT on the inflammatory component of neurodegenerative diseases like in PD and AD. Based upon the wide range of observed HT anti-inflammatory activities *in vitro* and *in vivo*, the mechanism of HT was postulated to include, at least in part, the modulation of the NF-kb cellular pathway (Killeen et al, 2013). This hypothesis was confirmed by a cellular experiment conducted by scientists at DSM (Basel) in 2007 (Richard et al, 2011). OVW was used and fractionated into its molecular components to identify and associate the anti-inflammatory activity to individual components of the natural formulation. Various fractions obtained by preparative hplc were tested on macrophages (RAW264.7 cells) stimulated with LPS for their effect on the production of NO and PGE2, cytokines, interleukins and chemokines. HT inhibited expression of NO and PGE2 with an IC50 of 11.4 and 19.5 uM, respectively, reflecting strong anti-inflammatory activity. In addition, secretion of cytokines (Il-1a, Il-1b, Il-6 Il-12 and TNF-alpha) and a variety of chemokines was diminished in a concentration-dependent manner, suggesting that the mechanism by which HT exerts its anti-inflammatory effects is partially mediated via the NF-kb cellular pathway. Curiously, the authors did not exclude the potential synergistic effects of minor components present in the OVW, a natural environment for HT, nor did they completely identify additional molecules with equally strong anti-inflammatory mechanism. Additional studies on endothelial cells (Scoditti et al, 2014) and human monocytic cell line (Zhang et al, 2009) have provided evidence as related to HT ability to down modulate NF-kB activation and its translocation in the nucleus. In keeping up with the above *in vitro* and *in vivo* studies and based upon the high safety profile of the OVW formulation (GRAS certification was obtained by CreAgri in 2004), HT, in the form of freeze dry powder, was orally administered in capsules to 90 humans in a double blinded, placebo-controlled clinical trial with patients affected by osteo and rheumatoid arthritis. The trial was a five-week treatment conducted in Arizona (Bitler et al, 2007). The amount of hydroxytyrosol provided by the 4 capsules/day, administered orally twice daily, was approximately 10 mg/daily or ca. 0.13 mg/kg body weight. The results of the trial confirmed the potent anti-inflammatory activity of HT/olive polyphenols in humans, manifested as reduction of swelling, pain and improved daily motilities with an associated reduction of two inflammation biomarkers, CRP and homocysteine. (Bitler et al, 2007). Hydroxytyrosol, in its natural environment (OVW, Olivenol ™or HIDROX™) was also tested positively on humans in two additional international studies, the first involving 100 healthy volunteers in Malaysia (Visioli et al, 2009) and the second including 50 patients in Japan (Numano et al, unpublished results). The first study was for monitoring HT effect on the total plasma antioxidant activity and the second to monitor HT effect on lipid profile. In the first study, a single dose (1 ml OWV, 2.5 mg HT) was administered orally to monitor the increase of the total antioxidant activity in plasma. Although the dosage was not sufficient to provide any evidence of an increased antioxidant index, there was a significant increase of total (reduced and oxidized) glutathione in the blood. The study conducted in Japan included patients with high cholesterol and confirmed the positive effect of olive polyphenols and HT on lipid profile. Doses as low as 5 mg HT/day produced a dramatic reduction of high MW cholesterol (VDL fraction 3,4 and 5) while HDL cholesterol concentration was unchanged or improved. In addition, the Japanese study provided a serendipitous discovery with some of the patients affected by skin disorders. The same HT dosage of 5-10 mg/daily produced some dramatic improvements in 5 patients with skin disorders (psoriasis purulenta, atopic, allergic and seborrheic dermatitis) that can only be explained by a positive effect of HT on the immune system (Numano et al, 2007). In 2011, EFSA, the European Food Safety Authority, on the strength of clinical trials conducted in Europe (Covas et al, 2006; Marrugat, 2004) approved a health claim that links olive polyphenols, especially hydroxytyrosol, to the protection of LDL from oxidative damage. During the past decade, hydroxytyrosol, formulated as enriched olive oil (FVOO) has been tested on humans in several trials (Valls et al, 2015; Castaner et al, 2012).

Hydroxytyrosol (OWV) was also studied for its effects on mitochondria. The first pivotal study was conducted by Liu and colleagues (Liu et al, 2007) on retinal pigment epithelial cells, ARPE-19, challenged

with acrolein, a compound that mimics smoke-induced oxidative stress. Mitochondria protection by a formulation of hydroxytyrosol produced from the OVW was monitored. The conclusions by the authors was that HT protects from oxidative damage and mitochondria dysfunction by multiple mechanisms and that dietary feeding of HT may be an effective strategy for reducing and/or preventing cigarette smoke-induced or age related macular degeneration. A subsequent publication (Hao et al, 2010) suggested that HT is able to promote mitochondrial function by stimulating mitochondrial biogenesis. HT over the concentration range of 0.1-10 micromol/L stimulated promoter transcriptional activation and protein expression of peroxisome proliferator-activated receptor (PPAR) coactivator 1 alpha (PPARGC1 alpha, the central factor for mitochondrial biogenesis and its downstream targets including nuclear respiration factors 1 and 2 and mitochondrial transcription factor A, which leads to an increase in mitochondrial DNA (mtDNA) and in the number of mitochondria. Consistent with these results, a proposed mechanism on HT protection activity against oxidative stress includes the simultaneous activation of two critically important pathways, i.e., induction of phase II detoxifying enzymes and stimulation of mitochondrial biogenesis. Hydroxytyrosol treatment was shown to simultaneously protect against acrolein-induced inhibition of nuclear factor-E2-related factor 2 (Nrf2) and peroxisome proliferator-activated receptor coactivator 1 alpha (PPARGC1α) in ARPE-19 cells. The activation of Nrf2 led to activation of phase II detoxifying enzymes, including γ-glutamyl-cysteinyl-ligase, NADPH (nicotinamide adenine dinucleotide phosphate)-quinone-oxidoreductase 1, heme-oxygenase-1, superoxide dismutase, peroxiredoxin and thioredoxin, as well as other antioxidant enzymes,

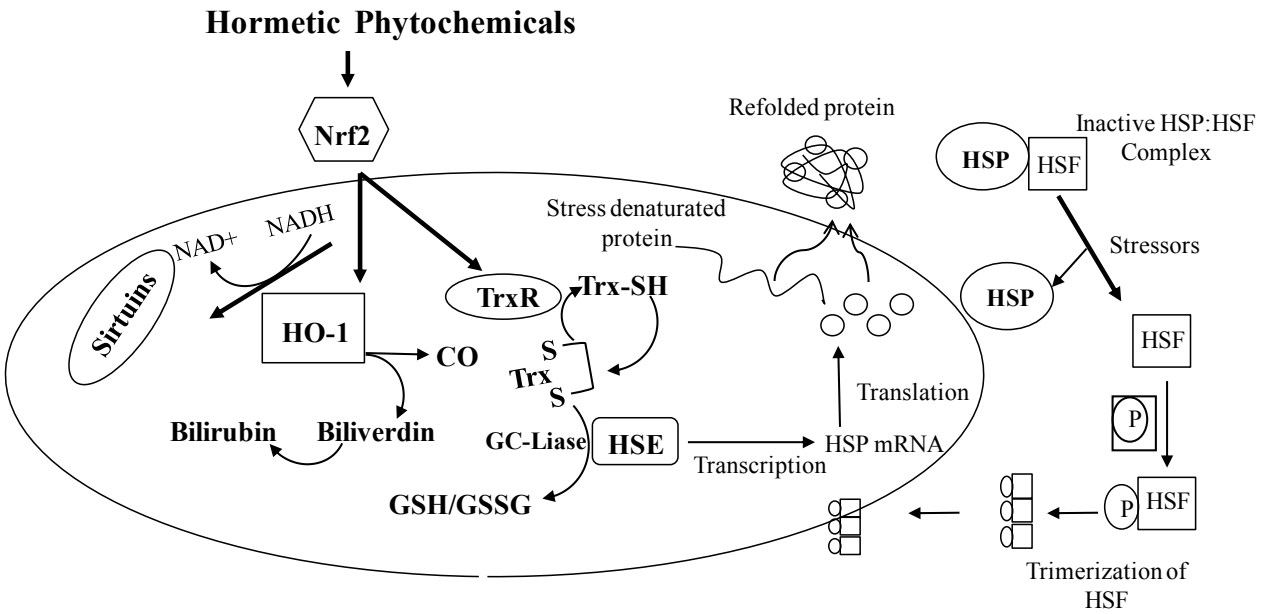

Fig. 2. *The Vitagene network of cellular stress response. Proteotoxic stresses causing accumulation of misfolded proteins is trigger for cellular stress response, where HSPs, normally bound to HSF1 are competitively dislocated by damaged proteins, which results in activation of HSF-1. Multi-step activation of HSF1 involves post-translational modifications, such as hyperphosphorylation, which allow HSF1 to trimerize, translocate into the nucleus, and bind to heat-shock elements (HSEs) in the promoter regions of its target hsp genes. Nutritional antioxidants, are able to activate vitagenes, such as heme oxygenase, Hsp70, thioredoxin reductase and sirtuins which represent an integrated system for cellular stress tolerance. During inflammaging, including aged-associated pathologies a gradual decline in potency of the heat shock response occur and this may prevent repair of protein damage, leading to neurodegeneration. Phytochemicals, such as Hydroxytyrosol, act through the activation of transcription factor Nrf2, which after binding to the antioxidant responsive element up-regulates vitagenes.*

while the activation of PPARGC1α led to increased protein expression of mitochondrial transcription factor A, uncoupling protein 2 and mitochondrial complexes (Zhu et al, 2010). More recently, one study (Arunsundar et al, 2015) tested HT with an AD C57BL/6 mouse model, where soluble oligomeric amyloid β1-42 plus ibotenic acid (oA42i)-induced neuro-behavioral dysfunction. Notably, oA42i administration altered the Bcl-2/Bad levels and activated the caspase-dependent mitochondria-mediated apoptotic pathway involving cytochrome c, apoptotic protease activating factor-1, and caspase-9/3. On the contrary, HT administration stabilized the disregulated activities of these apoptotic/anti-apoptotic markers and preserved the mitochondrial ultra-architecture. Moreover, it was observed that oA42i intoxication substantially down-regulated the expression of genes involved in the regulation of survival and memory functions including sirtuin-1, cyclic AMP response element-binding protein (CREB), CREB-target genes (BDNF, c-Fos, Nurr1, and Egr1) and a disintegrin and metalloprotease 10. HT treatment significantly diminished these aberrations when compared to the oA42i group In the same study, Aβ-induced down-regulation of the expression of survival genes involved in the regulation of memory functions, such as sirtuin-1, cyclic AMP response element-binding protein (CREB), CREB-target genes (BDNF, c-*Fos*, *Nurr1*, and *Egr1*) and a disintegrin, and metalloprotease 10 was considerably minimized. In addition, altered Bcl-2/Bad levels and activated caspase-dependent mitochondria-mediated apoptotic pathways with cytochrome *c release* and apoptotic protease activating factor-1, as well as caspase-9/3 activation induced by toxic Aβ, was significantly prevented.

Finally, a study by Zheng and colleagues (Zheng et al, 2015) suggested that HT is an efficient maternal nutrient protecting neurogenesis and cognitive function in prenatally stressed offspring. HT significantly increased transcription factors FOXO1 and FOXO3, as well as phase II enzyme-related proteins, including Nrf2 and HO-1, which may contribute to the decreased oxidative stress and increased mitochondrial function. Taken together, these findings suggest that HT may be an effective agent in AD and PD by a multifactorial mechanism.

In AD numerous pathways are involved in the onset and progression of the disease: amyloid precursor protein processing, amyloid-beta (Aβ) peptide and tau aggregation, autophagy impairment, neuroinflammation, etc. (Féart et al, 2013; Berr et al, 2009). Recent data show that oleuropein aglycone (OLE) interferes with APP processing (Kostomoiri et al, 2013), amyloid aggregation of Aß and tau protein, thus preventing the growth of toxic oligomers both *in vitro* (Rigacci et al, 2015; Daccache et al, 2011; Ladiwala et al, 2011) in *C. elegans* (Diomede et al, 2013) and in TgCRND8 mice, a model of Aß deposition (Grossi et al, 2013; Luccarini et al, 2014). In the latter, a diet supplemented with OLE displays strong protection against cognitive deterioration, improves synaptic function, tissue alterations and neuroinflammation, stimulates cell defenses against plaque-induced neurodegeneration and triggers autophagy, a function normally impaired in neurodegenerative conditions (Rubinsztein et al, 2015), with the possible involvement of epigenetic modifications (Luccarini et al, 2014). However, much less information and mechanistic data are presently available on the anti-neurodegenerative properties of hydroxytyrosol in humans. Today, two known sources of HT are readily available: (i) the ingestion of natural products that contain HT or its precursors both as dietary supplement and HT enriched olive oil (FVOO), and (ii) derivation from dopamine oxidative metabolism in the brain (De la Torre et al. 2006). Indeed, HT can be endogenously produced as a product of dopamine oxidation, as a component of 3,4-dihydroxyphenylethanol (DOPET); (Schröder et al, 2009). Recently, the potential of HT (3,4-dihydroxyphenylethanol, DOPET), both a dopamine metabolite, and a polyphenol from olive oil, was investigated in C57BL/6 mice (Arunsundar et al, 2015). Moreover, in the general metabolism of dopamine by monoamine oxidase (MAO), oxidative-deamination of dopamine generates DOPAL (3,4-dihydroxyphenylacetaldehyde), which is further oxidized to the carboxylic acid (3,4-dihydroxyphenylacetic acid, DOPAC) by aldehyde dehydrogenase. Although DOPAC is the major metabolite of dopamine in brain, a small portion of DOPAL is reduced to DOPET by aldehyde or aldose reductase (Rodríguez-Morató et al, 2015). DOPAL is a highly reactive neurotoxic metabolite, suggested to

operate *in vivo* in the pathogenesis of PD (Goldstein et al, 2015). In AD, disruption of dopamine metabolism is associated with increasing oxidative stress conditions, in which highly reactive aldehydes, such as 4-hydroxynonenal and malondialdehyde, are formed and which can inhibit aldehyde dehydrogenase 2 (ALDH2). Consistent with this notion, as the soluble forms of oligomeric amyloid beta are involved in the loss of synaptic plasticity and memory, especially in early phases of Alzheimer'sdisease, interestingly, stimulation of dopamine D1/D5 receptors (D1R/D5R) increases surface expression of synaptic α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate subtype glutamate and N-methyl-D-aspartate subtype glutamate receptors, thus facilitating the induction of the late phase of long-term potentiation (LTP), probably via a related mechanism. (Xiang et al, 2016). In addition, aberrant promoter methylation of multiple genes has been associated with increased AD risk in males. (Ji et al, 2016). Given these findings, viable HT /DOPET can be of value to reduce levels of DOPAL in AD patients. We speculate that this putative link between oxidative stress and the generation of endogenous will be important to further research focusing on mechanisms involved in neurotoxicity and neurodegeneration which are particularly relevant in the study of AD, as well as to PD and more in general in neuropsychiatric spectrum disorders.

CONCLUSIONS

Neurodegenerative disease pathogenesis is multifactorial, recognizing a complex combination of genetic and environmental factors. Some common, well established components, are oxidative stress and inflammation, where underlying molecular mechanisms such as toxic reactions, mitochondrial dysfunction, glutamate excitotoxicity and proteasomal alterations generally concur to activate apoptosis pathways and neuronal cell death. Polyphenol antioxidants have emerged as potential natural molecules with neuroprotective properties able therefore to treat neurodegenerative diseases. The majority of these natural compounds are not only active scavengers of free radical species and exert anti-inflammatory activities, but also are capable of modulating pro-survival and anti-apoptotic signaling pathways (Murugaiyah and Mattson, 2015; Kim et al, 2016; Mattson, 2014; Texel and Mattson, 2011). In respect to synthetic drugs showing generally only one mechanism of action, these compounds have a greater potential based on multiple lines of neuroprotection, such as: i) direct scavenging of free radicals; ii) enhancement of endogenous cellular antioxidant potential by nuclear factor erythroid-derived 2-related factor 2 (Nrf2) transcription factor pathway activation; iii) modulation of signal transduction cascades; iv) modulators of neuroinflammation via the NF-kb; and v) regulation of gene expression. A lot of funding and research have been dedicated to treatment of AD and PD, yet these pathologies have only symptomatic therapies, none capable of slowing or curing the underlying physiopathology of the disease. In this context, olive polyphenols, such as hydroxytyrosol, might represent a promising choice, at least in the short term, since they show an excellent safety profile even at high concentration and are subject to fewer regulations than traditional pharmaceuticals, therefore more promptly available to patients. Olive polyphenols are also readily available for large medical applications and their cost of production is relatively low, if compared to biologics. Consistent to this, is the initiation of several clinical trials with other plant polyphenols. For instance, EGCG (green tea) is currently being tested in phase II trials for PD and early stage AD (Charite University, Berlin, Germany), also resveratrol is being tested in a phase II trial to improve memory performance in the elderly. Yet, no trial has currently been initiated with HT/olive polyphenols for neuroprotection. It is interesting to consider that the process of evolutionary reciprocal adaptation of plants and animals consuming them fostered the generation of a wide variety of bioactive phytochemicals, with concomitant development of novel detoxifying mechanisms and, as a consequence, adaptive stress signaling pathways in animal cells. Thus, the genetic variation underlying molecular diversity in enzymes involved in the production of phytochemicals as secondary plant metabolites was the co-evolutionary consequence of the nutritional use of

plants by animals (Mattson, 2011). Consistently, studies on animal and human models have provided considerable evidence that regular exercise and dietary energy restriction protect various organisms against major diseases, including neurodegenerative disorders. The ability of phytochemicals, such as hydroxytyrosol, to activate the same adaptive stress response pathways activated by exercise and energy restriction, represents compelling evidence of their therapeutic potential to improve brain health and, hence, decrease neurodegenerative disorders (Fig. 2). The natural origin of hydroxytyrosol, its safety profile, bioavailability and its multitarget activity make it a potentially attractive tool in dealing with the multifactorial etiology of PD and AD.

ACKNOWLEDGEMENTS

We wish to acknowledge Dr. Alice Alario for reviewing and editing the manuscript.

CONFLICT OF INTEREST STATEMENT

One of the authors, Roberto Crea, is associated with CreAgri, Inc. a Californian company that commercially produces proprietary Hydroxytyrosol-rich formulations, Hidrox™, and focuses on the development of olive polyphenols for health applications.

ABBREVIATIONS

AD: Alzheimer's disease; amyotrophic lateral sclerosis (ALS); multiple sclerosis (MS); Parkinson's disease (PD); CNS: central nervous system; (CSF): cerebrospinal fluid; EEG: electroencephalography; EAE: experimental autoimmune encephalomyelitis; HDAC1: histone deacetylase 1; HNE: 4-hydroxynonenal; HO-1: Heme oxygenase-1; HSE: cis-acting heat shock elements; HSF1: heat shock transcription factor 1; Hsps: heat shock proteins; HSR: heat shock response; MSU: monosodium urate; Keap1: Kelch-like ECH-associated protein 1; Nrf2: nuclear factor erythroid 2-related factor 2; MAO: monoamine oxidase; MCI: mild cognitive impairment; ROS, reactive oxygen species; NOS: nitric oxide synthase; HO-1: heme oxygenase-1; CO: carbon monoxide; BR: bilirubin; OLE: oleuropein aglycone; HT: hydroxytyrosol; 6-OHDA: 6-hydroxydopamine; H2O2: hydrogen proxide; GSH: reduced glutathione; SOD2: mitochondrial superoxide dismutase; DOPET: 3,4-dihydroxyphenylethanol; DOPAL: 3,4-dihydroxyphenylacetaldehyde; ALDH2: aldehyde dehydrogenase 2; BACE-1: AβPP cleaving enzyme 1; TNF-α tumour necrosis factor α; , (IL)-1β: interleukin-1β; IFN-γ: interferon-γ; JNK: c-Jun NH2-terminal kinase; MAPKs: Mitogen-activated protein kinases; CREB: cyclic AMP response element-binding protein; NFkB: nuclear factor-kB; ETC: electron transport chain; SOD: superoxide dismutase; PRRs: Pathogen Recognition Receptors; PAMPs: Pathogen-associated molecular patterns; DAMPs: damage-associated molecular patterns; ASC: apoptotic speck containing protein with a CARD; CARD: Caspase recruit domain; AIM2: absent in melanoma-2; NLRP3: NOD-like receptor family, pyrin domain-containing-3; TLRs: Toll-like Receptors; CTLs: C-type lectins; AP-1: activator protein- 1; TNF-α: Tumor Necrosis Factor α; NFTs: intracellular neurofibrillary tangles; PUFAs: polyunsaturated fatty acids; HIF: hypoxia inducible factor; MAPK: mitogen-activated protein kinase; MMPs: matrix metalloproteinase; Hcy: Hyperhomocysteine; 6-OHDA: 6-hydroxydopamine; SIRT1: Silent Information Regulator Two; TrxR: thioredoxin reductase; UPR: unfolded protein response; Aβ: Amyloid-β peptide; APP/PS1: amyloid precursor protein (APP)/ presenilin mice.

REFERENCES

Anathy V, Roberson EC, Guala AS, Godburn KE, Budd RC, Janssen-Heininger YM (2012) Redox-based regulation of apoptosis: S-glutathionylation as a regulatory mechanism to control cell death. *Antioxid Redox Signal* **16**: 496-505.

Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? *Nat Med* **10**: 18-25.

Alfieri A, Srivastava S, Siow RC, Cash D et al and Manna GE (2013) Sulforaphane preconditioning of the Nrf2/HO-1 defense pathway protects the cerebral vasculature against blood–brain barrier disruption and neurological deficits in stroke. *Free Rad Biol Med* **65**: 1012-1022.

Arunsundar M, Shanmugarajan TS, Ravichandran V (2015) 3,4-Dihydroxyphenylethanol attenuates spatio-cognitive deficits in an Alzheimer's disease mouse model: modulation of the molecular signals in neuronal survival-apoptotic programs. *Neurotox Res* **27**: 143-155.

Bhat AH, Dar KB, Anees S, Zargar MA, Masood A, Sofi MA, Ganie SA (2015) Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. *Biomed Pharmacother* **74**: 101-10.

Bechtold DA, Brown IR (2003) Induction of Hsp27 and Hsp32 stress proteins and vimentin in glial cells of the rat hippocampus following hyperthermia. *Neurochem Res* **28**(8): 1163-73.

Bellucci A, Mercuri NB, Venneri A, Faustini G et al and Spano P (2016) Parkinson's disease: from synaptic loss to connectome dysfunction. *Appl Neurobiol* 42(1): 77-94.

Beitz JM (2014) Parkinson's disease: a review. *Frontiers Biosci Neuropathol* **S6**: 65-74,

Berr C, Portet F, Carriere I, Akbaraly TN et al and Ritchie K (2009) Olive oil and cognition: results from the three-city study. *Dement Geriatr Cogn Disord* **28**: 357-364.

Beschorner RD, Adjodah JM, Schwab M, Mittelbronn I et al and Meyermann J (2000) Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. *Act Neuropat* **4**: 377–384

Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR (2015) Oxidative stress and Parkinson's disease. *Front Neuroanat* **8**: 90-91.

Bharath S, Andersen JK. (2005) Glutathione depletion in a midbrain-derived immortalized dopaminergic cell line results in limited tyrosine nitration of mitochondrial complex I subunits: implications for Parkinson's disease. *Antioxid Redox Signal* 7: 900 – 910 .

Bitler CM, Viale TM, Damaj B, Crea R (2005) Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. *J Nutr* **135**(6): 1475-9.

Bitler, CM., Matt K, Irving M, Hook G et al and Crea R (2007) Olive extract supplement decreases pain and improves daily activities in adults with osteoarthritis and decrease plasma homocysteine in those with rheumatoid arthritis. *Nutrition Res* **27**: 470-477

Brieger K, Schiavone S, Miller FJ Jr, Krause KH (2012) Reactive oxygen species: from health to disease. *Swiss Med Wkly* **142**: w13659

Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement* **3**: 186-191

Butterfield DA, Gnjec A, Poon HF, Castegna A, Pierce WM, Klein JB, Martins RN (2006) Redox proteomics identification of oxidatively modified brain proteins in inherited Alzheimer's disease: an initial assessment. *J Alzheimer's Dis* **10**: 391-397.

Calabrese EJ, Baldwin LA. (2002) Defining hormesis. *Hum Exp Toxicol*.; 21(2):91-7.

Calabrese EJ (2008a) Neuroscience and hormesis: Overview and general findings; *Crit Rev Toxicol* **38**(4): 249-252.

Calabrese EJ (2008b) Dose-response features of neuroprotective agents: An integrative summary. *Crit Rev Toxicol* **38**(4): 253-348.

Calabrese EJ (2008c) Pharmacological enhancement of neuronal survival. *Crit Rev Toxicol* **38**(4): 349-389.

Calabrese EJ, Blain R (2011) The hormesis database: The occurrence of hermetic dose responses in the toxicological literature. *Regulatory Toxicol Pharm* **61**: 73-81.

Calabrese EJ (2016a) Preconditioning is hormesis part I: Documentation, dose-response features and mechanistic foundations. *Pharmacological Res* **110**: 242-264

Calabrese EJ (2016b). Preconditioning is hormesis part II: How the conditioning dose mediates protection: Dose optimization within temporal and mechanistic frameworks. *Pharmacological Res* **110**: 265-275.

Calabrese EJ, Dhawan G, Kapoor R, Iavicoli I, Calabrese V (2016c) Hormesis: A Fundamental Concept with Widespread Biological and Biomedical Applications.

Geront **62**: 530-535.

Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB (2001) Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. *Neurochem Res* **26**: 739-764.

Calabrese V, Scapagnini G, Ravagna A, Fariello RG, Giuffrida Stella AM, Abraham NG (2002) Regional distribution of heme oxygenase, HSP70, and glutathione in brain: relevance for endogenous oxidant/antioxidant balance and stress tolerance. *J Neurosc Res* **68**(1): 65-75.

Calabrese V, Scapagnini G, Ravagna A, Colombrita C, Spadaro F, Butterfield DA, Giuffrida Stella AM (2004) Increased expression of heat shock proteins in rat brain during aging: relationship with mitochondrial function and glutathione redox state. *Mech Ageing Dev* **125**(4):3 25-35.

Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Giuffrida Stella AM (2007°) Nitric Oxide in the CNS: Neuroprotection versus Neurotoxicity. *Nature Neurosci* **8**: 766-775.

Calabrese V, Mancuso C, Ravagna A, Perluigi M, Cini C, De Marco C, Butterfield DA, Stella AM (2007b) In vivo induction of heat shock proteins in the substantia nigra following L-DOPA administration is associated with increased activity of mitochondrial complex I and nitrosative stress in rats: regulation by glutathione redox state. *J Neurochem* **101**: 709-717.

Calabrese V, Cornelius C, Rizzarelli E, Owen JB, Dinkova-Kostova AT, Butterfield DA. 2009. Nitric oxide in cell survival: a Janus molecule. *Antioxid Redox Sign* **11**: 2717-2739.

Calabrese V, Cornelius C, Mancuso C, Lentile R, Stella AM, Butterfield DA. (2010) Redox homeostasis and cellular stress response in aging and neurodegeneration. *Methods Mol Biol* **610**: 285-308.

Calabrese V, Cornelius C, Stella AM, Calabrese EJ (2010) Cellular stress responses, mitostress and carnitine insufficiencies as critical determinants in aging and neurodegenerative disorders: role of hormesis and vitagenes. *Neurochem Res* **35**: 1880-1915.

Calabrese V, Cornelius C, Cuzzocrea S, Iavicoli I, Rizzarelli E, Calabrese EJ (2011) Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. *Mol Aspects of Med* **32**: 279-304.

Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I et al and Calabrese EJ (2012) Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. *Biochim Biophys Acta* **1822**: 753-783.

Calabrese V, Dattilo S, Petralia A, Parenti R et al and Calabrese EJ (2015°) Analytical approaches to the diagnosis and treatment of aging and aging-related disease: redox status and proteomics. *Free Radic Res* **49**(5): 511-24.

Calabrese V, Cighetti R, Peri F (2015) Molecular simplification of lipid A structure: TLR4-modulating cationic and anionic amphiphiles. *Mol Immunol* **63**(2): 153-61.

Campisi J (2013) Aging, cellular senescence, and cancer. *Annu. Rev. Physiol* **75**: 685-705.

Campolo J, De Maria R, Cozzi L, Parolini M et al and Marraccini P.(2016) Antioxidant and inflammatory biomarkers for the identification of prodromal Parkinson's disease. *J Neurol Sci* **370**: 167-172.

Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A et al and Fernández-Gutiérrez A (2005) Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. *J Agric Food Chem* **53**(23): 8918-25.

Castaner O, Covas MI, Khymenets O, Nyyssonen K et al and Fito' M (2012) Protection of LDL from oxidation by olive oil pholyphenols is associated with a downregulation of CD40-ligand expression and its downstream products in vivo in humans. *Am J Clin Nutr* **95**(5): 1238-44.

Chakrabarti S, Sinha M, Thakurta IG, Banerjee P, Chattopadhyay M (2013) Oxidative stress and amyloid beta toxicity in Alzheimer's disease: intervention in a complex relationship by antioxidants. *Curr Med Chem* **20**: 4648-4664.

Chen H, Yen GC. (2007) Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L.) leaves. *Food Chemistry*; 101 (2): 686–694

Chiurchiù V, Orlacchio A, Maccarrone M (2016) Is Modulation of Oxidative Stress an Answer? The State of

the Art of Redox Therapeutic Actions in Neurodegenerative Diseases. *Oxid Med Cell Longev* **2**: 7909380.

Christian MS, Sharper VA, Hoberman AM, Seng Jeb et al and Crea R (2004) The toxicity profile of hydrolyzed aqueous olive pulp extract. *Drug Chem Toxicol* **27**: 309-330.

Cobb CA, Cole MP (2015) Oxidative and nitrative stress in neurodegeneration. *Neurobiol Dis* 84: 4-21.

Cornelius C, Perrotta R, Graziano A, Calabrese EJ, Calabrese V (2013) Stress responses, vitagenes and hormesis as critical determinants in aging and longevity: Mitochondria as a "chi". *Immun Ageing* **10**: 15

Cornwell DG, Ma J (2008) Nutritional benefit of olive oil: the biological effects of hydroxytyrosol and its arylating quinone adducts. *J Agric Food Chem* **56**(19): 8774-86.

Covas MI, de la Torre K, Farre'-Albaladejo M, Kaikkonen J et al and De la Torre R (2006) Post-brandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Radic Biol Med* **40**(4): 608-16

Crea R (2002) Method of obtaining a hydroxytyrosol-rich composition from vegetation water. United States Patent # 6,416,808.

Crea R, Bitler CM, Bolin LM, Pontoniere (2012) Anti-inflammatory activity of hydroxytyrosol. *AgroFOOD Industry Hi-tec* **23**(2): 26-29.

D'Angelo S, Manna C, Migliardi V, Mazzoni O et al and Zappia V (2001) Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. *Drug Metab Dispos* **29**(11): 1492-8.

Di Bona D, Scapagnini G, Candore G, Castiglia L et al and Vasto S (2010) Immune-inflammatory responses and oxidative stress in Alzheimer's disease: therapeutic implications. *Curr Pharm Des* **16**: 684-691

Di Domenico F, Barone E, Perluigi M, Butterfield DA (2016) The "Triangle of Death" in Alzheimer disease brain: the aberrant cross talk among energy metabolism, mTOR signaling and protein homeostasis revealed by redox proteomics. *Antioxid Redox Signal* Epub. 27626216.

Daccache A, Lion C, Sibille N, Gerard M, Slomianny C, Lippens G, Cotelle P (2011) Oleuropein and derivatives from olives as Tau aggregation inhibitors. *Neurochem Int* **58**: 700-707.

Danielson SR, Andersen JK (2008) Oxidative and nitrative protein modifications in Parkinson's disease. *Free Radical Biology and Medicine* **44**(10): 1787-1794

Dattilo S, Mancuso C, Koverech G, Di Mauro P et al and Calabrese V (2015) Heat shock proteins and hormesis in the diagnosis and treatment of neurodegenerative diseases. *Immun Ageing* **12**: 20.

Dawson TM, Dawson VL (2002) Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nat Neurosci* Suppl **5**: 1058-61.

Dawson TM, Dawson VL (1995) ADP-ribosylation as a mechanism for the action of nitric oxide in the nervous system. *New Horiz* **3**(1):85-92.

Dexter DT, Carayon A, Javoy-Agid F, Agid Y et al and Marsden CD (1991) Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* **114**(Pt 4): 1953-75.

Dias V, Junn E, Mouradian MM (2013) The Role of Oxidative Stress in Parkinson's Disease. *J Parkinson's Dis* **3**(4): 461-491

Dickinson BC, Chang CJ (2011) Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol* **7**: 504-511.

Diomede L, Rigacci S, Romeo M, Stefani M, Salmona M (2013) Oleuropein aglycone protects transgenic C. elegans strains expressing Aβ42 by reducing plaque load and motor deficit. *PLoS One* **8**(3): e58893.

Dringen R (2000) Metabolism and functions of glutathione in brain. *Prog Neurobiol* **62**: 649 -671 .

Eriksen JL, Dawson TM, Dickson DW, Petrucelli L (2003) Caught in the Act: α-Synuclein Is the Culprit in Parkinson's Dis. *Neuron* **4**(3): 453-456.

Fahn S (1992) A pilot trial of high-dose alpha-tocopherol and ascorbate in early Parkinson's disease. *Ann Neurol* **32** Suppl: S128-32.

Féart C, Samieri C, Allès B, Barberger-Gateau P (2013) Potential benefits of adherence to the Mediterranean diet on cognitive health. *Proc Nutr Soc* **72**: 140-152.

Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E (2012) Mitochondria, oxidative stress and neurodegeneration. *J Neurol Sci* 322: 254-262.

Finkel T. (2011) Signal transduction by reactive oxygen species. *J Cell Biol* **194**: 7-15.

Fisher-Wellman KH, Gilliam LA, Lin CT, Cathey BL, Lark DS, Neufer PD (2013) Mitochondrial glutathione depletion reveals a novel role for the pyruvate dehydrogenase complex as a key H2O2-emitting source under conditions of nutrient overload. *Free Radic Biol Med* **65**: 1201-1208.

France-Lanord V, Brugg B, Michel PP, Agid Y, Ruberg M (1997) Mitochondrial free radical signal in ceramide-dependent apoptosis: a putative mechanism for neuronal death in Parkinson's disease. *J Neurochem* 69(4): 1612-21.

Giri M, Zhang M, Lü Y (2016) Genes associated with Alzheimer's disease: an overview and current status. *Clin Interv Aging* **11**: 665-681.

Goldstein DS, Sullivan P, Cooney A, Jinsmaa Y, Kopin IJ, Sharabi Y (2015) Rotenone decreases intracellular aldehyde dehydrogenase activity: implications for the pathogenesis of Parkinson's disease. *J Neurochem* **133**: 14-25.

Goldstein DS, Holmes C, Sullivan P, Jinsmaa Y, Kopin IJ, Sharabi Y (2016) Elevated cerebrospinal fluid ratios of cysteinyl-dopamine/3,4-dihydroxyphenylacetic acid in parkinsonian synucleinopathies. *Parkinsonism Relat Disord* **31**: 79-86.

Grossi C, Ed Dami T, Rigacci S, Stefani M, Luccarini I, Casamenti F (2013) Employing Alzheimer disease animal models for translational research: focus on dietary components. *Neurodegener* **13**(2-3): 131-4.

Hare D, Ayton S, Bush A, Lei P (2013) A delicate balance: Iron metabolism and diseases of the brain. *Front Aging Neurosci* **18**: 5-34

Hastings TG (2009) The role of dopamine oxidation in mitochondrial dysfunction: implications for Parkinson's disease. *J. Bioenerg Biomembr* **41**(6); 469-72.

Hao J, Shen W, Yu G, Jia H et al and Liu J (2010) Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. *J Nutr Biochem*.; 21(7):634-44.

Huang WJ, Zhang X, Chen WW (2016) Role of oxidative stress in Alzheimer's disease. *Biomed Rep* **4**: 519-522.

Hunot S, Brugg B, Ricard D, Michel PP et al and Hirsch EC (1997) Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with Parkinson disease. *Proc Natl Acad Sci U S A* **94**(14): 7531-6

Hyun DH, Lee M, Hattori N, Kubo S, Mizuno Y, Halliwell B, Jenner P (2002) Effect of wild-type or mutant Parkin on oxidative damage, nitric oxide, antioxidant defenses, and the proteasome. *J Biol Chem* **277**(32): 28572-7.

Islam MT (2016) Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurol Res* **3**: 1-10. [Epub ahead of print]

Janaky R, Shaw CA, Oja SS, Saransaari P (2008) Taurine release in developing mouse hippocampus is modulated by glutathione and glutathione derivatives. *Amino Acids* **34**: 75– 80.

Ji H, Wang Y, Jiang D, Liu G et al and Wang Q (2016) Elevated DRD4 promoter methylation increases the risk of Alzheimer's disease in males. *Mol Med Rep* 14: 2732-2738.

Javed H, Azimullah S, Abul Khair SB, Ojha S, Haque ME (2016) Neuroprotective effect of nerolidol against neuroinflammation and oxidative stress induced by rotenone. *BMC Neurosci* **17**: 58-63.

Jomova K, Vondrakova D, Lawson M, Valko M (2010) Metals, oxidative stress and neurodegenerative disorders. *Cell Biochem* **345**(1-2): 91-104.

Judy ME, Nakamura A, Huang A, Grant H et al and Kao AW (2013) A shift to organismal stress resistance in programmed cell death mutants. *PLoS Genet* **9**(9): e1003714

Kannan R, Mittur A, Bao Y, Tsuruo T, Kaplowitz (1999) GSH transport in immortalized mouse brain endothelial cells: evidence for apical localization of a sodium-dependent GSH transporter. *J Neurochem* **73**(1): 390-9.

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R A (1993) C. elegans mutant that lives twice as long as wild type. *Nature* 66(6454):461-4.

Killeen MJ, Linder M, Pontoniere P, Crea R (2014) NF-κβ signaling and chronic inflammatory diseases: exploring the potential of natural products to drive new therapeutic opportunities. *Drug Discov Today* **19**(4): 373-8.

Kim DK, Kim TH, Lee SJ (2016) Mechanisms of aging-related proteinopathies in Caenorhabditis elegans. *Exp Mol Med* **48**(10): e263.

Kostomoiri M, Fragkouli A, Sagnou M, Skaltsounis LA, Pelecanou M, Tsilibary EC, Tzinia AK (2013) Oleuropein, an anti-oxidant polyphenol constituent of olive promotes α-secretase cleavage of the amyloid precursor protein (AβPP). *Cell Mol Neurobiol* **33**: 147-154.

Ladiwala AR, Mora-Pale M, Lin JC, Bale SS, Fishman ZS, Dordick JS, Tessier PM (2011) Polyphenolic glycosides and aglycones utilize opposing pathways to selectively remodel and inactivate toxic oligomers of amyloid β. *Chembiochem* **12**: 1749-1758.

Lalla R, Donmez G (2013) The role of sirtuins in Alzheimer's disease. *Front Aging Neurosci* **5:** 16.

Lee HP, Zhu X, Casadesus G, Castellani RJ et al and Perry G (2010) Antioxidant approaches for the treatment of Alzheimer's disease. *Expert Rev Neurother* **10**: 1201-1208.

LeVault KR, Tischkau SA, Brewer GJ (2015) Circadian Disruption Reveals a Correlation of an Oxidative GSH/GSSG Redox Shift with Learning and Impaired Memory in an Alzheimer's Disease Mouse Model. *J Alzheimers Dis* **49**: 301-316.

Li H, Dryhurst G (1997) Irreversible inhibition of mitochondrial complex I by 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxyli c acid (DHBT-1): a putative nigral endotoxin of relevance to Parkinson's disease. *J Neurochem* **69**(4): 1530-4

Li J, Le W (2013) Modeling neurodegenerative diseases in Caenorhabditis elegans. *Exp Neurol* **250**: 94-103

Liu Z, Sun L, Zhu L, Jia X et al and Liu J (2007) Hydroxytyrosol protects retinal pigment epithelial cells from acrolein-induced oxidative stress and mitochondrial dysfunction. *J Neurochem* **103**(6): 2690-700.

Lockyer S, Rowland I, Spencer JP, Yaqoob P, Stonehouse W (2016) Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomized controlled trial. *Eur J Nutr* Epub. 26951205.

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hall-marks of aging. *Cell* **153**: 1194-1217.

Luccarini I, Grossi C, Rigacci S, Coppi E et al and Casamenti F (2015) Oleuropein aglycone protects against pyroglutamylated-3 amyloid-ß toxicity: biochemical, epigenetic and functional correlates. *Neurobiol Aging* **36**(2): 648-63.

Mancuso C, Scapagnini G, Currò D, Giuffrida Stella AM, De Marco C, Butterfield DA, Calabrese V (2007) Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. *Front Biosci* **12**: 1107-1123.

Marrugat J, Covas MI, Fito' M, Schroder H et al and Farre' M.(2004) Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation—a randomized controlled trial. *Eur J Nutr* **43**(3): 140-7.

Martín-Peláez S, Covas MI, Fitó M, Kušar A, Pravst I (2013) Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims. *Mol Nutr Food Res* **57**(5): 760-71.

Martínez-Lapiscina EH, Clavero P, Toledo E, San Julián B et al and Martínez-Gonzalez MÁ (2013) Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. *J Nutr Health Aging* **17**(6): 544-52.

Mateos R, Martínez-López S, Baeza Arévalo G, Amigo-Benavent M, Sarriá B, Bravo-Clemente L (2016) Hydroxytyrosol in functional hydroxytyrosol-enriched biscuits is highly bioavailable and decreases oxidized low density lipoprotein levels in humans. *Food Chem* **205**: 248-256.

Mattson MP (2002) Contributions of mitochondrial alterations, resulting from bad genes and a hostile environment, to the pathogenesis of Alzheimer's disease. *Int Rev Neurobiol* 53:387-409

Mattson MP (2011) Commentary: proteo-oxidotoxic process of aggregation. *Neuromolecular Med* **13**(2): 91-2

Mattson MP (2014) Interventions that improve body and brain bioenergetics for Parkinson's disease risk reduction and therapy. *J Parkinson Dis* **4**(1): 1-13.

Maybodi L, Pow DV, Kharazia VN, Weinberg RJ (1999) Immunocytochemical demonstration of reduced glutathione in neurons of rat forebrain. *Brain Res* **817**: 199-205

McNaught KS, Olanow CW, Halliwell B, Isacson O, Jenner P (2001) Failure of the ubiquitin-proteasome system in Parkinson's disease. *Nat Rev Neurosci* **2**(8): 589-94.

Meister A (1994) Glutathione, ascorbate, and cellular protection. *Cancer Res* **54**: 1969s-1975s

Mendiola-Precoma J, Berumen LC, Padilla K, Garcia-Alcocer G (2016) Therapies for Prevention and Treatment of Alzheimer's Disease. *Biomed Res Int* 2589276. Epub 2016

Meyer M, Kovács AD, Pearce DA (2016) Decreased sensitivity of palmitoyl protein thioesterase 1-deficient neurons to chemical anoxia - *Metabolic Brain Disease* Epub 27722792.

Mischley LK, Vespignani MF, Finnell JS (2013) Safety survey of intranasal glutathione. *J Altern Complement Med* **19**(5): 459-63.

Montedoro G, Servili M, Baldioli M, Miniati E (1992) Simple and hydrolyzable phenolic compounds in virgin olive oil. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *J. Agric Food Chem* **40**: 1571-1576.

Moore DJ, Dawson VL, Dawson TM (2003) Role for the ubiquitin-proteasome system in Parkinson's disease and other neurodegenerative brain amyloidoses. *Neuromolecular Med* **4**(1-2): 95-108.

Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* **417**: 1-13.

Murugaiyah V, Mattson MP (2015) Neurohormetic phytochemicals: An evolutionary-bioenergetic perspective. *Neurochem Int* **89**: 271-80.

Newman SF, Sultana R, Perluigi M, Coccia R et al and Butterfield DA (2007) An increase in S-glutathionylated proteins in the Alzheimer's disease inferior parietal lobule, a proteomics approach. *J Neurosci Res* **85**(7): 1506-14.

Nkuipou-Kenfack E, Koeck T, Mischak H, Pich A, Schanstra JP, Zürbig P, Schumacher B (2014a) Proteome analysis in the assessment of ageing. *Ageing Res Rev* **18**C: 74-85.

Nkuipou-Kenfack E, Duranton F, Gayrard N, Argilés À et al and Mischak H (2014b) Assessment of metabolomic and proteomic biomarkers in detection and prognosis of progression of renal function in chronic kidney disease. *PLoS One* 9(5): e96955.

Numano F, Crea R, Wiedemann T, Bitler C (2007) Vegetation water composition for treatment of inflammatory skin conditions. US Patent. Appl. 20070020350.

Olanow CW (2015) Levodopa: effect on cell death and the natural history of Parkinson's disease. *Mov Disord* **30**(1): 37-44.

Peng Y, Hou C, Yang Z, Li C et al and Liu J (2016) Hydroxytyrosol mildly improve cognitive function independent of APP processing in APP/PS1 mice. *Mol Nutr Food Res* Epub 27287957

Perluigi M, Joshi G, Sultana R, Calabrese V et al and Butterfield DA (2006) In vivo protective effects of ferulic acid ethyl ester against amyloid-beta peptide 1-42-induced oxidative stress. *J Neurosci Res* **84**(2): 418-26.

Perry TL, Godin DV, Hansen S (1982) Parkinson's disease: a disorder due to nigral glutathione deficiency? *Neurosci Lett* **33**(3):305-10.

Peterson DR, Sukowski EJ, Zikos D (2013) Blood-brain barrier transport pathways for cytoprotective thiols. *Am J Ther* **20**: 469-479.

Petroni A, Blasevich M, Salami M, Servili M, Montedoro GF, Galli C (1994) A phenolic antioxidant extracted from olive oil inhibits platelet aggregation and arachidonic acid metabolism in vitro. *World Rev Nutr Diet* **75**: 169-72.

Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N (2013) Mediterranean diet, stroke, cognitive impairment, and depression: A meta-analysis. *Ann Neurol* **74**: 580-591.

Puzzo D, Privitera L, Palmeri A (2012) Hormetic effect of amyloid-beta in synaptic plasticity and memory. *Neurobiol of Aging 33*: 1484e15-1484e24.

Querfurth HW, LaFerla FM (2010) Alzheimer's disease. *N Engl J Med* **362**: 329-344.

RajaSankara S, Manivasagam T, Sankard V, Prakashd

S, Muthusamy R, Krishnamurti A, Surendrand S (2009) Withania somnifera root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. *J Ethnopharm* **125**(3): 369–373.

Ren J, Fan C, Chen N, Jiagui Huang, Yang Q (2011) resveratrol pretreatment attenuates cerebral ischemic injury by upregulating expression of Transcription Factor Nrf2 and HO-1 in rats. *Neurochem Res* **36**: 2352

Richard N, Arnold S, Hoeller U, Kilpert C, Wertz K, Schwager J (2011) Hydroxytyrosol is the major anti-inflammatory compound in aqueous olive extracts and impairs cytokine and chemokine production in macrophages. *Planta Med* **77**(17): 1890-7.

Rietjens SJ, Bast A, Haenen GR (2007) New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. *J Agric Food Chem* **555**(18): 7609-14.

Rigacci S (2015) Olive oil phenols as promising multi-targeting agents against Alzheimer's disease. *Adv Exp Med Biol* **863**: 1-20.

Rodríguez-Morató J, Xicota L, Fitó M, Farré M, Dierssen M, de la Torre R (2015) Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases. *Molecules* **20**(3): 4655-4680

Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, Obeso JA (2009) Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. *Lancet Neurol* **8**(12): 1128-39.

Rubinsztein DC, Bento CF, Deretic V (2015) Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. *J Exp Med* **212**: 979-990.

Safouris A, Tsivgoulis G, Sergentanis TN, Psaltopoulou T (2015) Mediterranean Diet and Risk of Dementia. *Curr Alzheimer Res* **12**: 736-744.

Samuelsson M, Vainikka L, Ollinger K (2011) Glutathione in the blood and cerebrospinal fl uid: a study in healthy male volunteers. *Neuropep* **45**: 287-292 .

Santos CX, Tanaka LY, Wosniak J, Laurindo FR (2009) Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxid Redox Sign* **11**: 2409-2427.

Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. (2006) Mediterranean diet and risk for Alzheimer's disease. *Ann Neurol* **59**(6): 912-21.

Schaffer S, Podstawa M, Visioli F, Bogani P, Muller WE, Eckert GP (2007) Hydroxytyrosol-rich olive mill wastewater extract protects brain cells in vitro and ex vivo. *J.Agric Food Chem* **55**(13): 5043-9

Schaffer S, Müller WE, Eckert GP (2010) Cytoprotective effects of olive mill wastewater extract and its main constituent hydroxytyrosol in PC12 cells. *Pharmacol Res* **62**: 322-327.

Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. (1990) Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* **54**(3): 823-7.

Schipper HM (2000) Heme oxygenase-1: role in brain aging and neurodegeneration. *Experimental Gerontology* **35**(6-7): 821-830

Schipper H M, Bennett D A, Liberman A, Bieniase J L, Schneider J A, Kelly J, Arvanitakisc Z (2006) Glial heme oxygenase-1 expression in Alzheimer disease and mild cognitive impairment. *Neurobiol of Aging* **27**(2): 252-261

Schröder H, de la Torre R, Estruch R, Corella D et al and Covas MI (2009) PREDIMED Study Investigators. Alcohol consumption is associated with high concentrations of urinary hydroxytyrosol. *Am J Clin Nutr* **90**(5): 1329-35.

Segev-Amzaleg N, Trudler D, Frenkel D (2013) Preconditioning to mild oxidative stress mediates astroglial neuroprotection in an IL-10-dependent manner. *Brain Behav Immun* **30**: 176-85.

Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM (2016). Alzheimer's disease. *Lancet* **388**: 505-517.

Schulz JB, Lindenau J, Seyfried J, Dichgans (2000) Glutathione, oxidative stress and neurodegeneration. *J.Eur J Biochem* **267**(16): 4904-11.

Schwartz M, Deczkowska A (2016) neurological disease as a failure of brain-immune crosstalk: the multiple faces of neuroinflammation. *Trends Immunol* **37**: 668-679.

Scoditti E, Capurso C, Capurso A, Massaro M (2014) Vascular effects of the Mediterranean diet-part II: role of omega-3 fatty acids and olive oil polyphenols.*Vascul Pharmacol* **63**(3): 127-34.

Segura-Aguilar J, Paris I, Muñoz P, Ferrari E, Zecca L, Zucca F (2014) A Protective and toxic roles of dopamine in Parkinson's disease. *J Neurochem* **129**: 898-915.

Sian J, Dexter DT, Lees AJ, Daniel S, Jenner P, Marsden CD (1994) Glutathione-related enzymes in brain in Parkinson's disease. *Ann Neurol* **36**(3): 356-61.

Smith MA, Kutty RK, Richey PL, Yan SD et al and Perry G (1994) Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am J Pathol* **145**(1): 42-47

Soni MG, Burdock GA, Christian MS, Bitler CM, Crea R (2006) Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. *Food Chem Toxicol* **44**(7): 903-15.

Spencer JP, Jenner A, Aruoma OI, Evans PJ et al and Halliwell B (1994) Intense oxidative DNA damage promoted by L-dopa and its metabolites. Implications for neurodegenerative disease. *FEBS Lett* **353**(3): 246-50.

Spencer JP, Whiteman M, Jenner P, Halliwell B (2002) 5-s-Cysteinyl-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases in caspase-3 activity in neurons. *J Neurochem* 81(1):122-9.

Sultana R, Poon HF, Cai J, Pierce WM et al and Butterfield DA (2006) Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. *Neurobiol Dis* **22**(1): 76-87.

Sulzer D, Zecca L (2000) Intraneuronal dopamine-quinone synthesis: a review. *Neurotox Res* **1**(3): 181-95.

Sun YM, Wang YT, Jiang L, Xue MZ (2016) The effects of deferoxamine on inhibition for microglia activation and protection of secondary nerve injury after intracerebral hemorrhage in rats Pak. *J Pharm Sci* **29**(3 Suppl): 1087-93.

Swomley AM, Butterfield DA (2015) Oxidative stress in Alzheimer disease and mild cognitive impairment: evidence from human data provided by redox proteomics. *Arch Toxicol* **89**: 1669-1680

Takeda A, Perry G, Abraham NG, Dwyer BE et al and Smith MA (2000) Overexpression of heme oxygenase in neuronal cells, the possible interaction with Tau. *J Biol Chem* **275**(8): 5395-9.

Texel SJ, Mattson MP (2011) Impaired adaptive cellular responses to oxidative stress and the pathogenesis of Alzheimer's disease. *Antioxid Redox Sign* **14**(8): 1519-34.

Tramutola A, Di Domenico F, Barone E, Giorgi A et al and Perluigi M (2016) Poly-Ubiquitinylation Profile in Down Syndrome Brain before and after the Development of Alzheimer Neuropathology. *Antioxid Redox Signal* Epub PMID: 27627691

Trovato A, Siracusa R, Di Paola R, Scuto M et al and Calabrese V. (2016) Redox modulation of cellular stress response and lipoxin A4 expression by Hericium Erinaceus in rat brain: relevance to Alzheimer's disease pathogenesis. *Immun Ageing* **13**: 23.

Tuck KL, Freeman MP, Hayball PJ, Stretch GL, Stupans I (2001) The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *J Nutr* **131**(7): 1993-1996.

Uttara B, Singh AV, Zamboni P, Mahajan RT (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* (**1**): 65-74.

Valavanidis A, Vlachogianni T, Fiotakis K (2009) Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int J Environ Res Public Health* **6**: 445-462.

Valls RM, Farras M, Suarez M, Fernandez-Castillejo S et al and Sola R (2015) Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients. A randomized controlled trial. *Food Chem* **167**: 30-35.

Valls-Pedret C, Lamuela-Raventós RM, Medina-Remón A, Quintana M et al and Ros E (2012) Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. *J Alzheimers Dis* **29**(4): 773-82

Vanella L, Barbagallo I, Tibullo D, Forte S, Zappalà

A, Li Volti G (2016) The non-canonical functions of the heme oxygenases. *Oncotarget* Epub PMID 27626166.

Vaubel RA, Raghunathan A, Erickson LA (2016) Parkinson disease. *Mayo Clin Proc* **91**: e155-e156.

Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. *Biochem Biophys Res Commun* **247**(1): 60-64.

Visioli F, Wolfram R, Richard D, Abdullah MI, Crea R (2009) Olive phenolics increase glutathione levels in healthy volunteers. *J Agric Food Chem* **57**(5): 17939-6.

Von Bernhardi R, Eugenín J. (2012) Alzheimer's disease: redox dysregulation as a common denominator for diverse pathogenic mechanisms. *Antioxid Redox Signal* **16**: 974-1031.

Wang X, Thomas B , Sachdeva R , Arterburn L et al and Ma J (2003) Mechanism of arylating quinone toxicity involving Michael adduct formation and induction of endoplasmic reticulum stress Wenk GL. Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry* **64**: 7-10.

Xiang PY, Janc O, Grochowska KM, Kreutz MR, Reymann KG (2016) Dopamine agonists rescue Aβ-induced LTP impairment by Src-family tyrosine kinases. *Neurobiol Aging* **40**: 98-102.

Xin W, Shen XM, Li H, Dryhurst G (2000) Oxidative metabolites of 5-S-cysteinylnorepinephrine are irreversible inhibitors of mitochondrial complex I and the alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase complexes: possible implications for neurodegenerative brain disorders. *Chem Res Toxicol* **13**(8): 749-60.

Yamamori T, Yasui H, Yamazumi M, Wada Y, Nakamura Y, Nakamura H, Inanami O (2012) Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. *Free Radic Biol Med* **53**: 260-270.

Yu X, Song N, Jiang H, Xie J (2013) HO-1 participates differentially in astroglia and neurons vulnerability. *Proceedings of The Phys Society*.

Yingru Liu, Bing Z, Liqing L, Ping L, Donald P W. Max CS (2001) Heme oxygenase-1 plays an important protective rol e in experimental autoimmune encephalomyelitis. *Clin Neurosci Neuropathol* **12**(9): 1841-1845

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR. (2004) Iron, brain ageing and neurodegenerative disorders. *Nat Rev Neurosci* **5**(11): 863-73.

Zhang JY, Deng YN, Zhang M, Su H, Qu QM (2016) SIRT3 Acts as a neuroprotective agent in rotenone-induced Parkinson cell model. *Neurochem Res* **41**: 1761-1773.

Zhang X, Cao J, Zhong L (2009) Hydroxytyrosol inhibits pro-inflammatory cytokines, iNOS, and COX-2 expression in human monocytic cells. *Naunyn Schmiedebergs Arch Pharmacol* **379**(6): 581-6.

Zheng A, Li H, Cao K, Xu J et al and Feng Z (2015) Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring. *J Nutr Biochem* **26**(2): 190-9.

Zhu L, Liu Z, Feng Z, Hao J et al and Liu J (2010) Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. *J Nutr Biochem* **21**(11): 1089-98.

Ziegler-Graham K, Brookmeyer R, Johnson E, Arrighi HM (2008) Worldwide variation in the doubling time of Alzheimer's disease incidence rates. *Alzheimers Dement* **4**: 316-323

Zimprich A, Müller-Myhsok B, Farrer M, Leitner P et al and Gasser T (2004) The PARK8 locus in autosomal dominant parkinsonism: confirmation of linkage and further delineation of the disease-containing interval. *Am J Hum Genet* **74**(11): 11-9.

Zucca FA, Segura-Aguilar J, Ferrari E, Muñoz P et al and Zecca L (2015) Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. *Prog Neurobiol* Epub PMID: 26455458

Zuo L, Hemmelgarn BT, Chuang CC, Best TM (2015) The Role of Oxidative Stress-Induced Epigenetic Alterations in Amyloid-β Production in Alzheimer's Disease. *Oxid Med Cell Longev* Epub PMID: 26523231.

INSIGHTS INTO STRESS RESILIENCE

A. CARUSO[1], D. MANGO[1,2], F. NICOLETTI[1,3] and S. SCACCIANOCE[1]

[1]*Department of Physiology and Pharmacology, University of Rome "Sapienza", Rome, Italy* [2]*European Brain Research Institute, Rita Levi-Montalcini Foundation, Rome, Italy;* [3]*I.N.M. Neuromed, Pozzilli, Italy*

The land-mark paper published 80 years ago by Hans Selye, in which he introduced the concept of the general adaptation syndrome, set the scene of research into the biology of stress. However, the mechanism conferring to an individual the ability to efficiently cope with stress is still unclear. Here, we review the current knowledge of stress resilience. Animal research has indicated that active resilience is achieved through a mechanism of molecular adaptation finalized to counteract maladaptive molecular changes seen in susceptible animals, thus offering potential biomarkers of human resilience. Both early experiences and environmental conditions in adulthood can influence resilience. In preclinical research, those experimental paradigms have indicated a pivotal role of hippocampal glutamatergic synapses; its derangement could be an etiological determinant of psychiatric disorders. Finally, since epigenetic mechanisms are involved in conferring resiliency, in searching for pharmacotherapeutic interventions attention has been devoted to agents able to produce what has been termed as epigenetic allostasis.

The 1980 movie *Mon oncle d'Amérique* Henri Laborit, directed by Alain Resnais, clearly demonstrated that the same stressor could have different outcomes, either stomach ulcers or no pathological problems. The aim of this review is to describe the present knowledge, originating from preclinical studies, on the biological mechanism characterizing the ability to efficiently cope with stress.

Resilience can be defined as the ability of something to return to its original shape after it has been pulled, stretched, pressed or bent. In biomedical sciences, its meaning is not much different because it refers to the capacity of an individual to avoid negative social, psychological, and biological consequences of severe stress that would otherwise compromise their psychological or physical fitness (Russo et al, 2012). Resilience has been identified as a protective factor against the development of mental disorders and a risk factor for a number of clinical conditions as it relates to post-traumatic stress disorder (PTSD) and major depressive disorder (MDD). Several human studies have indicated that resilience does not represent the absence of pathological responses that occur in susceptible individuals but an active, adaptive process (Charney, 2004; Feder et al, 2009). The former can be defined as passive resilience while the latter as active resilience.

ANIMAL RESEARCH

Experimental research in animal models has demonstrated that active resilience is achieved through a mechanism of molecular adaptation finalized to counteract maladaptive molecular changes seen in susceptible animals (McEwen et al,

Key words: stress, resilience, hippocampus, glutamate, psychiatric disorders

Corresponding author:
Prof. Sergio Scaccianoce,
Dipartimento di Fisiologia e Farmacologia ,
University of Rome "Sapienza", Piazzale Aldo Moro, 5 - Roma
Tel.: +39 06 49912511
e-mail: sergio.scaccianoce@uniroma1.it

2279-5855 (2016)

2015). These studies not only provide mechanistic insight into the biological basis of active coping but could also indicate potential biomarkers of human resilience (Walker et al, 2016).

Research on the biochemical basis of resilience is hampered by strain and gender differences. For example, when C57BL6/J mice are subjected to social defeat, an experimental model widely used in rodents to explore the neurobiology of defeat-induced depressive-like behaviors, resilience is observable in ~35% mice, whereas resilience is closer to 100% in other strains such as CD1 or FVB (Golden et al, 2011). Gender differences in the resilience profile have been observed in response to chronic unpredictable stress. This experimental condition induces depression-associated behaviors in female mice, whereas males are resilient because they do not develop these behavioral abnormalities (Hodes et al, 2015; LaPlant et al, 2009). However, the issue is intrigued by the recognition that in response to chronic restraint stress, stressed female rats performed better than males in spatial memory tasks (Conrad et al, 2003; Galea et al, 1997; Bowman et al, 2003). As a result, several studies have focused on the role of sexual hormones on different aspects of resilience (see Luine for a recent review on this topic). Before reviewing molecular and cellular mechanisms that underlie resilience in rodent models, it is necessary to offer a precise definition of resilience in experimental settings. Unfortunately, there is no unequivocal definition of resilience in animal studies. The group led by Eric Nestler in their seminal work defined resilience to chronic social defeat stress "as the absence of social avoidance, anhedonia, and metabolic syndrome, which are highly correlated with one another" (Russo et al, 2012). Evidence indicates that resilience is promoted by the ability/possibility to exert a form of "control" over cessation of the stress. For example, we have demonstrated that rats who learned how to avoid an electric foot-shock stimulation in a two-compartment shuttle box, resulted in an increase of hippocampal brain-derived neurotrophic factor (BDNF) although their plasma levels of corticosterone (an hormone that normally down regulates BDNF) were highly increased (Scaccianoce et al, 2003). Moreover, studies on mice characterized by a good ability to avoid an inescapable stress led to the identification of substance P in the ventrolateral periaqueductal gray as a key factor involved in the resilience endophenotype (Berton et al, 2007). Other forms of stress were used in order to investigate the biochemical basis of resilience. For example, exposure of a rat to predator odor induces anxiety-like behavior and increases acoustic startle response. Using this experimental approach, it has been demonstrated that the most behaviorally affected rats show a reduced neuropeptide Y expression in several brain areas (Cohen et al, 2012). In rodents, chronic mild stress induces anhedonia-like behavior (as measured by reduced sucrose consumption). Combining this paradigm with structural magnetic resonance imaging and spectroscopy, Delgado et al. (Delgado y Palacios, 2011) demonstrated that subjects (ca. 30%) which did not exhibit behavioral disturbances did not show a reduction in hippocampal volume or alterations in glutamate metabolism, as instead observed in susceptible mice. The role of glutamate receptors in mechanisms of resilience to stress has been recently investigated by Carla Nasca and her colleagues in the lab of Prof. Bruce McEwen (Nasca et al, 2015). Their research describes a mechanism by which glucocorticoids, acting via mineralocorticoid receptors (MRs), decrease resilience to stress by down-regulating mGlu2 receptors in the hippocampus. mGlu2 receptors exert an inhibitory control on glutamate release; hence, a reduced expression of mGlu2 receptors may result in an enhanced glutamate release. In addition, Nasca et al. (2015) were able to demonstrate the existence of an epigenetic link between MRs and mGlu2 receptors. More precisely, MRs inhibit K27 acetylation of H3 histone mediated by the histone-acetyltransferase (HAT), p300, on the GRM2 gene promoter, thereby reducing the expression of mGlu2 receptors.

Pro–resilience adaptation mechanisms have been examined using immediate-early gene activation. In particular, recent studies have shown a greater degree of c–Fos, FosB, or ΔFosB expression in glutamatergic neurons of the medial prefrontal cortex (PFC) (infralimbic, paralimbic PFC) of resilient

mice following chronic predator or social defeat stress (Adamec et al, 2012; Covington et al, 2010). These data would suggest that increased neuronal activation within this brain region, as reflected by the expression of immediate early genes, might represent a pro-resilience adaptation. While an increased glutamatergic tone on medium spiny neurons in the nucleus accumbens (NAc) (i.e., increased frequency of excitatory currents and number of glutamatergic synapses) is associated with vulnerability to chronic social defeat stress, recent findings suggest a form of active resilience which counteracts this susceptibility mechanism (Christoffel et al, 2011; Vialou et al, 2010). Specifically, ΔFosB induction in the NAc of resilient mice induces the expression of the GluA2 subunit of AMPA receptors. This subunit restrains Ca^{2+} permeability of AMPA-gated ion channels, thereby decreasing the responsiveness of NAc neurons to glutamate. As hypothesized by Vialou and colleagues, this active neuroadaptation might render a salient stimulus, such as a novel mouse in the social defeat paradigm, less able to activate NAc neurons, thereby enabling goal-directed behavior to continue despite the stress (Vialou et al, 2010). The ventral tegmental area (VTA) and its forebrain projection regions, in particular the NAc, have been actively investigated in order to identify the molecular mechanisms underlying resilience. This VTA-NAc circuit plays a critical integrative role in reward- and emotion-related behaviors. In response to chronic social defeat stress, an experimental paradigm commonly used in rodents, the firing rate of VTA dopamine neurons is increased in susceptible mice, while it is unaffected in resilient subjects. By combining behavioral testing, gene knockdown, gene expression microarrays and electrophysiology, Krishnan and colleagues have demonstrated the presence of active, independent process occurring in resilient mice that normalizes VTA firing to control levels (Krishnan et al, 2007). They found that in both susceptible and resilient mice, the hyper–excitability of VTA dopamine neurons is mediated by induction of hyperpolarization–activated cation current (Ih). However, in resilient mice, they identified an increase of mRNAs encoding three K^+ channels that hyperpolarize neurons, thus decreasing their excitability. Corticotrophin-releasing hormone (CRH), expressed by, and secreted from the parvocellular neurons of the paraventricular nucleus (PVN) in the hypothalamus, is the key mediator of the neuroendocrine stress response. Sustained and prolonged activation of the CRH system has been linked to stress-related emotional disorders such as anxiety, anorexia nervosa and depression (Gold, 2015). After chronic social defeat stress, *Crh* gene expression is increased in the PVN of hypothalamus of susceptible adult mice, and this parallels the development of social avoidance. In contrast, in a subset of animals, termed "resilient for the lack of social avoidance", the *Crh* gene is hypermethylated and silenced to prevent *Crh* induction (Elliot et al, 2010). Based on these results, it could be suggested that stress-induced changes in chromatin-regulating enzymes may modulate DNA methylation, reinforcing a link between experience-based and epigenetic factor. Clearly, these findings offer a potential usefulness to understand stress vulnerabilities in humans and how the regulation of genomic DNA methylation of key stress-response genes may influence psychological susceptibility or resilience to environmental stressors (Zannas and West, 2014).

VULNERABILITY AND RESILIENCE IN INFANCY

There is no doubt that childhood trauma, particularly maltreatment, increases the risk for psychopathology (Maccari et al, 2016; Maccari et al, 2014; McLaughlin et al, 2013). The parent-infant relationship is an important context for the characterization of very early risk and resilience factors and for the identification of new targets for the development of preventative interventions. In non-human primates, intermittent mother-offspring separation induces a behavioral and biochemical phenotype which is indicative of a better coping strategy towards stress, or, in other words, promotes resilience (Parker et al, 2004; Parker et al, 2005). Of note, that intermittent exposure to early life stress induces resilience has been proposed by Levine and his colleagues in the mid-1950s (Levine

et al, 1956; Levine and Moody, 2003). On this specific topic, several researches have used rodent models. For example, when maternal deprivation is combined with chronic unpredictable stress (CUS) in the juvenile age, the offspring shows a greater degree of behavioral resilience in respect to maternal deprivation alone or administration of CUS in adulthood (Rincon et al, 2012). Meaney and co-workers have extensively investigated the effects of early maternal care in rats on the behavioral and neuroendocrine profile of the adult progeny (Francis and Meaney, 1999). More precisely, they focused on central (mainly hippocampal) regulation of the hypothalamic-pituitary-adrenal (HPA) axis, a neuroendocrine axis systematically studied over several decades by McEwen and colleagues. In particular, the latter group tackled key aspects, including how stress positively or negatively influences resilience (McEwen and Gianaros, 2011). On the other hand, the group led by Meaney has characterized several aspects related to mother-infant relationship: among them, they demonstrated that high levels of maternal care (licking/grooming and arched-back nursing) are associated with decreased DNA methylation of the glucocorticoid receptor gene, higher levels of glucocorticoid receptor expression, greater feedback inhibition of the HPA axis and resilient stress responses in adulthood (see Szyf et al, 2005 for a review). Notably, they also offered potential translational aspects that are based on socioeconomic context and childhood socioeconomic status (Hackman et al, 2010).

ENVIRONMENTAL CONDITIONS AND RESILIENCE

Housing a rat in a context of "enriched environment" produces several biochemical and behavioral effects (Chapillon et al, 2002). In particular, enriched environmental (EE) housing reduces stress-induced alterations in neurobiological systems, promotes adaptability, and extinguishes submissive behavioral traits developed during social defeat stress (Schloesser et al, 2010; Takuma et al, 2011). An infralimbic cortex-dependent neuroanatomical pathway has been characterized as the key neurochemical pathway responsible for the EE-dependent induction of psychological resilience to chronic social defeat (Lehmann and Herkenham, 2011). Of note, recent research has stigmatized that the source of "enriching" stimuli may differentially affect the brain and behavior (Bardi et al, 2016; Lambert et al, 2016). Thus, the nature of elements placed in the complex environment (i.e., paper or plastic) must be carefully chosen in order to perform studies aimed at elucidating the relationship between EE conditions and biochemical and behavioral resilience.

THERAPEUTIC IMPLICATIONS

Without any doubt, the knowledge gained through the use of animal models raises the possibility to untangle the intricate mechanism conferring to an individual the ability to prevent or reverse the deleterious effect of stress. However, at present in the clinical setting the main therapeutic options are represented by the use of antidepressants frequently associated with psychological therapies, although in patients suffering from PTSD, evidence to support the use of pharmacotherapy combined with psychological therapy over either treatment method separately is insufficient (Hetrick et al, 2010; Bisson et al, 2015). Pharmacological studies over the past decades have shed light on the mechanism(s) of action of antidepressant drugs. Nevertheless, data from preclinical studies have indicated that most of the stress-induced changes in gene expression are reversed by chronic imipramine treatment, and that resilient mice show patterns of chromatin regulation that overlap dramatically with those seen with imipramine treatment (Wilkinson et al, 2009). As described above, in a mouse model of resiliency it was possible to observe a specific ΔFosB induction in the NAc. Interestingly, ΔFosB induction is also required for the ability of the standard antidepressant, fluoxetine, to reverse behavioral pathology induced by social defeat (Vialou et al, 2010). These findings raise the possibility that antidepressant drugs promote their clinical effect by inducing in depressed patients some of the adaptations naturally occurring in resilient individuals. Epigenetic

mechanisms are involved in conferring resiliency (see above). Among possible pharmacotherapeutic interventions suitable for patients in whom stress is responsible for the induction of neuropsychiatric disorders, attention has been devoted to agents able to produce what has been termed epigenetic allostasis (McEwen et al, 2015). Among candidate drugs with such characteristics, considerable attention has been addressed to l-acetylcarnitine (LAC). Indeed, experiments conducted by Nasca and colleagues, have elucidated the possible mechanism of action of LAC (Bigio et al, 2016; Lau et al, 2016; Nasca et al, 2013). In particular, LAC enhances the acetylation of NF-κB-p65 subunit and H3 histone, thereby increasing transcription of the *Grm2* gene encoding for the mGlu2 receptor in hippocampus and prefrontal cortex. These findings reinforce a pivotal role of malfunctioning glutamatergic synapses (i.e. reduced presynaptic negative feed-back tone) as an etiological determinant of psychiatric disorders. Although at present readily accessible biomarkers of resilience in humans are lacking, it could be concluded that if preclinical research will be so fruitful as it was in recent years, it might be possible to offer novel therapeutic targets as well as identify environmental factors threatening resilience in human beings.

REFERENCES

Adamec R, Toth M, Haller J, Halasz J, Blundell J (2012) A comparison of activation patterns of cells in selected prefrontal cortical and amygdala areas of rats which are more or less anxious in response to predator exposure or submersion stress. *Physiol Behav*. **105** :628-38.

Bardi M, Kaufman C, Franssen C, Hyer MM, Rzucidlo A, Brown M, Tschirhart M, Lambert KG (2016) Paper or Plastic? Exploring the Effects of Natural Enrichment on Behavioural and Neuroendocrine Responses in Long-Evans Rats. *J Neuroendocrinol.* **28**:1-12.

Berton O, Covington HE 3rd, Ebner K, Tsankova NM, Carle TL, Ulery P, Bhonsle A, Barrot M, Krishnan V, Singewald GM, Singewald N, Birnbaum S, Neve RL, Nestler EJ (2007) Induction of deltaFosB in the periaqueductal gray by stress promotes active coping responses. *Neuron.* **55:**289-300.

Bigio B, Mathé AA, Sousa VC, Zelli D, Svenningsson P, McEwen BS, Nasca C (2016) Epigenetics and energetics in ventral hippocampus mediate rapid antidepressant action: Implications for treatment resistance. *Proc Natl Acad Sci U S A*. Jul 12;113(28):7906-11. doi: 10.1073/pnas.1603111113. Epub 2016 Jun 27.

Bisson JI, Cosgrove S, Lewis C, Robert NP (2015) Post-traumatic stress disorder. *BMJ.* Nov 26;351:h6161. doi: 10.1136/bmj.h6161.

Bowman RE, Beck KD, Luine VN (2003) Chronic stress effects on memory: sex differences in performance and monoaminergic activity. *Horm Behav.* **43**:48-59.

Chapillon P, Patin V, Roy V, Vincent A, Caston J (2002) Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. *Dev Psychobiol.* **41**: 373-87.

Charney DS (2004) Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. *Am J Psychiatry.* **161**:195-216.

Christoffel DJ, Golden SA, Dumitriu D, Robison AJ et al and Russo SJ (2011) IκB kinase regulates social defeat stress-induced synaptic and behavioral plasticity. *J Neurosci.* **31**: 314-21.

Cohen H, Liu T, Kozlovsky N, Kaplan Z, Zohar J, Mathé AA (2012) The neuropeptide Y (NPY)-ergic system is associated with behavioral resilience to stress exposure in an animal model of post-traumatic stress disorder. *Neuropsychopharmacology.* **37**:350-63.

Conrad CD, Grote KA, Hobbs RJ, Ferayorni A (2003) Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiol Learn Mem.* **79**:32-40.

Covington HE 3rd, Lobo MK, Maze I, Vialou V, Hyman JM et al and Nestler EJ (2010) Antidepressant effect of optogenetic stimulation of the medial prefrontal cortex. *J Neurosci.* **30**:16082-90.

Delgado y Palacios R, Campo A, Henningsen K, Verhoye M et al and Van der Linden A (2011) Magnetic resonance imaging and spectroscopy reveal differential hippocampal changes in anhedonic and resilient subtypes of the chronic mild stress rat model. *Biol Psychiatry.* **70**:449-57.

Elliott E, Ezra-Nevo G, Regev L, Neufeld-Cohen A,

Chen A (2010) Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. *Nat Neurosci.* **13**:1351-3.

Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci.* **10**:446-57.

Francis DD, Meaney MJ (1999). Maternal care and the development of stress responses. *Curr Opin Neurobiol.* **9**:128-34.

Galea LA, McEwen BS, Tanapat P, Deak T, Spencer RL, Dhabhar FS (1997) Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience.* **81**:689-97.

Gold PW (2015) The organization of the stress system and its dysregulation in depressive illness. *Mol Psychiatry.* **20**:32-47.

Golden SA,Covington HE 3rd, Berton O, Russo SJ (2011) A standardized protocol for repeated social defeat stress in mice. *Nat Protoc.* **6**: 1183-91

Hackman DA, Farah MJ, Meaney MJ (2010) Socioeconomic status and the brain: mechanistic insights from human and animal research. *Nat Rev Neurosci.* **11**:651-9.

Hetrick SE, Purcell R, Garner B, Parslow R (2010) Combined pharmacotherapy and psychological therapies for post traumatic stress disorder (PTSD). *Cochrane Database Syst Rev.* 2010 Jul 7;(7):CD007316. doi: 10.1002/14651858.CD007316.pub2.

Hodes GE, Pfau ML, Purushothaman I, Ahn HF et al and Russo SJ (2015). Sex differences in nucleus accumbens transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress. *J Neurosci.* **35**:16362-76.

Krishnan V, Han MH, Graham DL, Berton O et al and Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* **131**:391-404.

Lambert K, Hyer M, Bardi M, Rzucidlo A et al and Kinsley C (2016) Natural-enriched environments lead to enhanced environmental engagement and altered neurobiological resilience. *Neuroscience.* **330**:386-94.

LaPlant Q, Chakravarty S, Vialou V, Mukherjee S et al and Russo SJ (2009) Role of nuclear factor kappaB in ovarian hormone-mediated stress hypersensitivity in female mice. *Biol Psychiatry.* **65**:874-80

Lau T, Bigio B, Zelli D, McEwen BS, Nasca C (2016) Stress-induced structural plasticity of medial amygdala stellate neurons and rapid prevention by a candidate antidepressant. *Mol Psychiatry*. 2016 May 31. doi: 10.1038/mp.2016.68.doi: 10.1038/mp.2016.68.

Lehmann ML, Herkenham M (2011) Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. *J Neurosci.* **31**:6159-73.

Levine S, Chevalier JA, Korchin SJ (1956) The effects of early shock and handling on later avoidance learning. *J Pers.* **24**:475-93.

Levine S, Mody T (2003) The long-term psychobiological consequences of intermittent postnatal separation in the squirrel monkey. *Neurosci Biobehav Rev.* **27**:83-9.

Luine V (2016) Estradiol: Mediator of memories, spine density and cognitive resilience to stress in female rodents. *J Steroid Biochem Mol Biol.* **160**:189-95.

Maccari S, Krugers HJ, Morley-Fletcher S, Szyf M, Brunton PJ (2014) The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. *J Neuroendocrinol.* **26**:707-23.

Maccari S, Polese D, Reynaert ML, Amici T, Morley-Fletcher S, Fagioli F (2016) Early-life experiences and the development of adult diseases with a focus on mental illness: The Human Birth Theory. *Neuroscience.* 2016 May 26. pii: S0306-4522(16)30201-9. doi: 10.1016/j.neuroscience.2016.05.042.

McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C (2015) Mechanisms of stress in the brain. *Nat Neurosci.* **18**:1353-63.

McEwen BS, Gianaros PJ (2011) Stress- and allostasis-induced brain plasticity. *Annu Rev Med.* **62**:431-45.

McLaughlin KA, Koenen KC, Hill ED, Petukhova M, Sampson NA, Zaslavsky AM, Kessler RC (2013) Trauma exposure and posttraumatic stress disorder in a national sample of adolescents. *J Am Acad Child Adolesc Psychiatry* **52**:815-830.e14.

Nasca C, Bigio B, Zelli D, Nicoletti F, McEwen BS

(2015) Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility. *Mol Psychiatry.* **20**:755-63.

Nasca C, Xenos D, Barone Y, Caruso A, Scaccianoce S, Matrisciano F, Battaglia G, Mathé AA, Pittaluga A, Lionetto L, Simmaco M, Nicoletti F (2013) L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. *Proc Natl Acad Sci U S A.* **110**:4804-9.

Parker KJ, Buckmaster CL, Justus KR, Schatzberg AF, Lyons DM (2005) Mild early life stress enhances prefrontal-dependent response inhibition in monkeys. *Biol Psychiatry.* **57**:848-55.

Parker KJ, Buckmaster CL, Schatzberg AF, Lyons DM (2004) Prospective investigation of stress inoculation in young monkeys. *Arch Gen Psychiatry.* **61**:933-41.

Ricon T, Toth E, Leshem M, Braun K, Richter-Levin G (2012) Unpredictable chronic stress in juvenile or adult rats has opposite effects, respectively, promoting and impairing resilience. *Stress.* **15**:11-20.

Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ (2012) Neurobiology of resilience. *Nat Neurosci.* **15**:1475-84.

Scaccianoce S, Del Bianco P, Caricasole A, Nicoletti F, Catalani A (2003) Relationship between learning, stress and hippocampal brain-derived neurotrophic factor. *Neuroscience.* **121**:825-8.

Schloesser RJ, Lehmann M, Martinowich K, Manji HK, Herkenham M (2010) Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. *Mol Psychiatry.* **15**:1152-63.

Szyf M, Weaver IC, Champagne FA, Diorio J, Meaney MJ (2005) Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrinol.* **26**:139-62.

Takuma K, Ago Y, Matsuda T (2011) Preventive effects of an enriched environment on rodent psychiatric disorder models. *J Pharmacol Sci.* **117** :71-6.

Mouzon E, Rush AJ 3rd, Watts EL, Wallace DL et al and Nestler EJ (2010) DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat Neurosci.* **13**:745-52.

Walker FR, Pfingst K, Carnevali L, Sgoifo A, Nalivaiko E (2016) In the search for integrative biomarker of resilience to psychological stress. *Neurosci Biobehav Rev.* 2016 May 11. pii: S0149-7634(15)30360-2.doi:10.1016/j.neubiorev.2016.05.003.

Wilkinson MB, Xiao G, Kumar A, LaPlant Q, Renthal W, Sikder D, Kodadek TJ, Nestler EJ (2009) Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models. *J Neurosci.* **29**:7820-32.

Zannas AS, West AE (2014) Epigenetics and the regulation of stress vulnerability and resilience. *Neuroscience.* **264**:157-70.

THE THERAPEUTIC POTENTIAL OF AUTOPHAGY IN DUCHENNE MUSCULAR DYSTROPHY

I. DI RENZO[1], F. PROIETTI SERAFINI[2], A. GIAVAZZI[3], S. ZECCHINI[3], M. GIOVARELLI[1], D. CERVIA[1,2], C. PERROTTA[1], C. DE PALMA[3] and E. CLEMENTI[1,3,4]

[1]Department of Biomedical and Clinical Sciences "Luigi Sacco" (DIBIC), Università degli Studi di Milano, Italy
[2]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy
[3]Unit of Clinical Pharmacology, University Hospital "Luigi Sacco"-ASST Fatebenefratelli Sacco, Milano, Italy
[4]Scientific Institute IRCCS Eugenio Medea, Bosisio Parini, Italy

Muscular dystrophies are heterogeneous, genetically based diseases affecting mainly skeletal muscle. The most severe form of this group of disorders is Duchenne muscular dystrophy (DMD), caused by mutations in the dystrophin gene leading to chronic local inflammation, muscle weakness and wasting, exhaustion of satellite cell pool and impaired regenerative capacity accounting for substitution of myofibres by connective and adipose tissue. DMD boys show progressive skeletal muscle damage finally resulting in paralysis and death, usually for cardiac and respiratory problems. Currently, there is no cure for DMD, but preclinical and clinical settings with different drugs are ongoing in order to ameliorate the course of the disease and delay the muscle degeneration. Autophagy is a proteolytic cell mechanism with a key role in the elimination of misfolded or aggregated proteins and damaged organelles. The autophagic process is constantly operating in skeletal muscle and maintaining its balance is fundamental in regulating tissue homeostasis: when highly activated, autophagy may be unfavorable and induce muscle wasting, while when switching off it can trigger weakness and muscle degeneration, following the unrestrained accumulation of damaged organelles and proteins. Recent studies have begun to investigate the role of dysfunctional autophagy in DMD pathogenesis, identifying the Akt-mTOR axis as one of the key pathways that are disregulated. However, the molecular events underlying this dysregulation need to be investigated further. Here, we provide evidence on the clinical potential of autophagy signalling in DMD. This picture may pave the way for the development of new strategies to boost muscle growth thus limiting dystrophic muscle damage.

PATHOLOGY OF DYSTROPHIN DEFICIENCY

Duchenne muscular dystrophy (DMD) is a fatal X-linked disease due to a wide range of mutations in the dystrophin gene, the largest gene inside the human genome, and represents one of the most severe and diffuse type of muscular dystrophies with a high incidence (1 out of 3,500 newborns) (Govoni et al, 2013). At present, more than 4,700 mutations on dystrophin gene have been detected: deletions (65.8%) and duplications (13.6%) account for the majority of gene mutations, whereas point mutations

Key words: autophagy signalling, muscle impairment, mdx mice, Duchenne muscular dystrophy, Akt-mTOR axis

Corresponding author:
Prof. Emilio Clementi,
Department of Biomedical and Clinical Sciences "Luigi Sacco" (DIBIC),
Università degli Studi di Milano,
via G.B. Grassi 74, 20157 Milano, Italy
Tel.: +39 02 50319683 - Fax: +39 02 50319682
e-mail: emilio.clementi@unimi.it

2279-5855 (2016)

(micro-insertions, microdeletions, nonsense, missense, and splicing mutations) represent the smaller part (20.6%) (Magri et al, 2011). In DMD muscles the dystrophin mutations typically create premature stop codons or change the reading frame of the gene resulting in the complete loss of the protein. Nonetheless, other mutations can generate smaller forms of dystrophin or can account for low protein production, thus leading to Becker muscular dystrophy (BMD), an allelic dystrophinopathy disorder less severe than DMD and affecting 1 of 18,518 male births (Emery, 1991).

The diagnosis of DMD and BMD requires the analysis of clinical symptoms supported by additional tests such as muscle biopsy and genetic assays. Typically, DMD arises early, before the third year of age, manifesting ambulation loss between 10 and 14 years of age; usually patients die between 20 and 30 years of age (Davies et al, 1988). In fact, reduced wall and lung compliance, hypoxemia, hypercapnia, and hypoventilation associated with diaphragm wasting (the most affected muscle in DMD) determines respiratory failure and relative death (Fayssoil et al, 2010; Mosqueira et al, 2013). Other muscles involved in DMD degeneration are the cardiac muscle and, to a small extent, smooth muscles (Fayssoil et al, 2010; Mosqueira et al, 2013). Cardiomyopathy is a common trait of DMD disease (affecting more than 90% of patients) (Fayssoil et al, 2010; Mosqueira et al, 2013) inducing a progressive impairment of the ejection fraction which might lead to arrhythmias and heart failure (Jefferies et al, 2005). Usually, the onset of cardiomyopathy in DMD patients starts from 18 years of age (Spurney, 2011; Politano and Nigro, 2012), whereas BMD patients show a stronger cardiac decline, in addition to skeletal muscle wasting (Bushby et al, 2010).

Dystrophic muscle fibres are more sensitive to eccentric contraction than their healthy counterparts (Lynch et al, 2000); in response to contractile stress, they undergo continued cycles of necrosis and regeneration processes during which satellite cells (the multipotent myogenic precursor pool into skeletal muscle fibres) trigger muscle regeneration (Le Grand and Rudnicki, 2007; Biressi and Rando, 2010). In dystrophic condition, the repeated cycles of necrosis and regeneration exhaust the skeletal muscle regenerative potential leading to an inefficient process, ultimately resulting in satellite cell number drop and gradual substitution of muscle with fat and connective tissue. Thus, the loss of dystrophin is responsible for a chain of events reinforcing a progressive muscle wasting and weakness.

The integrity and the functionality of the sarcolemma is ensured by the multimeric dystrophin-associated glycoprotein complex; dystrophin constitutes the essential cytoskeletal scaffolding protein of this complex, recruiting crucial structural and signalling factors to the muscle membrane and creating a highly organized network. The dystrophin-associated glycoprotein complex consists of a large number of tissue specific transmembrane and peripheral proteins, including several sarcoplasmic proteins (α- and β-dystrobrevins, α1-, β1-, β2-, γ1-, and γ2-syntrophins, and neuronal nitric oxide synthase (nNOS)), transmembrane proteins (β-dystroglycan, α-, β-, γ-, δ-, ε-, and ζ-sarcoglycans, sarcospan, and caveolin-3) and extracellular proteins (α-dystroglycan and laminin). During muscle contraction the sarcolemma of fibres is mechanically stabilized by a complex of glycoproteins associated to Dystrophin, acting there as a scaffold for other proteins implicated in intracellular signalling transduction (Straub and Campbell, 1997). The dystrophin-associated complex is composed of glycoproteins tightly connected to each other and any modification in one of these proteins affects the others, leading to different muscular defects.

In particular, the disruption of the dystrophin-associated glycoprotein complex in DMD alters the localization of the nNOS in the sarcolemma, thus generating lower NO production.

NO plays a crucial role in skeletal muscle physiology, balancing energy consumption and excitation-contraction coupling (De Palma and Clementi, 2012). The loss of sarcolemmal nNOS and, consequently, of the NO-dependent signalling pathways significantly reduces muscle bulk and force generation, causing increased fatigability (De

Palma and Clementi, 2012). Another consequence of nNOS loss in the sarcolemma region is the increase of muscle activity-related injury and damage, and the increased risk of ischemia during exercise (Thomas, 2013).

Mouse models

Different mouse models have been generated to deep inside the DMD physiopathology, characterized by mutations in different loci within the dystrophin gene. The *mdx* mouse model of DMD is generated on a C57BL/10ScSn genetic background (C57BL/10ScSn-*Dmd*^{mdx}, referred as "*mdx* mouse") (Coulton et al, 1988) carrying a point mutation in exon 23, generating an early stop codon causing the absence of full-length dystrophin (Willmann et al, 2009). The other models of DMD are the *mdx*2cv, *mdx*3cv, *mdx*4cv, or *mdx*5cv mouse (Im et al, 1996), *mdx*52 with mutation on exon 52 (Araki et al, 1997), and *mdx*/utr-/- (*mdx* mouse with additional knockout of utrophin), which are characterized by different mutations (Deconinck et al, 1997).

The *mdx* mouse model of DMD is the most widely studied (Nakamura and Takeda, 2011) as it resembles the human pathology where the mutation on exon 23 is present in one-third of DMD patients (Willmann et al, 2009).The *mdx* mice show a shorter life compared to wild type and decreased relative muscle force, whereas the absolute muscle force is not affected. Muscles isolated from *mdx* mice belonging to different experimental groups show a reduced contractility force, although this type of analysis requires standardization. Young *mdx* mice (2-4 weeks) are characterized by degeneration, regeneration and necrosis resulting in increased number of regenerating-centronucleated fibres and in heterogeneous myofibre structures (McGeachie et al, 1993). Starting from 18 months, muscles of *mdx* mice show large areas of necrotic fibres that can be found at any age with very high frequency (Nakamura and Takeda, 2011). Muscles of *mdx* mice are characterized by frequent cycles of necrosis/regeneration, associated with weakness and muscle loss. These cycles of regeneration lead to a milder muscle phenotype than in human patients. Fibrosis appears at a lower extent compared to DMD patients, except in the diaphragm muscle (Nakamura and Takeda, 2011). Respiratory complications are visible only in 16-month-old mice (Stedman et al, 1991; Nakamura and Takeda, 2011) although the *mdx* mouse diaphragm reproduces the degenerative changes of DMD. Forced exercise worsened muscular phenotype in *mdx*, leading to a suitable experimental strategy to unravel the efficacy of new therapies in DMD.

Another interesting pathological feature is that the *mdx* mouse displays cardiomyopathy, characterized by fibrosis, necrosis and inflammation, resembling the cardiomyopathy in DMD-patients, thus representing a valid preclinical model to investigate cardio-protective pharmacological approaches (Quinlan et al, 2004).

ROLE OF AUTOPHAGY IN SKELETAL MUSCLE

Autophagy is a critical mechanism involved in the replacement of cell components both in constitutive and catabolic conditions, such as stress responses, nutrient deprivation, cytokines, amino acid deprivation. Autophagy is a physiological process to degrade cytoplasmic constituents, proteins, protein aggregates and whole organelles, and is also a regulator of cellular homeostasis. This process commonly protects the eukaryotic cells from death, and defective autophagy can be related to several human diseases including cancer, neurodegenerative diseases, infectious diseases, and metabolic diseases (Cervia et al, 2013; Jiang and Mizushima, 2014; Schneider and Cuervo, 2014). To date, three different mechanisms of autophagy have been described: macroautophagy, microautophagy and chaperone-mediated autophagy. The first one, usually simply referred to as autophagy, is the most common pathway in skeletal muscle, and is characterised by membranes that gradually grow in size to generate double membrane-structures called autophagosomes, that surround organelles, a small portion of cytosol or protein aggregates. In particular, during macroautophagy, small ubiquitin-like proteins (LC3, GABARAP, GATE-16, and ATG12) are required for the formation of autophagosomes, and these are covalently bound

to phosphatidylethanolamine. Initially, autophagy was considered as a non-selective degradation mechanism, but it is now clear that selective forms of autophagy exist. For example, removal of damaged mitochondria and peroxisomes represent two specific types of autophagy, that are known as mytophagy and pexofagy, respectively

Muscle damage

The role of autophagy in skeletal muscle has been recently investigated (Sandri, 2010; Neel et al, 2013). Muscle mass constitutes 40-50% of the human body and represents one of the major sites for checking metabolism. Recent data suggest that excessive protein degradation in the skeletal muscle is harmful for the maintenance of muscle mass and it can lead to death. In order to identify the role of autophagy in muscles, many studies have been performed on mice with muscle-specific inactivation of autophagic genes (Sandri, 2010; Neel et al, 2013). Briefly, specific removal of Atg7 gene causes a pathological phenotype characterised by accumulation of protein aggregates and polyubiquitinated proteins, presence of abnormal mitochondria and concentric membranous structures, that are assembled between the myofibrils or under the sarcolemma, enhanced oxidative stress and activation of unfolded protein response (Masiero et al, 2009). These effects lead to muscle weakness, atrophy and other marks of myopathy, such as an irregular distribution of fiber shape, as well as fibres with fragmented and vacuolated cytosol. Generally, lack of basal autophagy in muscle enhances the accumulation of damaged and dysfunctional mitochondria, suggesting that mitophagy, which is critical to maintain muscle homeostasis, is impaired (Masiero et al, 2009). Furthermore, muscle-specific Atg5 knockout mice displayed an atrophic phenotype in the fast fibres (II type), associated to accumulation of autophagic substrates, as for instance ubiquitinated proteins (Raben et al, 2008). In addition, in fast muscles fibres of Atg5-/- mice, the size and density of lysosomes result increased and their distribution is unexpectedly altered, as well as the distribution of microtubules. In fact, microtubules displayed a more linear organisation than the wild-type mice, demonstrating that autophagy is crucial also for the correct arrangement of microtubules, which are in turn essential for the formation and movement of autophagosomes (Raben et al, 2008).

Furthermore, in other studies it has been seen that autophagy-lysosome system was enhanced when the transcription of FoxO3 was upregulated, especially during muscle wasting enhancing the autophagy process and starting atrophy.(Mammucari et al, 2007). Interestingly, a new study showed that mice lacking the nutrient-deprivation autophagy factor-1 displayed muscle weakness, connected with an enhanced basal autophagy, dysregulation of calcium flux and accumulation of elongated mitochondria (Chang et al, 2012). Therefore, based on these results, the dual role appears evident of the autophagy-lysosome system in skeletal muscle homeostasis: at high levels it can be harmful and contribute to muscle degeneration, while at low levels it can cause weakness and wasting, due to the inefficient removal of damaged proteins and dysfunctional organelles (Sandri, 2010; Sandri et al, 2013). Accordingly, a correct autophagic process is essential for both functional skeletal muscle, which controls the support and movement of the skeleton, and muscle metabolism (Neel et al, 2013).

DMD AND AUTOPHAGY IMPAIRMENT

To investigate whether a connection exists between disregulated autophagy and degenerative muscle disease, genetic studies of muscular dystrophy have been carried out (Neel et al, 2013; Sandri et al, 2013). The findings of the last 5 years have greatly enhanced our understanding of the role of defective autophagy in different forms of inherited muscular dystrophies, including Bethlem myopathy, Ullrich congenital muscular dystrophy, merosin-deficient congenital muscular dystrophy, and Emery-Dreyfuss muscular dystrophy (Sandri et al, 2013).

The fact that deficient autophagy is involved in DMD pathogenesis was initially postulated. When autophagy is reduced, cyto-pathological hallmarks of DMD are often observed, such as the presence of swollen and damaged mitochondria, protein aggregation, and distension of sarcoplasmic reticulum

(Culligan et al, 2002; Zhao et al, 2007). In muscles from *mdx* mice and dystrophin-deficient primary myotubes, activation of Akt was significantly higher therefore proposing a defective autophagic process (Dogra et al, 2006; Peter and Crosbie, 2006). DMD patients were found to exhibit a similar pattern of Akt activation in accordance with these observations (Peter and Crosbie, 2006).

Recent studies investigated the causal relationship between DMD pathogenesis and dysfunctional autophagy. Biochemical and ultrastructural analyses in muscles from patients affected by DMD and *mdx* mice definitely demonstrated a serious impairment of autophagy. A significant reduction in the lipidated form of the protein LC3, which is a common marker of autophagy induction, was shown in these muscles (De Palma et al, 2012; Bibee et al, 2014). The diminution in lipidated LC3 has been found to be associated by actual signs of impaired autophagy at the ultrastructural level, *i.e.* by the existence of damaged organelles, anincrease in the signalling adaptor p62 protein (a marker inversely correlated with autophagic flux), and the decrease of Bnip3, a mitochondrial protein which recruits LC3 to mitochondria (De Palma et al, 2012; Bibee et al, 2014). Other studies shed light on a role for the TNF receptor-associated factor 6 (TRAF6) as an important regulator of autophagy. The activity of TRAF6 in skeletal muscle of *mdx* mice is increased and its absence correlates with a reduced autophagy (Hindi et al, 2014). The same study also reported that, at later stages of disease progression, the inhibition of TRAF6 signalling deteriorates muscle pathology but it has been thus hypothesised that the initial inhibition of autophagy in young *mdx* mice in the absence of TRAF6 gene may be a protective mechanism to preserve skeletal muscle mass. Autophagy emerges therefore as an essential process for the clearance of dead cellular organelles and an enduring inhibition of autophagy amplifies dystrophic phenotype (Hindi et al, 2014). The overexpression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1a), an important mediator of muscle plasticity (Hollinger et al, 2013) and the treatment with a drug against the energy sensor AMPK, *i.e.* AICAR (5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside) (Pauly et al, 2012), confirmed the beneficial effects of activating autophagy in *mdx* mice. The autophagy is significantly activated by AICAR treatment, as indicated by the characteristic biochemical changes of increased lipidated LC3 content, an up-regulation of other prototypical autophagy-associated proteins, and to significant improvements in both muscle structure and maximum force-generating capacity (Pauly et al, 2012).

After behavioral studies, it has been shown that voluntary exercise in *mdx* mice enhances markers of autophagy to or above the levels observed in healthy mice, thus suggesting that beneficial autophagy can be induced by exercise (Hulmi et al, 2013). In agreement with these data, fasting induced autophagy in diaphragms of wild-type and *mdx* mice (Spitali et al, 2013). It is interesting to underline that in *mdx* mice the capacity of upregulating autophagy appears selective to certain muscles: *tibialis anterior* muscle of *mdx* mice are unable to enhance autophagy in response to fasting, which induces autophagy in wild-type mouse *tibialis anterior* muscle.

Molecular features

The persistent activation of the Akt-mTOR axis and its related autophagy-inhibiting pathways is another feature of the defect in autophagy of *mdx* mouse muscles, and the coincident downregulation of several autophagy-inducing genes. (Dogra et al, 2006; Peter and Crosbie, 2006; De Palma et al, 2012). Indeed, during pre-necrotic stages of disease pathogenesis, Akt activation occurs with a progressive increase with disease aggravation (Peter and Crosbie, 2006). The mTOR-dependent pathways is in turn stimulated from this high activation of Akt while the mTOR-independent axis is not significantly altered (Dogra et al, 2006; Peter and Crosbie, 2006).

The effects of the mTOR-targeting, immunosuppressant drug rapamycin demonstrates the involvement of the mTOR axis in the pathogenesis of *mdx* mice. The onset of histopathological features of dystrophy has been shown after the treatment with oral or injected rapamycin (Eghtesad et al, 2011) and the rapamycin-loaded nanoparticle treatment increases skeletal muscle strength in both young and

adult mice and has been shown to simultaneously increase mTOR-dependent autophagy (Bibee et al, 2014). Constantly, a long-term low-protein diet reactivates autophagy in muscle fibres of *mdx* mice treated with increased lipidation of LC3, reduced levels of p62, normalization of Akt and mTOR signalling, a reduced accumulation of damaged organelles, and a significant recovery of muscle inflammation, fibrosis, myofibre damage and muscle function (De Palma et al, 2012). The regenerating ability of *mdx* mouse muscle is preserved by the long-term low-protein diet, therefore the physiological levels of autophagy maintain the number and function of myogenic precursor cells.

There are still some controversies and issues remaining to be solved to shed light on the complete picture. The advancing of age with a reduction in mTOR signalling in dystrophic muscles is correlated in a study on Akt-mTOR axis activation in very old *mdx* mice (Mouisel et al, 2010).

In another study it is reported that autophagy levels and Akt axis activation in *mdx* mice is similar to wild-type control mice (Spitali et al, 2013). Instead it was also reported that in diaphragm muscles of wild-type mice, but not in *mdx* mice, mTOR activation is increased by age, while similar levels of mTOR activation were found in tibialis anterior muscles (Eghtesad et al, 2011). Although difficult to reconcile, these data may be explained by the existence of different confounding factors, for instance the extensive muscle regeneration of *mdx* mice between 6 and 12 weeks. In addition, the regulation of autophagy in *mdx* mice may vary in different muscle types (Spitali et al, 2013), with the glycolytic muscles showing more vesicle formation than oxidative muscles (Mizushima et al, 2004). Autophagy can also be triggered without the direct participation of mTOR mechanisms. Indeed, in AICAR-treated *mdx* mice there was significant upregulation of Ulk1 and an increased expression of Bnip3 independent of mTOR inhibition (Pauly et al, 2012). It has been thus proposed that TNF receptor-associated factor 6 (TRAF6) regulation of Akt signaling is independent of TRAF6 regulation of autophagy (Hindi et al, 2014). Autophagy is the main regulated catabolic mechanism that eukaryotic cells use to degrade and recycle intracellular components. Therefore, its increase may mean an adaptive response to endeavor to rid dystrophic myofibres of detrimental and/or defective constituents e.g. dysfunctional mitochondria (Ljubicic and Jasmin, 2013). Given the above evidence, the autophagic machinery in *mdx* mice is more complex than in wild-type mice, and for this reason a systematic analysis is necessary in order to be able to give a detailed characterization of each stage of disease progression.

DMD: THERAPEUTIC EFFECTS OF CORRECTIVE AUTOPHAGY

DMD is a progressive, life-limiting muscle-wasting disease for which no curative treatment is yet available. Different therapeutic approaches are being studied. Standard management of DMD requires multidisciplinary care that includes the use of corticosteroids as well as respiratory, cardiac and orthopedic interventions. Corticosteroids slow the progression of muscle weakness and delay some of the complications of the disease, but they do not treat or correct the underlying causes of DMD. The use of corticosteroids comes with a high frequency of significant side effects, including behavioural change, obesity, immune suppression and bone demineralization (Bushby et al, 2010). These effects are often temporary and correlate with important side effects: more than 25% of patients are not treated with glucocorticoids due to adverse effects (obesity, immune suppression, bone demineralization, negative behavioural changes, lack of response) (Bushby et al, 2010). This strongly indicates an urgent need for new clinical interventions for DMD patients.

At present, the genetic approaches, e.g viral vector-mediated gene delivery, exon skipping and cell therapy (Pichavant et al, 2011; Mendell et al, 2012), are being investigated and some of them have been granted orphan status by either the European Medicines Agency or the Food and Drug Administration.

These approaches cannot fully repair the damage caused by the disease to the muscle, therefore

identification of suitable therapeutic targets is needed. A possible strategy could be the modulation of autophagy in order to delay muscle degeneration. The first pharmacological approach involves the role of AICAR. The beneficial effects of AICAR in dystrophic muscle may be due to a well-known effect in stimulating the slow, oxidative phenotype (Ljubicic et al, 2011). It may also be due to induction of autophagic pathways, since AICAR stimulates the removal of damaged mitochondria via mitophagy (Pauly et al, 2012). The AICAR-activated AMPK ameliorated phenotypic and functional features of dystrophic muscle (Pauly et al, 2012). AMPK pharmacological agonists are clinically approved and AICAR has been tested for ischemic damage in the heart and used in clinical trials for metabolic disorder.

Bibee and co-workers have proposed a second pharmacological approach that targets autophagy (Bibee et al, 2014) in which rapamycin-loaded nanoparticles (RNPs) were used to successfully enhance grip strength and the left-ventricular ejection fraction in *mdx* mice. This approach ameliorated both physical and cardiac performance. These effects with RNPs have been obtained after administration of only 8 doses of RNPs, with the final dose within the range of recommended oral doses for immune-suppressive therapy in patients. Moreover, oral administration of rapamycin at pharmacological doses had no effect on muscle strength. It would seem that the way RNPs worked was via autophagy induction, dependent on nanoparticle delivery of the drug: rapamycin was locally delivered at high concentration, thus enabling autophagy to be triggered (Bibee et al, 2014). It should also be noted that corticosteroids induce autophagy in *mdx* mice, thus contributing to their beneficial effects. However autophagy cannot be envisaged as a stand-alone therapeutic option but in combination with pharmacological treatment and cell and gene therapies. In this respect, a low protein diet regimen has been shown to be safely usable in the treatment of DMD patients (De Palma et al, 2012). A low protein diet has been demonstrated safe and useful also in Col6a1-deficient mouse, considered an animal model of Bethlem myopathy (Grumati et al, 2010). Moreover, RPNs and a low protein diet might also be used in combination with corticosteroids to act as steroid sparing drugs, reducing their toxic effects. A very recent and noteworthy clinical study suggests that also in humans a strategy of autophagy reactivation is applicable. This study, indeed, shows a clear induction of autophagy with a low protein diet regimen in muscles from Becker and Ulrich patients (Merlini and Nishino, 2014).

CONCLUSIONS

The dysregulation of autophagy has emerged as a key event involved in the pathogenesis of several muscular dystrophies. Indeed, a normalization of this process by pharmacological approaches leads to an amelioration of the dystrophic phenotype. At present, the drugs targeting autophagy have good perspective in terms of therapy, however, in order to design selected modulating drugs it is necessary to refine them and to identify specific targets in the autophagic pathway.

REFERENCES

Araki E, Nakamura K, Nakao K, Kameya S, Kobayashi O, Nonaka I, Kobayashi T, Katsuki M (1997) Targeted disruption of exon 52 in the mouse dystrophin gene induced muscle degeneration similar to that observed in Duchenne muscular dystrophy. *Biochem Biophys Res Commun* **238**: 492-497.

Bibee KP, Cheng YJ, Ching JK, Marsh JN, Li AJ, Keeling RM, Connolly AM, Golumbek PT, Myerson JW, Hu G, Chen J, Shannon WD, Lanza GM, Weihl CC, Wickline SA (2014) Rapamycin nanoparticles target defective autophagy in muscular dystrophy to enhance both strength and cardiac function. *Faseb J* **28**: 2047-2061.

Biressi S, Rando TA (2010) Heterogeneity in the muscle satellite cell population. *Semin Cell Dev Biol* **21**: 845-854.

Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, Kaul A, Kinnett K, McDonald C, Pandya S, Poysky J, Shapiro F, Tomezsko J, Constantin C (2010) Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol* **9**: 77-93.

Cervia D, Perrotta C, Moscheni C, De Palma C, Clementi E (2013) Nitric oxide and sphingolipids control apoptosis and autophagy with a significant impact on Alzheimer's disease. *J Biol Regul Homeost Agents* **27**: 11-22.

Chang NC, Nguyen M, Bourdon J, Risse PA, Martin J, Danialou G, Rizzuto R, Petrof BJ, Shore GC (2012) Bcl-2-associated autophagy regulator Naf-1 required for maintenance of skeletal muscle. *Hum Mol Genet* **21**: 2277-2287.

Coulton GR, Curtin NA, Morgan JE, Partridge TA (1988) The mdx mouse skeletal muscle myopathy: II. Contractile properties. *Neuropathol Appl Neurobiol* **14**: 299-314.

Culligan K, Banville N, Dowling P, Ohlendieck K (2002) Drastic reduction of calsequestrin-like proteins and impaired calcium binding in dystrophic mdx muscle. *J Appl Physiol* **92**: 435-445.

Davies KE, Smith TJ, Bundey S, Read AP, Flint T, Bell M, Speer A (1988) Mild and severe muscular dystrophy associated with deletions in Xp21 of the human X chromosome. *J Med Genet* **25**: 9-13.

De Palma C, Clementi E (2012) Nitric Oxide in Myogenesis and Therapeutic Muscle Repair. *Molecular Neurobiology* 1-11.

De Palma C, Morisi F, Cheli S, Pambianco S, Cappello V, Vezzoli M, Rovere-Querini P, Moggio M, Ripolone M, Francolini M, Sandri M, Clementi E (2012) Autophagy as a new therapeutic target in Duchenne muscular dystrophy. *Cell Death Dis* **3**: e418.

Deconinck AE, Rafael JA, Skinner JA, Brown SC, Potter AC, Metzinger L, Watt DJ, Dickson JG, Tinsley JM, Davies KE (1997) Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. *Cell* **90**: 717-727.

Dogra C, Changotra H, Wergedal JE, Kumar A (2006) Regulation of phosphatidylinositol 3-kinase (PI3K)/Akt and nuclear factor-kappa B signaling pathways in dystrophin-deficient skeletal muscle in response to mechanical stretch. *J Cell Physiol* **208**: 575-585.

Eghtesad S, Jhunjhunwala S, Little SR, Clemens PR (2011) Rapamycin ameliorates dystrophic phenotype in mdx mouse skeletal muscle. *Mol Med* **17**: 917-924.

Emery AE (1991) Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord* **1**: 19-29.

Fayssoil A, Nardi O, Orlikowski D, Annane D (2010) Cardiomyopathy in Duchenne muscular dystrophy: pathogenesis and therapeutics. *Heart Fail Rev* **15**: 103-107.

Govoni A, Magri F, Brajkovic S, Zanetta C, Faravelli I, Corti S, Bresolin N, Comi GP (2013) Ongoing therapeutic trials and outcome measures for Duchenne muscular dystrophy. *Cell Mol Life Sci* **70**: 4585-4602.

Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, Blaauw B, Urciuolo A, Tiepolo T, Merlini L, Maraldi NM, Bernardi P, Sandri M, Bonaldo P (2010) Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. *Nat Med* **16**: 1313-1320.

Hindi SM, Sato S, Choi Y, Kumar A (2014) Distinct roles of TRAF6 at early and late stages of muscle pathology in the mdx model of Duchenne muscular dystrophy. *Human Molecular Genetics* **23**: 1492-1505.

Hollinger K, Gardan-Salmon D, Santana C, Rice D, Snella E, Selsby JT (2013) Rescue of dystrophic skeletal muscle by PGC-1alpha involves restored expression of dystrophin-associated protein complex components and satellite cell signaling. *Am J Physiol Regul Integr Comp Physiol* **305**: R13-23.

Hulmi JJ, Oliveira BM, Silvennoinen M, Hoogaars WM, Pasternack A, Kainulainen H, Ritvos O (2013) Exercise restores decreased physical activity levels and increases markers of autophagy and oxidative capacity in myostatin/activin-blocked mdx mice. *Am J Physiol Endocrinol Metab* **305**: E171-182.

Im WB, Phelps SF, Copen EH, Adams EG, Slightom JL, Chamberlain JS (1996) Differential expression of dystrophin isoforms in strains of mdx mice with different mutations. *Hum Mol Genet* **5**: 1149-1153.

Jefferies JL, Eidem BW, Belmont JW, Craigen WJ, Ware SM, Fernbach SD, Neish SR, Smith EO, Towbin JA (2005) Genetic predictors and remodeling of dilated cardiomyopathy in muscular dystrophy. *Circulation* **112**: 2799-2804.

Jiang P, Mizushima N (2014) Autophagy and human diseases. *Cell Res* **24**: 69-79.

Le Grand F, Rudnicki MA (2007) Skeletal muscle satellite cells and adult myogenesis. *Curr Opin Cell Biol* **19**: 628-633.

Ljubicic V, Jasmin BJ (2013) AMP-activated protein kinase at the nexus of therapeutic skeletal muscle plasticity in Duchenne muscular dystrophy. *Trends Mol Med* **19**: 614-624.

Ljubicic V, Miura P, Burt M, Boudreault L, Khogali S, Lunde JA, Renaud JM, Jasmin BJ (2011) Chronic AMPK activation evokes the slow, oxidative myogenic program and triggers beneficial adaptations in mdx mouse skeletal muscle. *Hum Mol Genet* **20**: 3478-3493.

Lynch GS, Rafael JA, Chamberlain JS, Faulkner JA (2000) Contraction-induced injury to single permeabilized muscle fibres from mdx, transgenic mdx, and control mice. *Am J Physiol Cell Physiol* **279**: C1290-1294.

Magri F, Govoni A, D'Angelo MG, Del Bo R, Ghezzi S, Sandra G, Turconi AC, Sciacco M, Ciscato P, Bordoni A, Tedeschi S, Fortunato F, Lucchini V, Bonato S, Lamperti C, Coviello D, Torrente Y, Corti S, Moggio M, Bresolin N, Comi GP (2011) Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. *J Neurol* **258**: 1610-1623.

Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J, Goldberg AL, Schiaffino S, Sandri M (2007) FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab* **6**: 458-471.

Masiero E, Agatea L, Mammucari C, Blaauw B, Loro E, Komatsu M, Metzger D, Reggiani C, Schiaffino S, Sandri M (2009) Autophagy is required to maintain muscle mass. *Cell Metab* **10**: 507-515.

McGeachie JK, Grounds MD, Partridge TA, Morgan JE (1993) Age-related changes in replication of myogenic cells in mdx mice: quantitative autoradiographic studies. *J Neurol Sci* **119**: 169-179.

Mendell JR, Rodino-Klapac L, Sahenk Z, Malik V, Kaspar BK, Walker CM, Clark KR (2012) Gene therapy for muscular dystrophy: lessons learned and path forward. *Neurosci Lett* **527**: 90-99.

Merlini L, Nishino I (2014) 201st ENMC International Workshop: Autophagy in muscular dystrophies - Translational approach, 1-3 November 2013, Bussum, The Netherlands. *Neuromuscul Disord* **24**: 546-561.

Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y (2004) In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol Biol Cell* **15**: 1101-1111.

Mosqueira M, Zeiger U, Forderer M, Brinkmeier H, Fink RH (2013) Cardiac and respiratory dysfunction in Duchenne muscular dystrophy and the role of second messengers. *Med Res Rev* **33**: 1174-1213.

Mouisel E, Vignaud A, Hourde C, Butler-Browne G, Ferry A (2010) Muscle weakness and atrophy are associated with decreased regenerative capacity and changes in mTOR signaling in skeletal muscles of venerable (18-24-month-old) dystrophic mdx mice. *Muscle Nerve* **41**: 809-818.

Nakamura A, Takeda S (2011) Mammalian models of Duchenne Muscular Dystrophy: pathological characteristics and therapeutic applications. *J Biomed Biotechnol* **2011**: 184393.

Neel BA, Lin Y, Pessin JE (2013) Skeletal muscle autophagy: a new metabolic regulator. *Trends Endocrinol Metab* **24**: 635-643.

Pauly M, Daussin F, Burelle Y, Li T, Godin R, Fauconnier J, Koechlin-Ramonatxo C, Hugon G, Lacampagne A, Coisy-Quivy M, Liang F, Hussain S, Matecki S, Petrof BJ (2012) AMPK activation stimulates autophagy and ameliorates muscular dystrophy in the mdx mouse diaphragm. *Am J Pathol* **181**: 583-592.

Peter AK, Crosbie RH (2006) Hypertrophic response of Duchenne and limb-girdle muscular dystrophies is associated with activation of Akt pathway. *Exp Cell Res* **312**: 2580-2591.

Pichavant C, Aartsma-Rus A, Clemens PR, Davies KE, Dickson G, Takeda S, Wilton SD, Wolff JA, Wooddell CI, Xiao X, Tremblay JP (2011) Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. *Mol Ther* **19**: 830-840.

Politano L, Nigro G (2012) Treatment of dystrophinopathic cardiomyopathy: review of the literature and personal results. *Acta Myol* **31**: 24-30.

Quinlan JG, Hahn HS, Wong BL, Lorenz JN, Wenisch AS, Levin LS (2004) Evolution of the mdx mouse cardiomyopathy: physiological and morphological findings. *Neuromuscul Disord* **14**: 491-496.

Raben N, Hill V, Shea L, Takikita S, Baum R, Mizushima N, Ralston E, Plotz P (2008) Suppression of autophagy in skeletal muscle uncovers the accumulation of ubiquitinated proteins and

their potential role in muscle damage in Pompe disease. *Hum Mol Genet* **17**: 3897-3908.

Sandri M (2010) Autophagy in skeletal muscle. *FEBS Lett* **584**: 1411-1416.

Sandri M, Coletto L, Grumati P, Bonaldo P (2013) Misregulation of autophagy and protein degradation systems in myopathies and muscular dystrophies. *J Cell Sci* **126**: 5325-5333.

Schneider JL, Cuervo AM (2014) Autophagy and human disease: emerging themes. *Curr Opin Genet Dev* **26C**: 16-23.

Spitali P, Grumati P, Hiller M, Chrisam M, Aartsma-Rus A, Bonaldo P (2013) Autophagy is Impaired in the Tibialis Anterior of Dystrophin Null Mice. *PLoS Curr* **5**:

Spurney CF (2011) Cardiomyopathy of Duchenne muscular dystrophy: current understanding and future directions. *Muscle Nerve* **44**: 8-19.

Stedman HH, Sweeney HL, Shrager JB, Maguire HC, Panettieri RA, Petrof B, Narusawa M, Leferovich JM, Sladky JT, Kelly AM (1991) The mdx mouse diaphragm reproduces the degenerative changes of Duchenne muscular dystrophy. *Nature* **352**: 536-539.

Straub V, Campbell KP (1997) Muscular dystrophies and the dystrophin-glycoprotein complex. *Curr Opin Neurol* **10**: 168-175.

Thomas GD (2013) Functional muscle ischemia in Duchenne and Becker muscular dystrophy. *Front Physiol* **4**: 381.

Willmann R, Possekel S, Dubach-Powell J, Meier T, Ruegg MA (2009) Mammalian animal models for Duchenne muscular dystrophy. *Neuromuscul Disord* **19**: 241-249.

Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S, Lecker SH, Goldberg AL (2007) FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metab* **6**: 472-483.