COMPARTMENTALIZED CAMP SIGNALING IN NEURODEGENERATIVE DISEASES

M. SEPE, L. RINALDI, M. PORPORA, R. DELLE DONNE, S. SAUCHELLA and A. FELICIELLO

Dipartimento di Medicina Molecolare e Biotecnologie Mediche, University "Federico II", Naples, Italy

Neurodegeneration is becoming a major health problem for ageing population worldwide. The high morbidity and mortality for neurodegenerative disorders demand earlier diagnosis and better taylored treatment. Neurodegeneration occurs as consequence of progressive deterioration of the neuronal structure and activity that eventually leads to neuronal dysfunction and cell death. Recent discoveries highlighted the existence of common mechanisms underlying the onset and progression of a variety of neurodegenerative disorders. Understanding the pathogenic mechanisms of neurodegenerative diseases and interfering with aberrant neural activity represent the principal aims of many investigators in the field. Reversible modification of proteins, such as phosphorylation and ubiquitination, are the most common and important modes to control protein function. In neurons, protein modification induced by the second messenger cAMP at subcellular compartments is emerging as a key mechanism to control the generation and dissemination of neutrophin signals from cell membrane to target substrates. Dysregulation of such a mechanisms could promote neuronal dysfunction and disease. Here, we will focus on the role of deranged cAMP signaling in neurodegenerative disorders.

Protein phosphorylation is an evolutionarily conserved postranslational mechanism that eukaryotic cells adopt to control complex biological activities, as growth, differentiation, apoptosis, ion channel activity and synaptic transmission. cAMP is an ancient second messenger that plays a major role in a wide array of biological processes, as growth and development, metabolism, differentiation and neuronal activity (Tasken and Aandahl, 2004, Taylor et al., 2008). cAMP accumulates in response to the activation of adenylyl cyclase by extracellular ligands acting through G-protein coupled receptors (GPCR). In eukayotes, most of the effects elicited by cAMP are consequences of the activation of protein kinase A (PKA). Binding of cAMP to the regulatory subunit (R) of PKA holoenzyme releases the catalytic subunit (PKAc), which in turn phosphorylates a wide array of cellular substrates, controlling fundamental aspects of cell physiology (Taylor et al., 1992) (Fig. 1). The biochemical and functional features of PKA holoenzymes are largely determined by the structure, properties and relative abundance of the R subunits (McKnight et al., 1998). In this context, the activation rate, the persistence and magnitude of cAMP signals contribute to enhance specificity and sensitivity of a given tissue to distinct GPCR ligands. In mammalian cells, PKA is concentrated in membranes and subcellular compartments through interactions with A-kinase anchor proteins (AKAPs). AKAPs contain a PKA-binding motif that tethers the regulatory subunit of PKA holoenzyme and a targeting domain that directs the kinase complex to subcellular

Key words: neurodegeneration, cAMP, AKAP, NOGO-A, ubiquitin

Corresponding Author:
Dr A. Feliciello,
Dipartimento di Medicina Molecolare e Biotecnologie Mediche,
University "Federico II",
Naples, Italy
e-mail: feliciel@unina.it

2279-5855 (2014)

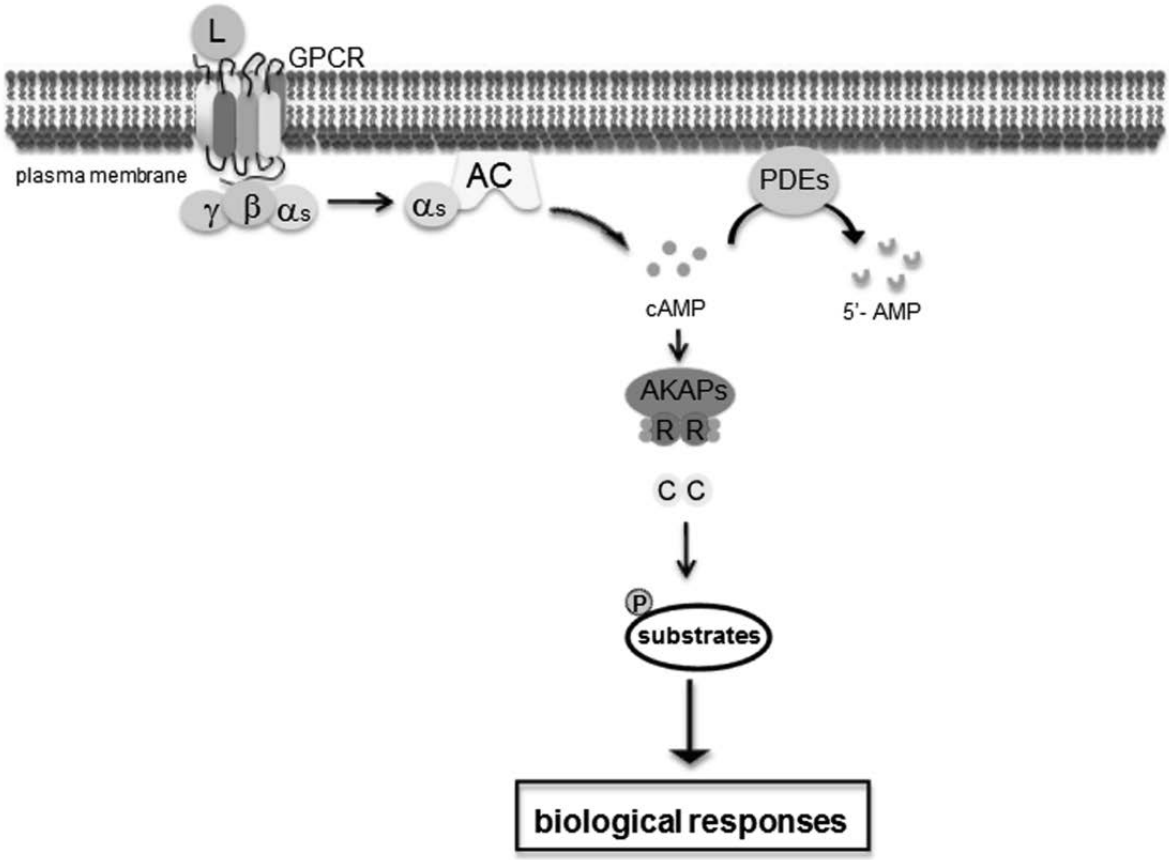

Fig. 1. *Schematic representation of cAMP/PKA pathway. Activation of adenylate cyclase (AC) by G-protein coupled receptors (GPCRs) converts ATP in the second messenger cAMP. cAMP binding to R subunit of PKA causes dissociation of inactive PKA holoenzyme. The free catalytic subunit (C) phosphorylates a wide array of substrates exerting several biological responses. Phosphodiesterases (PDEs) hydrolyze cAMP ensuring the attenuation of the signaling.*

compartments and organelles. As consequence of PKA targeting by AKAPs, cAMP signals generated at cell membrane efficiently travel from sites of signal generation to distal compartments. This system provides a mechanism that efficiently couples stimulation of membrane receptors to activation of downstream PKA substrates/effectors (Feliciello et al., 2001, Tasken and Aandahl, 2004). AKAPs form a transduceosome that assembles components of the cAMP generating systems (receptors and adenylate cyclase), effectors (PKA and Epac) and attenuating enzymes, as cAMP phosphodiesterases (PDEs) and phosphatases (PPs). This implies that complexes nucleated by AKAPs create intracellular sites where distinct signaling pathways converge and are locally attenuated or amplified, optimizing the biological response to extracellular stimuli (Feliciello et al., 2001, Beene and Scott, 2007). It appears that AKAPs play an important role in key neuronal duties. Thus, manipulation of AKAP signaling complexes *in vivo* profoundly impacts on highly specialized brain functions, as learning and memory. Emerging evidences, discussed here, indicate that derangement of compartmentalized cAMP signaling is a pathogenic mechanism relevant for the development and progression of neurodegenerative disorders.

Intracellular targeting of cAMP signaling in neurons

Compartmentalized cAMP signaling plays a major role in neurons. Genetic and pharmacological interference with cAMP signaling in the brain is often associated to defects in ion channel activities,

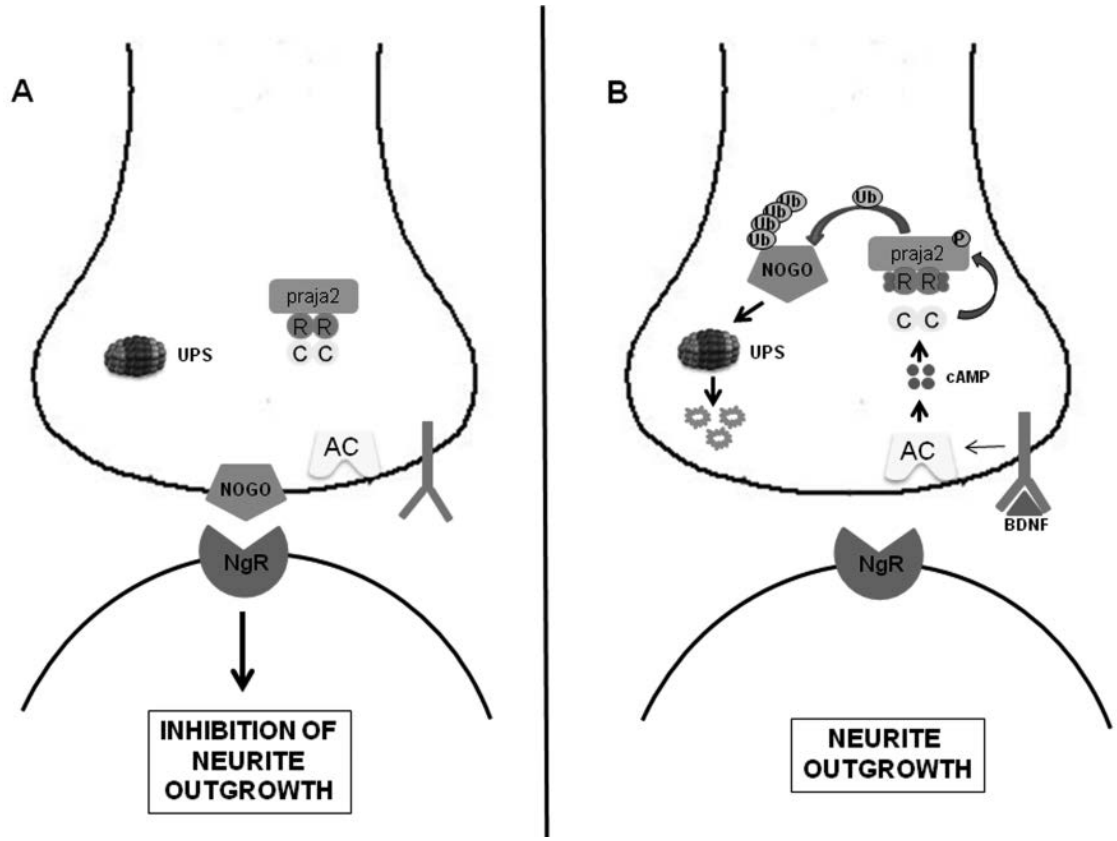

Fig. 2. *Proteolysis of NOGO-A by cAMP-praja2 pathway promotes neurite outgrowth. Activation of adenylate cyclase (AC) by BDNF induces PKA-mediated phosphorylation of praja2, which ubiquitinates and degrades NOGO-A through the proteasome. Decrease of NOGO-A levels promotes neurite outgrowth.*

neuronal transmission, synaptic plasticity, learning and memory (Abel and Nguyen, 2008, Sanderson and Dell'Acqua, 2011). Several AKAPs have been isolated from neurons and functionally characterized. Although all share common ability to bind PKA, their relative abundance in distinct brain areas, their localization at discrete subcellular compartment and the property of each family of neuronal AKAP to assemble distinct multimeric complexes confer enormous specificity in the regulation of different brain activities. In this context, AKAP5 (also known as AKAP79/150) is a prototypic neuronal AKAP broadly expressed in different brain areas. AKAP5 forms a complex which includes not only PKA, but also protein phosphatase 2B/calcineurin (PP2B/CaN) and protein kinase C (PKC). Through these different modular interactions, AKAP5 controls important aspects of neuronal activity, such as learning and memory (Bauman et al., 2004, Tunquist et al., 2008, Sanderson and Dell'Acqua, 2011). AKAP12 (also known as AKAP250/Gravin) is a scaffold protein that, in response to catecholamine stimulation, rapidly and reversibly recruits PKA, PKC, calmodulin and phosphodiesterase 4D (PDE4D) in proximity of β2-adrenergic receptor (β2-AR). By recruiting this multivalent complex at membrane, AKAP12 controls resensitization and recycling of the activated β2-AR (Lin et al., 2000). Interestingly, mice bearing mutation of AKAP12 gene exhibit deficits in PKA-dependent synaptic plasticity and memory storage (Havekes et al., 2012). WAVE1 is another member of the neuronal AKAPs that binds and targets PKA and other signaling enzymes to the actin cytoskeleton. Actin remodeling is a

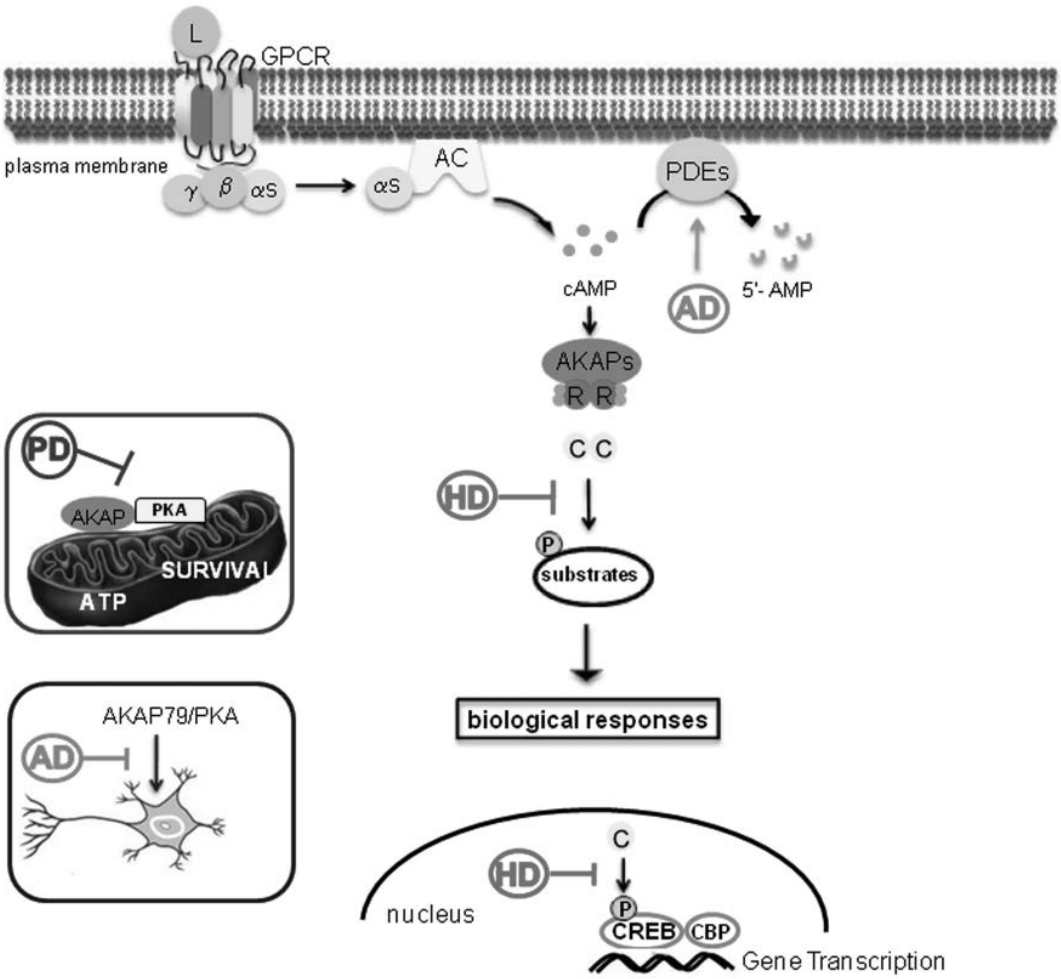

Fig. 3. *Perturbation of cAMP signaling can contribute to development and progression of neurodegenerative disorders. In course of Alzheimer disease (AD), upregulation of phosphodiesterases (PDEs) decreases cAMP signaling. Moreover, hyperphosphorylation of tau by AKAP79-anchored PKA induces the formation of aggregates that impair synaptic activity, leading to neuronal cell death. In Parkinson's disease (PD), upregulation of AKAP1•PKA axis reverses mitochondrial defects, suggesting a pathogenic role of deranged cAMP signaling in the disease. In Huntington disease (HD), poly(Q)-expanded HTT (mHTT) inhibits CREB-dependent transcription of genes involved in synaptic plasticity. Furthermore, inhibition of PKA is linked to mHTT aggregates and progression of the disease.*

fundamental step in neurite outgrowth. It ensures the formation of appropriate synaptic connections during the development and a more dynamic cytoskeleton structure that favors neuronal plasticity (Higgs, 2001, Nozumi et al., 2003). As expected, WAVE1 knockout mice show cognitive and behavioral defects as impairment of hippocampal learning and memory and deficit in locomotor activity (Bauman et al., 2004). MAP2 (microtubule-associated protein-2) is an AKAP highly expressed in neurons and in non-neuronal cells. MAP2 tethers and targets PKA on microtubules. In neurons, MAP2 is localized at dendrites and it has been implicated in neuritogenesis and synapse formation (Johnson and Jope, 1992). Mice bearing a mutation within the PKA binding domain of MAP2 show morphological alterations of the hippocampal architecture and significant deficit in memory processing (Khuchua et al., 2003, Weisenhaus et al., 2010).

Ubiquitin control of cAMP signaling in neurons

Ubiquitin-directed proteolysis of adenylate

cyclase, PDEs, PKA, AKAPs and downstream targets is emerging as important mechanism to finely modulate the extent and duration of the activated cascade. Regulation of the proteasome activity by PKA constitutes an auto-regulatory loop among components of the cAMP pathway and the ubiquitin•proteasome system (UPS). This cross-talk shapes the wave of cAMP signaling, ensuring efficient and temporally-monitored propagation of the messages evoked by hormones at cell membranes. Dysregulation of any component of this circuitry may lead to cell dysfunction and human disorders, including neurodegenerative diseases (Carlucci et al., 2008b). The levels of AKAPs and PKA can be regulated at the post-translational level by the UPS. As an example, ubiquitin-dependent proteolysis of the mitochondrial scaffold AKAP121/149 in course of hypoxic conditions rapidly reduces mitochondrial respiration and oxidative ATP synthesis, providing a mechanism of attenuation of the cAMP/PKA cascade that occurs at sites distal to signal generation (Carlucci et al., 2008a). Similarly, delocalization of PKA from mitochondria affects the physiology of the organelles, promoting oxidative stress and mitochondrial fragmentation, eventually leading to mitophagy and cell death of cardiomyocyte and neurons. Accordingly, forced relocalization of PKA by overexpressing AKAP1 prevents mitochondrial damage in course of ischemic insult or Parkinson's disease (Dagda et al., 2011). This constitutes an important mechanism to finely modulates the amplitude and duration of the signaling cascade, positively impacting on cell physiology.

A novel member of the AKAP family, namely praja2, has been recently characterized as essential relay in the cAMP cascade. praja2 is a prototypic AKAP with intrinsic E3 ubiquitin ligase activity abundantly expressed in different brain areas. praja2 controls the stability of intracellular substrates and plays an essential role in different aspects of cell signaling. Under basal conditions, praja2 controls the bulk levels of compartmentalized PKA holoenzyme. In course of GPCR stimulation, praja2 optimally couples cAMP signaling to proteolysis of PKA-R subunits, reducing the R/PKAc ratio and sustaining substrate phosphorylation by the locally activated kinase. Removing the inhibitory R subunits, praja2 enhances CREB phosphorylation and cAMP-directed gene transcription, positively impacting on synaptic plasticity and long-term memory (Lignitto et al., 2011a, Lignitto et al., 2011b).

Role of cAMP and the ubiquitin pathway in neurite outgrowth

Neurite extension constitutes a major mechanism that underlies the development and activity of the central nervous system (CNS). Dynamic remodeling of neurites and synaptic terminals is a complex process that requires coordinated activation of distinct signaling pathways by neurotrophins (NTFs) and neurotransmitters (Huang and Reichardt, 2001, Poo, 2001). Inhibitory constraints of glial cells and of myelin-associated inhibitors on neurite extension prevent uncontrolled formation of new circuits, contributing to the maintenance and dynamic remodeling of brain activity, easily adapting the neural network to fluctuations of external stimuli. However, in course of brain injury, inhibitors of neuritogenesis oppose spontaneous regeneration of damaged neurites (Horner and Gage, 2000). This is a major limitation for therapeutic initiatives aimed to restore the perturbed brain activity.

Neurite outgrowth inhibithor NOGO-A, is a membrane protein abundantly expressed in oligodendrocytes and in distinct neuronal subpopulations. NOGO-A acts as the principal inhibitor of neuritogenesis in central nervous system (GrandPre et al., 2000). In the adult brain and in injured neurons, NOGO-A limits the axonal growth and regeneration. Deletion of NOGO-A gene promotes neuritogenesis and fasciculation of dorsal root ganglion neurons and oligodendrocytes and favors the neuronal network and recovery from post-ischemic adult brain jnjury (Bongiorno and Petratos, 2010). In response to neurotrophin stimulation, NOGO-A undergoes to ubiquitination and proteasomal degradation. praja2 was identified as the E3-ligase that ubiquitinates NOGO-A. praja2 acts in response to cAMP-PKA stimulation by NTFs, as PKA phosphorylation of praja2 stimulates its ubiquitin ligase activity. Proteolysis of NOGO-A promotes neurite outgrowth, both in differentiating neurons and brain (Fig. 2) (Sepe et al., 2014). These findings highlight the existence of a negative regulation of NOGO-A stability by neurothrophins which act through compartmentalized cAMP

signaling. This UPS-driven signalling circuit targeting NOGO-A would promote and sustain biological processes underlying neurite extension, well impacting on neural network and synaptic activity.

Deranged cAMP signaling in neurodegenerative diseases

As previously described, cAMP balance in neurons is crucial for learning, memory and physiological events. So, perturbation of local cAMP signaling can contribute to development and progression of neurodegenerative diseases. Evidences show potential involvement of the cAMP pathway in a growing list of neurodegenerative disorders, like Alzheimer's disease, Parkinson's disease and Huntington's disease. Alzheimer's disease (AD) is characterized by the progressive loss of cognitive function and memory and is the fourth cause of death of old people. The disease is characterized by accumulation of β-amyloid (Aβ) which forms plaques and intracellular neurofibrillary tangles, causing cholinergic transmission defects and neuronal loss (Sonkusare et al., 2005). It is generally assumed that inflammation is linked to the pathogenesis of the disease, and that the amyloid plaques seem to trigger this inflammatory process (McGeer and McGeer, 1995, Martinez et al., 1999, Halliday et al., 2000, Rogers et al., 2008). Cumulating evidences suggest that progression of AD is associated with a limitation of cAMP signaling. Studies show that, at early stage of the disease, cAMP phosphodiesterases (PDE4B, PDE7 and PDE8) are upregulated **(Figure 3**) (Perez-Torres et al., 2003). By increasing the activity of cAMP-hydrolyzing enzymes, Aβ accumulation causes a rapid and long-lasting decrease in the activity of PKA in cultured hippocampal neurons, with consequent inhibition of neurothrophin-dependent CREB phosphorylation (Vitolo et al., 2002). Derangement of compartmentalized cAMP also contributes to the pathogenesis of AD. Thus, AKAP79-mediated anchoring of PKA is responsible of neurofibrillary degeneration, one of the main features of AD patients (Scott et al., 1993, Leger et al., 1997). In particular, AKAP79 promotes hyperphosphorylation of tau by PKA. Hyperphosphorylated tau cannot be degraded and accumulates in the brain, forming aggregates. These proteinaceous aggregates damage neuronal structure and impair synaptic activity (Jicha et al., 1999). Hence, PKA elicits the shift from normal tau towards hyperphosphorylated tau and it well correlates with the initial step of the disease (Fig. 3). These studies predicted that enhancing cAMP/PKA pathway could reverse the AD neuronal phenotype. Accordingly, selective inhibition of phosphodiesterases or prolonged exposure to an enriched environment that activates β2-adrenergic receptors ameliorated the cognitive functions and synaptic activity in mouse models of AD (Gong et al., 2004, Smith et al., 2009a, Li et al., 2013).

Parkinson's disease (PD) is the most common movement disorder, caused by the loss of dopaminergic (DA) neurons in the *substantia nigra*. PD also causes substantial alterations of the dendritic spine morphology and function of striatal projection neurons (SPNs) (Smith et al., 2009b, Schapira and Tolosa, 2010, Hirsch et al., 2013)**.** Leucine-rich repeat kinase 2 (LRRK2) is a gene pathogenetically linked with both, familiar and sporadic PD (Singleton et al., 2013). LRRK2 acts as an AKAP-like protein and controls intracellular distribution of PKARIIβ in striatal neurons (Parisiadou et al., 2014). During synaptogenesis or in response to activation of dopamine receptor, LRRK2 negatively regulates PKA activity in the SPNs. The loss of function of LRRK2 gene induces synaptic translocation of PKA, where it increases phosphorylation of actin-disassembling enzyme cofilin and of glutamate receptor GluR1. All this, eventually, leads to altered synaptogenesis and reduced synaptic activity in the developing SPNs (Muda et al., 2014, Parisiadou et al., 2014) . As envisioned, inhibition of adenylate cyclase reversed the PD phenotype in mice (Park et al., 2014).

Mitochondrial dysfunctions and disruption of mitochondrial homeostasis are implicated in the pathogenesis of PD (Abeliovich and Flint Beal, 2006, Burte et al., 2014). PKA plays a crucial role in the mitochondrial homeostasis. Local activation of PKA leads to the efficient phosphorylation of several mitochondrial substrates. This modification modulates the effects of cAMP on distinct mitochondrial functions. The balance between fusion and fission requires a tight control by factors that participate in mitochondrial dynamics and

homeostasis. Mitochondrial fission is regulated by dynamin-related protein 1 (Drp1). Drp1 is a direct substrate of PKA. Phosphorylation of Drp1 by PKA causes the suppression of fission, increase the stability of mitochondrial network (Cribbs and Strack, 2007). Genetic silencing of PTEN-induced kinase1, PINK1, a protein that exert a protective role in neuron, induces mitochondrial fragmentation, reduction of mitochondrial membrane potential and accumulation of mitochondrial ROS (Haque et al., 2008). Forced relocalization of PKA on the outer mitochondrial membrane by AKAP1 restores mitochondrial respiratory activity, reduces mitophagy and promotes neuronal survival (Dagda et al., 2011). These data suggest a pathogenic role of deranged mitochondrial cAMP signaling in PD (Fig. 3).

A positive feedback regulation between PKA signaling and the proteasome activity is critical for the pathogenesis of Huntington's disease (HD). HD is a neurodegenerative disorder caused by the expansion of a CAG repeat in the Hungtintin (HTT) gene. Components of the cAMP/PKA pathway are abnormally regulated during HD progression. Thus, poly(Q)-expanded mutant HTT (mHTT) downregulates the expression of the striatum-enriched A2A adenosine receptor (A2A-R) by preventing the binding of CREB to the A2A-R core promoter. Conversely, stimulation of A2A-R restores CREB binding and significantly reduces aggregation of mutant HTT (Fig. 3) (Chiang et al., 2005). Moreover, in mouse models of HD, mHTT is linked to impairment of the proteasome pathway. Decreased turnover of R subunits within the striatum favors the reconstitution of PKA holoenzyme, limiting local activation of PKA. Conversely, phosphorylation of components of the proteasome (Rpt6) by PKA rescued the proteasome activity, negatively impacting on the formation of mHTT aggregates (Lin et al., 2013). Altogether, these findings indicate that perturbation of local cAMP signaling, as consequence of PKA inhibition or activation of specific subsets of PDEs or by a combination of both events, could contribute to the development and progression of neurodegenerative disorders.

Concluding remarks and future perspectives

Emerging evidences point to a role of deranged cAMP signaling in the pathogenesis of neurodegenerative disorders. In the last years, it emerged that dissemination of signals from cell membrane to distinct intracellular compartments by AKAPs controls essential aspects of cell biology. Functional analysis of cAMP in cells and mouse lines carrying genetic inactivation of distinct classes of AKAPs revealed a major contribution of these proteins in differentiation, growth, development and metabolism. In this context, special attention has been drained by the characterization of the role of AKAPs in a variety of neuronal functions, as ion channel activity, synaptic transmission, learning and memory. Genetic defects affecting compartmentalized cAMP signaling in several neurological disorders are now contributing to define a pathogenic role of AKAPs in human diseases. Therefore, the structural and functional analysis of macromolecular complexes nucleated by AKAPs in neurons, the identification of pathogenic alterations of these signaling networks *in vivo* and the generation of appropriate mouse models of human disease will likely impact on the early diagnosis and better treatment of brain disorders.

ACKNOWLEDGEMENTS

AF wrote the review with contributions from the co-authors. We apologize to those whose work was not mentioned in this review due to space limitations.

REFERENCES

Abel T, Nguyen PV (2008) Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. Prog Brain Res 169:97-115.

Abeliovich A, Flint Beal M (2006) Parkinsonism genes: culprits and clues. J Neurochem 99:1062-1072.

Bauman AL, Goehring AS, Scott JD (2004) Orchestration of synaptic plasticity through AKAP signaling complexes. Neuropharmacology 46:299-310.

Beene DL, Scott JD (2007) A-kinase anchoring proteins take shape. Curr Opin Cell Biol 19:192-198.

Bongiorno D, Petratos S (2010) Molecular regulation of Nogo-A in neural cells: Novel insights into structure and function. Int J Biochem Cell Biol 42:1072-1075.

Burte F, Carelli V, Chinnery PF, Yu-Wai-Man P (2014) Disturbed mitochondrial dynamics and neurodegenerative

disorders. Nat Rev Neurol.

Carlucci A, Adornetto A, Scorziello A, Viggiano D, Foca M, Cuomo O, Annunziato L, Gottesman M, Feliciello A (2008a) Proteolysis of AKAP121 regulates mitochondrial activity during cellular hypoxia and brain ischaemia. EMBO J 27:1073-1084.

Carlucci A, Lignitto L, Feliciello A (2008b) Control of mitochondria dynamics and oxidative metabolism by cAMP, AKAPs and the proteasome. Trends Cell Biol 18:604-613.

Chiang MC, Lee YC, Huang CL, Chern Y (2005) cAMP-response element-binding protein contributes to suppression of the A2A adenosine receptor promoter by mutant Huntingtin with expanded polyglutamine residues. J Biol Chem 280:14331-14340.

Cribbs JT, Strack S (2007) Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. EMBO Rep 8:939-944.

Dagda RK, Gusdon AM, Pien I, Strack S, Green S, Li C, Van Houten B, Cherra SJ, 3rd, Chu CT (2011) Mitochondrially localized PKA reverses mitochondrial pathology and dysfunction in a cellular model of Parkinson's disease. Cell Death Differ 18:1914-1923.

Feliciello A, Gottesman ME, Avvedimento EV (2001) The biological functions of A-kinase anchor proteins. J Mol Biol 308:99-114.

Gong B, Vitolo OV, Trinchese F, Liu S, Shelanski M, Arancio O (2004) Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. J Clin Invest 114:1624-1634.

GrandPre T, Nakamura F, Vartanian T, Strittmatter SM (2000) Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 403:439-444.

Halliday G, Robinson SR, Shepherd C, Kril J (2000) Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. Clin Exp Pharmacol Physiol 27:1-8.

Haque ME, Thomas KJ, D'Souza C, Callaghan S, Kitada T, Slack RS, Fraser P, Cookson MR, Tandon A, Park DS (2008) Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. Proc Natl Acad Sci U S A 105:1716-1721.

Havekes R, Canton DA, Park AJ, Huang T, Nie T, Day JP, Guercio LA, Grimes Q, Luczak V, Gelman IH, Baillie GS, Scott JD, Abel T (2012) Gravin orchestrates protein kinase A and beta2-adrenergic receptor signaling critical for synaptic plasticity and memory. J Neurosci 32:18137-18149.

Higgs HN (2001) Actin nucleation: nucleation-promoting factors are not all equal. Curr Biol 11:R1009-1012.

Hirsch EC, Jenner P, Przedborski S (2013) Pathogenesis of Parkinson's disease. Mov Disord 28:24-30.

Horner PJ, Gage FH (2000) Regenerating the damaged central nervous system. Nature 407:963-970.

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677-736.

Jicha GA, Weaver C, Lane E, Vianna C, Kress Y, Rockwood J, Davies P (1999) cAMP-dependent protein kinase phosphorylations on tau in Alzheimer's disease. J Neurosci 19:7486-7494.

Johnson GV, Jope RS (1992) The role of microtubule-associated protein 2 (MAP-2) in neuronal growth, plasticity, and degeneration. J Neurosci Res 33:505-512.

Khuchua Z, Wozniak DF, Bardgett ME, Yue Z, McDonald M, Boero J, Hartman RE, Sims H, Strauss AW (2003) Deletion of the N-terminus of murine map2 by gene targeting disrupts hippocampal ca1 neuron architecture and alters contextual memory. Neuroscience 119:101-111.

Leger J, Kempf M, Lee G, Brandt R (1997) Conversion of serine to aspartate imitates phosphorylation-induced changes in the structure and function of microtubule-associated protein tau. J Biol Chem 272:8441-8446.

Li S, Jin M, Zhang D, Yang T, Koeglsperger T, Fu H, Selkoe DJ (2013) Environmental novelty activates beta2-adrenergic signaling to prevent the impairment of hippocampal LTP by Abeta oligomers. Neuron 77:929-941.

Lignitto L, Carlucci A, Sepe M, Stefan E, Cuomo O, Nistico R, Scorziello A, Savoia C, Garbi C, Annunziato L, Feliciello A (2011a) Control of PKA stability and signalling by the RING ligase praja2. Nat Cell Biol 13:412-422.

Lignitto L, Sepe M, Carlucci A, Feliciello A (2011b) An intimate connection between ubiquitination and compartmentalized cAMP signaling. Cell Cycle 10:2051-2052.

Lin F, Wang H, Malbon CC (2000) Gravin-mediated formation of signaling complexes in beta 2-adrenergic receptor desensitization and resensitization. J Biol Chem 275:19025-19034.

Lin JT, Chang WC, Chen HM, Lai HL, Chen CY, Tao MH, Chern Y (2013) Regulation of feedback between protein kinase A and the proteasome system worsens Huntington's disease. Mol Cell Biol 33:1073-1084.

Martinez M, Fernandez E, Frank A, Guaza C, de la Fuente M, Hernanz A (1999) Increased cerebrospinal fluid cAMP levels in Alzheimer's disease. Brain Res 846:265-267.

McGeer PL, McGeer EG (1995) The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Brain Res Rev 21:195-218.

McKnight GS, Cummings DE, Amieux PS, Sikorski MA, Brandon EP, Planas JV, Motamed K, Idzerda RL (1998) Cyclic AMP, PKA, and the physiological regulation of adiposity. Recent Prog Horm Res 53:139-159; discussion 160-131.

Muda K, Bertinetti D, Gesellchen F, Hermann JS, von Zweydorf F, Geerlof A, Jacob A, Ueffing M, Gloeckner CJ, Herberg FW (2014) Parkinson-related LRRK2 mutation R1441C/G/H impairs PKA phosphorylation of LRRK2 and disrupts its interaction with 14-3-3. Proc Natl Acad Sci U S A 111:E34-43.

Nozumi M, Nakagawa H, Miki H, Takenawa T, Miyamoto S (2003) Differential localization of WAVE isoforms in filopodia and lamellipodia of the neuronal growth cone. J Cell Sci 116:239-246.

Parisiadou L, Yu J, Sgobio C, Xie C, Liu G, Sun L, Gu XL, Lin X, Crowley NA, Lovinger DM, Cai H (2014) LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. Nat Neurosci 17:367-376.

Park HY, Kang YM, Kang Y, Park TS, Ryu YK, Hwang JH, Kim YH, Chung BH, Nam KH, Kim MR, Lee CH, Han PL, Kim KS (2014) Inhibition of adenylyl cyclase type 5 prevents L-DOPA-induced dyskinesia in an animal model of Parkinson's disease. J Neurosci 34:11744-11753.

Perez-Torres S, Cortes R, Tolnay M, Probst A, Palacios JM, Mengod G (2003) Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. Exp Neurol 182:322-334.

Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24-32.

Rogers JT, Bush AI, Cho HH, Smith DH, Thomson AM, Friedlich AL, Lahiri DK, Leedman PJ, Huang X, Cahill CM (2008) Iron and the translation of the amyloid precursor protein (APP) and ferritin mRNAs: riboregulation against neural oxidative damage in Alzheimer's disease. Biochem Soc Trans 36:1282-1287.

Sanderson JL, Dell'Acqua ML (2011) AKAP signaling complexes in regulation of excitatory synaptic plasticity. Neuroscientist 17:321-336.

Schapira AH, Tolosa E (2010) Molecular and clinical prodrome of Parkinson disease: implications for treatment. Nat Rev Neurol 6:309-317.

Scott CW, Spreen RC, Herman JL, Chow FP, Davison MD, Young J, Caputo CB (1993) Phosphorylation of recombinant tau by cAMP-dependent protein kinase. Identification of phosphorylation sites and effect on microtubule assembly. J Biol Chem 268:1166-1173.

Sepe M, Lignitto L, Porpora M, Delle Donne R, Rinaldi L, Belgianni G, Colucci G, Cuomo O, Viggiano D, Scorziello A, Garbi C, Annunziato L, Feliciello A (2014) Proteolytic control of neurite outgrowth inhibitor NOGO-A by the cAMP/PKA pathway. Proc Natl Acad Sci U S A 111:15729-15734.

Singleton AB, Farrer MJ, Bonifati V (2013) The genetics of Parkinson's disease: progress and therapeutic implications. Mov Disord 28:14-23.

Smith DL, Pozueta J, Gong B, Arancio O, Shelanski M (2009a) Reversal of long-term dendritic spine alterations in Alzheimer disease models. Proc Natl Acad Sci U S A 106:16877-16882.

Smith Y, Villalba RM, Raju DV (2009b) Striatal spine plasticity in Parkinson's disease: pathological or not? Parkinsonism Relat Disord 15 Suppl 3:S156-161.

Sonkusare SK, Kaul CL, Ramarao P (2005) Dementia of Alzheimer's disease and other neurodegenerative disorders--memantine, a new hope. Pharmacol Res 51:1-17.

Tasken K, Aandahl EM (2004) Localized effects of cAMP mediated by distinct routes of protein kinase A. Physiol Rev 84:137-167.

Taylor SS, Kim C, Cheng CY, Brown SH, Wu J, Kannan N (2008) Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. Biochim Biophys Acta 1784:16-26.

Taylor SS, Knighton DR, Zheng J, Ten Eyck LF, Sowadski JM (1992) Structural framework for the protein kinase family. Annu Rev Cell Biol 8:429-462.

Tunquist BJ, Hoshi N, Guire ES, Zhang F, Mullendorff K, Langeberg LK, Raber J, Scott JD (2008) Loss of AKAP150 perturbs distinct neuronal processes in mice. Proc Natl Acad Sci U S A 105:12557-12562.

Vitolo OV, Sant'Angelo A, Costanzo V, Battaglia F, Arancio O, Shelanski M (2002) Amyloid beta -peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signaling. Proc Natl Acad Sci U S A 99:13217-13221.

Weisenhaus M, Allen ML, Yang L, Lu Y, Nichols CB, Su T, Hell JW, McKnight GS (2010) Mutations in AKAP5 disrupt dendritic signaling complexes and lead to electrophysiological and behavioral phenotypes in mice. PLoS One 5:e10325.

MAMMALIAN CARBONIC ANHYDRASE FAMILY OF ENZYMES IN THE NERVOUS SYSTEM: A FOCUS ON CARBONIC ANHYDRASE IX

M. VITALE[1,2], F. MONTELEONE[1,2], E. SASSO[1,2,3], C. GARBI[1], S. PARISI[1,2] and N. ZAMBRANO[1,2,3]

[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy; [2]CEINGE Biotecnologie Avanzate, Napoli, Italy; [3]Associazione Culturale DiSciMuS RFC, Casoria (NA), Italy

MV and FM contributed equally to this review

Mammalian carbonic anhydrases (CAs; also known as Carbonate Dehydratases EC 4.2.1.1) constitute a wide and complex family of enzymes, due to their peculiar schemes of expression and localizations in tissues and cells. Their fundamental activities are involved in transport of CO_2 and bicarbonate, pH balance, gas exchange, ion transport. However, besides their enzymatic activities, CAs are emerging as key regulators in several cellular processes. Among CAs, CA IX is a classical target of the hypoxia-induced factor HIF1A, ensuring proper response of cells to hypoxic stresses. Although CA IX is widely recognized as a prognostic factor and a therapeutic target in human cancer, its known properties, as well as its recently described novel activities, may be relevant to the physiopathology of the nervous system. In this review we describe the general properties of the several members of the carbonic anhydrase family of enzymes and their involvement in the physiopathology of the nervous system. We then conclude with a focus on the recently described, novel molecular functions of CA IX, highlighting the potential involvement of this peculiar member of the family in the neuronal responses and adaptation to hypoxic stress.

Carbonic Anhydrases are ubiquitous zinc metalloenzymes, present in prokaryotes and eukaryotes, that catalyze the reversible hydration reaction of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and protons (H^+). They are encoded by five evolutionarily unrelated gene families: the α-CAs (present in vertebrates, bacteria, algae and cytoplasm of green plants); the β-CAs (expressed predominantly in bacteria, algae and chloroplasts of monocotyledons and dicotyledons); the γ-CAs (mainly represented in archaea and some eubacteria); the δ-CAs and ζ-CAs (present in some marine diatoms) (Supuran et al., 2003, 2004 and 2008a; Scozzafava et al., 2006; Pastorekova et al., 2004a). In mammals, 16 α-CA isozymes or CA-related proteins are present: thirteen are active isozymes, while three lack classical CA activity because of the absence of one or more of the three functionally important histidines, the zinc binding residues, required for CA catalytic activity (Tashian et al., 2000; Nishimori et al., 2004). Fifteen CA isoforms are expressed in humans, as the CA XV gene is expressed in rodents, but it appears to have become a pseudogene in primates (Saari et al., 2010). They can be classified according to various criteria, including subcellular localization, catalytic activity and expression pattern. So, we can now

Key words: hypoxia, nucleolus, rDNA, nucleolar stress

Corresponding Author: Prof. Nicola Zambrano,
Dipartimento di Medicina Molecolare e Biotecnologie Mediche,
Via S. Pansini, 5
80131 Napoli, Italy
Te:. +39 081 3737 877 Fax: +39 081 3737 808
e--mail zambrano@unina.it

2279-5855 (2014)

distinguish intracellular (CA I-III, VA, VB, VII, VIII, X, XI, XIII) and extracellular (CA IV, VI, IX, XII, XIV), catalytically active (CAI-VII, IX, XII-XIV) and inactive (CA VIII, X, XI), wide-spread (CA II, IV, VB, XII, XIV) and restricted to few tissues (CA I, III, VA, VI, VII) isoforms (Pastorekova et al., 2004b). CAs catalytically active can be further divided into 3 broad categories: CA II, IV, VB and VII are the fastest human CAs; CA VA, VI, IX and XII show relatively intermediate catalytic activity, while CA III, XIII and XIV are the slower enzymes (Aggarwal et al., 2013; Alterio et al., 2012). The inactive isoforms are known as carbonic anhydrase related proteins (CARPs) VIII, X and XI as their sequence is similar to that of active α-CA isozymes. Furthermore, there are two CARPs which exist as domains of protein tyrosine phosphate receptor (PTPR) ζ or β and γ (Ohradanova et al., 2007).

CAs are usually expressed in well differentiated metabolically active cells and tissues. Their enzymatic activity is involved in many physiological and pathological processes based on gas exchange, ion transport and pH balance, such as respiration and transport of CO_2 and bicarbonate between metabolizing tissues and lungs; pH and CO_2 homeostasis; electrolyte secretion in various tissues and organs; biosynthetic reactions (gluconeogenesis, lipogenesis and ureagenesis); bone resorption; calcification; production of body fluids; digestion; renal acidification and tumorigenicity (Nishimori et al., 2005; Vullo et al., 2005; Kohler et al., 2007; Supuran et al., 1998). Cytosolic CA I (together with CA II) is expressed in red blood cells and works maintaining physiological pH of the blood through production of HCO_3^- (Maren et al., 1980). CA II is the most widely distributed, being almost ubiquitous, and it is one of the most efficient catalyst, showing a K_{cat} of 1.4×10^6. Among tissues in which it is expressed there are kidney (Brown et al., 1983), bone and also ocular tissues (Gilmour et al., 2010). CA III shows a 200-fold lower catalytic activity (Supuran et al., 2008b) and it is expressed only in skeletal muscle and adipose (both white and brown) tissue (Carter et al., 1991; Stanton et al., 1991; Lyons et al., 1991). Membrane-bound CA IV is expressed in the lung and in kidney (Zhu et al., 1990); in the latter, CA IV catalytic activity is involved in bicarbonate resorption and therefore necessary for its normal function (Sly et al., 1983). CA VA and VB are the unique mitochondrial isoforms and they are found in the matrix of mitochondria of hepatocytes and adipocytes, respectively (Shah et al., 2000); they play a key role in several metabolic pathways (Lynch et al., 1995). CA VI is the only secreted CA isoform; it is expressed in tears, respiratory airways, epithelial lining of the alimentary canal, enamel organs, and most significantly in human saliva (Ogawa et al., 2002; Leinonen et al., 2004; Kaseda et al., 2006; Smith et al., 2006; Feldstein et al., 1984). Its physiological function is likely associated to maintenance of pH homeostasis of the mouth (Ship et al., 2003). CA VII is primarily expressed in colon, liver, skeletal muscle, and in the brain (Bootorabi et al., 2010); its physiological role is unclear but it seems to play a role in neuronal excitement by way of HCO_3^- production (Thiry et al., 2007). Also CA IX is a transmembrane isoform, but it shows a unique distribution pattern among all of the CAs. It is expressed only in a few normal tissues, but it is highly expressed in several cancers (Pastorekova et al., 1997). It is a well known marker of hypoxia and is involved in pH regulation, migration/invasion and survival in hypoxic cancer cells. It is indicative of a poor prognosis in many cancer types and is associated with resistance to conventional therapy. CA XII is yet another tumor-associated CA (Ivanov et al., 2001). However, unlike CA IX, it is widely expressed also in normal tissues such as the kidney, lung, prostate, ovaries, uterine endometrium, breast, and basolaterial membrane of gut epithelium (Ivanov et al., 2001; Parkkila et al., 2000; Hynninen et al., 2012; Kivela et al., 2000). Furthermore, it seems to be important for normal kidney function (Muhammad et al., 2011). CA XIII has been found in the thymus, kidney, submandibular gland, small intestine, and predominantly in both male and female reproductive organs (Lehtonen et al., 2004.); it seems to play a significant role in pH regulation of reproductive processes including sperm motility (Kummola et al., 2005). Membrane-bound CA XIV is expressed in most parts of the brain, colon, small intestine, urinary bladder, kidney, and retina (Fujikawa-Adachi et al., 1999a; Ochrietor et al., 2005); its sequence is highly similar to that of CA XII and its expression pattern is strongly correlated with that of CA IV, suggesting a functional overlap

between them (Kaunisto et al., 2002). Among processes in which CA XIV is involved there are pH balance in muscle and erythrocytes in response to chronic hypoxia and pH regulation in the retina (Juel et al., 2003; Vargas et al., 2012; Linser et al., 1984). Finally, we conclude this general presentation of CA gene family members with the CARPs VIII, X, and XI, that show a wide expression profile in all parts of the brain in both humans and mice (Fujikawa-Adachi et al., 1999b; Okamoto et al., 2001; Taniuchi et al., 2002a; Akisawa et al., 2003). However, their expression was also found in many other organs: CARP VIII is expressed in the liver, lung, heart, gut, thymus, and kidney (Hirota et al., 2003; Akisawa et al., 2003); CA X is expressed in the human testis, salivary glands, and kidney (Okamoto et al., 2001); CA XI expression is found in the kidney, liver, and salivary glands (Fujikawa-Adachi et al., 1999b).

FUNCTIONS OF CARBONIC ANHYDRASES IN THE CENTRAL NERVOUS SYSTEM

Maintenance of pH homeostasis is a key factor in the functioning of the Central Nervous System (CNS). Among processes affected by pH shifts there are cell volume regulation, metabolism and transport of lactate, glutamine and glutamate and neuronal excitability. Specifically, protons modulate neuronal excitability by conditioning synaptic transmitter release, conductance of ionic channels and gap junctional communication. It has been also suggested that pH gradients may be important in neuronal differentiation, development of growth cones and neurites, regulation of pH in dendrite spines, learning and memory (Obara et al., 2008). Importance of pH regulation in the CNS becomes even greater considering that neuronal activity itself, or application of specific membrane ligands, cause rapid extracellular and intracellular pH shifts, that take place in time frames from milliseconds to minutes involving both neurons and glia. Other factors determining pH shifts in the CNS cells are amino acid neurotransmitters, GABA and glycine (Obara et al., 2008). The mechanisms responsible for the regulation of intracellular pH in brain are analogous to those present in other tissues and include principally transport of acid/base equivalents across cell membranes (Na^+/H^+ exchanger, Na^+/HCO_3^- cotransporters, Na^+-independent Cl^-/HCO_3^- exchangers, Na^+-driven Cl^-/HCO_3^- exchangers, V-ATPase proton pump, monocarboxylic acid transporters), acidification of organelles and metabolic production of protons. Carbonic anhydrases play a key role in pH regulation along with the mechanisms listed above. They convert protons to bicarbonate, thus buffering intracellular pH. In the CNS of mammals, all CA isozymes except CA I were shown to be expressed (Ghandour et al., 2000; Parkkila et al., 2001; Nogradi et al., 2003; Lehtonen et al., 2004; Hilvo et al., 2005; Rivera et al., 2005; Halmi et al., 2006; Kida et al., 2006; Kallio et al., 2006; Wang et al., 2006). In the brain, they are primarily expressed in glial cells (Giacobini 1962; Sapirstein et al. 1984; Cammer 1991), and to a lesser degree in neurones and in the extracellular spaces (Diaz et al. 1982; Agnati et al. 1995). Glial cells, comprising astrocytes, oligodendrocytes and microglia, have an important role in the regulation of ion concentrations in the intracellular and extracellular spaces in the brain (Maragakis et al., 2006). They are primarily glycolytic and convert glucose to lactic acid: pyruvate, instead of being metabolized through the tricarboxylic acid (TCA) cycle, is converted into lactate by lactate deydrogenase (LDH) isoform 5 (Dringen et al. 1993; Tsacopoulos and Magistretti 1996; Schousboe et al. 1997). Lactate leaves glial cells through the monocarboxylate transporter MCT-1, using protons produced through hydration reaction of CO_2 by intracellular CAs (on the other hand bicarbonate produced through the same reaction activates the sodium-bicarbonate cotransport). The lactate is subsequently taken up into neurons via MCT-2 in a cotransport with a proton and converted to pyruvate by lactate deydrogenase (LDH) isoform 1 to be then channelled into the TCA cycle and ultimately to generate ATP and CO_2 by oxidative metabolism; protons are extruded from neurons through Na^+/H^+ exchange (Deitmer, 2002). So doing the intracellular and extracellular CAs act cooperatively, coupling intercellular CO_2 shuttling to the acid/base transporter activities (Obara et al., 2008). Several studies support the release of lactate from glial cells and the uptake of lactate into neurones (Walz and Mukieri 1988; Dringen et al. 1993; Poitry-Yamate et al. 1995; Hu and Wilson 1997; Schurr et al. 1997; Bouzier et al. 2000), processes that are both favoured by the different affinities for lactate of the glial MCT-1 and the neuronal MCT-2 (Bröer et al. 1997; Halestrap and

Price 1999). Therefore, in the brain the main source of CO_2 are active neurones, which generate their ATP mainly by oxidative metabolism (Sokoloff 1993) to maintain their electrical and synaptic activity (Schurr et al. 1988, 1999; Izumi et al. 1997). After leaving the neurones by diffusion, CO_2 may be hydrated to bicarbonate and protons by intracellular CA activity of glial cells to be then regenerated by extracellular CA activity on glial plasma membrane (Deitmer, 2002), that also allows buffering of extracellular pH.

Experiments with CA IV/CA XIV knockout mice allowed to find that in the hippocampus extracellular space CA activity is due mainly to isoforms CA IV and CA XIV (Shah et al., 2005). CA IX, an additional membrane-bound isoform, is lowly expressed in normal human brain tissue; it has been found in the ventricular lining cells and in the choroid plexus (Ivanov et al. 2001). In the mouse brain, CA IX was found in some neuronal axons and Purkinje cells (Hilvo et al., 2004). It may represent another transmembrane isozyme, specifically present on neurons, in addition to CA XIV (Pan et al., 2012). CA IX is also expressed in the eye during development (Liao et al. 2003).

CA II and CAVII are the only cytosolic isoforms present in both somata and dendrites of mature hippocampal CA1 pyramidal neurons (Hubner et al., 2013). CA II is the main isozyme of the CA family also in the human brain. It is expressed in neurons and glia (Ghandour et al., 1980); specifically, it is located mainly in oligodendroglia and less in astrocytes (Cammer et al 1977). CA II has multiple functions: production of HCO_3^-, that is involved in regulation of membrane transport of Na^+/water and contribute to cerebrospinal fluid formation; pH regulation, HCO_3^- reabsorption and CO_2 exhalation. Moreover, it seems to take part in the processes of myelination and to play a key role in signal processing, long-term synaptic transformation and attentional gating of memory storage (Sun et al., 2002). Interestingly, CA II has also been shown to be associated to several acid-base transporters, suggesting that it is involved in some metabolic pathways by providing the substrates for these various transporters (McMurtrie et al., 2004). Differently, CA VII is expressed only in neurons; together with CA II, they promote HCO_3^--dependent GABAergic depolarization and excitation triggered by intense $GABA_AR$ activation in mature neurons (Ruusuvuori et al., 2013). Both CA II and CA VII allow the fast replenishment of HCO_3^- and consequent net uptake of Cl^-, which are key mechanisms in the generation of excitatory HCO_3^--dependent GABAergic responses. As anticipated, CARPs were also found to be expressed in the human and mouse brain, suggesting for them important roles in the brain development and/or neural functions (Taniuchi et al., 2002a). However, their precise physiological roles are poorly understood. CARP VIII is predominantly expressed in the mouse and human cerebellum, especially in the Purkinje cells; differently, CARP X and XI have revealed a lower level of expression in the cerebellum (Nishimori et al., 2003; Taniuchi et al., 2002a) and showed the strongest mRNA expression in the nervous tissues. CA X is highly expressed in the parietal cortex and the frontal cortex, lowly expressed in the midbrain, and extremely low expressed in the eye. CA XI mRNA is expressed in all parts of the human brain. As suggested by their distribution patterns, CARPs may contribute to the development of the nervous system and motor coordination functions (Aspatwar et al., 2010).

CARBONIC ANHYDRASES IN NEUROLOGICAL DISORDERS

As a consequence of CA distribution in the CNS and of its key role in pH balance, the alteration of CA activity has been associated with some neurological disorders and behavioural abnormalities. CA II deficiency has been associated with pathological consequences such as mental retardation and brain calcification (Vlkolinsky et al., 2001). Carbonic anhydrase II deficiency syndrome (CADS) is an uncommon autosomal recessive disease; it represents the only known symptomatic inherited deficiency of a carbonic anhydrase. Three different structural gene mutations have been identified in patients with CADS: a missense mutation (H107Y), a splice junction mutation in intron 5 (G-to-C) and a splice junction mutation in intron 2 (Arabic Mutation). Clinical phenotype of subjects with CADS include osteopetrosis and renal tubular acidosis (Borthwick et al., 2003), reduced vision (Sly et al., 1985), sometimes decreased hearing (Zakzouk et al., 1995), possibly due to cranial nerve compression within narrowed bony foramina (Ohlsson et al., 1980),

and also short stature, a large cranial vault, multiple skeletal fractures, developmental delay and cognitive defects varying from mild learning disabilities to severe mental retardation, anaemia, splenomegaly and secondary erythropoiesis. A proteomic study carried out on inferior parietal lobule (IPL) from subjects affected by mild cognitive impairment (MCI) hig ted increased levels of carbonylated CA II associated with a parallel enzyme activity decline, suggesting that dysfunction of CA II, due to its oxidative modifications, impairs cognition and might be associated with decreased cognition in Alzheimer disease (AD) (Sultana et al., 2010). In fact, previous studies reported a decreased activity and oxidative modification of CA II in AD brain (Butterfield et al., 2007; Meier-Ruge et al., 1984; Sultana et al., 2006). Increased levels of CA II were also found in the brain of Ts65Dn mice, a mouse model for Down syndrome (DS), using a 2-D gel proteomic approach. CA II up-regulation in the brain of Ts65Dn mice was confirmed by western blot; CA II levels were also investigated in infants and young children with DS, in comparison with age-matched controls; CA II resulted overexpressed in the frontal and temporal cortices and white matter (Palminiello et al., 2008). Finally, CA II has also been associated with glaucoma (Aggarwal et al., 2013).

Mutant forms of CA IV have been shown to be associated with an autosomal dominant form of *retinitis pigmentosa*, despite intrinsic levels of wild-type CA IV not being observed in ocular tissue (Rebello et al., 2004).

In 2012, Price et al. published a study in which they had obtained rescues of oxidative stress-induced pericyte loss in the diabetic mouse by inhibiting mitochondrial CA V with topomirate. A later study carried out by Shah et al. and published in 2013 showed that topomirate was able to rescue both intracellular oxidative stress of pericytes, determined by exposure to high-glucose, and also apoptosis response, suggesting that CA V could be targeted in oxidative stress-related illnesses of the CNS.

Carbonic anhydrase VI may be involved in the pathogenesis of neurodegenerative diseases through the decrease of the intracellular pH during endoplasmic reticulum (ER) stress (Sok et al., 1999). In fact chronicization of Unfolded Protein Response (UPR) signalling leads to the induction of the multifunctional transcription factor, CHOP, through the ATF6- and PERK-mediated UPR pathways (Ma et al., 2002; Ron et al., 1992) and subsequently to activation of the transcription of several apoptotic genes among which there is CA VI. *Chop* -/- cells are protected from ER stress-induced apoptosis (Zinszner et al., 1998). Moreover, interestingly, CA VI was also associated with taste. Specifically, inhibition of CA VI was shown to cause irregularities in taste perception, or sometimes complete loss of taste (Ortho-McNeil, 2013). This effect was, however restored following exposure to high levels of zinc (Shatzman et al., 1981).

Inhibition of CA VII determines the interruption of the current-gated channel, so leading to suppression of neural excitement that translates into pain or seizures. (Ruusuvuori et al., 2004). So, CA VII was proposed as a target for treatment of seizures and neuropathic pain (Asiedu et al., 2010).

CA IX is best known as a tumor-associated isoform whose expression is a negative prognostic factor, also indicative of resistance to conventional therapy. To dissect CA IX function in the brain, Gut et al. in 2002 generated CA IX-deficient mice by the targeted disruption of the *Car9* gene. These mice showed distinct morphological and behavioural phenotypes. The brain tissue exhibited vacuolar degenerative changes and spongiform degeneration that were not visible in the wild-type mice. The brain tissue architecture was most severely disrupted. The *Car9-/-* mice also had several abnormal behavioural features and poorer memory function. These phenotypic changes seemed to be age-dependent, and the functional changes preceded the microscopic alterations in the brain. cDNA microarray analysis revealed 68 differentially transcribed genes, among which *Atp2b2*, *Maf*, *Gand*, *Cntfr* were genes of a special interest (Pan et al., 2012).

The nature of the CAs expressed and most likely involved in the development and function of the ciliary epithelium has been clarified, so allowing a better understanding of glaucoma pathogenesis (Liao et al., 2003). Initially, it was believed that CA IV was the only carbonic anhydrase to be involved in glaucoma. Conversely, in the study of Liao et al. CA IX and CA XII were found in anatomical structures of the human eye, namely, in embryonic, neonatal/infant, and adult eyes, under normal and

pathological conditions including angle closure glaucoma, whereas CA IV was not found in the ciliary epithelium (Hageman et al., 1991). CA IX and CA XII expression was also found in cultured human ciliary nonpigmented epithelial (NPE) cells from normal and glaucomatous eyes. The NPE from glaucoma eyes expressed higher levels of CA XII, but not CA IX, in comparison to normal eyes. So, transmembrane CA IX and CA XII seem to be really involved in aqueous humour production. Moreover, as overexpressed, CA XII may be a target gene in glaucoma. *CA9* gene hypermethylation (Cho et al., 2001) may determine overexpression CA XII in the ciliary NPE cells, which may in turn cause overproduction of the aqueous humour and subsequently high intraocular pressure and hence lead to glaucoma; alternatively the overexpression of CA XII in glaucoma patients may be caused by mutated allele(s) of this gene.

Many CAs are important therapeutic targets that may be inhibited to treat a range of disorders including edema, glaucoma, obesity, cancer, epilepsy and osteoporosis (Sethi et al., 2011). Glaucoma is a complex eye disorder, clinically and genetically heterogeneous (Sheffield et al., 2001; Lichter et al., 2001; Wang et al., 2001). Multiple causative and modifying susceptibility genes are involved in its transmission. To date, at least 10 loci that potentially confer susceptibility to glaucoma have been mapped and so far only two causative candidate genes have been identified (WuDunn et al., 2002; Stone et al., 1997; Rezaie et al., 2002). It is treated using carbonic anhydrase inhibitors both locally (dorzolamide, brinzolamide) as well as general (acetazolamide). It has been proved that these drugs not only lower the intra ocular pressure by reducing the rate of aqueous humour secretion mediated by the CAs in the ciliary epithelium, but also improve the flow in eye blood vessels. Moreover, acetazolamide increases the cerebral flow (Moss et al., 2010) and dorzolamide protects retinal ganglion cells (RGCs) against the influence of apoptosis-inducing factors (Rohit et al., 2008). Another therapeutic application of acetazolamide is as anticonvulsants for treatment of epilepsy, even though its side effects limit use for extended periods (Ruusuvuori et al., 2013).

CARP VIII plays an important role in motor coordination. The evidence for the involvement of *CA8* gene in neurodegeneration and ataxia came from waddles mice, which were originally discovered at The Jackson Laboratory (TJL); these mice show a 19-base pair deletion in *Car8* gene and are characterized by wobbly side-to-side ataxic movement. The deletion in *Car8* gene leads to the complete absence of CARP VIII protein and caused both structural and functional abnormalities of excitatory synapses of Purkinje cells, which may lead to motor coordination defect in these mice (Hirasawa et al., 2007). The involvement of *CA8* gene in human neurodevelopmental disorder was also suggested, based on the studies in members of Iraqi and Saudi Arabian families (Kaya et al., 2011; Turkmen et al., 2009), whose affected members had a phenotype similar to wdl mice (Jiao et al., 2005), with ataxia and lifelong gait disorder, because of a homozygous missense mutation in the gene encoding CARP VIII that caused the substitution a serine 100 by a proline residue. Their clinical features included cerebellar ataxia, dysarthria, mild mental retardation and tremor (Turkmen et al., 2009). *In vitro*, the mutation S100P induces proteasome-mediated degradation of CARP VIII protein, probably due to misfolding, leading to drastically reduced amounts of protein. It is well known that any expansion mutation in trinucleotide repeats can lead to neurodegenerative disorders in humans. Interestingly, unlike the other active isozymes belonging to the α-CA gene family, CARP VIII cDNAs of all mammals contain 19 GAG repeats which code for glutamic acid (Aspatwar et al., 2010).

At a cellular level CARP X protein is expressed in the myelin sheath. The expression of CARP X in the myelin sheath in the normal human and mouse brain and the loss of expression in the disease suggests the involvement of CARPX in myelin sheath organization (Taniuchi et al., 2002b). Moreover Okamoto et al. in 2001 reported that the *Car10* sequence contains seven CCG repeats in the 5'-untranslated region followed by two CCG repeats located 16 bp downstream. These repeats have been associated with various neurological disorders (Kleiderlein et al., 1998); so, the presence of the CCG repeats in the *Car10* gene makes it a potential candidate gene that might contribute to the development of neurodegenerative disorders. Specifically, CARP X could be involved in

demyelination disorders (Kleiderlein et al., 1998).

Recently, in 2013, Hsieh et al., studied the expression of CARP XI in cultured neuronal cells expressing mutant ataxin 3 and in humans and mice with defects in ataxin 3 protein that, as known, contains CAG trinucleotide repeats whose expansion cause a neurodegenerative disorder known as spinocerebellar ataxia 3 /Machado-Joseph disease (SCA3/MJD) in both humans and mice (Kawaguchi et al., 1994) showing that CA11 mRNA was overexpressed and CA11 protein present an altered cellular localization (Hsieh et al., 2013).

CARBONIC ANHYDRASE IX, AN UNUSUAL MEMBER OF THE CA FAMILY

CA IX has been described as a non-canonical carbonic anhydrase enzyme. In fact, even though its canonical subcellular localization is classically reported on plasma membrane, recent experimental evidences obtained in mammalian cells, including the neuroblastoma SH-SY5Y cells, have shown that CA IX is actively trafficking to, and from, the nuclei, via its interaction with importins and exportins (Buanne et al., 2013). Oxygen levels regulate nuclear and subnuclear CA IX localizations. In the nuclear compartment, CA IX binds to, and contributes to the active transcription of the repeated 45S rDNA genes in the nucleoli of normoxic cells. Contrariwise, in cells exposed to hypoxia, CA IX increases its presence in complexes with exportin-1 in the nucleoli. Such exportin-1 based mechanism of CA IX removal from nucleolar rDNA genes may act as a decoy mechanism, to down-regulate rDNA transcription (Sasso et al., 2015). We proposed that this mechanism may act as an attenuator of energy consumption in the hypoxic cells. The conventional function of nucleoli is to synthesize the 28S, 18S and 5.8S ribosomal RNAs from the unique 45S rRNA precursor, transcribed by RNA polymerase I, and to assemble them in the ribosomal subunits with the nucleoplasmic-derived rRNA 5S and ribosomal proteins. These events are among the most energy demanding processes for a cell; so, rDNA gene transcription needs to be tightly regulated in order to preserve the energy homeostasis of a hypoxic cell. Indeed, it's known that the nucleolar compartment can be used by cells in order to regulate different physiopathological conditions. These recent evidences shed light on a central role of CA IX on nucleolar response to hypoxic stress.

A fine regulation of rDNA transcription is also required during differentiation, cell survival, and embryonic development. In particular, just before neuronal differentiation, for example in brain and retina, the synthesis and maturation markers of rRNA are rapidly reduced, suggesting that nucleoli may play an important role both in neurogenesis and in neurodegeneration. Carbonic anhydrase IX function may be related to cell survival in the differentiating neuronal precursors, and the nuclear accumulation of this enzyme in hypoxic condition could shed light on new non-canonical role of CA IX also on nucleoplasmic transcription of DNA, rather than on nucleolar rDNA. Besides its function in nucleoli, further studies may indeed reveal CA IX functions in the nuclear transcription by RNA polymerase II as well. Recent evidence from our laboratory shows the potential involvement of CA IX in two models of neuronal differentiation. In fact, CA IX expression and its nuclear representation are dramatically increased in Sox1-negative, β3-tubulin positive neurons (Figure 1) originating from differentiating mouse ES cell system (Parisi et al., 2010). Additionally, CA IX expression and its nuclear representation are increased in SH-SY5Y cells differentiated upon retinoic acid treatment (Figure 2). Accordingly, retinoic acid-induced neuronal differentiation of SH-SY5Y neuroblastoma cells is accompanied by decreased association of the general factor UBF1 and CA IX itself to nucleolar chromatin (Figure 3), in agreement with the decreased rDNA transcription occurring in differentiating neuronal cells. This may be seen as a molecular mechanism ensuring removal of CA IX from nucleolar chromatin and its availability outside nucleoli, to regulate genes transcribed by RNA polymerase II, sustaining cell survival in differentiated cells. Further experiments on the search for CA IX gene targets will clarify this matter.

Over these physiological conditions, nucleoli are able to rebuild their function and structure in response to different kind of stresses. In fact adverse growth conditions, like oxidative stress, metabolic deficits and oncogene activations rapidly induce a down-regulation of rRNA synthesis and a nucleolar architectural reorganization by transcriptional and

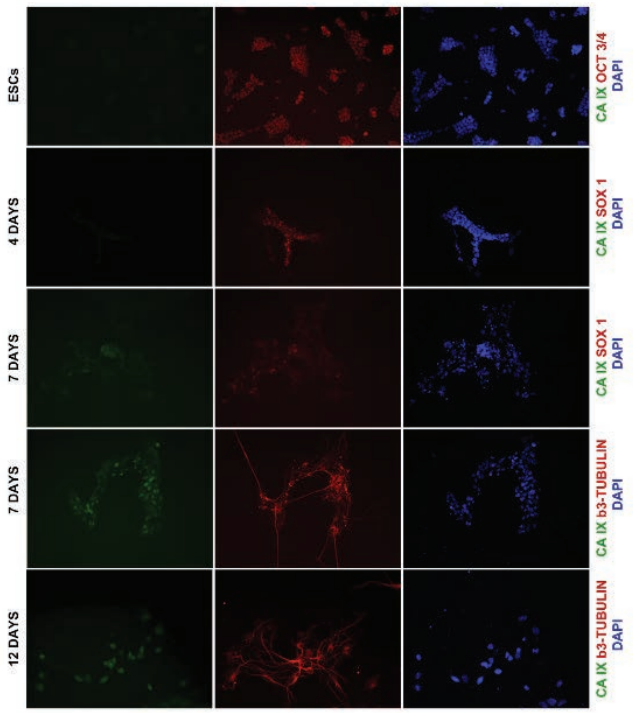

Fig. 1. ***CA IX expression in differentiating mouse ESCs.*** *CA IX (green) expression is not detectable in undifferentiated murine embryo stem cells (ESCs) which are positive to staining with self-renewal marker OCT 3/4 (red), neither in 4-days differentiated ESCs, positive to the neural progenitor marker SOX 1 (red). At 7 days a group of cells are stained for CA IX in the nucleus, but not for SOX 1 (red). At the same time point, another group of cells are positive for both CA IX (green) and β3-tubulin (red), a marker of post-mitotic neurons. At 12 days of differentiation, the nuclear CA IX expression was still evident in neuronal cells, but also in β3-tubulin negative cells. The nature of these cells is yet unclear. Nuclei are highlighted by DAPI staining (blue).*

epigenetic changes. The reprogramed function of nucleoli involves the canonical cell-cycle arrest factor p53. The loss of nucleolar structure, often evident in stress conditions, provokes the release of ribosomal proteins (RPs) such as L5, L11, L23 and S7 that act as a decoy to sequestrate the E3 ubiquitin ligase Mdm2. In normal conditions, Mdm2 can ubiquitylate p53, inducing its loss of function via degradation. Moreover, another released nucleolar factor, RPL11, can directly bind the p53 mRNA, improving its translation. In stress conditions the nucleolus seems to act as a sensor, inducing Mdm2 sequester and p53 nuclear stabilization (Boulon et al., 2010). The potential role of CA IX and of its complexes in nucleoli with CAND1, an inhibitor of cullin-RING E3 ubiquitin ligases (CRL) (Buanne et al., 2013), may also reveal emerging *scenarios* in the regulation of protein stability during nucleolar stress.

The great importance of a correct nucleolar activity and structure is also supported by its implication in several human diseases. Accordingly, decline in nucleolar size and functionality often occurs in neurodegenerative diseases. Silencing of rDNA was reported in the early stage of Alzheimer's disease (AD) through rDNA genes methylation (Parlato and Kreiner, 2013); accordingly, it was proposed to exploit the epigenetic pattern of rDNA as a marker for AD progression. Also in Parkinson's disease (PD) it was demonstrated that dopaminergic neurons are affected by nucleolar distruption (Parlato and Liss, 2014). In addition, nucleolin, a well-known marker rRNA synthesis, is dramatically reduced in the *substantia nigra pars compacta* from

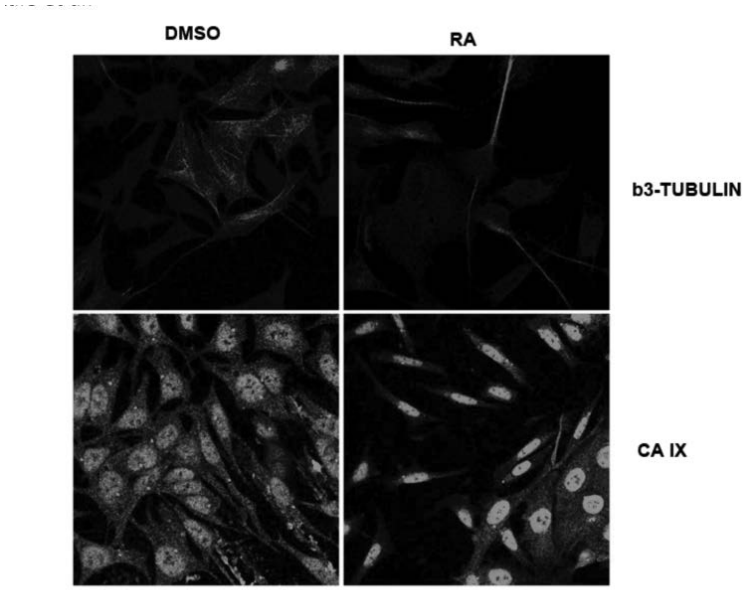

Fig. 2. ***Subcellular distribution of CA IX in undifferentiated and retinoic acid (RA) -differentiated SH-SY5Y cell line.*** *In differentiated neuroblastoma cells, CA IX is expressed in the cytoplasm, but it is much more abundant in the nucleus, compared to their undifferentiated counterpart.* β*3-tubulin is a differentiation marker and it is clearly highlighting the neurofilaments of differentiated cells.*

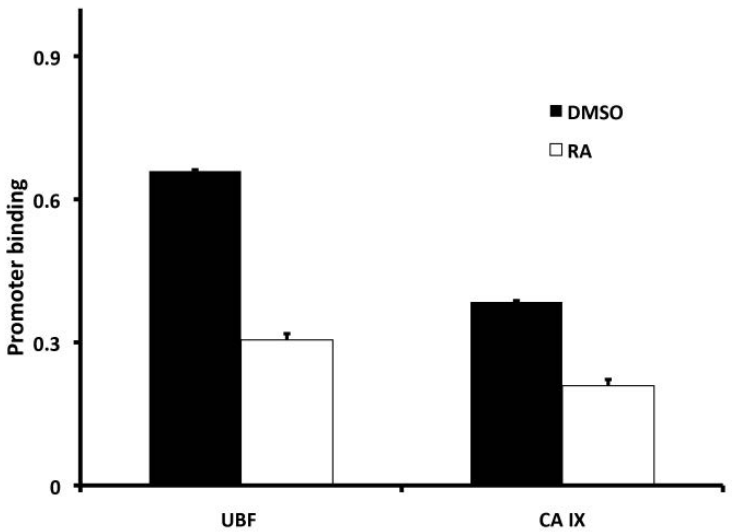

Fig. 3. Decreased CA IX occupancy of rDNA gene promoters in differentiated SH-SY5Y cells. *The Figure shows the results of a chromatin immunoprecipitation assay of UBF1 and CA IX on pre-45S rDNA genes. Neuroblastoma SH-SY5Y cells were treated with vehicle (DMSO, black bars), or differentiated to neurons with retinoic acid (white bars). Both the architectural UBF1 factor and CA IX decreased their association to rDNA chromatin in differentiated cells.*

post-mortem samples of PD (Parlato and Kreiner, 2013). Then, there is a potential for treatment of neurodegenerative disorders, including Alzheimer's disease, using carbonic anhydrase activators as it is the anhydrase function impairment that is connected with AD and brain function disorders (Sun et al., 2002; Supuran et al., 2008a). It is known that alterations in synaptic spines and loss of dendrites during aging are associated with a significant decline of CAs in the brain, and that this decline is even

more dramatic in brains of AD subjects (Scheff et al., 2006). Several CA isozymes are downregulated in the brain of patients affected by Alzheimer's disease (Meier-Ruge et al., 1984). Phenylalanine is a CA activator; when experimental animals are treated with it, a pharmacological enhancement of synaptic efficacy, spatial learning, and memory are observed, suggesting that its possible use for the management of conditions in which learning and memory are impaired, such as Alzheimer's disease or aging (Sun et al., 2001).

CONCLUSIONS

CA IX is a well known target of the hypoxia-induced HIF1A factor in mammalian cells of different origin, mostly characterized in human cancer. Membrane-localized CA IX does indeed contribute, together with intracellular CAs, to the metabolic adaptation of cells to hypoxic stress. A recently described, novel function of CA IX within hypoxic responses of mammalian cells, including the SH-SY5Y-based model of differentiation, shows the potential involvement of CA IX in the attenuation of rRNA transcription, to safeguard the delicate energetic state of cells and to guarantee their survival during hypoxia and differentiation. While the main efforts of the researchers involved in CA IX studies are dedicated to find its selective inhibitors for applications in oncology, the discussed evidences underline a therapeutic potential for CA IX activation in brain ischemia and neurodegenerative disorders.

REFERENCES

Aggarwal M, Boone CD, Kondeti B, and McKenna R (2013) Structural annotationof human carbonic anhydrases. J. Enzyme Inhib. Med. Chem,. 28(2): 267–277.

Agnati LF, Tinner B, Staines WA, Vaananen K and Fuxe K (1995) On the cellular localization and distribution of carbonic anhydrase II immunoreactivity in the rat brain. Brain Res. 676: 10-24.

Akisawa Y, Nishimori I, Taniuchi K, Okamoto N, Onishi S et al. (2003) Expression of carbonic anhydrase-related protein CA-RP VIII in non-small cell lung cancer. Virchows Arch, 442(1):66-70.

Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, and De Simone G, (2012) Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms?. Chem. Rev., 112(8): 4421–4468.

Asiedu M, Ossipov MH, Kaila K, and Price TJ (2010) Acetazolamide and midazolam act synergistically to inhibit neuropathic pain. Pain, 148(2): 302–308.

Aspatwar A, Tolvanen MEE, Parkkila S. (2010) Phylogeny and expression of carbonic anhydrase-related proteins. BMC Molecular Biology, 11(25).

Bootorabi F, Jänis J, Smith E, Waheed A, Parkkila S, et al. (2010) Analysis of a shortened form of human carbonic anhydrase VII expressed in vitro compared to the full-length enzyme, Biochimie, 92(8): 1072–1080.

Borthwick KJ, Kandemir N, Topaloglu R, Kornak U, Karet FE, et al. (2003) A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. J Med Genet, 40: 115–21.

Boulon S, Westman BJ, Hutten S, Boisvert FM, Lamond AI. (2010) The nucleolus under Stress. Molecular Cell. 40(2): 216–227.

Bouzier A-K, Thiaudiere E, Biran M, Rouland R, Canioni P and Merle M (2000) The metabolism of [3-(13)C]lactate in the rat brain is specific of a pyruvate carboxylase deprived compartment. J. Neurochem. 75: 480-486.

Bröer S, Rahman B, Pellegri G, Pellerin L, Magistretti PJ et al. (1997) Comparison of lactate transport in astroglial cells and monocarboxylate transporter (MCT 1) expressing Xenopus laevis oocytes. J. Biol. Chem. 272: 30096-30102.

Brown D, Kumpulainen T, Roth J, Orci L, (1983) Immunohistochemical localization of carbonic anhydrase in postnatal and adult rat kidney. Am. J. Physiol., 245(1), F110–118.

Buanne P, Renzone G, Monteleone F, Vitale M, Zambrano N et al. (2013) Characterization of carbonic anhydrase IX interactome reveals proteins assisting its nuclear localization in hypoxic cells. J Proteome Res 12: 282–292.

Butterfield DA, Reed T, Newman SF, and Sultana R. (2007) Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive

impairment. Free Radic Biol Med, 43: 658–677.

Cammer W (1991) Immunostaining of carbamoylphosphate synthase II and fatty acid synthase in glial cells in rat, mouse, and hamster brains suggests roles for carbonic anhydrase in biosynthetic processes. Neurosci. Lett. 129, 247-250.

Cammer W, Bieler L, Fredman T, and Norton WT. (1977) Quantitation of myelin carbonic anhydrase-development and subfractionation of rat brain myelin and comparison with myelin from other species. Brain Res 138: 17–28.

Carter ND (1991) "The carbonic anhydrases: cellular physiology and moledular genetics.," in Hormonal and neuronal control of carbonic anhydrase III gene expression in skeletal muscle. Dodgson SJ, Tashian RE, Gross G, Carter ND (eds)., New York: Plenum Publishing, 247–256.

Cho M, Uemura H, Kim SC, Kawada Y, Yoshikawa K et al. (2001) Hypomethylation of the MN/CA9 promoter and upregulated MN/CA9 expression in human renal cell carcinoma. Br JCancer, 85: 563-7.

Deitmer JW (2002) A role for CO(2) and bicarbonate transporters in metabolic exchanges in the brain. J Neurochem., 80(5):721-6.

Diaz E, Sandblom JP Wistrand PJ (1982) Selectivity properties of channels induced by a reconstituted membrane-bound carbonic anhydrase. Acta Physiol. Scand. 116: 461-463.

Dringen R, Gebhardt R, Hamprecht B (1993) Glycogen in astro-cytes: Possible function as lactate supply for neighbouring cells. Brain Res. 623: 208-214.

Feldstein JB and Silverman DN (1984) Purification and characterization of carbonic anhydrase from the saliva of the rat. J. Biol. Chem., 259(9): 5447–5453.

Fujikawa-Adachi K, Nishimori I, Taguchi T, and Onishi S (1999a) Human carbonicanhydrase XIV (CA14): cDNA cloning, mRNA expression, and mapping tochromosome 1. Genomics, 61(1): 74–81.

Fujikawa-Adachi K, Nishimori I, Taguchi T, Yuri K, Onishi S (1999b) cDNA sequence, mRNA expression, and chromosomal localization of human carbonic anhydrase-related protein, CA-RP XI. Biochim Biophys Acta, 1431(2):518-524.

Ghandour MS, Langley OK, Vincendon G, Gombos G, Laurent G et al. (1980) Immunochemical and immunohistochemical study of carbonic anhydrase-II in adult rat cerebellum—a marker for oligodendrocytes. Neuroscience 5: 559–571.

Ghandour MS, Parkkila, Waheed, Sly, (2000). Mitochondrial carbonic anhydrase in the nervous system: expression in neuronal and glial cells. J. Neurochem. 75: 2212–2220.

Giacobini E (1962) A cytochemical study of the localization of carbonic anhydrase in the nervous system. J. Neurochem. 9: 169-177.

Gilmour KM (2010) Perspectives on carbonic anhydrase. Comp. Biochem. Physiol. A. Mol. Integr. Physiol., 157(3): 193–197.

Gut MO, Parkkila S, Vernerova Z, Rohde E, Pastorekova S (2002) et al Gastric hyperplasia in mice with targeted disruption of the carbonic anhydrase gene Car9. Gastroenterology 123:1889–1903.

Hageman GS, Zhu XL, Waheed A, Sly WS. (1991) Localization of carbonicanhydrase IV in a specific capillary bed of the human eye. Proc NatlAcad Sci USA, 88:716-20.

Halestrap AP and Price NT (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochem. J. 343: 281-299.

Halmi P, Parkkila S and Honkaniemi J (2006) Expression of carbonic anhydrases II, IV, VII, VIII and XII in rat brain after kainic acid induced status epilepticus. Neurochemistry International, 48: 24-30.

Hilvo M, Rafajova M, Pastorekova S, Pastorek J, Parkkila S (2004) Expression of carbonic anhydrase IX in mouse tissues. J Histochem Cytochem, 52:1313–1322.

Hilvo M, Tolvanen M, Clark A, Shen B, Parkkila S et al. (2005) Characterization of CA XV, a new GPI-anchored form of carbonic anhydrase. Biochem J 392:83–92.

Hirasawa M, Xu X, Trask RB, Maddatu TP, Ikeda A et al. (2007) Carbonic anhydrase relatedprotein 8 mutation results in aberrant synaptic morphology and excitatorysynaptic function in the cerebellum. Mol Cell Neurosci, 35(1): 161-70.

Hirota J, Ando H, Hamada K, Mikoshiba K. (2003) Carbonic anhydraserelated protein is a novel binding protein for inositol 1,4,5-trisphosphate receptor type 1. Biochem J; 372(Pt 2): 435-41.

Hsieh M, Chang WH, Hsu CF, Nishimori I, Kuo CL, and Minakuchi T (2013) Altered expression of carbonic

anhydrase-related protein XI in neuronal cells expressing mutant ataxin-3. Cerebellum 12:338–349.

Hu Y and Wilson GS (1997) A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. J. Neurochem. 69: 1484-1490.

Hübner CA and Holthoff K (2013) Anion transport and GABA signalling. Front Cell Neurosci., 7: 177.

Hynninen P, Parkkila S, Huhtala H, Pastorekova S, Tomas E et al. (2012) Carbonic anhydrase isozymes II, IX, and XII in uterine tumors, APMIS Acta Pathol. Microbiol. Immunol. Scand., 120(2): 117–129.

Ivanov S, Liao SY, Ivanova A, Danilkovitch-Miagkova A, Stanbridge EJ et al. (2001) Expression of hypoxia inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am. J. Pathol., 158(3): 905–919.

Izumi Y, Benz AM, Katsuki H and Zorumski CF (1997) Endogenous monocarboxylates sustain hippocampal synaptic function and morphological integrity during energy deprivation. J. Neurosci. 17: 9448-9457.

Jiao Y, Yan J, Zhao Y, Donahue LR, Gu W et al. (2005). Carbonic anhydrase-related protein VIII deficiency is associated with adistinctive lifelong gait disorder in waddles mice. Genetics 171: 1239-1246.

Juel C, Lundby C, Sander M, Calbet JA, Hall Gv (2003) Human skeletal muscle and erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. J Physiol, 548(Pt 2): 639–648.

Kallio H, Pastorekova S, Pastorek J, Waheed A, Parkkila S et al. (2006) Expression of carbonic anhydrases IX and XII during mouse embryonic development. BMC Dev. Biol. 23: 6–22.

Kaseda M, Ichihara N, Nishita T, Amasaki H, and Asari M (2006) Immunohistochemistry of the bovine secretory carbonic anhydrase isozyme (CA-VI) in bovine alimentary canal and major salivary glands. J. Vet. Med. Sci. Jpn. Soc. Vet. Sci., 68(2): 131–135.

Kaunisto K, Parkkila S, Rajaniemi H, Waheed A, Grubb J, and Sly WS (2002) Carbonic anhydrase XIV: luminal expression suggests key role in renalacidification. Kidney Int., 61(6): 2111–2118.

Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Akiguchi I et al. (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8: 221-228.

Kaya N, Aldhalaan H, Al-Younes B, Colak D, Al-Bakheet A et al. (2011) Phenotypical spectrum of cerebellar ataxia associated with a novel mutation in the CA8 gene, encoding carbonicanhydrase (CA) VIII. Am J Med Genet B Neuropsychiatr Genet 156: 826-834.

Kida E, Palminiello S, Golabek AA, Walus M, Wisniewski KE et al. (2006) Carbonic anhydrase II in the developing and adult human brain. J. Neuropathol. Exp. Neurol. 65: 664–674.

Kivelä A, Parkkila S, Saarnio J, Karttunen TJ, Rajaniemi H et al. (2000) Expression of a novel transmembrane carbonic anhydrase isozyme XII in normal human gut and colorectal tumors. Am. J. Pathol. 156(2): 577–584.

Kleiderlein JJ, Nisson PE, Jessee J, Li WB, Margolis RL et al. (1998) CCG repeats in cDNAs from human brain. Hum Genet, 103(6): 666-673.

Kohler K, Hillebrecht A, Schulze Wischeler J, Innocenti A, Heine A, Supuran CT, Klebe G (2007) Saccharin inhibits carbonic anhydrases: possible explanation for its unpleasant metallic aftertaste. Angew Chem Int Ed Engl, 46: (40), 7697-9.

Kummola L, Hämäläinen JM, Kivelä J, Kivelä AJ, Saarnio J, Karttunen T, and Parkkila S (2005) Expression of a novel carbonic anhydrase, CA XIII, in normaland neoplastic colorectal mucosa. BMC Cancer, 5:41.

Lehtonen J, Shen B, Vihinen M, Casini A, Parkkila AS et al. (2004) Characterization of CA XIII, a novel member of the carbonic anhydraseisozyme family. J. Biol. Chem., 279(4): 2719–2727.

Leinonen JS, Saari KA, Seppänen JM, Myllylä HM, Rajaniemi HJ et al. (2004) Immunohistochemical demonstration of carbonic anhydrase isoenzyme VI (CA VI) expression in rat lower airways and lung. J. Histochem. Cytochem. Off. J. Histochem. Soc., 52(8): 1107–1112.

Liao SY, Ivanov S, Ivanova A, Ghosh S, Lerman MI et al. (2003) Expression of cell surface transmembrane carbonic anhydrase genes CA9 and CA12 in the human eye: overexpression of CA12 (CAXII) in glaucoma. J MedGenet 40:257–26

Lichter PR (2001) Genetics of the glaucomas. J Glaucoma; 5(suppl 1):S13-15.

Linser P and Moscona AA (1984) Variable CA II compartmentalization invertebrate retina. Ann. N. Y. Acad. Sci., 429: 430–446.

Lynch CJ, Fox H, Hazen SA, Stanley BA, Dodgson S, and Lanoue KF (1995) Role of hepatic carbonic anhydrase in de novo lipogenesis, Biochem. J., 310: (Pt 1) 197–202.

Lyons GE, Buckingham ME, Tweedie S, and Edwards YH (1991) Carbonic anhydrase III, an early mesodermal marker, is expressed in embryonic mouse skeletal muscle and notochord, Dev. Camb. Engl., 111(1): 233–244.

Ma Y, Brewer JW, Diehl JA, and Hendershot LM. (2002) Two distinctstress signaling pathways converge upon the CHOP promoter duringthe mammalian unfolded protein response. J Mol Biol 318:1351–1365.

Maragakis NJ and Jeffrey D Rothstein (2006) Mechanisms of Disease: astrocytes in neurodegenerative disease. Nature Clinical Practice Neurology 2: 679-689.

Maren TH and Swenson ER (1980) A comparative study of the kinetics of the Bohr effect in vertebrates. J. Physiol., 303: 535–547.

McMurtrie HL, Cleary HJ, Alvarez BV, Loiselle FB, Casey JR et al. (2004) The bicarbonate transport metabolon. J. Enzyme Inhib. Med. Chem., 19(3): 231–236.

Meier-Ruge W, Iwangoff P, and Reichlmeier K. (1984) Neurochemicalenzyme changes in Alzheimer's and Pick's disease. Arch Gerontol Geriatr 3: 161–165.

Moss AM, Harris A, Siesky B, Rusia D, Williamson KM, Shoshani Y (2010) Update and critical appraisal of combined timolol and carbonic anhydrase inhibitors and the effect on ocular blood flow in glaucoma patients. Clin Ophthalmol; 4: 233-241.

Muhammad E, Leventhal N, Parvari G, Hanukoglu A, Parvari R et al. (2011) Autosomal recessive hyponatremia due to isolated salt wasting in sweat associated with a mutation in the active site of Carbonic Anhydrase 12, Hum. Genet., 129(4): 397–405.

Nishimori I (2004) Acatalytic CAs: Carbonic anhydrase-related proteins. In Carbonic anhydrases: Its inhibitors and activators. CRC enzyme inhibitor series Edited by: Supuran CT, Scozzfava A, Conway J. CRC press; 25-43.

Nishimori I, Takeuchi H, Morimoto K, Taniuchi K, Okamoto N, Onishi S, Ohtsuki Y (2003) Expression of carbonic anhydrase-related protein VIII, X andXI in the enteric autonomic nervous system. Biomed Res,14(1):69-73.

Nishimori I, Vullo D, Innocenti A, Scozzafava A, Mastrolorenzo A, Supuran CT (2005) Carbonic anhydrase inhibitors. The mitochondrial isozyme VB as a new target for sulfonamide and sulfamate inhibitors. J Med Chem, 48(24):7860-7866.

Nogradi A, Domoki F, Degi R, Borda S, Pakaski M, Szabo A, Bari F (2003) Up-regulation of cerebral carbonic anhydrase by anoxic stress inpiglets. J. Neurochem. 85: 843–850.

Obara M Szeliga M, Albrecht J (2008) Regulation of pH in the mammalian central nervous system under normal and pathological conditions: Facts and hypotheses. Neurochemistry International 52: 905–919.

Ochrietor JD, Clamp MF, Moroz TP, Grubb JH, PJ Linser et al. (2005) Carbonic anhydrase XIV identified as the membrane CA in mouse retina: strong expression in Müller cells and the RPE. Exp. EyeRes., 81(4): 492–500.

Ogawa Y, Matsumoto K, Maeda T, Tamai R, Suzuki T, Sasano H, and Fernley RT (2002) Characterization of lacrimal gland carbonic anhydrase VI, J. Histochem. Cytochem. Off. J. Histochem. Soc., 50: 6821–827.

Ohlsson A, Stark G, Sakati N. (1980) Marble brain disease: recessive osteopetrosis, renal tubular acidosis and cerebral calcification in three Saudi Arabian families. Dev Med Child Neurol, 22: 72–84.

Ohradanova A, Vullo D, Kopacek J, Temperini C, Supuran CT et al. (2007) Reconstitution of carbonic anhydrase activity of the cell-surface-binding protein of vaccinia virus. Biochem J 407: 61-67

Okamoto N, Fujikawa-Adachi K, Nishimori I, Taniuchi K, Onishi S (2001) cDNA sequence of human carbonic anhydrase-related protein, CA-RP X: mRNA expressions of CA-RP X and XI in human brain. Biochim Biophys Acta, 1518(3): 311-316.

Ortho-McNeil-Janssen Pharmaceuticals I (2013) Topomax: drug summary. Physicians' desk reference.

Palminiello S, Kidaa E, Kaura K, Walusa M, Golabeka AA et al. (2008) Increased levels of carbonic anhydrase II in the developing Down syndrome brain. Brain Research 1190:193 – 205.

Pan PW, Parkkila AK, Autio S, Hilvo M, Sormunen RH, Parkkila S (2012)

Brain phenotype of carbonic anhydrase IX-deficient mice. Transgenic Res. 21(1):163-76.

Parisi S, Tarantino C, Paolella G, Russo T. (2010) A flexible method to study neuronal differentiation of mouse embryonic stem cells. Neurochem Res. 35:2218-2225.

Parkkila S, Parkkila AK, Rajaniemi H, Shah GN, Grubb JH, Waheed A, Sly WS (2001). Expression of membrane-associated carbonic anhydrase XIV on neurons and axons in mouse and human brain. Proc. Natl. Acad. Sci. U.S.A. 98: 1918–1923.

Parkkila S, Rajaniemi H, Parkkila AK, Kivela J, Sly AWS et al. (2000) Carbonic anhydrase inhibitor suppresses invasion of renal cancer cells in vitro. Proc. Natl. Acad. Sci. U.S.A., 97(5): 2220–2224.

Parlato R, Kreiner G (2013) Nucleolar activity in neurodegenerative diseases: a missing piece of the puzzle? J Mol Med (Berl); 91(5): 541–547.

Parlato R, Liss B (2014) How Parkinson's disease meets nucleolar stress. Biochim. Biophys. Acta 1842(6): 791–797.

Pastorekova S Parkkila S, Pastorek J, Supuran CT (2004a) Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib Med Chem, 19(3): 199-229.

Pastorekova S, Zavada J (2004b) Carbonic anhydrase IX (CA IX) as a potential target for cancer therapy. Cancer Therapy 2: 245-262.

Pastorekova S, Parkkila S, Parkkila AK, Opavsky R, Zelnik V, Saarnio J, Pastorek J (1997) Carbonic anhydrase IX, MN/CA IX: analysis of stomach complementary DNA sequence and expression in human and rat alimentary tracts. Gastroenterology 112:398–408.

Poitry-Yamate C L, Poitry S and Tsacopoulos M (1995) Lactate released by Muller glial cells is metabolized by photoreceptors from mammalia retina. J. Neurosci. 15: 5179-5191.

Price TO, Eranki V, Banks WA, Ercal N, and Shah GN (2012) Topiramate treatment protects blood-brain barrier pericytes from hyperglycemia-induced oxidative damage in diabetic mice. Endocrinology 153: 362–372.

Rebello G, Ramesar R, Vorster A, Roberts L, Sly WS et al. (2004) Apoptosis-inducing signal sequence mutation in carbonic anhydrase IV identified in patients with the RP17 form of retinitis pigmentosa, Proc. Natl. Acad. Sci. U. S. A., 101(17): 6617–6622.

Rezaie TR, Child A, Hitchings R, Brice G, Sarfarazi M et al. (2002) Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science, 295: 1077-9.

Rivera C, Voipio J, Kaila K, (2005). Two developmental switches in GABAergic signalling: the K+–Cl_ cotransporter KCC2 and carbonic anhydrase CAVII. J. Physiol. 562.1: 27–36.

Rohit V, Peeples P, Walt JG, Bramley TJ (2008) Disease Progression and the Need for Neuroprotection in Glaucoma Management. Am J Manag Care; 14: 15-19.

Ron D, and Habener JF (1992) CHOP, a novel developmentally regulatednuclear protein that dimerizes with transcription factorsC/EBP and LAP and functions as a dominant-negative inhibitorof gene transcription. Genes Dev 6: 439–453.

Ruusuvuori E, Huebner AK, Kirilkin I, Yukin AY, Kaila K et al. (2013) Neuronal carbonic anhydrase VII provides GABAergic excitatory drive to exacerbate febrile Seizures The EMBO Journal 32: 2275–2286.

Ruusuvuori E, Li H, Huttu K, Palva JM, Voipio J, et al. (2004) Carbonic anhydrase isoform VII acts as a molecular switch in the development of synchronous gamma-frequency firing of hippocampal CA1 pyramidal cells. J. Neurosci. Off. J. Soc. Neurosci., 24(11): 2699–2707.

Saari S Hilvo M, Pan P, Gros G, Parkkila S et al. (2010) The most recently discovered carbonic anhydrase, CA XV, is expressed in the thick ascending limb of Henle and in the collecting ducts of mouse kidney. PLoS One, 5 (3): e9624.

Sapirstein VS, Strocchi P and Gilbert JM (1984) Properties and function of brain carbonic anhydrase. Ann. NY Acad. Sci. 429: 481-493.

Sasso E, Vitale M, Monteleone F, Boffo FL, Zambrano N et al., (2015) Binding of Carbonic Anhydrase IX to 45S rDNA Genes Is Prevented by Exportin-1 in Hypoxic Cells BioMed Research International, vol. 2015, Article ID 674920, 10 pages, 2015. doi:10.1155/2015/674920

Scheff SW, Price DA, Schmitt FA, and Mufson EJ. (2006) Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 27: 1372–1384.

Schousboe A, Westergaard N, Waagepetersen HS, Larsson OM, Bakken IJ and Sonnewald U (1997) Trafficking between glia and neurons of TCA cycle intermediates and related metabolites. Glia 21: 99-105.

Schurr A, Payne RS, Miller JJ and Rigor BM (1997) Glia are the main source of lactate utilized by neurons for recovery of function posthypoxia. Brain Res. 774: 221-224.

Schurr A, Miller JJ, Payne RS and Rigor BM (1999) An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. J. Neurosci. 19(1): 34-9.

Schurr A, West CA and Rigor BM (1988) Lactate-supported synaptic function in the rat hippocampal slice preparation. Science 240: 1326-1328.

Scozzafava A, Mastrolorenzo A, Supuran CT (2006) Carbonic anhydrase inhibitors and activators and their use in therapy. Expert. Opin. Ther. Pat, 16: 1627-1664.

Sethi KK, Verma SM, Kumar PM, Mishra R, Supuran CT (2011) Carbonic Anhydrase I and II Inhibition with Natural Products: Leucas cephalotes. Pharmacognosy Communications 1 (2): 41-46.

Shah GN, Hewett-Emmett D, Grubb JH, Migas MC, Fleming RE, Waheed A and Sly WS (2000) Mitochondrial carbonic anhydrase CA VB: differences in tissue distribution and pattern of evolution from those of CA VA suggest distinct physiological roles, Proc. Natl. Acad. Sci. U.S.A., 97(4): 1677–1682.

Shah GN, Price TO, Banks WA, Morofuji Y, Sheibani N et al. (2013) Pharmacological Inhibition of Mitochondrial Carbonic Anhydrases Protects Mouse Cerebral Pericytes from High Glucose-Induced Oxidative Stress and Apoptosis J Pharmacol Exp Ther 344 :637–645.

Shah GN, Ulmasov B, Waheed A, Becker T, Sly WS et al. (2005). Carbonic anhydrase IV and XIV knockout mice: roles of the respective carbonic anhydrases in buffering the extracellular space in brain. Proc. Natl. Acad. Sci. U.S.A. 102: 16771–16776.

Shatzman R and Henkin RI (1981) Gustin concentration changes relative to salivary zinc and taste in humans. Proc. Natl. Acad. Sci. U.S A., 78(6): 3867–3871.

Sheffield VC, Alward WLM., Stone EM. (2001). The Glaucomas. In: Scriver CR, Beaudet al., Sly WS, Valle D, eds. The metabolic and molecular bases of inherited diseases. New York: Medical Publishing Division, 6063-6075.

Ship JA (2003) Diabetes and oral health: an overview. J. Am. Dent. Assoc. 1939, 134 Spec No, 4S–10S.

Sly WS, Hewett-Emmett D, Whyte MP, Yu YS, and Tashian RE (1983) Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc. Natl. Acad. Sci. U.S.A., 80(9): 2752–2756.

Sly WS, Whyte MP, Sundaram V, Tashian RE, Ohlsson A et al. (1985) Carbonic anhydrase II deficiency in 12 families with the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. N Engl J Med; 313: 139–45.

Smith CE, Nanci A, and Moffatt P (2006) Evidence by signal peptide trap technology for the expression of carbonic anhydrase 6 in rat incisor enamel 27 organs. Eur. J. Oral Sci., 114 Suppl 1, pp. 147–153; discussion 164–165, 380–381.

Sok J, Wang XZ, Batchvarova N, Kuroda M, Harding H, and Ron D (1999) CHOP-Dependent stress-inducible expression of a novel form of carbonic anhydrase VI. Mol Cell Biol 19: 495–504.

Sokoloff L. (1993) Sites and mechanisms of function-related changes in energy metabolism in the nervous system. Dev. Neurosci. 15,194-206.

Stanton LW, Ponte PA, Coleman RT, and Snyder MA (1991) Expression of CA 25 III in rodent models of obesity. Mol. Endocrinol. Baltim. Md, 5(6): 860–866.

Stone EM, Fingert JH, Alward WLM, Nguyen TD, Sheffield VCl et al. (1997) Identification of a gene that causes primary open angle glaucoma. Science, 275: 668-70.

Sultana R, Boyd-Kimball D, Poon HF, Cai J, Butterfield DA et al. (2006) Redox proteomics identification of oxidized proteins in Alzheimer's disease hippocampus and cerebellum: An approach to understand pathological and biochemical alterationsin AD. Neurobiol Aging 27: 1564–1576.

Sultana R, Perluigi M, Newman SF, Pierce WM , Butterfield A, et al. (2010) Redox Proteomic Analysis of Carbonylated Brain Proteins in Mild Cognitive Impairment and Early Alzheimer's Disease. Antioxidants & Redox Signaling, 12(3).

Sun MK and Alkon DL (2002) Carbonic anhydrase gating of attention: Memory therapy and enhancement. Trends Pharmacol Sci 23: 83–89.

Sun MK, Alkon DL (2001) Pharmacological enhancement of synaptic efficacy, spatial learning and memory through carbonic anhydrase activation in rats. J.

Pharmacol. Exp. Ther. 297: 961-967.

Supuran CT (2008a) Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov, 7(2): 168-81.

Supuran CT (2008b) Carbonic anhydrases an overview, Curr. Pharm. Des.,14(7): 603–614.

Supuran CT Scozzafava A, Casini A (2003) Carbonic anhydrase inhibitors. Med Res Rev, 23(2): 146-89

Supuran CT, Scozzafava A, Conway J (2004) Carbonic Anhydrase - Its inhibitors and Activators. CRC, Boca Raton, P 1-363.

Supuran et al. (1998) Carbonic anhydrase inhibitors. Part 29. Interaction of isozymes I, II and IV with benzolamide-like derivatives. Eur. J. Med. Chem, 33: 739-752.

Taniuchi K, Nishimori I, Takeuchi T, Ohtsuki Y, Onishi S (2002a) cDNA cloning and developmental expression of murine carbonic anhydrase-related proteins VIII, X, and XI. Brain Res Mol Brain Res, 109(1-2): 207-215.

Taniuchi K, Takeuchi T, Fujikawa-Adachi K, Ohtsuki Y, Onishi S (2002b) Developmental expression of carbonic anhydrase-related proteins VIII, X, and XI in the human brain. Neuroscience, 112:93-99.

Tashian REH-ED, Carter ND, Bergenhem NCH, (ed) (2000) Carbonic anhydrase (CA)-related proteins (CA-RPs), and trans-membrane proteins with CA or CA RP membrane proteins with CA or CA RP domains. Basel: New Horizons, Birkhauser.

Thiry A, Dogné JM, Supuran CT, Masereel B (2007) Carbonic anhydrase inhibitors as anticonvulsant agents. Curr. Top. Med. Chem., 7(9): 855–864.

Tsacopoulos M and Magistretti PJ (1996) Metabolic coupling between glia and neurons. J. Neurosci. 16: 877-885.

Turkmen S, Guo G, Garshasbi M, Hoffmann K, Robinson PN et al. (2009) CA8 mutations cause a novelsyndrome characterized by ataxia and mild mental retardation with predisposition toquadrupedal gait. PLoS Genet 5, e1000487.

Vargas LA and Alvarez BV (2012) Carbonic anhydrase XIV in the normal andhypertrophic myocardium. J. Mol. Cell. Cardiol., 52(3): 741–752.

Vlkolinsky R, Cairns N, Fountoulakis M, and Lubec G (2001) Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. Neurobiol Aging 22: 547–553.

Vullo D, Voipio J, Innocenti A, Rivera C, Ranki H, Supuran CT, et al. (2005) Carbonic anhydrase inhibitors. Inhibition of the human cytosolic isozyme VII with aromatic and heterocyclic sulfonamides. Bioorg Med Chem Lett, 15(4): 971-976.

Walz W and Mukieri S (1988) Lactate release from cultured astrocytes and neurons: a comparison. Glia 1: 366-370.

Wang HL, Zhu ZM, Wang H, Yang SL, Zhao SH, Li K (2006) Molecular characterization and association analysis of porcine CA3. Cytogenet.Genome Res. 115: 129–133.

Wang N, Chintala SK, Fini ME, Schuman JS (2001) Activation of a tissue-specific stress response in the aqueous outflow pathway of the eye defines the glaucoma disease phenotype. Nat Med, 7:304-9.

WuDunn D (2002) Genetic basis of glaucoma. Curr Opin Ophthalmol; 13:55-60.

Zakzouk SM, Sobki SH, Mansour F, Al Anazy FH. (1995) Hearing impairment in association with distal renal tubular acidosis among Saudi children. J Laryngol Otol; 109: 930–4.

Zhu XL and Sly WS (1990) Carbonic anhydrase IV from human lung. Purification, characterization, and comparison with membrane carbonic anhydrase from human kidney, J. Biol. Chem., 265(15): 8795–8801.

Zinszner H, Kuroda M, Wang X, Batchvarova N, Ron D et al. (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 12: 982–995.

ASTROGLIOPATHOLOGY IN NEURODEGENERATION

A.VERKHRATSKY[1,2], R. ZOREC[3,4], J.J. RODRIGUEZ[2] and V. PARPURA[5,6]

[1]Faculty of Life Sciences, The University of Manchester, Manchester, UK; [2]Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, Bilbao, Spain and Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED, Leioa, Spain; [3]University of Ljubljana, Institute of Pathophysiology, Laboratory of Neuroendocrinology and Molecular Cell Physiology, Ljubljana, Slovenia; [4]Celica, BIOMEDICAL, Ljubljana, Slovenia; [5]Department of Neurobiology, Civitan International Research Center and Center for Glial Biology in Medicine, Evelyn F. McKnight Brain Institute, Atomic Force Microscopy and Nanotechnology Laboratories, University of Alabama at Birmingham, Birmingham, AL, USA; [6]Department of Biotechnology, University of Rijeka, Rijeka, Croatia

Neurodegenerative diseases lead to the progressive death of the brain tissue which results in cognitive deficits. Astroglial cells that are responsible for the homoeostasis, defence and regeneration of the central nervous system significantly contribute to neurodegeneration. Changes in astroglia in neurodegeneration are highly heterogeneous and region-specific. Both astroglial atrophy with the loss of function and astroglial reactivity have been detected in virtually all forms of neurodegenerative pathologies. Targeting astroglia in neurodegeneration may open new avenues for novel therapeutic strategies aimed at preventing and delaying the progression of neurodegenerative disorders.

NEUROLOGICAL DISEASES: THE SCOURGE OF MANKIND

Diseases of the nervous system are the least understood and the least curable disorders known to us. The ultimate reason for this is an extraordinary complexity of the human nervous system, in which hundreds of billions of cells connected through many trillions of contacts create a sublime organ of computation, emotions and creativity. Evolution of the nervous system progressed through cell diversification and cell specialisation; this resulted in the emergence of many hundreds of types of neurones which are capable of generating and propagating action potentials, which in combination with synaptic machinery underlie fast signalling within neural networks. The second class of cells that evolved in parallel is represented by a hugely heterogeneous neuroglia that assumes full responsibility for nervous system homeostasis and defence. These two classes of neural cells differ in their biochemistry and physiology and yet they are combined to form nervous tissue, which functions because of continuous intimate communications between all cellular elements.

Pathological insults trigger homeostatic failure which manifests in neurological diseases. This definition instantly highlights the fundamental role of neuroglia, that protects the nervous system through multiple homeostatic mechanisms and are

Key words: neuroglia, astrocytes, astrogliopathology, neurology, neurodegeneration, Alzheimer's disease, Huntington's disease

Corresponding Author:
Prof. Alexei Verkhratsky,
The University of Manchester,
Oxford Road, Manchester, M13 9PT, UK.
Telephone +44 (0)161-2757324,
e-mail: alexej.verkhratsky@manchester.ac.uk

2279-5855 (2014)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

capable of mounting an evolutionary conserved and multifaceted defensive reaction known as reactive gliosis. Although glial reactivity is known for almost 100 years [defined in seminal works of Pío Del Río Hortega and Wilder Penfield (Del Rio Hortega and Penfield, 1927; Del Rio-Hortega, 1932)] only very recently the potential of neuroglia in defining the progression of neuropathology begun to be acknowledged (De Keyser et al., 2008; Parpura et al., 2012; Pekna and Pekny, 2012; Verkhratsky et al., 2012; Verkhratsky et al., 2013; Burda and Sofroniew, 2014; Verkhratsky et al., 2014d). In this essay, we shall present a concise overview of the general astrogliopathology and narrate the role of astrocytes in various forms of neurodegeneration.

INTRODUCING ASTROCYTES

Neuroglia in the nervous system is represented by several types of peripheral glia (Schwann cells of the peripheral nerves, satellite cells of peripheral ganglia, enteric glia and olfactory ensheathing glia) and by glial cells of the central nervous system (CNS). The CNS glia is classified into microglia (cells of myeloid origin that enter the CNS very early in development and are responsible for innate immunity of the nervous tissue) and the macroglia represented by astroglia, oligodendroglia and NG2 glia (Kettenmann and Ransom, 2013; Verkhratsky and Butt, 2013). Astrocytes, distributed throughout the grey and the white matter of the brain and the spinal cord are, arguably, the most heterogeneous (in form and function) type of neuroglia, providing for virtually every homeostatic need of the CNS. Astrocytes are highly heterogeneous in form and function; the main types of astroglial cells are (i) protoplasmic astrocytes of the grey matter; (ii) fibrous astrocytes of the white matter; (iii) radial glia of the embryonic CNS; (iv) "stem" astrocytes of neurogenic niches of the subvetricular and subgranular zones (v) vela astrocytes of the cerebellum; (vi) surface-associated astrocytes, which outline the cortical surface in the posterior prefrontal and amygdaloid cortices; (vii) interlaminar, polarized and varicose projection astrocytes which are found only in the brains of high primates and humans and functions of which remain unknown; (viii) Müller glial cells of the retina; (ix) Bergmann glial cells localised in the Purkinje neurones layer of the cerebellum; (x) tanycytes of the hypothalamus; (xi) pituicytes of the neuro-hypophysis; (xii) perivascular and marginal astrocytes, which form the *glia limitans* barrier at the pia mater; (xiii) ependymocytes, choroid plexus cells and retinal pigment epithelial cells. All these many cell types have distinct physiological properties that are defined by their respective positions in different regions of the brain and the spinal cord; and yet their common and major function remains maintenance of CNS homeostasis

Astrocytic homeostatic functions are exceptionally diverse. In the development the radial glia acts as the pluripotent precursor cell that through asymmetric division gives rise to neuronal and glial progenitors. In the perinatal period, characterised by a massive wave of astrogliogenesis, astrocytes support synaptogenesis through secretion of numerous trophic factors such as trombospondins, hevin, cholesterol and apolipoprotein E. Astrocytes control the structural organisation of the nervous tissue by organising the grey matter into relatively independent neurovascular units, associated with astroglial territorial domains. Astroglial cells regulate the appearance and function of blood-brain and cerebrospinal fluid-brain barriers and form the blood-brain barrier in neurohypophysis. Astrocytes, which express numerous specific transporters, regulate ion homeostasis of the CNS, being particularly important for extracellular buffering of K^+ ions, which is critical for neuronal excitability. Astrocytes are fundamental elements of neurotransmission being central elements in the regulation of turnover of neurotransmitters; the astroglial cells, for example, take up glutamate, γ-aminobutyric acid (GABA), glycine and adenosine by specific transporters, catabolise glutamate by glutamine synthetase and adenosine by adenosine kinase; both of these enzymes are expressed almost exclusively in astroglia. Astrocytes supply neurones with glutamine, which is a compulsory precursor for glutamate and GABA; inhibition of astroglial-neuronal glutamate/GABA - glutamine shuttle invariably suppresses both excitatory and inhibitory neurotransmission. Astrocytes regulate water transport in the CNS by specific water channels of aquaporine 4 type, which are expressed only in astroglia. Astrocytes also represent the major

buffering system for reactive oxygen species, being the source of main anti-oxidants glutathione and ascorbic acid (for detailed account of astroglial functions and relevant references see (Iadecola and Nedergaard, 2007; Kriegstein and Alvarez-Buylla, 2009; Kimelberg and Nedergaard, 2010; Zhang and Barres, 2010; Kirischuk et al., 2012; Nedergaard and Verkhratsky, 2012; Oberheim et al., 2012; Parpura and Verkhratsky, 2012; Clarke and Barres, 2013; Kettenmann and Ransom, 2013; Verkhratsky and Butt, 2013; Verkhratsky and Nedergaard, 2014; Verkhratsky et al., 2014a).

INTRODUCING ASTROGLIOPATHOLOGY

The neurono-centric doctrine, which considers neurones as main substrates of neuropathology, dominates contemporary neurological thoughts and practices. It is universally acknowledged that neuronal damage or aberrant neuronal processes are both the causes and engines of neurological disorders. This point of view is in striking contrast with generalised observations indicating that the first and the only cells that respond to pathological insults with complex and disease-specific reactions are neuroglia.

Above, neuroglial cells posses an evolutionary conserved defensive programme, the reactive gliosis, that encodes profound cellular metamorphosis in response to polyaetiological lesions of the CNS (Pekna and Pekny, 2012; Burda and Sofroniew, 2014; Verkhratsky et al., 2014d). The reactive gliosis is represented by reactive astrogliosis, proliferative response of NG2 cells and the activation of microglia; all these processes being required in neuropathology. Reactive astrogliosis is a fundamentally survival event; it is a complex and multistage process aimed at neuroprotection and regeneration. Reactive astrocytes change their morphology, biochemistry and physiology in a disease-specific context (Zamanian et al., 2012); multiple phenotypes of activated astroglia are indispensable to contain the damage (for example by making the glial scar), and for post-lesion regeneration (Sofroniew and Vinters, 2010; Pekna and Pekny, 2012; Burda and Sofroniew, 2014; Pekny et al., 2014).

Besides reactive response, astroglial cells may also undergo degeneration and atrophy with a loss of function. These changes are observed in many chronic diseases and may co-exist with astroglial reactivity, i.e., in pathological tissue several population of atrophic and reactive astrocytes may be present. Astroglial atrophy and asthenia have been detected in major neuropsychiatric, neurodevelopmental and neurodegenerative diseases (Rossi et al., 2008; Staats and Van Den Bosch, 2009; Verkhratsky et al., 2010; Rajkowska and Stockmeier, 2013; Williams et al., 2013; Verkhratsky et al., 2014c; Verkhratsky et al., 2014b; Zeidan-Chulia et al., 2014). Alternatively astroglial cells may undergo pathological remodelling which changes their functional properties that may contribute to pathological progression. Astroglial atrophy and pathological remodelling decrease overall homoeostatic reserves in the CNS, and may result in a reduced synaptic coverage and hence the weakening of the synaptic transmission. Functional changes in astroglia may reduce neuroprotection, whereas in certain conditions astrocytes may secrete neurotoxic factors hence contributing to neuronal damage.

Astrogliopathololgy can be primary or secondary. In many neurological disorders astrocytes represent the primary pathological target and underlie pathological progression. The primary genetic astrogliopathology is Alexander disease, in which astrocytes express sporadically mutated glial fibrillary acidic protein (GFAP); this leads to an early and profound leukomalacia (Messing et al., 2012)), Astrocytes are primary targets in numerous toxic brain injuries, such as poisoning with heavy metals; these metals being accumulated by astroglia disrupt astrocytic glutamate uptake, resulting in excitotoxic neuronal damage (Verkhratsky et al., 2013). Similarly, a profound inhibition of astroglial glutamate uptake lies at the core of neuronal death underlying Wernicke-Korsakoff encephalopathy (Hazell, 2009; Hazell et al., 2009). Astrocytes are also primary targets in hyperammoniemia (which is the main pathogenetic factor of acute and chronic hepatoencephalopathies); astrocytes accumulate ammonia which interferes with glutamine synthetase and profoundly disrupts astroglial homeostatic cascades responsible for K^+, Na^+, pH and Ca^{2+} homeostasis and induces pathological secretion of glutamate from astroglia (Kelly et al., 2009; Haack et al., 2014; Liang et al., 2014; Montana et al., 2014). The inability of astrocytes to maintain a balance

between synaptic and extra-synaptic glutamate is arguably a leading mechanism in addictive disorders (Scofield and Kalivas, 2014).

Astrogliopathology often develops as a secondary process, being in essence a reaction to various lesions. Reactive astrogliosis is an example of this secondary astrogliopathology that is manifested in many neurological diseases such as neurotrauma, stroke, infection, or later stages of neurodegeneration (Heneka et al., 2010; Burda and Sofroniew, 2014; Pekny et al., 2014). Activation of astrocytes is a heterogeneous process which depends on the disease and produces many distinct phenotypes of activated cells. Reactive astrogliosis is fundamental for defining progression and resolution of neuropathology, and suppressing astroglial reactivity increases neuronal vulnerability, exacerbates pathological development and alters post-lesion regeneration (Burda and Sofroniew, 2014; Pekny et al., 2014).

INTRODUCING NEURODEGENERATION

Neurodegenerative diseases, which afflict almost exclusively humans, are chronic neurological disorders that result in a progressive loss of function, structure and number of neural cells, ultimately resulting in the atrophy of the brain and profound cognitive deficits. The causes of neurodegenerative diseases are many and they may include physical, chemical or infectious trauma, genetic predisposition, metabolic deficits or the combination of the above likely with some other, yet unidentified factors. Molecular and cellular mechanisms of neurodegeneration remains generally unknown; although certain mutant genes responsible for various forms of neurodegeneration have been identified, highlighting certain proteins that might be involved (Bekris et al., 2010; Bertram et al., 2010). Neurodegeneration is often associated with abnormal protein synthesis that results in an accumulation of pathological proteins either inside or outside the cells. These proteins are, for example, represented by β-amyloid or α-synuclein; extracellularly, these pathological proteins are often aggregated and represent the cores of histopathological lesions known as senile plaques, Lewy bodies or Rosenthal fibres. These lesions are preferential for various diseases and have complex morphology which often includes reactive astrocytes and activated microglia. In many neurodegenerative disorders the synaptic weakness, synaptic loss and misbalance in neurotransmission develop at the early stages (Terry, 2000; Knight and Verkhratsky, 2010); later in the disease progression neurones die and the brain atrophy develops.

NEUROGLIA IN NEURODEGENERATIVE DISEASES

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) also known as motor neurone disease or Lou Gehrig disease is a specific degeneration , with death of motor neurones localised in the cortex, brain stem and spinal cord. Astrocytes are intimately associated with the development of ALS; reactive astrogliosis and astroglial degeneration with functional asthenia have been described. At the early stages of the disease astrocytes undergo degeneration and many die through apoptosis; these degenerated astrocytes have a deficient glutamate transport, which arguably, contributes to glutamate excitotoxicity and neuronal death (Rossi and Volterra, 2009; Staats and Van Den Bosch, 2009). At later stages of ALS, dying neurones initiate astroglial reactivity; this, however, never results in the scar production. In animal models of ALS, in mice which expresses mutant human gene for superoxide dismutase 1 (hSOD1 G93A), astrodegeneration and astroglial death precede neuronal death and clinical symptoms (Rossi et al., 2008). Astrocytes in ALS animal models have a decreased expression of plasmalemmal glutamate transporters, whereas the genetic deletion of excitatory amino acid transporter 2 (EAAT2, also known as GLT-1 in rodents) caused the death of motoneurones, thus, mimicking the ALS pathological evolution (Staats and Van Den Bosch, 2009). Finally, selective silencing of the SOD1 mutant gene expression in astrocytes significantly slowed the progression of the ALS in transgenic mice (Yamanaka et al., 2008; Wang et al., 2011).

Human immunodeficiency virus-1 (HIV-1) associated dementia

HIV-1 infects microglial cells, which are the main CNS target. The activation of microglia by HIV-

1 with subsequent secretion of pro-inflammatory and neurotoxic factors defines neuronal death and development of cognitive deficits characteristic for HIV-associated dementia (HAD) (Kaul and Lipton, 2006). In HAD, both astrodegeneration and reactive astrogliosis have been detected. A rather severe decrease in the number of astrocytes in HAD was identified in the basal ganglia, with a correlation between the progression of cognitive impairments and the degree of astroglial death (Thompson et al., 2001). Reactive astrogliosis seems to be the most prominent in the entorhinal cortex and hippocampus (Vanzani et al., 2006).

Parkinson's disease

Roles of astrocytes in Parkinson's disease (PD) are poorly characterised. Experiments *in vitro* have demonstrated that astrocytes are important for protection and survival of dopaminergic neurones *in vitro* (Mena et al., 2002; Mena and Garcia de Yebenes, 2008). Experiments in neuronal glial co-cultures also demonstrated that astrocytes convert L-DOPA, the dopamine precursor, to dopamine,(Mena et al., 1996). Astrocytes are also important for dopamine metabolism and transport of dopamine and its precursors from the blood to the brain. Dopamine is transported into astrocytes by a large plasmalemmal neutral amino acids transporter encoded by the *SLC7A5* gene. The dopamine precursors tyrosine and L-DOPA are taken from the blood by LAT1/4F2hc complex, which is expressed in astroglia. Astrocytes may also express functional dopamine transporter DAT1/SLC6A3. L-DOPA has been shown to be transported by an organic cation transporter 1, which has also been identified in astrocytes. In the striatum, astrocytes act as a reservoir for L-DOPA, which they release to be subsequently transported to neurones (Asanuma et al., 2014) (Asanuma et al., 2014).

Alzheimer's disease

Alzheimer's disease (AD), named so after Alois Alzheimer who described the first case of early familial form of dementia (Alzheimer, 1907), characterised by progressive dementia and specific histopathological lesions represented by senile plaques (extracellular depositions of β-amyloid) and interneuronal tangles resulting from abnormal phosphorylation of tau protein (Braak et al., 1998; Armstrong, 2009). The most popular contemporary hypothesis of AD pathogenesis emphasises the role of β-amyloid (the β-amyloid cascade hypothesis - (Korczyn, 2008; Karran et al., 2011), although numerous clinical trials based on this hypothesis had failed and criticism of ubiquitous pathological significance of β-amyloid is mounting (Hardy, 2009; Castellani et al., 2010; Castellani and Smith, 2011).

Astrocytes and β-amyloid

The question of whether astrocytes can participate in production and/or degradation of β-amyloid in the context of AD remains open. There were several reports indicating the ability of reactive astrocytes to accumulate and degrade β-amyloid (Guenette, 2003; Nicoll and Weller, 2003), and β-amyloid was detected in astroglial cells from the entorhinal cortex of AD patients (Nagele et al., 2003), although it was rarely found in astrocytes from the triple transgenic model model of AD (3xtg-AD) (Olabarria et al., 2010). Reactive astrocytes, in a different model of AD, the transgenic mice expressing mutant amyloid precursor protein (APP), were found to express the amyloid degrading enzyme neprilysin (Apelt et al., 2003). At the same time the pathology may affect the ability of astrocytes to scavenge β-amyloid; active accumulation was found in cultured primary astrocytes isolated from healthy mice brain, while astrocytes isolated from mutant APP transgenic mice could not take up β-amyloid (Wyss-Coray et al., 2003).

Similarly obscure remains a possibility of astrocytes to produce β-amyloid. Normal, healthy astroglial cells do not express β-secretase and hence do not produce β-amyloid. When astrocytes were exposed to chronic stress, however, the expression of β-secretase was induced, endowing astrocytes with the β-amyloid producing capability (Rossner et al., 2005). Astroglial expression of β-secretase was detected also in various AD mice models (Rossner et al., 2001; Heneka et al., 2005).

Astrogliosis in AD

Reactive astrocytes (defined by the increased expression of GFAP and hypertrophic morphology) are often found in the post-mortem tissues from

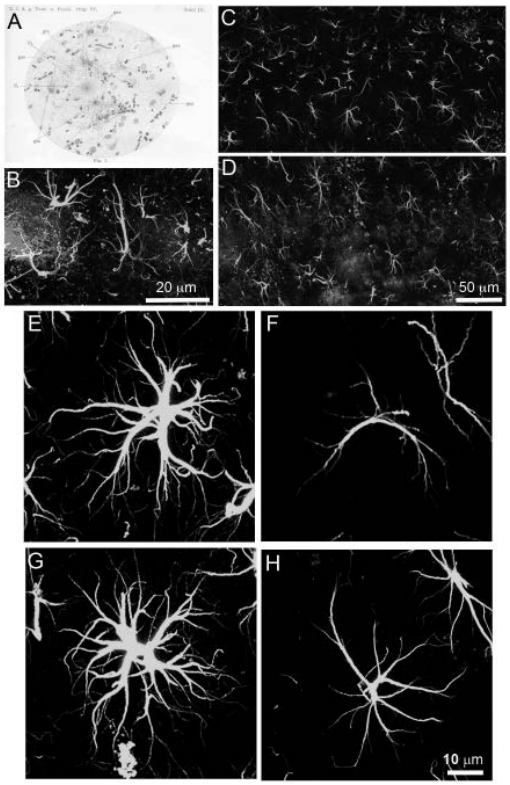

Fig. 1. *Reactive astroglia in association with senile plaques in the hippocampus.* ***A)*** *Neuritic Aβ plaques (P in A) as seen and drawn by Alois Alzheimer (Alzheimer, 1910); the plaque core (P1) is surrounded by activated glial cells (glz).* ***B***, ***C)*** *Confocal images of β-amyloid (red) and reactive astrocytes (green) associated with senile plaques (B) and diffuse amyloid deposits (C) in the hippocampus of the 3xTg-AD animal model.* ***D)*** *From the same preparation as shown in (C) confocal images of astrocytes in β-amyloid-free area; these astroglial profiles are atrophic when compared to controls and much smaller that reactive cells.* ***E-H)*** *High magnification of normal astrocytes (D, G) compared to the atrophic ones (F, H) that appear in AD within the hippocampal dentate gyrus (E-F) and CA1 region (G-H). Modified and adapted from (Rodríguez et al., 2015; Verkhratsky et al., 2014c).*

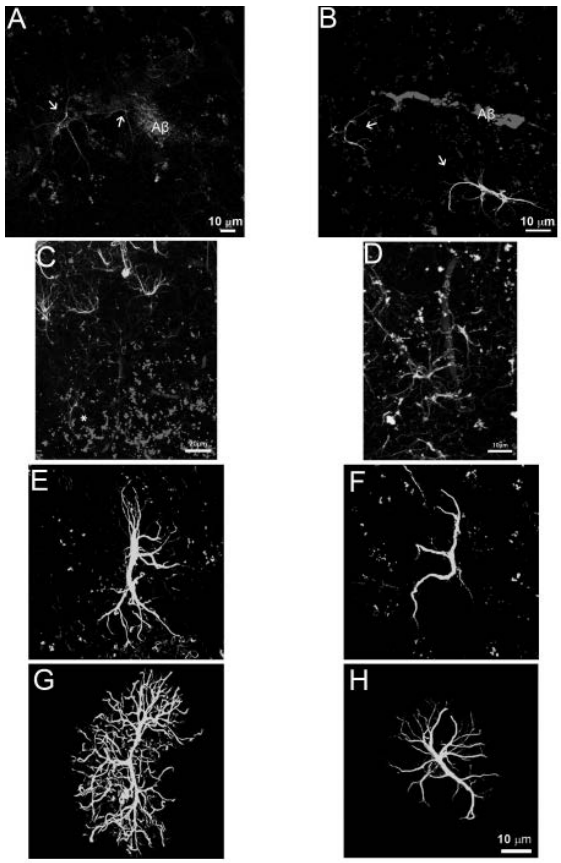

Fig. 2. *3xTg-AD confocal 3-dimensional reconstructed images labelled in green for GFAP and in red for Aβ (****A-D****). It is evident that in either entorhinal cortex (A-B) or prefrontal cortex (C-D) independently of Aβ accumulation the majority of GFAP labelled astrocytes are atrophic and not reactive, which is different in the hippocampus (Fig. 1). In both cortices the presence of astrocytes is rarely associated with Aβ deposits either in plaques (A-C) or blood vessels (B-D); astrocytes show clear atrophic signs (in B, asterisk).* ***E-H)*** *High magnification of normal astrocytes (E, G) compared to the atrophic ones (F, G) that appear in AD in entorhinal cortex (E-F) and prefrontal cortex (G-H). Modified and adapted from (Rodríguez et al., 2015;Verkhratsky et al., 2014c; Kulijewicz-Nawrot et al., 2012 and Yeh et al., 2011).*

AD patients, where they are usually associated with senile plaques (Beach and McGeer, 1988; Griffin et al., 1989; Meda et al., 2001; Mrak and Griffin, 2005; Rodriguez et al., 2009). Similarly, reactive astroglial cells were found in association with plaques in animal models of AD (Verkhratsky et al., 2010). Hypertrophic GFAP-positive astroglial cells surrounding the plaques preserve their

territorial domain, indicating the isomorphic, rather than anisomorphic gliosis. Similarly, there are no signs of astroglial scar in the AD nervous tissue. Reactive astrocytes in AD animal models show aberrant physiology, manifested by spontaneous Ca^{2+} oscillations and abnormal intercellular Ca^{2+} waves (Kuchibhotla et al., 2009; Lim et al., 2014). There are indications for a direct link between β-amyloid, astroglial Ca^{2+} signalling and initiation of astrogliotic response. Exposure of primary hippocampal cultured astrocytes and astrocytes in organotypic hippocampal slice cultures to β-amyloid triggers Ca^{2+} oscillations, originating from intracellular Ca^{2+} release from the endoplasmic reticulum (ER) store, as well as astroglial reactivity. Inhibition of this β-amyloid-induced Ca^{2+} release suppresses astrogliotic response (Alberdi et al., 2013). Importantly, astrogliotic response to β-amyloid and to AD-type pathology differed between brain regions. In 3xTG-AD mice (Oddo et al., 2003) the accumulation of β-amyloid triggered astrogliotic response in the hippocampus (Fig. 1.), but not in prefrontal or entorhinal cortices (Fig. 2.) (Olabarria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012). This absence of astrogliotic response correlated with the absence of β-amyloid remodelling of Ca^{2+} signalling toolkits in the entorhinal astrocytes, which is in contrast to hippocampal astroglia where β-amyloid substantially up-regulated the expression of plasmalemmal glutamate metabotropic receptors and inositol 1,4,5 trisphosphate receptors of the ER (Grolla et al., 2013).

Astroglial atrophy in AD

At the early stages the progression of the AD type pathology in genetic models of the disease involves astroglial atrophy. Recent studies of transgenic AD mice models revealed significant astrodegeneration that occurs at the early stages of AD progression and leads to an emergence of atrophic astrocytes (Olabarria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012; Beauquis et al., 2013), see also (Verkhratsky et al., 2014c).

A reduction of GFAP-positive and glutamine synthetase (GS)-positive astroglial profiles have been found in various regions of the aforementioned 3xTg-AD mice (Olabarria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012). Atrophy of astroglia was quantified by decreased surface area and volume of GFAP/GS-positive profiles, decreased volume of cell somata, decreased number of primary processes and reduction in number of primary processes. The overall number of GFAP-positive astrocytes, however, remained stable in the hippocampus, entorhinal and prefrontal cortices of AD mice at all ages (1 – 24 month of age) (Olabarria et al., 2010; Yeh et al., 2011; Kulijewicz-Nawrot et al., 2012). Rather similar atrophic morphology was observed in hippocampal astrocytes from another AD animal model, the mutant APP (PDAPP-J20) mice carrying the Swedish and Indiana APP human mutations (Beauquis et al., 2013).

The timing of astroglial atrophy in the 3xTG-AD mice was region-dependent; significant reduction in astroglial profiles was detected at very early stages (at 1 months of age) in the entorhinal cortex, somewhat later in the prefronatal cortex (~ 6 months) and substantially later in the hippocampus (~9 – 12 months) (Beauquis et al., 2013). In all regions, however, astroglial atrophy preceded an emergence of β-amyloid deposition and the formation of senile plaques.

The atrophic morphology of astrocytes in the brains of 3xTG-AD animals may indicate a reduced astroglial coverage of the grey matter. Atrophic astrocytes, arguably, provide less synaptic coverage, which coincides with a decrease in astroglial homeostatic capacity. Astrodegeneration may also affect the neuro-vascular unit and could impact upon astroglia-dependent neuroprotection. Astrocytes are also critical for maintaining normal neurotransmission by supplying neurones with glutamine that is indispensable for glutamatergic and GABA-ergic pathways. This astroglial asthenia may trigger deficits in the synaptic strength and even contribute to a decrease in the number of active synapses which is observed at the early stages of AD (Terry, 2000). Astroglial atrophy may also affect the brain circulation, as the deficiency in the blood flow is a characteristic feature of AD (Bell and Zlokovic, 2009). Astrocytes are central elements of neurovascular units that integrate neurones with local circulation. Astrocytes secrete various factors that mediate vasoconstriction and vasodilation by acting on pericytes or smooth muscle cells of arterioles; astrocytes also communicate with endothelial cells

and support the blood-brain barrier (Zonta et al., 2003; Mulligan and MacVicar, 2004; Takano et al., 2006). Thus, an early astroglial atrophy and the later astrogliosis may contribute to vascular pathology and deficient circulation. Progression of AD is also associated with a developing metabolic deficiency; reduced glucose utilisation has been identified in functional brain imaging in AD patients starting from the very early stages of the disease (Mosconi et al., 2008). Exposure of cultured astrocytes to β-amyloid affects their metabolism, which may have pathological significance (Soucek et al., 2003; Allaman et al., 2010).

Huntington's disease

Huntington's disease (HD) was described by George Huntington, in 1872 as a chorea-type motor disorder (Huntington, 1872). HD is an inherited, autosomal dominant and progressive neurodegeneration, at the core of which lies genetic aberration associated with the triplet nucleotide repeat cytosine-adenine-guanine (CAG), which encodes glutamine, in exon 1 of the widely expressed huntingtin gene (Zuccato and Cattaneo, 2014). Expression of this mutant gene results in the synthesis of mutant huntingtin protein (mhtt) containing an expanded polyglutamine section in its N-terminal portion; the higher is the number of glutamine repeats the more severe is the disease progression (Zoghbi and Orr, 2000). The mutant protein is expressed throughout the CNS in both neurones and astrocytes. Astroglial expression of mhtt leads to a substantial decrease in the density of astroglial plasmalemmal glutamate transporters with consequent diminution of the efficacy of astroglial glutamate uptake. This decrease in astroglial expression of EAAT2/GLT-1 transporter has been identified in post-mortem human tissues and in a mouse genetic model of HD (Lievens et al., 2001; Behrens et al., 2002; Hassel et al., 2008; Faideau et al., 2010). Deficient astroglial glutamate uptake leads to elevated glutamate concentration in the brain and could be a leading factor in excitotoxicity and neuronal death (Lievens et al., 2001; Shin et al., 2005; Hassel et al., 2008). Astrocytes may also contribute to glutamate excitotoxicity in HD through pathological glutamate release, which was found in astrocytes isolated from an HD animal model (Lee et al., 2013), known as BACHD mouse (Gray et al., 2008). Cultured astrocytes, prepared from the cortex of BACHD mouse demonstrated an enhanced Ca^{2+}-dependent exocytotic release of glutamate, which appeared to be the result of an increased expression of pyruvate carboxylase, the critical enzyme for *de novo* synthesis of glutamate. Increase in glutamate synthesis stipulated and increased glutamate content in the exocytotic vesicles. In addition, astrocytes in a different HD mouse model show deficient K^+ buffering which may further contribute to pathogenesis of the disease (Tong et al., 2014).

CONCLUSIONS

Neurodegenerative diseases are associated with substantial pathological changes in astroglial cells, which surprisingly include both atrophic changes and reactivity, albeit in region- and time-dependent domain. These changes may decrease the homeostatic reserve of the CNS and contribute to evolution of pathology. Targeting astroglia in neurodegeneration may result in new therapeutic strategies aimed at preventing and delaying the disease progression.

ACKNOWLEDGEMENTS

The authors research was supported by the Alzheimer's Research Trust (UK) Programme Grant (ART/PG2004A/1) to AV and JJR; by National Institutes of Health (The Eunice Kennedy Shriver National Institute of Child Health and Human Development award HD078678) to VP, by the grants P3 310, J3 4051, J3 3632, J36790 and J3 4146 from the Slovenian Research Agency (ARRS) and the EduGlia ITN EU grant to RZ and AV, and by Plan Nacional de I+D+I 2008–2011 and ISCIII-Subdirección General de Evaluación y Fomento de la investigación co-financed by FEDER (grant PI10/02738 to J.J.R and AV); and the Government of the Basque Country grants AE-2010-1-28, AEGV10/16 and GV- 2011111020 to J.J.R.

REFERENCES

Alberdi E, Wyssenbach A, Alberdi M, Sanchez-Gomez MV, Cavaliere F, Rodriguez JJ, Verkhratsky A, Matute C (2013) Ca^{2+} -dependent endoplasmic reticulum

stress correlates with astrogliosis in oligomeric amyloid beta-treated astrocytes and in a model of Alzheimer's disease. Aging Cell 12:292-302.

Allaman I, Gavillet M, Belanger M, Laroche T, Viertl D, Lashuel HA, Magistretti PJ (2010) Amyloid-b aggregates cause alterations of astrocytic metabolic phenotype: impact on neuronal viability. J Neurosci 30:3326-3338.

Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde. Allg Z Psychiat Psych-Gericht Med 64:146-148.

Alzheimer A (1910) Beiträge zur Kenntnis der pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgängen im Nervengewebe. In: Histologische und histopathologische Arbeiten über die Grosshirnrinde mit besonderer Berücksichtigung der pathologischen Anatomie der Geisteskrankheiten. (Nissl F, Alzheimer A, eds), pp 401-562. Jena: Gustav Fischer.

Apelt J, Ach K, Schliebs R (2003) Aging-related down-regulation of neprilysin, a putative b-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of b-amyloid plaques. Neurosci Lett 339:183-186.

Armstrong RA (2009) The molecular biology of senile plaques and neurofibrillary tangles in Alzheimer's disease. Folia Neuropathol 47:289-299.

Asanuma M, Miyazaki I, Murakami S, Diaz-Corrales FJ, Ogawa N (2014) Striatal Astrocytes Act as a Reservoir for L-DOPA. PLoS One 9:e106362.

Beach TG, McGeer EG (1988) Lamina-specific arrangement of astrocytic gliosis and senile plaques in Alzheimer's disease visual cortex. Brain Res 463:357-361.

Beauquis J, Pavia P, Pomilio C, Vinuesa A, Podlutskaya N, Galvan V, Saravia F (2013) Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of Alzheimer's disease. Exp Neurol 239:28-37.

Behrens PF, Franz P, Woodman B, Lindenberg KS, Landwehrmeyer GB (2002) Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. Brain 125:1908-1922.

Bekris LM, Yu CE, Bird TD, Tsuang DW (2010) Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol 23:213-227.

Bell RD, Zlokovic BV (2009) Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol 118:103-113.

Bertram L, Lill CM, Tanzi RE (2010) The genetics of Alzheimer disease: back to the future. Neuron 68:270-281.

Braak H, de Vos RA, Jansen EN, Bratzke H, Braak E (1998) Neuropathological hallmarks of Alzheimer's and Parkinson's diseases. Prog Brain Res 117:267-285.

Burda JE, Sofroniew MV (2014) Reactive gliosis and the multicellular response to CNS damage and disease. Neuron 81:229-248.

Castellani RJ, Smith MA (2011) Compounding artefacts with uncertainty, and an amyloid cascade hypothesis that is 'too big to fail'. J Pathol 224:147-152.

Castellani RJ, Rolston RK, Smith MA (2010) Alzheimer disease. Dis Mon 56:484-546.

Clarke LE, Barres BA (2013) Emerging roles of astrocytes in neural circuit development. Nat Rev Neurosci 14:311-321.

De Keyser J, Mostert JP, Koch MW (2008) Dysfunctional astrocytes as key players in the pathogenesis of central nervous system disorders. J Neurol Sci 267:3-16.

Del Rio-Hortega P (1932) Microglia. In: Cytology and cellular pathology of the nervous system (Penfield W, ed), pp 482-534. New York: Hoeber.

Del Rio Hortega P, Penfield W (1927) Cerebral cicatrix. reaction of neuroglia and microglia to brain wounds. Johns Hopkins Hosp Bull 41:278 - 303.

Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G, Dufour N, Guillermier M, Brouillet E, Hantraye P, Deglon N, Ferrante RJ, Bonvento G (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. Hum Mol Genet 19:3053-3067.

Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE, Li XJ, Levine MS, Yang XW (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182-6195.

Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CL, 3rd, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in

Down syndrome and Alzheimer disease. Proc Natl Acad Sci U S A 86:7611-7615.

Grolla AA, Sim JA, Lim D, Rodriguez JJ, Genazzani AA, Verkhratsky A (2013) Amyloid-b and Alzheimer's disease type pathology differentially affects the calcium signalling toolkit in astrocytes from different brain regions. Cell Death Dis 4:e623.

Guenette SY (2003) Astrocytes: a cellular player in Ab clearance and degradation. Trends Mol Med 9:279-280.

Haack N, Dublin P, Rose CR (2014) Dysbalance of astrocyte calcium under hyperammonemic conditions. PLoS One 9:e105832.

Hardy J (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem 110:1129-1134.

Hassel B, Tessler S, Faull RL, Emson PC (2008) Glutamate uptake is reduced in prefrontal cortex in Huntington's disease. Neurochem Res 33:232-237.

Hazell AS (2009) Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy. Neurochem Int 55:129-135.

Hazell AS, Sheedy D, Oanea R, Aghourian M, Sun S, Jung JY, Wang D, Wang C (2009) Loss of astrocytic glutamate transporters in Wernicke encephalopathy. Glia 58:148-156.

Heneka MT, Rodriguez JJ, Verkhratsky A (2010) Neuroglia in neurodegeneration. Brain Res Rev 63:189-211.

Heneka MT, Sastre M, Dumitrescu-Ozimek L, Dewachter I, Walter J, Klockgether T, Van Leuven F (2005) Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APPV717I transgenic mice. J Neuroinflam 2:22.

Huntington G (1872) On Chorea. Medical and Surgical Reporter of Philadelphia 26:317-321.

Iadecola C, Nedergaard M (2007) Glial regulation of the cerebral microvasculature. Nat Neurosci 10:1369-1376.

Karran E, Mercken M, De Strooper B (2011) The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 10:698-712.

Kaul M, Lipton SA (2006) Mechanisms of neuronal injury and death in HIV-1 associated dementia. Curr HIV Res 4:307-318.

Kelly T, Kafitz KW, Roderigo C, Rose CR (2009) Ammonium-evoked alterations in intracellular sodium and pH reduce glial glutamate transport activity. Glia 57:921-934.

Kettenmann H, Ransom BR, eds (2013) Neuroglia. Oxford: Oxford University Press.

Kimelberg HK, Nedergaard M (2010) Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics 7:338-353.

Kirischuk S, Parpura V, Verkhratsky A (2012) Sodium dynamics: another key to astroglial excitability? Trends Neurosci 35:497-506.

Knight RA, Verkhratsky A (2010) Neurodegenerative diseases: failures in brain connectivity? Cell Death Differ 17:1069-1070.

Korczyn AD (2008) The amyloid cascade hypothesis. Alzheimers Dement 4:176-178.

Kriegstein A, Alvarez-Buylla A (2009) The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149-184.

Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 323:1211-1215.

Kulijewicz-Nawrot M, Verkhratsky A, Chvatal A, Sykova E, Rodriguez JJ (2012) Astrocytic cytoskeletal atrophy in the medial prefrontal cortex of a triple transgenic mouse model of Alzheimer's disease. J Anat 221:252-262.

Lee W, Reyes RC, Gottipati MK, Lewis K, Lesort M, Parpura V, Gray M (2013) Enhanced Ca^{2+}-dependent glutamate release from astrocytes of the BACHD Huntington's disease mouse model. Neurobiol Dis 58:192-199.

Liang C, Du T, Zhou J, Verkhratsky A, Peng L (2014) Ammonium increases Ca^{2+} signalling and up-regulates expression of TRPC1 gene in astrocytes in primary cultures and in the *in vivo* brain. Neurochem Res 39:2127-2135.

Lievens JC, Woodman B, Mahal A, Spasic-Boscovic O, Samuel D, Kerkerian-Le Goff L, Bates GP (2001) Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. Neurobiol Dis 8:807-821.

Lim D, Ronco V, Grolla AA, Verkhratsky A, Genazzani AA (2014) Glial calcium signalling in Alzheimer's

disease. Rev Physiol Biochem Pharmacol 167:45-65.

Meda L, Baron P, Scarlato G (2001) Glial activation in Alzheimer's disease: the role of Abeta and its associated proteins. Neurobiol Aging 22:885-893.

Mena MA, Garcia de Yebenes J (2008) Glial cells as players in parkinsonism: the "good," the "bad," and the "mysterious" glia. Neuroscientist 14:544-560.

Mena MA, Casarejos MJ, Carazo A, Paino CL, Garcia de Yebenes J (1996) Glia conditioned medium protects fetal rat midbrain neurones in culture from L-DOPA toxicity. Neuroreport 7:441-445.

Mena MA, de Bernardo S, Casarejos MJ, Canals S, Rodriguez-Martin E (2002) The role of astroglia on the survival of dopamine neurons. Mol Neurobiol 25:245-263.

Messing A, Brenner M, Feany MB, Nedergaard M, Goldman JE (2012) Alexander disease. J Neurosci 32:5017-5023.

Montana V, Verkhratsky A, Parpura V (2014) Pathological role for exocytotic glutamate release from astrocytes in hepatic encephalopathy. Curr Neuropharmacol 12:324-333.

Mosconi L, Pupi A, De Leon MJ (2008) Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. Ann N Y Acad Sci 1147:180-195.

Mrak RE, Griffin WS (2005) Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 26:349-354.

Mulligan SJ, MacVicar BA (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 431:195-199.

Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY (2003) Astrocytes accumulate A b 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. Brain Res 971:197-209.

Nedergaard M, Verkhratsky A (2012) Artifact versus reality--how astrocytes contribute to synaptic events. Glia 60:1013-1023.

Nicoll JA, Weller RO (2003) A new role for astrocytes: b-amyloid homeostasis and degradation. Trends Mol Med 9:281-282.

Oberheim NA, Goldman SA, Nedergaard M (2012) Heterogeneity of astrocytic form and function. Methods Mol Biol 814:23-45.

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Ab and synaptic dysfunction. Neuron 39:409-421.

Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 58:831-838.

Parpura V, Verkhratsky A (2012) Homeostatic function of astrocytes: Ca^{2+} and Na^{+} signalling. Transl Neurosci 3:334-344.

Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG, Stout RF, Jr., Spray DC, Reichenbach A, Pannicke T, Pekny M, Pekna M, Zorec R, Verkhratsky A (2012) Glial cells in (patho)physiology. J Neurochem 121:4-27.

Pekna M, Pekny M (2012) The neurobiology of brain injury. Cerebrum 2012:9.

Pekny M, Wilhelmsson U, Pekna M (2014) The dual role of astrocyte activation and reactive gliosis. Neurosci Lett.

Rajkowska G, Stockmeier CA (2013) Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. Curr Drug Targets 14:1225-1236.

Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A (2009) Astroglia in dementia and Alzheimer's disease. Cell Death Differ 16:378-385.

Rodriguez-Arellano JJ, Parpura V, Zorec R, Verkhratsky A (2015) Astrocytes in physiological aging and Alzheimer's disease. Neuroscience. in press; doi: 10.1016/j.neuroscience.2015.01.007

Rossi D, Volterra A (2009) Astrocytic dysfunction: Insights on the role in neurodegeneration. Brain Res Bull 80:224-232.

Rossi D, Brambilla L, Valori CF, Roncoroni C, Crugnola A, Yokota T, Bredesen DE, Volterra A (2008) Focal degeneration of astrocytes in amyotrophic lateral sclerosis. Cell Death Differ 15:1691-1700.

Rossner S, Lange-Dohna C, Zeitschel U, Perez-Polo JR (2005) Alzheimer's disease b-secretase BACE1 is not a neuron-specific enzyme. J Neurochem 92:226-234.

Rossner S, Apelt J, Schliebs R, Perez-Polo JR, Bigl V (2001) Neuronal and glial b-secretase (BACE) protein expression in transgenic Tg2576 mice with amyloid

plaque pathology. J Neurosci Res 64:437-446.

Scofield MD, Kalivas PW (2014) Astrocytic dysfunction and addiction: consequences of impaired glutamate homeostasis. Neuroscientist 20:610-622.

Shin JY, Fang ZH, Yu ZX, Wang CE, Li SH, Li XJ (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol 171:1001-1012.

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7-35.

Soucek T, Cumming R, Dargusch R, Maher P, Schubert D (2003) The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide. Neuron 39:43-56.

Staats KA, Van Den Bosch L (2009) Astrocytes in amyotrophic lateral sclerosis: direct effects on motor neuron survival. J Biol Phys 35:337-346.

Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, Nedergaard M (2006) Astrocyte-mediated control of cerebral blood flow. Nat Neurosci 9:260-267.

Terry RD (2000) Cell death or synaptic loss in Alzheimer disease. J Neuropathol Exp Neurol 59:1118-1119.

Thompson KA, McArthur JC, Wesselingh SL (2001) Correlation between neurological progression and astrocyte apoptosis in HIV-associated dementia. Ann Neurol 49:745-752.

Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Haustein MD, Anderson MA, Mody I, Olsen ML, Sofroniew MV, Khakh BS (2014) Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci 17:694-703.

Vanzani MC, Iacono RF, Caccuri RL, Troncoso AR, Berria MI (2006) Regional differences in astrocyte activation in HIV-associated dementia. Medicina (B Aires) 66:108-112.

Verkhratsky A, Butt AM (2013) Glial Physiology and Pathophysiology. Chichester: Wiley-Blackwell.

Verkhratsky A, Nedergaard M (2014) Astroglial cradle in the life of the synapse. Philos Trans R Soc Lond B Biol Sci 369:20130595.

Verkhratsky A, Rodriguez JJ, Parpura V (2013) Astroglia in neurological diseases. Future Neurol 8:149-158.

Verkhratsky A, Nedergaard M, Hertz L (2014a) Why are Astrocytes Important? Neurochem Res. 40: 389-401

Verkhratsky A, Rodriguez JJ, Steardo L (2014b) Astrogliopathology: a central element of neuropsychiatric diseases? Neuroscientist 20:576-588.

Verkhratsky A, Marutle A, Rodriguez-Arellano JJ, Nordberg A (2014c) Glial Asthenia and Functional Paralysis: A New Perspective on Neurodegeneration and Alzheimer's Disease. Neuroscientist. in press; doi 10.1177/1073858414547132

Verkhratsky A, Parpura V, Pekna M, Pekny M, Sofroniew M (2014d) Glia in the pathogenesis of neurodegenerative diseases. Biochem Soc Trans 42:1291-1301.

Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ (2010) Astrocytes in Alzheimer's disease. Neurotherapeutics 7:399-412.

Verkhratsky A, Sofroniew MV, Messing A, deLanerolle NC, Rempe D, Rodriguez JJ, Nedergaard M (2012) Neurological diseases as primary gliopathies: a reassessment of neurocentrism. ASN Neuro 4: e00082.

Wang L, Gutmann DH, Roos RP (2011) Astrocyte loss of mutant SOD1 delays ALS disease onset and progression in G85R transgenic mice. Hum Mol Genet 20:286-293.

Williams MR, Hampton T, Pearce RK, Hirsch SR, Ansorge O, Thom M, Maier M (2013) Astrocyte decrease in the subgenual cingulate and callosal genu in schizophrenia. Eur Arch Psychiatry Clin Neurosci 263:41-52.

Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J (2003) Adult mouse astrocytes degrade amyloid-b *in vitro* and *in situ*. Nat Med 9:453-457.

Yamanaka K, Chun SJ, Boillee S, Fujimori-Tonou N, Yamashita H, Gutmann DH, Takahashi R, Misawa H, Cleveland DW (2008) Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. Nat Neurosci 11:251-253.

Yeh CY, Vadhwana B, Verkhratsky A, Rodriguez JJ (2011) Early astrocytic atrophy in the entorhinal cortex of a triple transgenic animal model of Alzheimer's disease. ASN Neuro 3:271-279.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32:6391-6410.

Zeidan-Chulia F, Salmina AB, Malinovskaya NA,

Noda M, Verkhratsky A, Moreira JC (2014) The glial perspective of autism spectrum disorders. Neurosci Biobehav Rev 38:160-172.

Zhang Y, Barres BA (2010) Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr Opin Neurobiol 20:588-594.

Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217-247.

Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat Neurosci 6:43-50.

Zuccato C, Cattaneo E (2014) Huntington's disease. Handb Exp Pharmacol 220:357-409.

MITOCHONDRIAL NCX3: A NEW PLAYER IN THE REGULATION OF MITOCHONDRIAL CALCIUM HANDLING IN NEURODEGENERATIVE DISEASES

M.J. SISALLI[1], K.M. OMURA[1], L. ANNUNZIATO[1,2] and A. SCORZIELLO[1]

[1]Division of Pharmacology, Department of Neuroscience, Reproductive and Odontostomatological Science, School of Medicine, Federico II University of Naples, Naples, Italy; [2]Fondazione IRCSS SDN, Naples, Italy

Mitochondria are intracellular membrane enclosed organelles found in most eukaryotic cells, which play important roles in several cellular functions, such as the production of energy by oxidative phosphorylation, the regulation of cellular calcium homeostasis, and the control of programmed cell death. The mitochondrial influx and efflux calcium pathways play a relevant role in cytosolic and mitochondrial calcium homeostasis and contribute to the regulation of mitochondrial functions. Furthermore, mitochondria are dynamic organelles that actively divide, fuse with one another, and undergo to regulated turnover, all of which are important for the maintenance of mitochondrial function and quality control. According to a widespread concept, neurons are critically dependent on mitochondrial integrity based on their specific morphological, biochemical, and physiological features. Indeed, neurons are characterized by high rates of metabolic activity and need to respond promptly to activity-dependent fluctuations in bioenergetic demand. The dimensions and polarity of neurons require efficient transport of mitochondria to hot spots of energy consumption, such as presynaptic and postsynaptic sites. Consequently, alterations in any of these mitochondrial features can potentially cause disease and have been linked to the pathogenesis of neurodegeneration. In this review particular emphasis will be devoted to the description of the role played by the newly identified mitochondrial proteins in the regulation of mitochondrial calcium dynamics as starting point for investigation of new molecular target responsible for mitochondrial dysfunctions leading to neuronal degeneration.

The calcium ion (Ca^{2+}) acts as an ubiquitous intracellular messenger to regulate a plead of physiological functions. In excitable cells and particularly in neurons of the central nervous system, that are continuously exposed to firing action potentials at various frequencies, Ca^{2+} ions undergo an endless cycling of Ca^{2+} influx through plasmalemmal Ca^{2+} channels, intracellular buffering by Ca^{2+} binding proteins (CBPs) and organelles, such as the endoplasmic reticulum (ER) and mitochondria, Ca^{2+} release from these organelles into the cytosol, and Ca^{2+} efflux through plasmalemmal Ca^{2+} transporters, namely, the ATPase Ca^{2+} pump and the Na^{+}/Ca^{2+} exchanger. As recently proposed by Fernández-Morales and collaborators it is possible to envisage two "Ca^{2+} circuits" referred as neuronal Ca^{2+} cycling (NCC) and mitochondrial Ca^{2+} cycling (MCC) respectively (Fernández-Morales et al., 2012). These Ca^{2+} circuits serve to regulate important neuronal functions such as the synaptic

Key words: Sodium Calcium Exchanger (NCX), mitochondria, mitochondrial calcium, neurodegenerative diseases

Corresponding Author: Antonella Scorziello
Department of Neuroscience, School of Medicine,
Federico II University of Naples,
Via S. Pansini 5, 80131 Naples, Italy
Tel.: +390817463330
Fax: +390817463323
e-mail: scorziel@unina.it

2279-5855 (2014)

release of neurotransmitters, or the respiration rate of mitochondria by Ca^{2+}-dependent dehydrogenases, that couple bioenergetics through ATP synthesis to neuronal activity. Disruption of NCC and/or MCC will enhance the vulnerability of neurons to various stressors, leading to necrotic and/or apoptotic death of the vulnerable neurons as it occurs in neurodegenerative diseases and stroke.

Indeed, as above mentioned Ca^{2+} is the main second messenger that helps to transmit depolarization status and synaptic activity to the biochemical machinery of a neuron. In neurons, Ca^{2+} have multiple complex and integrated functions, including the control of dendritic responses to neurotransmitters, signalling to the nucleus to regulate gene expression, and initiation of neurotransmitter release from presynaptic axon terminals (Gleichmann and Mattson, 2011). By these ways Ca^{2+} plays a pivotal roles in controlling neuronal excitability. Moreover, Ca^{2+} functions as a key regulator of electrochemical signalling, not only within individual neurons, but also among large populations of neurons that comprise neuronal networks (Gleichmann and Mattson, 2011).

The influx of Ca^{2+} through voltage-dependent and ligand-gated channels in the plasma membrane is a critical signal for the release of the neurotransmitters from presynaptic terminals and for the responses of the postsynaptic neuron (Mattson 2007). Glutamate, an excitatory neurotransmitter in the central nervous system, induces local and general increases of cytoplasmic Ca^{2+} through the activation of AMPA and NMDA receptors in the plasmamembrane, with consequent activation of voltage-dependent Ca^{2+} channels (VDCC) (Cali et al., 2011). In addition, the activation of metabotropic glutamate receptors coupled to the GTP-binding protein stimulates the release of inositol triphosphate (IP_3), which activates Ca^{2+} channels in the endoplasmic reticulum (Mattson, 2007). The cost for extensive neuronal Ca^{2+} signalling is an increased energy demand because all the Ca^{2+} that enters in neurons must be removed from the cytoplasm by ATP-dependent membrane calcium pumps in order to maintain Ca^{2+} homeostasis (Gleichmann and Mattson, 2011). Moreover, Ca^{2+} is removed from the cytoplasm thank to the activity of the plasma membrane Na^{2+}/Ca^{2+} Exchanger (NCX) (Blaustein and Lederer, 1999; Philipson and Nicoll, 2000; Annunziato et al., 2004, Lytton, 2007) the plasma membrane and endoplasmatic reticulum (ER) Ca^{2+}-ATPases (Berridge et al., 2003; Grienberger and Konnerth, 2012), and the Ca^{2+}-bindings proteins such as calbindin and parvalbulmin (Schwaller, 2010). Finally, Ca^{2+} can also be transported into and released from mitochondria (Mattson, 2007). In the dopaminergic neurons of the SNpc, that have most of Ca^{2+}channels opened much of the time, due to their pace making activity, for example, the magnitude of the Ca^{2+} influx appears to be much larger and the charge to the cell much greater compared to neurons in other different brain regions (Chan et al., 2010).

Mechanisms of neuronal calcium cycling

Because of the slow kinetics of the plasma membrane transporters and considering their restriction to the cellular surface, Ca^{2+} entering neurons must be rapidly sequestered either in organelles lying below the plasma membrane or through ionic interactions with cytosolic buffering proteins before being extruded from the cell. It is well known that highly localized and transient high $[Ca^{2+}]_c$ microdomains occurs underneath the plasmalemma, nearby the exocytotic machinery (Chad and Eckert, 1984; Simon and Llinas, 1985; Neher, 1998a; Neher, 1998b). The likelihood for generation of a high Ca^{2+} microdomain is strictly dependent by the opening of various Ca^{2+} channels upon action potential firing. These localized $[Ca^{2+}]_c$ transients may also be favored by Ca^{2+}-induced Ca^{2+} release (CICR) from the ER, through both ryanodine receptor (RyR) and inositol trisphosphate receptor (InsP3R) channels (Berridge et a., 2000). Furthermore, the formation of Ca^{2+} microdomains may also be favored by the subcellular disposition of ER, mitochondria, nucleus, secretory vesicles, or dendritic spines (Csordas et al., 1999; Csordas and Hajnoczky, 2003; Rizzuto et al., 1998; Szabadkai et al., 2003; Thomas et al., 1996). Thus, the Ca^{2+} signaling system is organized to favor the generation of large $[Ca^{2+}]_c$ microdomains that are highly localized in space and time. Indeed, at each moment of cell activity, Ca^{2+} homeostasis is finely tuned by fluxes between three compartments: the extracellular milieu, the cytosol, and the Ca^{2+}-storing organelles. At rest, these fluxes are small both at the plasma membrane and at ER membrane (Fernández-Morales et al., 2012). Mitochondrial Ca^{2+} uptake through the uniporter (MCU) is very slow because

of its low Ca^{2+} affinity and its exponential kinetics (Patron et al., 2013). The $[Ca^{2+}]_c$ at steady state is in the range of 10^{-7} M in the cytosol and in the mitochondrial matrix, and around 10^{-3} M at the extracellular milieu and at the ER lumen (Fernández-Morales et al., 2012). At low stimulation conditions, the $[Ca^{2+}]_c$ reaches the level of 10^{-6} M and clearance by the high-affinity Ca^{2+} pumps (plasma membrane and SERCA) dominates. At high stimulation rates, $[Ca^{2+}]_i$ may reach levels high enough to stimulate transport through MCU. Under this condition, most of the Ca^{2+} load is taken up by mitochondria (Herrington et al., 1996; Montero et al., 2000; Villalobos et al., 2002; Xu, et al., 1997). Ca^{2+} accumulated in mitochondria stimulates respiration and ATP synthesis (Gunter et al.,1994; Rizzuto et al., 2000). This may help to provide more extra energy for maintaining the exocytotic release of neurotransmitters under intense stimulation and to clear up the Ca^{2+} load, thus restoring cell homeostasis after the activity period of the neuron. Since high Ca^{2+} microdomains are particularly pronounced nearby the inner mouth of VDCCs, mitochondrial Ca^{2+} uptake could take place locally at these places during physiological stimulation. Therefore, it is possible to speculate that if the Ca^{2+} uptake properties of mitochondria could be modulated, this would be an effective strategy to regulate the exocytotic process and, hence, the release of neurotransmitters and synaptic plasticity. Thus, under pathological conditions, as well as, excitatory neurotoxicity, ischemia−reperfusion in stroke, aging, or neurodegenerative diseases, mitochondrial damage may reduce the ability of this compartment to take up Ca^{2+} leading to increased secretion of excitatory neurotransmitters and abnormal neuronal activation. These events trigger a vicious circle that in turn stimulate Ca^{2+}-dependent processes driving the cells to necrosis or apoptosis.

Mechanisms of mitochondrial calcium cycling

As above mentioned ER and mitochondria are the principal organelles involved in sequestering Ca^{2+} in neurons (Rizzuto and Pozzan, 2006; Verkhratsky, 2005). In the last few years more attention has been devoted to the mechanisms responsible for calcium cycling between these two compartments in both physiological and in pathological conditions (Hajnoczky et al., 2000a; Hajnóczky et al. 2000b, Rizzuto et al., 2004). Indeed, ER plays an important role in the maintenance of Ca^{2+} homeostasis into neurons due to its ability to store Ca^{2+} within the cell thank to its intralumial Ca^{2+} capacity of about 0.5 mM. Ca^{2+} is pumped into the ER by sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA) and is extruded by IP_3-RS and RyRs channels (Gleichmann and Mattson, 2011). Indeed, as this store fills up, Ca^{2+} triggers the opening of ER Ca^{2+} channels that let the Ca^{2+} flow back into the cytoplasm. These channels are often found in close apposition to mitochondria and their opening creates a region of high-local Ca^{2+} concentration that drives influx of Ca^{2+} into the matrix of mitochondria through Ca^{2+} uniporters (Rizzuto and Pozzan, 2006). The regulation of Ca^{2+} release from ER is responsible for many neuronal functions, from plasmalemmal excitability to synaptic plasticity. Together with mitochondria, ER forms junctions that support signal transduction and biosynthetic pathways and affect distribution of the organelles. These junctions have a pivotal role in mediating Ca^{2+} signal propagation to the mitochondria (Zundorf and Reiser, 2011). Ca^{2+} accumulation in the mitochondrial matrix again comes at an energetic cost, as it dissipates the electrochemical gradient created by respiratory metabolism along the electron transport chain (ETC). In fact, an important feature of the mitochondrial Ca^{2+} transport pathway is that this organelle contains low calcium in resting cells, but is able to accumulate large amounts of calcium in condition stimulating Ca^{2+} entry, and to release this calcium loaded during the recovery phase (Nicholls, 2005). This is due to the ability of specific transporters localized on the inner mitochondrial membrane that allow calcium to cycle from mitochondrial matrix to the cytosol and from the cytosol to the mitochondrial matrix. Indeed, Ca^{2+} is removed from the matrix through the mitochondrial NCX (Kim and Matsuoka, 2008; Rizzuto and Pozzan, 2000), the Ca^{2+} proton exchanger (Williams and Fry, 1979), and the transient opening of the mitochondrial permeability transition pore (mPTP) (Hüser and Blatter, 1999). These events allow the maintenance of mitochondrial calcium concentrations within physiological range that are necessary for the neurons to adjust aerobic ATP production, to regulate synaptic transmission and excitability, to promote organelle dynamics and trafficking, to mediate signalling to nucleus,

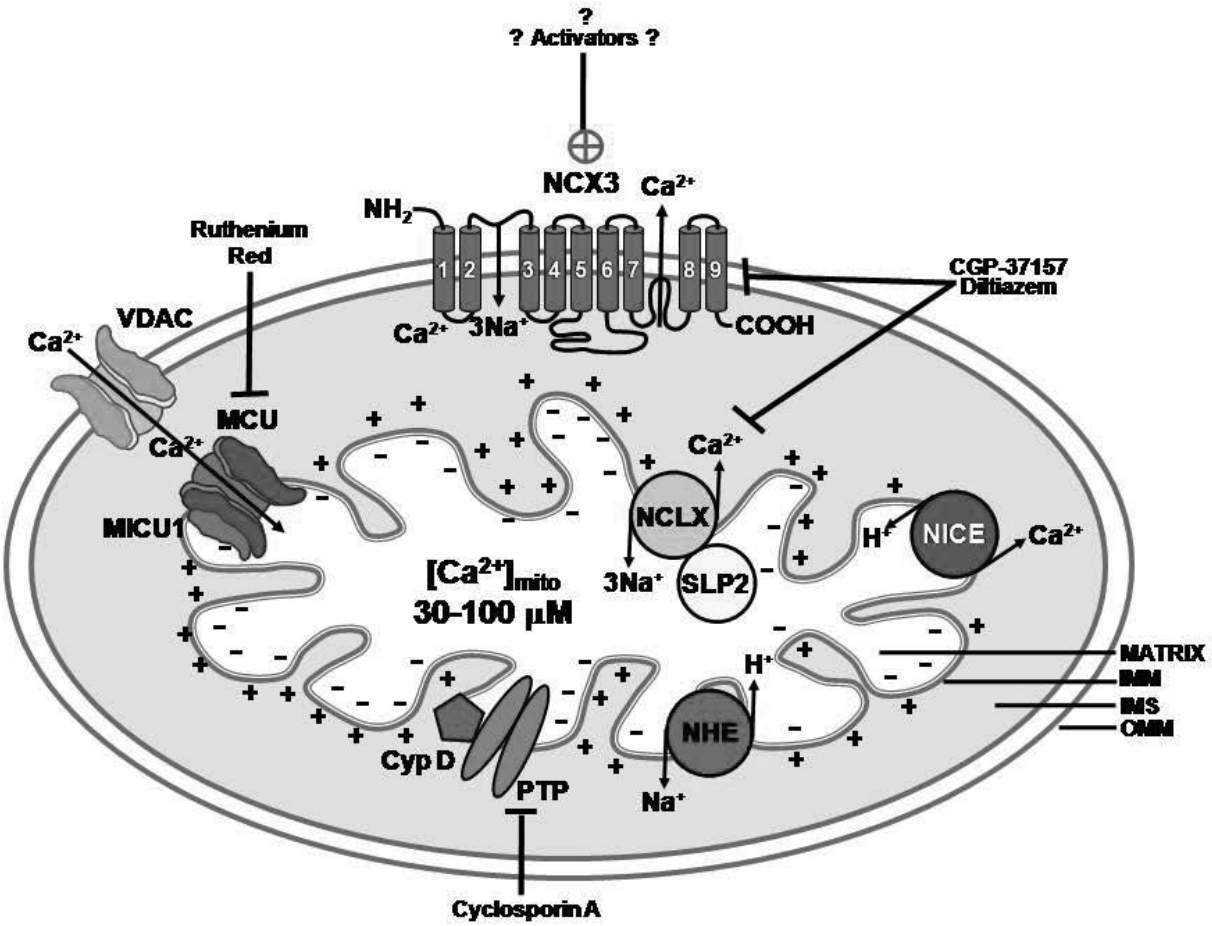

Fig. 1. *Schematic representation of mitochondrial calcium influx and efflux pathways and potential druggable targets regulating mitochondrial calcium handling. Influx transporters: mitochondrial calcium uniporter (MCU); voltage-dependent ion channel (VDAC). Efflux transporters: Na^+/Ca^{2+} exchanger Li-dependent on the inner mitochondrial membrane (NCLX); Ca^{2+}/H^+ exchanger (NICE); permeability transition pore (PTP); Na^+/Ca^{2+} exchanger on the outer mitochondrial membrane (NCX3). Ruthenium red: MCU inhibitor, CGP-37157 Diltiazem: NCLX and NCX3 inhibitor, and Cyclosporin A: PTP inhibitor. OMM outer mitochondrial membrane, IMM inner mitochondrial membrane, IMS intermembrane space, MATRIX mitochondrial matrix.*

to control the generation of ROS, and to preserve neuronal survival (Nicholls, 2005; Starkov, 2002; Chinopoulos and Adam-Vizi, 2010; Duchen, 2004; Mattson et al., 2008). We recently demonstrate that the nuclear encoded NCX3 is the only isoform of the Na^+/Ca^{2+}-exchanger localized within the OMM, where it forms a stable complex with the Protein Kinase A Anchoring protein (AKAP121) and plays a relevant role in the control of mitochondrial Ca^{2+} homeostasis both under physiological and hypoxic conditions (Scorziello et al., 2013) (Fig.1).

Owing to the activation the above mentioned mechanisms, the intracellular Ca^{2+} concentration increases only transiently during normal physiological activity, with no adverse effects on the neurons. However, in pathological conditions, and in normal aging, the ability of neurons to control Ca^{2+} effluxes and to recover from a Ca^{2+} load is compromised (Mattson, 2007). Perturbations in calcium homeostasis are, indeed, the common denominator in several neurodegenerative disorders (Surmeier, 2013). However, the molecular mechanisms responsible for the selective loss of neuronal population in the different neurodegenerative

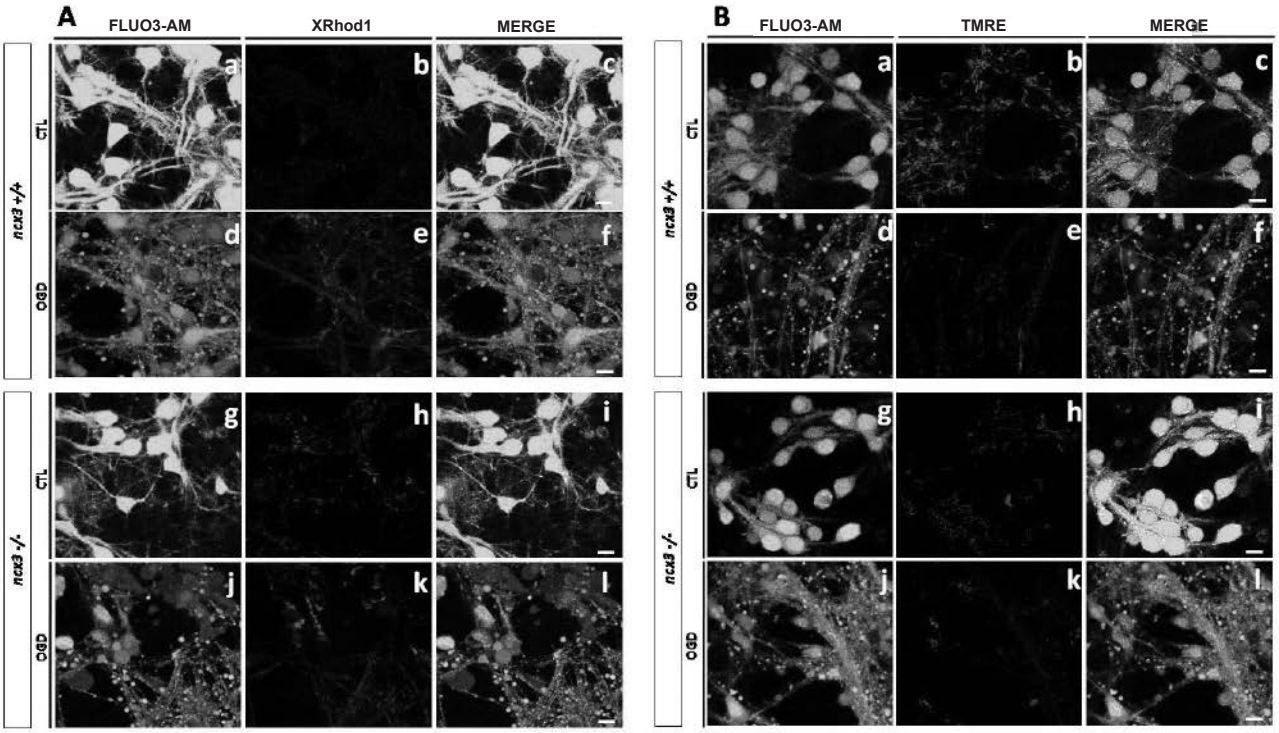

Fig. 2. ***A)*** *Mitochondrial calcium content measured in cortical neurons from ncx3+/+ and ncx3-/- mice brain exposed to Oxygen and Glucose Deprivation. Confocal images showing mitochondrial Ca^{2+} content measured by XRhod-1 probe (200nM, 15 minutes at RT, Fig 2A panels b, e, h, k) and cytosolic calcium concentration measured by Fluo 3 AM probe (5mM, 30 minutes at RT). Panels a–c: cortical ncx3+/+ neurons under control conditions (CTL); panels d–f: ncx3+/+ neurons exposed to 3 hrs Oxygen and glucose deprivation (OGD). Panels g–i: cortical ncx3-/- neurons under control conditions (CTL); panels j–l: ncx3-/- neurons exposed to OGD. Scale bars: 1 mm.* ***B)*** *Mitochondrial membrane potential measured in cortical neurons from ncx3+/+ and ncx3-/- mice brain exposed to Oxygen and Glucose Deprivation. Confocal images showing mitochondrial membrane potential measured by TMRE probe (20nM, 30 minutes at RT in redistribution mode, Fig 2B panels b,e, h, k) and cytosolic calcium concentration measured by Fluo 3 AM probe (5μM, 30 minutes at RT). Panels a–c: cortical ncx3+/+ neurons under control conditions (CTL); panels d–f: ncx3+/+ neurons exposed to 3 hrs Oxygen and Glucose Deprivation (OGD). Panels g–i: cortical ncx3-/- neurons under control conditions (CTL); panels j–l: ncx3-/- neurons exposed to OGD. Scale bars: 1 mμ.*

disorders are less clear. We recently demonstrated that an interaction between ER and mitochondria occurs in neurons during ischemic preconditioning. This effect is mediated by NCX1 and NCX3 activation and is responsible for neuroprotection observed in preconditioned neurons exposed to the subsequent OGD followed by reoxygenation (Sisalli et al., 2014).

Deregulation of neuronal calcium homeostasis and neurodegeneration

It has become clear that mitochondria, in addition to their role as the cell powerhouse, have a key role in several other processes of major importance in cell physiology and pathology (Rasola and Bernardi, 2007; Kroemer et al., 2007; McBride et al., 2006; Giacomello et al., 2007). Notably, great attention has been paid to the role of mitochondria in cell death and in neurodegeneration. Indeed, in the intrinsic pathway of apoptosis these organelles perform a pivotal role since they release a number of pro-apoptotic factors from the intermembrane space (IMS) responsible for caspase cascade activation (Kroemer et al., 2007; Galluzzi et al., 2009). The main mechanism leading

to the release of pro-apoptotic factors in the cytosol is the formation of the "permeability transition pore," also known as mitochondrial megachannel. Remarkably, its opening is favored by abnormal Ca^{2+} accumulation into mitochondria, especially when it occurs in concurrence with oxidative stress, high levels of phosphate and adenine nucleotide depletion (Rasola and Bernardi, 2007; Halestrap, 2009). The protein composition of PTP has been under debate until 2013 when it was discovered that dimers of mitochondrial ATP synthase form the permeability transition pore (Giorgio et al., 2013). Then it was also found that Cyp D interacts with the OSCP subunit of F-ATP synthase (Giorgio et al., 2013). Interestingly, in cells from Cyp D KO mice, the genetic ablation of Cyp D causes a delayed Cyclosporin A-insensitive PTP activation in response to Ca^{2+} overload. However, PTP opening triggered by stimuli other than Ca^{2+} overload, is similar in wild type and Cyp D or in KO cells, confirming that Cyp D plays a role as regulator of mitochondrial PTP rather than as structural constituent of this megachannel. (Baines et al., 2005; Basso et al., 2005; De Marchi et al., 2006; Nakagawa et al., 2005). Apoptosis is also modulated by a number of proteins belonging to the Bcl-2 family, among which the classical pro-apoptotic proteins Bax and Bak and the anti-apoptotic Bcl-2 and Bcl-XL. These proteins reside in the ER, cytosol and mitochondria as homo o heterodimers. During apoptosis permeabilization of the OMM causes the release from IMS of cytochrome c and other proapoptotic factors. As far as concern the mechanisms leading to OMM permeabilization, apart PTP, a pivotal role is played by Bid (Rasola and Bernardi, 2007; Upton et al., 2008). While Bid-dependent OMM permeabilization is insensitive to mitochondrial Ca^{2+}, in many other situations the apoptotic cascade and OMM permeability somehow relies on mitochondrial Ca^{2+} overload. The concept of mitochondrial Ca^{2+} overload, however, does not necessarily refer solely to a large increase in $[Ca^{2+}]_m$. Indeed, large, but short lasting, Ca^{2+} increases in mitochondrial matrix may occur under several physiological conditions without detrimental consequences for cell survival; whereas, much smaller, but prolonged, increases of mitochondrial Ca^{2+} may activate the apoptotic machinery.

An interesting aspect of mitochondrial calcium handling, relevant for physiopathology is that mitochondria may function as cellular detectors for the apoptotic process. Indeed, as recently reported (Pinton et al., 2008; Pizzo et al., 2007) an increase in $[Ca^{2+}]_m$ not toxic in itself because it increases the efficiency of ATP synthesis, if occurs synchronously with another toxic event, it may synergize with non toxic insult and in turn transforms a beneficial process into a death stimulus. This explanation justifies the emerging role of mitochondria as crucial players in the pathogenesis of many different diseases, both as primary or secondary executioners. From a pathological point of view, a cellular Ca^{2+} deregulation leading to mitochondrial Ca^{2+} overload and cell death through PTP opening followed by mitochondrial swelling has been described, as final step, for many neurodegenerative diseases (Panov et al., 2002; Sheehan et al., 1997; Sherer et al., 2002; Siklos et al., 1998). For instance, recent findings by Gandhi and co-workers reported that a impaired Ca^{2+} efflux from mitochondria through the mitochondrial Na^{+}/Ca^{2+} exchanger occurs in neurons lacking PINK1, a serine threonine kinase implicated in autosomal recessive early-onset parkinsonism. This led to increased Ca^{2+} uptake capacity, decreased membrane potential, and increased ROS production, all conditions leading to early triggering of the PTP opening and concomitant neuronal death (Gandhi et al., 2009). The blockade of this final process is thus emerging as an effective therapeutic strategy also in vivo. In this regard, the genetic ablation or pharmacological inhibition of Cyp D, a main regulator of the PTP, has been shown to decrease the mitochondrial alterations and ameliorate the pathology both in the case of Collagen VI deficiency and in the Scgd-/- mouse, a model for severe dystrophia (Millay et al., 2008; Irwin et al., 2003). Interestingly, the genetic ablation of CypD substantially improves the cognitive abilities of a mouse model of AD and alleviates Aβ-mediated reduction of long-term potentiation (Du et al., 2008). Moreover, intra-mitochondrial Aβ was demonstrated to directly interact with CypD, thus providing a molecular basis for the pathogenic mechanism leading to neuronal degeneration in AD (Du et al., 2008). Mitochondrial Ca^{2+} overload appeared the decisive commitment step also in Huntington's disease (Bezprozvanny et al., 2004). In

line with this view, it has been shown that mutated, but not wild type, Htt induces PTP opening in isolated mitochondria (Choo et al., 2004) as well as a facilitated opening of PTP in permeabilized polyQ-Htt expressing cells (Lim et al., 2008). Thus, also in this case PTP appears to be the final commitment step in a number of cellular stress conditions, with Ca^{2+} acting as a potent sensitizing factor (Lim et al., 2008).

THE SODIUM CALCIUM EXCHANGER

The Na^+/Ca^{2+} exchanger (NCX) represents a major transporter assuring Ca^{2+} efflux from mammalian cells (Blaustein and Lederer, 1999). Under physiologic conditions NCX provides the exchange of $3Na^+/1Ca^{2+}$ between cytoplasm and extracellular medium. In most tissues, it operates in a "forward" way corresponding to inward current and thus to calcium exit from the cell (Blaustein and Lederer, 1999). Under some conditions, however, a reverse mode of Na^+/Ca^{2+} exchange can be activated coupling the extrusion of three Na^+ ions with the influx of one Ca^{2+} ion (Blaustein and Lederer, 1999; Philipson and Nicoll, 2000; Annunziato et al., 2004).

NCX belongs to a multigene family comprising three isoforms, named NCX1, NCX2, and NCX3. To fulfill the physiological demands of various cell types, the NCX isoforms and their splice variants are expressed in a tissue-specific manner (Philipson and Nicoll, 2000; Lytton, 2007; Khananshvili, 2012). NCX1 is ubiquitously expressed in all tissues, NCX2 is mainly restricted to the brain, and NCX3 is expressed exclusively in brain and skeletal muscles (Quednau et al., 1997). In addition, NCX1 and NCX3 give rise to several splicing variants that appear to be selectively expressed in different regions and cellular populations of the brain (Quednau et al., 1997; Yu and Colvin, 1997). In fact, NCX1 mRNA can be detected in midbrain and in basal ganglia in which dopaminergic cell bodies are localized. Moreover, NCX1 protein isoform is present in the striatum, where the terminal projection fields of dopaminergic nigrostriatal neurons were found (Canitano et al., 2002; Papa et al., 2003). Several factors are involved in the regulation of NCX activity. Among them, the two transported ions Na^+ and Ca^{2+} play a crucial role. Indeed, a rise in cytosolic $[Na^+]$ rapidly stimulates and then inactivates the exchanger, whereas a rise in cytosolic $[Ca^{2+}]$ activates NCX and relieves the Na^+-dependent inactivation (Hilgemann et al., 1992a, b). Moreover, NCX is extremely sensitive to cytosolic acidification, redox status and metabolic state (DiPolo and Beauge, 1982, 2006; Doering and Lederer, 1994; Doering et al., 1996). These factors imply, in some cases, modifications of the exchange activity and, in others, alterations of the protein expression and docking into the membrane where, associated with other transporters such as Na^+/K^+-ATPase and Na^+/H^+ exchanger as well as enzymes like kinases and phosphatases, they form functional supra molecular complexes (Bers and Despa, 2009; Schulze et al., 2003; Hilgemann, 2007; McLaughlin et al., 2002; Berberián et al., 2009).

The activity of NCX is important especially in some neurophysiological conditions. In fact, the level of expression of NCX in neurons is predominantly high in those sites where a large movement of Ca^{2+} ions occurs across the plasma membrane, as it happens at the level of synapses (Juhaszova et al., 1996; Canitano et al., 2002). The Na^+/Ca^{2+}exchanger becomes the dominant Ca^{2+} extrusion mechanism when $[Ca^{2+}]_i$ is higher than 500nM, as it happens when a train of action potentials reaches the nerve terminals. It has been calculated that for these $[Ca^{2+}]_i$ values, more than 60% of Ca^{2+} extrusion is mediated by Na^+/Ca^{2+}exchanger families. In such physiological conditions, NCX activation is consistent with its low-affinity (K_d 500nM) and high-capacity ($5x10^3 Ca^{2+}/s$) function. In contrast, in resting conditions or after a single action potential, when $[Ca^{2+}]_i$ slightly increases, requiring, therefore, a more fine control, the high-affinity (K_d100 nM) and low-capacity ($10^2 Ca^{2+}/s$) pump, the plasmamembrane Ca^{2+}ATPase, assumes a predominant function, thus making the involvement of NCX less relevant (Blaustein and Lederer, 1999). On the other hand, dysregulation of $[Ca^{2+}]_i$ and $[Na^+]_i$ homeostasis is involved in neuronal injury occurring in *in vitro* and *in vivo* models of hypoxia-anoxia and in several neurodegenerative diseases. More specifically, in the early phase of neuronal anoxic insult, the Na^+/K^+-ATPase blockade causes a increase of $[Na^+]_i$, which in turn induces NCX to reverse its mode of operation. Although during this phase NCX causes an increase in $[Ca^{2+}]_i$, its effect on neurons appears beneficial for two reasons. First, by

promoting Ca^{2+} influx, NCX promotes Ca^{2+} refilling into the ER, which is depleted by anoxia followed by reoxygenation, thus allowing neurons to delay ER stress (Sirabella et al., 2009). Second, by eliciting the decrease in $[Na^+]_i$ overload, NCX prevents cell swelling and death (Annunziato et al., 2007). Conversely, in the later phase of neuronal anoxia, when $[Ca^{2+}]_i$ overload takes place, the NCX forward mode of operation contributes to the lowering of $[Ca^{2+}]_i$, thus protecting neurons from $[Ca^{2+}]_i$-induced neurotoxicity (Annunziato et al., 2004). Interestingly, in the last few years our laboratory gave an important contribution to the understanding of the differences in the functional properties of each of the three isofoms of NCX as well as to their differential subcellular distribution (Secondo et al., 2007; Scorziello et al., 2013). Indeed, it has been demonstrated that NCX1 and NCX2 are more sensitive to changes in ATP since NCX1 and NCX2 forward mode of operation is impaired by ATP depletion. Conversely, the functional properties of NCX3 are not affected by ATP depletion. These findings are consistent with the differential distribution of NCX3 in the brain (Canitano et al., 2002; Papa et al., 2003), as well as with its subcellular localization not only at plasmamembrane level but also on the outer mitochondrial membrane (Scorziello et al., 2013). Moreover, these finding let to hypothesize that each of the three isoforms might play a different role in the pathogenesis of a cellular damage (Secondo et al., 2007; Bano et al., 2005). In line with this hypothesis the treatment of ischemic rats with NCX1 or NCX3 antisense correlates with a remarkable enlargement of the infarct volume (Pignataro et al., 2004) thus suggesting a crucial role of these two isoforms in the pathogenesis of ischemic damage.

It is also demonstrated that NCX plays an important role during aging, since the impairment of Ca^{2+}homeostasis in neuronal cells is considered to be the major triggering event that leads to the development of brain aging (Canzoniero et al., 1992). Studies performed on the cerebro-cortex nerve endings of aged rats have shown that the activity of NCX is markedly reduced in the forward and in the reverse mode of action (Michaelis et al., 1984; Canzoniero et al., 1992). NCX functional decline seems to be the consequence of a reduced affinity of the antiporter for Ca^{2+}ions (Michaelis et al., 1984).

In this sense, during aging and also during neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease in which a neuronal calcium dysfunction occurs, NCX might play a relevant role. In fact, a study performed in the synaptic terminals obtained from the brain cortex of AD patients (Colvin et al., 1994) or in neurons treated with beta amyloid (Pannaccione et al., 2012) showed that NCX activity was increased.

mNCX AND NEURODEGENERATION

The role played by mNCX in neurodegeneration has been object of extensive investigation in the last few years (Palty et al., 2010; Celsi et al., 2013; Scorziello et al., 2013). It has been reported that during Parkinson's disease the activation of mNCX is the primary mechanism by which mitochondrial calcium concentrations ($[Ca^{2+}]_m$) is returned to the cytoplasm and therefore it is critical to a multitude of Ca^{2+}-dependent processes including neurotransmitter release, synaptic plasticity, bioenergetics and mitochondrial NO and free radical generation (Castaldo et al., 2009). Recently, it was found that in the absence of PINK1, mNCX activity was severely impaired, leading to mitochondrial calcium overload, permeability transition pore opening and cell death (Gandhi et al., 2009). Moreover, a recent study performed in human dopaminergic neurons showed that the plasmalemal NCX2 and NCX3 contribute to mitochondrial Na^+ and Ca^{2+} exchange and may act downstream of PINK1 in the prevention of neurodegeneration by $[Ca^{2+}]_m$ accumulation (Wood-Kaczmar et al., 2013). Furthermore, experiments performed in primary midbrain neurons obtained from A53T transgenic mice embryos showed that among the three isoforms of NCX, NCX3 is the only one whose expression was reduced. In these cells cytosolic calcium concentration was increased. Interestingly, a significant mitochondrial hyperpolarisation was observed in midbrain neurons obtained from A53T transgenic mice accompanied by a sustained increase in mitochondrial calcium content (personal unpublished observation).

Furthermore, the NCX3 isoform, which is selectively expressed in the brain and skeletal muscle (Papa et al., 2003), plays a fundamental role in buffering intracellular Ca^{2+} and Na^+ overload

occurring not only under physiological conditions but also in pathophysiological conditions such as ischemia (Condrescu et al., 1995; Linck et al., 1998; Secondo et al., 2007). Indeed, neurons silenced with siRNA-NCX3 or derived from *ncx3-/-* mice display a remarkable vulnerability in both *in vivo* and *in vitro* hypoxic conditions (Pignataro et al., 2004; Molinaro et al., 2008). These findings suggest that NCX-induced buffering of Ca^{2+} overload during exposure of neurons to hypoxic conditions may exert a neuroprotective function. Interestingly, we demonstrated that the exposure of wt neurons to OGD caused an increase in mitochondrial calcium content, whereas, it returned to values comparable to control during reoxygenation (Scorziello et al., 2013) (Fig 2A). In these experimental conditions mitochondrial inner membrane is depolarized in wt neurons exposed to OGD (Fig 2B). These experimental conditions correlated with changes in the expression of mNCX3 which decreased in OGD and returned to the basal level during the reoxygenation phase (Sirabella et al., 2009). Moreover, mitochondrial calcium content in ncx3-/- neurons is higher compared to that measured in wt neurons (Fig 2A).

We recently demonstrated that neurons treated with Aβ1–42 showed an upregulation of NCX3 activity that promoted ER Ca^{2+} refilling, thus helping neurons to mitigate ER stress and thus delaying neuronal death (Pannaccione et al., 2012). Since it is known that mitochondria associate with ER and their networks are important for the maintenance of calcium homeostasis (Raturi and Simmen, 2013) it is possible to speculate that the increased activity of mNCX3 might contribute to effect above described. This hypothesis is in line with the findings observed in preconditioned neurons exposed to OGD/ Reoxygenation (Sisalli et al., 2014). The exposure of cortical neurons to a sublethal hypoxic insult caused an increase in NCX3 expression and a reduction in mitochondrial calcium content in comparison with neurons exposed to OGD/Reoxygenation. Interestingly, in these conditions the ER calcium content is increased compared with untreated neurons. Collectively, these findings strongly support the hypothesis that mNCX3 might play a key role in the regulation of mitochondrial calcium homeostasis thus representing a new promising pharmacological target in neurodegenerative diseases and ischemia.

REGULATION OF MITOCHONDRIAL CALCIUM CYCLING AS NEW STRATEGY TO DEVELOP NEUROPROTECTIVE COMPOUNDS

As above largely described mitochondrial dysfunction and alterations of Ca^{2+} homeostasis has long been implicated in the pathogenesis of neurodegenerative illnesses such as AD (Mattson, 2007), PD (Yao et al., 2009), ALS (von Lewinski and. Keller, 2005; Grosskreutz et al., 2010) or HD (Damiano et al.,2010; Oliveira, 2010; Quintanilla and Johnson, 2009) and cerebral ischemia (Scorziello et al., 2013; Sisalli et al., 2014). Despite extensive research into the causes of these diseases, clinical researchers have had very limited progress and, there is still no cure for any of these diseases. One of the main obstacles in the way of creating treatments for these disorders is the fact that their etiology and pathophysiology still remain unclear. In this regard, the preservation of the mitochondrial proton gradient represents an optimal upstream target to counteract irreversible apoptosis occurring in neurodegeneration. Indeed, strategies targeting the mPTP and its regulation by CypD have been shown to confer significant protection in hearts (Griffiths et al., 1993) or brains (Camara et al., 2010). Moreover, the administration of cyclosporine, which is able to inhibit CypD, during percutaneous coronary intervention reduced infarct size in a cohort of patients (Piot et al., 2008). Unfortunately cyclosporine causes immunosuppression and nephrotoxicity and the benefits of mPTP inhibition are balanced by its adverse effects since, the loss of mPTP mediated-Ca^{2+} efflux increases mitochondrial Ca^{2+} content in the matrix (Di Lisa et al., 2010; Elrod et al., 2010). On the other hand, inhibition of mitochondrial Ca^{2+} uptake, is expected to reduce the long-lasting mitochondrial calcium elevations that occur during ischemia and to prevent PTP opening. Therefore, the MCU is a further key target since drugs that inhibit this Ca^{2+} uptake system should retain the beneficial effects conferred by mPTP inhibition but not its adverse effects. Accordingly, inhibition of the MCU by ruthenium red protects hearts against ischemic injury (Miyamae et al., 1996). Unfortunately ruthenium red is a very unspecific inhibitor that also inhibit several classes of ion channels and that interfere with the binding of Ca^{2+} to calmodulin (Santo-Domingo et al., 2010). However, the molecular identification of the MCU as a forthy

Kd protein containing two transmembrane domains localized in the inner mitochondrial membrane which markedly enhances mitochondrial Ca^{2+} uptake into the mitochondrial matrix, (De Stefani et al., 2011) and the identification of its ancillary subunits (Mallilankaraman et al., 2012) opens the way to the rational design of drugs targeting specifically the MCU. Another strategy to reduce mitochondrial Ca^{2+} content during neurodegeneration might be addressed to potentiate the activity of mitochondrial efflux pathways either on the IMM or on the OMM. The discovery of the benzothiazepine derivative CGP37157 (CGP) as a blocker of the mNCX constituted a breakthrough for the functional characterization of the mNCX (Chiesi et al.,1988). CGP causes a sustained elevation of the $[Ca^{2+}]_m$, thereby causing the augmentation of Ca^{2+}-dependent activity of dehydrogenases, ATP synthesis, and heart contraction (Cox et al., 1993; Cox and Matlib, 1993). The identification of NCLX and NCX3 as new player in the regulation of mitochondrial calcium efflux might help to develop new therapeutic strategy potentially able to delay neuronal loss or, at the best, to arrest the disease progression. More interestingly, the demonstration that an increase in NCX1 and NCX3 activity is responsible for Ca^{2+} cycling from ER and mitochondria and represents the molecular mechanism underling neuroprotection occurring in ischemic preconditioning support this hypothesis (Sisalli et al., 2014). In this scenario, it has to be underlined that a compound able to selectively stimulate the activity of NCX1 has been recently synthesized and demonstrated able to prevent neuronal degeneration in an *in vitro* model of cerebral ischemia (Molinaro et al., 2013). Moreover, compounds able to stimulate NCX3 activity are in progress of development in our Laboratory. Therefore, based on this early observation, these studies have potentially revealed a new molecular target in cerebral ischemia and neurodegenerative diseases pathogenesis, which ultimately may open up new avenues for future therapeutic intervention (Fig 1).

CONCLUSIONS

As emerging from the above described findings the hypothesis that is being consolidating in the last few years is that the pathogenesis of neurodegerative diseases is multifactorial. Therefore, it is important to approach their treatment from different angles, as well as environmental factors, oxidative stress, and mitochondrial dysfunction, including ER and mitochondrial interaction. Considering that these diseases are devastating not only due to their economic impact on the society in terms of costs of healthcare and loss of productivity, but also because they literarily deprive individuals from its identity, it is important to search for new targets that may be pharmacologically engaged, to curtail the progress of neurodegeneration. Indeed, despite societies around the world invest billions of dollars in the search for drugs that would stop, or at least significantly slow-down neurodegeneration, the results are unsatisfactory at best. Thus, compounds acting on specific disease proteins and pathways such as dysregulation of neuronal and mitochondrial Ca^{2+} cycling, and impairment of mitochondrial bioenergetics, could be more efficacious to rescue vulnerable neurons from death in neurodegenerative diseases. In this regard, the molecular identification of NCLX and NCX as proteins able to regulate mitochondrial Na^+/Ca^{2+} exchange might result as new promising targets for the development of therapeutic strategies aimed to prevent mitochondrial dysfunction occurring during ischemia and neurodegenerative diseases. However, further efforts have to be performed to improve the knowledge on the functional properties of these transporters in order to finely tune their activity to preserve mitochondrial function during neurodegeneration.

REFERENCES

Annunziato L, Cataldi M, Pignataro G, Secondo A, and Molinaro P. (2007) Glutamate- independent calcium toxicity: introduction. Stroke. 38 (Suppl 2):661–664.

Annunziato L, Pignataro G, and Di Renzo GF. (2004) Pharmacology of brain Na+/Ca2+ exchanger: from molecular biology to therapeutic perspectives. Pharmacol Rev. 56:633–654.

Baines CP, Kaiser RA, Sheiko T, Craigen WJ and Molkentin.JD. (2007) Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat. Cell Biol. 9: 550-555.

Bano D, Young KW, Guerin CJ, Lefeuvre R, Rothwell NJ, Naldini L, Rizzuto R, Carafoli E, Nicotera P. (2005)

Cleavage of the plasma membrane Na+ /Ca2+ exchanger in excitotoxicity. Cell. 120, 275–285.

Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. (2005) Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. J Biol Chem. 280(19):18558-61.

Berberián G, Forcato D, Beaugé L. (2009) Key role of PTDIns-4,5P2 micro domain in ionic regulation of the mammalian heart Na/Ca exchanger. Cell Calcium. 45, 546–553.

Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodeling. Nat Rev Mol Cell Biol 4: 517–529.

Berridge MJ, Lipp P, and Bootman MD (2000) The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1:11−21.

Bers D, Despa S. (2009) Na+ /K+ -ATPase, an integral player in the adrenergic fight-or- flight response. Trends Cardiovasc Med. 19:111–118.

Bezprozvanny I, Hayden MR (2004) Deranged neuronal calcium signaling and Huntington disease. Biochem Biophys Res Commun 322:1310.

Blaustein MP, Lederer WJ. (1999) Sodium/calcium exchange: its physiological implications. Physiol. Rev. 79: 763–854.

Calì T, Ottolini D, Brini M (2011) Mitochondria, calcium, and endoplasmic reticulum stress in Parkinson's disease. Biofactors 37:228-240.

Camara AK, Lesnefsky EJ, Stowe DF (2010) Potential therapeutic benefits of strategies directed to mitochondria. Antioxid Redox Signal. 13(3):279-347.

Canitano A, Papa M, Boscia F, Castaldo P, Sellitti S, Taglialatela M, and Annunziato L (2002) Brain distribution of the Na/Ca2 exchanger-encoding genes NCX1, NCX2 and NCX3 and their related proteins in the central nervous system. Ann N Y Acad Sci. 976:394–404.

Canzoniero LM, Rossi A, Taglialatela M, Amoroso S, Annunziato L, Di Renzo G (1992) The Na+-Ca2+ exchanger activity in cerebrocortical nerve endings is reduced in old compared to young and mature rats when it operates as a Ca2+ influx or efflux pathway. Biochim Biophys Acta 1107:175-178.

Castaldo P, Cataldi M, Magi S, Lariccia V, Arcangeli S, Amoroso S. (2009) Role of the mitochondrial sodium/cal- 456 cium exchanger in neuronal physiology and in the 457 pathogenesis of neurological diseases. Prog Neurobiol. 458 (87):58–79.

Celsi F, Pizzo P, Brini M, Leo S, Fotino C, Pinton P, and Rizzuto R (2009) Mitochondria, calcium and cell death: A deadly triad in neurodegeneration. Biochim Biophys Acta 1787: 335–344

Chad JE and Eckert R (1984) Calcium domains associated with individual channels can account for anomalous voltage relations of Ca-dependent responses. Biophys J 45:993−999.

Chan CS, Gertler TS, Surmeier DJ (2010) A molecular basis for the increased vulnerability of substantia nigra dopamine neurons in aging and Parkinson's disease. Mov. Disord. 25 Suppl 1:S63-S70.

Chiesi M, Schwaller R, Eichenberger E, (1988) Structural dependency of the inhibitory action of benzothiazepines and related compounds on the mitochondrial Na+–Ca2+ exchanger. Biochem Pharmacol 37:4399–4403.

Chinopoulos C & Adam-Vizi V (2010) Mitochondrial Ca2+ sequestration and precipitation revisited. FEBS. 277: 3637–3651.

Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M (2004) Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 13:1407.

Colvin RA, Davis N, Wu A, Murphy CA, Levengood J. (1994) Studies of the mechanism underlying increased Na+/Ca2+ exchange activity in Alzheimer's disease brain. Brain Res. 665:192–200.

Condrescu M, Gardner JP, Chernaya G, Aceto JF, Kroupis C, Reeves JP. (1995) ATP-dependent regulation of sodium-calcium exchange in Chinese hamster ovary cells transfected with the bovine cardiac sodium-calcium exchanger. J Biol Chem. 270(16):9137-46.

Cox DA, Conforti L, Sperelakis N, Matlib MA (1993) Selectivity of inhibition of Na+–Ca2+ exchange of heart mitochondria by benzothiazepine CGP-37157. J Cardiovasc Pharmacol 21: 595–599.

Cox DA, Matlib MA (1993) Modulation of intramitochondrial free Ca2+concentration by antagonists of Na+–Ca2+ exchange. Trends Pharmacol Sci 14: 408–413.

Csordas G, and Hajnoczky G (2003) Plasticity of mitochondrial calcium signaling. J Biol Chem 278: 42273−42282.

Csordas G, Thomas AP, and Hajnoczky G (1999) Quasisynaptic calcium signal transmission between endoplasmic reticulum and mitochondria. EMBO J 18:96−108.

Damiano M., Galvan L., Déglon N., Bronillet E., (2010). Mitochondria in Huntington's disease. Biochim Biophys Acta 1802:52–61.

De Marchi U, Basso E, Szabo I, Zoratti M. (2006) Electrophysiological characterization of the Cyclophilin D-deleted mitochondrial permeability transition pore. Mol. Membr. Biol. 23:521-530.

De Stefani D, Raffaello A, Teardo E, Szabo I, and Rizzuto R (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature. 476:336-40.

Di Lisa F, Carpi A, Giorgio V, Bernardi P (2011) The mitochondrial permeability transition pore and cyclophilin D in cardioprotection. Biochim Biophys Acta. 1813:1316-1322.

Di Polo R, Beauge L. (1982)The effects of pH on Ca2+ extrusion mechanisms in dialyzed squid axons. Biochim. Biophys. Acta. 688: 237–245.

Doering AE, Eisner DA, Lederer WJ. (1996) Cardiac Na-Ca exchange and pH. Ann. N. Y. Acad. Sci. 779: 182–198.

Doering AE, Lederer WJ. (1994) The action of Na+ as a cofactor in the inhibition by cytoplasmic protons of the cardiac Na+ -Ca2+ exchanger in the guinea-pig. J. Physiol. 480:9–20.

Du H, Guo L, Fang F, Chen D, Sosunov A, McKhann M, Yan Y, Wang C, Zhang H, Molkentin JD,Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD (2008) Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. Nat Med 14:1097.

Duchen MR. (2004) Mitochondria in health and disease: perspectives on a new mitochondrial biology. Mol Aspects Med. 25:365–451.

Elrod JW, Wong R, Mishra S, Vagnozzi RJ, Sakthievel B, Goonasekera SA, Karch J, Gabel S, Farber J, Force T, Brown JH, Murphy E, Molkentin JD (2010) Cyclophilin D controls mitochondrial pore-dependent Ca2+ exchange, metabolic flexibility, and propensity for heart failure in mice. J Clin Invest. 120:3680-3687.

Fernández-Morales JC, Arranz-Tagarro JA, Calvo-Gallardo E, Maroto M, Padín JF, and García AG (2012) Stabilizers of Neuronal and Mitochondrial Calcium Cycling as a Strategy for Developing a Medicine for Alzheimer's Disease. ACS Chem Neurosci 3: 873−883.

Galluzzi L, Blomgren K, Kroemer G (2009) Mitochondrial membrane permeabilization in neuronal injury. Nat Rev Neurosci. 10:481–494.

Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, Downward J, Latchman DS, Tabrizi SJ, Wood NW, Duchen MR, Abramov AY. (2009) PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. Mol. Cell. 33(5):627-38.

Giacomello M, Drago I, Pizzo P, Pozzan T (2007) Mitochondrial Ca2+ as a key regulator of cell life and death. Cell Death Differ. 14:1267–1274.

Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabó I, Lippe G, Bernardi P. (2013) Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci U S A. 9;110(15):5887-92.

Gleichmann M, and Mattson MP (2011) Neuronal Calcium homeostasis and dysregulation. Antiox and Redox Sig 7: 1261-1273.

Grienberger C, and Konnerth A (2012) Imaging Calcium in Neurons. Neurons 73: 862-885.

Griffiths EJ, Halestrap AP (1993) Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. J Mol Cell Cardiol. 25:1461-1469.

Grosskreutz J, Van Den Bosch L, Keller BU, (2010) Calcium dysregulation in amyotrophic lateral sclerosis. Cell Calcium 47: 165–174.

Gunter TE, Gunter KK, Sheu SS, and Gavin CE (1994) Mitochondrial calcium transport: physiological and pathological relevance. Am J Physiol 267:C313−C339.

Hajnoczky G, Csordas G, Madesh M, Pacher P (2000a) The machinery of local Ca2+ signalling between sarco-endoplasmic reticulum and mitochondria. J Physiol 529 Pt 1:69-81.

Hajnóczky G, Csordás G, Madesh M, Pacher P (2000b) Control of apoptosis by IP(3) and ryanodine receptor driven calcium signals. Cell Calcium 28(5-6):349-363.

Halestrap AP (2009) What is the mitochondrial permeability transition pore? J Mol Cell. Cardiol. 46:821–831.

Herrington J, Park YB, Babcock DF, and Hille B (1996) Dominant role of mitochondria in clearance of large Ca2+ loads from rat adrenal chromaffin cells. Neuron 16: 219−228.

Hilgemann DW, Collins A, Matsuoka S. (1992a) Steady-state and dynamic properties of cardiac sodium-calcium exchange. Secondary modulation by cytoplasmic calcium and ATP. J. Gen. Physiol. 100: 933–961.

Hilgemann DW, Matsuoka S, Nagel GA, Collins A. (1992b) Steady-state and dynamic properties of cardiac sodium-calcium exchange. Sodium-dependent inactivation. J. Gen. Physiol. 100: 905–932.

Hilgemann DW. (2007) On the physiological roles of PIP 2 at cardiac Na+ /Ca2+ exchangers and K(ATP) channels: a long journey from membrane biophysics into cell biology. J. Physiol. 582: 903–909.

Hüser J and Blatter LA. (1999) Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore. J Biochem. 343: 311–317.

Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P. (2003) Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. Nat Genet 35: 367–371.

Juhaszova M, Shimizu H, Borin ML, Yip RK, Santiago EM, Lindenmayer GE, Blaustein MP. (1996) Localization of the Na(+)-Ca2+ exchanger in vascular smooth muscle, and in neurons and astrocytes. Ann N Y Acad Sci.779:318-35.

Khananshvili D. (2012) The SLC8 gene family of sodium-calcium exchangers (NCX)–structure, function, and regulation in health and disease. Mol. Asp. Med. 34(2-3):220-35.

Kim B and Matsuoka S. (2008) Cytoplasmic Na+-dependent modulation of mitochondrial Ca2+ via electrogenic mito-chondrial Na+-Ca2+ exchange. J Physiol. 586: 1683–1697.

Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. Physiol Rev. 87: 99–163.

Lim D, Fedrizzi L, Tartari M, Zuccato C, Cattaneo E, Brini M, Carafoli E (2008) Calcium homeostasis and mitochondrial dysfunction in striatal neurons of Huntington disease. J Biol Chem 283:5780.

Linck B., Qiu Z., He Z., Tong Q., Hilgemann DW., Philipson KD. (1998) Functional comparison of the three isoforms of the Na+/Ca2+ exchanger (NCX1, NCX2, NCX3). Am J Physiol. 274(2 Pt 1):C415-23.

Lytton J. (2007) Na+/Ca2+ exchangers: three mammalian gene families control Ca2+ transport. J. Biochem. 406, 365–382.

Mallilankaraman K, Cárdenas C, Doonan PJ, Chandramoorthy HC, Irrinki KM, Golenár T, Csordás G, Madireddi P, Yang J, Müller M, Miller R, Kolesar JE, Molgó J, Kaufman B, Hajnóczky G, Foskett JK, Madesh M. (2012) MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism. Nat Cell Biol. 14(12):1336-43.

Mattson MP (2007) Calcium and neurodegeneration, Aging Cell 6: 337–350.

Mattson MP, Gleichmann M, Cheng A (2008) Mitochondria in neuroplasticity and neurological disorders. Neuron. 60: 748–766.

McBride HM, Neuspiel M, Wasiak S, (2006) Mitochondria: more than just a powerhouse. Curr Biol. 16: R551–R560.

McLaughlin S, Wang J, Gambhir A, Murray D. (2002) PIP(2) and proteins: interactions, organization, and information flow. Annu. Rev. Biophys. Biomol. Struct. 31:151–175.

Michaelis ML, Johe K, and Kitos TE (1984) Age-dependent alterations in synaptic membrane systems for Ca2+ regulation. Mech. Ageing Dev. 25:215–225.

Millay DP, Sargent MA, Osinska H, Baines CP, Barton ER, Vuagniaux G, Sweeney HL, Robbins J, Molkentin JD. (2008) Genetic and pharmacologic inhibition ofmitochondrial-dependent necrosis attenuates muscular dystrophy. Nat Med. 14:442–447.

Miyamae M, Camacho SA, Weiner MW, Figueredo VM (1996) Attenuation of postischemic reperfusion injury is related to prevention of [Ca2+]m overload in rat hearts. Am J Physiol. 271:H2145-H2153.

Molinaro P, Cantile M, Cuomo O, Secondo A, Pannaccione A, Ambrosino P, Pignataro G, Fiorino F, Severino B, Gatta E, Sisalli MJ, Milanese M, Scorziello A, Bonanno G, Robello M, Santagada V, Caliendo G, Di Renzo G, Annunziato L. (2013) Neurounina-1, a novel compound that increases Na+/Ca2+ exchanger activity, effectively protects against stroke damage. Mol Pharmacol. 83(1):142-56.

Molinaro P, Cuomo O, Pignataro G, Boscia F, Sirabella R, Pannaccione A, Secondo A, Scorziello A, Adornetto A, Gala R, Viggiano D, Sokolow S, Herchuelz A, Schurmans S, Di Renzo G, Annunziato L. (2008) Targeted disruption of Na+/Ca2+ exchanger 3 (ncx3) gene leads to a worsening of ischemic brain damage. J. Neurosci. 28:1179–1184.

Montero M, Alonso MT, Carnicero E, Cuchillo-Ibanez I, Albillos A, Garcia AG, Garcia-Sancho J, and Alvarez J (2000) Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca2+ transients that modulate secretion. Nat Cell Biol 2:57−61.

Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, Tsujimoto Y, (2005) Cyclophilin D-dependentmitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature. 434:652–658.

Neher E (1998a) Vesicle pools and Ca2+ microdomains: new tools for understanding their roles in neurotransmitter release. Neuron 20:389−399.

Neher E (1998b) Usefulness and limitations of linear approximations to the understanding of Ca+2 signals. Cell Calcium 24: 345−357.

Nicholls DG (2005) Mitochondria and calcium signaling. Cell Calcium. 38: 311–317.

Oliveira JMA (2010) Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum. J Neurochem 114:1–12.

Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, Nolte C, Fishman D, Shoshan-Barmatz V, Herrmann S, Khananshvili D, Sekler I (2010) NCLX is an essential component of mitochondrial Na+/Ca2+ exchange. Proc Natl Acad Sci 107:436-441

Pannaccione A, Secondo A, Molinaro P, D'Avanzo C, Cantile M, Esposito A, Boscia F, Scorziello A, Sirabella R, Sokolow S, Herchuelz A, Di Renzo G, Annunziato L. (2012). A new concept: Aβ1-42 generates a hyperfunctional proteolytic NCX3 fragment that delays caspase-12 activation and neuronal death. J. Neurosci. 32(31):10609-17.

Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci. 5:731-736.

Papa M, Canitano A, Boscia F, Castaldo P, Sellitti S, Porzig H, Taglialatela M, and Annunziato L (2003) Differential expression of the Na+-Ca2+ exchanger transcripts and proteins in rat brain regions. J. Comp. Neurol. 461:31–48.

Patron M, Raffaello A, Granatiero V, Tosatto A, Merli G, De Stefani D, Wright L, Pallafacchina G, Terrin A, Mammucari C and Rizzuto R. (2013) The Mitochondrial Calcium Uniporter (MCU): Molecular Identity and Physiological Roles- Journal Biological Chemistry 288: 10750–10758.

Philipson KD, Nicoll DA. (2000) Sodium-calcium exchange: a molecular perspective. Annu. Rev. Physiol. 62:111-33.

Pignataro G, Gala R, Cuomo O, Tortiglione A, Giaccio L, Castaldo P, Sirabella R, Matrone C, Canitano A, Amoroso S, Di Renzo G, Annunziato L. (2004) Two sodium/calcium exchanger gene products, NCX1 and NCX3, play a major role in the development of permanent focal cerebral ischemia. Stroke . 35: 2566–2570.

Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R, (2008) Calcium and apoptosis: ER–mitochondria Ca2+ transfer in the control of apoptosis. Oncogene. 27:6407–6418.

Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, Andre-Fouet X, Revel D, Kirkorian G, Monassier JP, Derumeaux G, Ovize M (2008) Effect of cyclosporine on reperfusion injury in acutemyocardial infarction. New Engl J Med. 359:473-481.

Pizzo P, Pozzan T. (2007) Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics. Trends Cell Biol. 17:511–517.

Quednau BD, Nicoll DA, and Philipson KD. (1997) Tissue specificity and alternative splicing of the Na1/Ca21 exchanger isoforms NCX1, NCX2, and NCX3 in rat. Am. J. Physiol. 272:C1250–C1261.

Quintanilla RA, Johnson GWW, (2009) Role of mitochondrial dysfunction in the pathogenesis of Huntington disease. Brain Res Bull 80: 242–247.

Rasola A and Bernardi P. (2007) The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. Apoptosis, 12(5):815-33.

Raturi A, Simmen T. (2013) Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). Biochim Bio-

phys 1833(1):213-24

Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, and Pozzan T (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. Science 280: 1763−1766.

Rizzuto R, Bernardi P, and Pozzan T (2000) Mitochondria as all-round players of the calcium game. J Physiol 529: 37−47.

Rizzuto R, Duchen MR, Pozzan T (2004) Flirting in little space: the ER/mitochondria Ca2+ liaison. Sci. STKE. (215):re1.

Rizzuto R, Pozzan T. (2006) Microdomains of intracellular Ca2+: molecular determinants and functional consequences. Physiol. Rev. 86:369–408.

Santo-Domingo J, Demaurex N (2010) Calcium uptake mechanisms of mitochondria. Biochim. Biophys. Acta. 1797:907-912.

Schulze DH, Muqhal M, Lederer WJ, Ruknudin AM. (2003) Sodium/calcium exchanger (NCX1) macromolecular complex. J. Biol. Chem. 278:28849–28855.

Schwaller B (2010) Cytosolic Ca2+ buffers. Cold Spring Harb Perspect Biol 2: a004051.

Scorziello A, Savoia C, Sisalli MJ, Adornetto A, Secondo A, Boscia F, Esposito A, Polishchuk EV, Polishchuk RS, Molinaro P, Carlucci A, Lignitto L, Di Renzo G, Feliciello A, Annunziato L. (2013) NCX3 regulates mitochondrial Ca2+ handling through the AKAP121-anchored signaling complex and prevents hypoxia-induced neuronal death. J. Cell Sci. 15:126 (Pt 24):5566-77.

Secondo A, Staiano RI, Scorziello A, Sirabella R, Boscia F, Adornetto A, Valsecchi V, Molinaro P, Canzoniero LM, Di Renzo G, Annunziato L. (2007) BHK cells transfected with NCX3 are more resistant to hypoxia followed by reoxygenation than those transfected with NCX1 and NCX2: Possible relationship with mitochondrial membrane potential. Cell Calcium. 42: 521–535.

Sheehan JP, Swerdlow RH, Miller SW, Davis RE, Parks JK, Parker WD, Tuttle JB (1997) Calcium homeostasis and reactive oxygen species production in cells transformed by mitochondria from individuals with sporadic Alzheimer's disease. J Neurosci. 17:4612-4622.

Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, Cookson MR, Greenamyre JT (2002) An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci. 22:7006-7015.

Siklos L, Engelhardt JI, Alexianu ME, Gurney ME, Siddique T, Appel SH (1998) Intracellular calcium parallels motoneuron degeneration in SOD-1 mutant mice. J Neuropathol Exp Neurol. 57:571-587.

Simon SM, and Llinas RR (1985) Compartmentalization of the submembrane calcium activity during calcium influx and its significance in transmitter release. Biophys J 48: 485−498.

Sirabella R, Secondo A, Pannaccione A, Scorziello A, Valsecchi V, Adornetto A, Bilo L, Di Renzo G, and Annunziato L (2009) Anoxia-induced NF-kappaB-dependent upregulation of NCX1 contributes to Ca2+ refilling into endoplasmic reticulum in cortical neurons. Stroke. 40:922–929.

Sisalli MJ, Secondo A, Esposito A, Valsecchi V, Savoia C, Di Renzo GF, Annunziato L, Scorziello A. (2014) Endoplasmic reticulum refilling and mitochondrial calcium extrusion promoted in neurons by NCX1 and NCX3 in ischemic preconditioning are determinant for neuroprotection. Cell Death Differ. 21(7):1142-9.

Starkov AA, Polster BM, Fiskum G. (2002) Regulation of hydrogen peroxide production by brain mitochondria by calcium and Bax. J Neurochem. 83(1):220-8.

Surmeier DJ, Schumacker PT (2013) Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. J Biol Chem 288:10736-41.

Szabadkai G, Simoni AM, and Rizzuto R (2003) Mitochondrial Ca2+ uptake requires sustained Ca2+ release from the endoplasmic reticulum. J Biol Chem 278: 15153−15161.

Thomas AP, Bird GS, Hajnoczky G, Robb-Gaspers LD, and Putney JWJr (1996) Spatial and temporal aspects of cellular calcium signaling. FASEB J 10:1505−1517.

Upton JP, Austgen K, Nishino M, Coakley KM, Hagen A, Han D, Papa FR, Oakes SA (2008) Caspase-2 cleavage of BID is a critical apoptotic signal downstream ofendoplasmic reticulum stress. Mol Cell Biol. 28:3943–3951.

Verkhratsky A. (2005) Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. Physiol Rev. 85:201–279.

Villalobos C, Nunez L, Montero M, Garcia AG, Alonso MT, Chamero P, Alvarez J, and Garcia-Sancho J (2002) Redistribution of Ca2+ among cytosol and organella during stimulation of bovine chromaffin cells. FASEB J

16: 343−353.

von Lewinski F, Keller BU, (2005) Ca2+, mitochondria and selective motoneuron vulnerability: implications for ALS. Trends Neurosci 28: 494–500.

Williams AJ and Fry CH. (1979) Calcium–proton exchange in cardiac and liver mitochondria. FEBS Lett. 97: 288–292.

Wood-Kaczmar A. (2013) The role of the mitochondrial NCX in the mechanism of neurodegeneration in Parkinson's disease. Adv. Exp. Med. Biol. 961:241–249.

Xu T, Naraghi M, Kang H, and Neher E (1997) Kinetic studies of Ca2+ binding and Ca2+ clearance in the cytosol of adrenal chromaffin cells. Biophys J 73: 532−545.

Yao Z, Wood NW, (2009) Cell death pathways in Parkinson's disease: role of mitochondria. Antioxid Redox Signal 11: 2135–2149.

Yu L, Colvin RA. (1997) Regional differences in expression of transcripts for Na+/Ca2+ exchanger isoforms in rat brain. Brain Res Mol Brain Res. 50(1-2):285-92.

Zundorf, G. and Reiser, G. (2011) Calcium dysregularion and homeostasis of neuronal calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. Antiox. and Redox. sig. 7:1276-1288.

POTASSIUM CHANNELS IN NEURONAL DEATH AND SURVIVAL

M.V. SOLDOVIERI[1], F. MICELI[2], V. BARRESE[2], M.MANCINI[1], F. BOSCIA[2] and M. TAGLIALATELA[1,2]

[1]Department of Health Science, University of Molise, Campobasso, Italy;
[2]Department of Neuroscience, University of Naples Federico II, Naples, Italy

Changes in the intracellular and extracellular concentration of several ion species have been shown to occur and to play a critical role in specific neurodegenerative diseases, and hyperactivation of glutamate receptors (excitotoxicity) appears as a primary mechanism for neuronal death occurring upon exposure to neurodegenerative stimuli. A large number of potassium channels are expressed in distinct neuronal type, each with specific biophysical, functional, and pharmacological properties; despite their role in controlling neuronal excitability has been widely explored, much less attention has been dedicated to investigating their participation in neuronal survival/death mechanisms. Therefore, the aim of the present work is to review the available preclinical data on potassium channels contribution in cell death triggered by various neurotoxic insults, and to provide explanations reconciling apparently contradictory conclusions present in the literature. Given that several new potassium channel modulators are currently being developed for the treatment of various neurological and non-neurological indications, it seems possible to envisage that these molecules may be optimized for the treatment of various neurodegenerative diseases where potassium channels specifically participate in disease pathogenesis.

The disruption of ionic homeostasis is a key trigger for neuronal injury and death occurring during exposure to several neurotoxic stimuli, including hypoxic/ischemic conditions (Chao and Xia, 2010), Alzheimer's disease Aβ fragments (Fraser et al., 1997), and prion-induced neurodegenerative processes (Biasini et al., 2013). In all these neurodegenerative disorders, a unifying hypotheses for neuronal death is the hyperactivation of glutamate receptors (excitotoxicity), leading to excessive stimulation of both NMDA and non-NMDA receptors, excessive calcium (Ca^{2+}) influx along with sodium (Na^{+}) and chloride (Cl^{-}) accumulation which disrupts the osmotic equilibrium resulting in cell lysis, one of the major contributing factor in neuronal death (Mehta et al., 2013). However, recent work has increasingly suggested that several other processes, in addition to excessive glutamate receptors activation, may contribute to a loss of ionic homeostasis, cellular energy failure, and Ca^{2+} accumulation during ischemia (Besancon et al., 2008). In the present review, we will concentrate on the relationship between the disruption of potassium (K^{+}) homeostasis and neuronal death. In comparison with the well-established harmful role of Ca^{2+} and Na^{+} overload, the role of K^{+} efflux appears to be much more complex. In fact, on one side the enhanced efflux of K^{+} ions is classically considered an adaptive mechanism to decrease neuronal excitability during hypoxia/ischemia; on the other hand, recent evidence suggests that an excessive loss of cellular K^{+} is causally linked to neuronal damage and apoptotic neuronal death in various conditions including

Key words: potassium channels, neurodegeneration, apoptosis, ischemic preconditioning

Corresponding Author:
Maurizio Taglialatela, MD PhD
Department of Health Sciences, University of Molise.
Via De Sanctis, 86100,
Campobasso, Italy
Tel.: +39 0874 404851 Fax: +39 0874-404778
e-mail: m.taglialatela@unimol.it

2279-5855 (2014)

hypoxia/ischemia (Yu et al., 1997; Huang et al., 2001; Liu et al., 2003; Zhao et al., 2006; Nistico et al., 2007; Karki et al., 2007; Chao and Xia, 2010). We will briefly review the experimental evidence suggesting a possible neuroprotective/neurodegenerative role for various classes of K^+ channels, concentrating in particular on voltage-gated K^+ channels of the K_v7 subfamily, which have recently gained a center-stage role in neuronal excitability control, etiology of several human epileptic diseases, and as targets for treatment of neuronal hyperexcitability disease states.

FUNCTIONAL AND STRUCTURAL ROLES OF K^+ CHANNELS

K^+ channels perform an extraordinary array of functional roles

K^+ channels are the largest and the most functionally heterogeneous class of ion channels; they are expressed in all eukaryotic cells and in prokaryotes. K^+ channels mostly perform inhibitory functions by a membrane-stabilizing action; their opening drives the membrane potential closer to the K^+ equilibrium potential, away from the action potential threshold in excitable cells. Moreover, activation of K^+ channels shortens the duration of the action potential, terminates periods of intense electrical activity, reduces neuronal firing frequency, and, in general terms, decreases the efficacy of cell excitatory inputs. Beside these roles, K^+ channels participate in solute transport across epithelial membranes, and to glial cells K^+ clearance from brain interstitial spaces. Such an extraordinary heterogeneity of functions in each cell type at various differentiation stages is accomplished through the expression of a specific pattern of K^+ currents, each with distinct sub-cellular localization, biophysical properties, modulation, and pharmacological profile. In fact, electrophysiological studies in a variety of cellular models have revealed an astonishing functional diversity of the K^+ current family, often anticipating the remarkable genetic and structural heterogeneity of K^+ channels (Wulff et al., 2009).

K^+ channels are complexes of subunits with 2, 4, or 6 transmembrane segments.

More than 70 genes encoding for K^+ channels have been identified in humans. Molecular cloning of these genes has allowed to classify K^+ channels on the basis of the presumed topology of their protein products deduced from primary sequences. As illustrated in Fig. 1, subunits belonging to three classes can have K^+ channel function: 1) the classical family with 6 transmembrane segments (6TM); voltage-gated K^+ channels (K_v channels; K_v1-K_v12) belong to this family. Segments S_1-S_4 form the voltage sensor domain (VSD); four to seven positively charged arginines, each separated by 2-3 mostly uncharged residues, in the S_4 segment play a critical role in voltage sensing. On the other hand, S_5, S_6, and the intervening linker contribute to the formation of the pore; within this region, a canonical GYGD sequence forms the K^+ selectivity filter. 2) the 2 transmembrane segment family (2TM), homologous to the S_5-S_6 segments of K_v channels. As the VSD is missing, channels formed by these subunits are voltage-independent; inward-rectifier channels (both constitutively-active and G-protein-gated) belong to this group, which is formed by at least seven gene families ($K_{IR}1$-$K_{IR}7$); 3) the 4 transmembrane segment family (4TM), formed by subunits encoded by at least 15 different genes (K2P1-K2P17). While channels formed by subunits of the first two groups are tetrameric, those of the third group are dimers. However, it should be reminded that the structural and functional heterogeneity of K^+ channel is not restricted to the three structural groups mentioned so far; as an example, large conductance Ca^{2+}-dependent K^+ channels (BK channels) assemble as tetramers of subunits containing seven transmembrane segments, which differ from K_v subunits for the presence of an extra transmembrane segment (S_0) at the N-terminus. Except in BK channels, both the N- and the C-terminal regions in other K^+ channels are located intracellularly; at these locations, sites determining homomeric and heteromeric assembly, interaction with regulatory molecules, subcellular localization, and binding of accessory proteins have been identified.

Plasma membrane mechanisms contributing to ionic homeostasis

In normal conditions, ions are not uniformly distributed between extracellular and intracellular milieu of neurons. Rather, a steep electrochemical gradient across the plasma membrane exists for most

of them, with the extracellular fluid being rich in Na^+ (concentrations up to 150 mM) and relatively poor in K^+ (around 3 mM). Conversely, the intracellular concentrations of these two ions are reversed, with up to 150 mM K^+ and 4-12 mM Na^+. Ca^{2+} and Cl^- are also asymmetrically distributed, with high levels in the extracellular space. Both active and passive transport mechanisms contribute to the maintenance of this steep electrochemical ionic gradient across the membrane. Passive processes transport ions from high to low electrochemical gradient. Two types of proteins are responsible for passive ion transport: facilitated transporters and ion channels, with very different transport mechanisms. Substrate binding to the transporter on one side of the membrane induces a conformational change exposing the substrate to the opposite side of the membrane. The substrate concentration gradient provides the energy required for such a process; as the substrate movement is coupled to protein conformational changes, transport rate is rather low. On the other hand, ion channel allow permeating ion transport through aqueous pores within the protein itself at very high rates (>10^6 ions/s, closer to the diffusion rate in water), thus generating significant currents which may rapidly change the resting membrane potential (V_{REST}) of a cell. Both these passive processes dissipate the energy gradient established by the active transporters, which pump ions through the membrane against their concentration gradient; this process requires an energy input generally provided by ATP hydrolysis (primary active transporters) or by coupling the net flux of the solute transported against the electrochemical gradient to that of another which moves following its electrochemical gradient (secondary active transporters); under this condition, the two solutes can be transported in the same ("cotransporter" or "synporter") or in the opposite ("counter-transporter" or "antiporter") direction. The electrogenic Na^+-K^+ ATPase (also called Na^+–K^+ pump or Na^+ pump) is the primary active transporter which functions to actively extrude three Na^+ outside the cell, and move two K^+ into the cell in each cycle of activity, thereby maintaining the high intracellular K^+ and low intracellular Na^+ concentrations (Jorgensen et al., 2003; Panayiotidis et al., 2006). The concentrations of ions are in dynamic balance under physiological conditions, and they only transiently fluctuate within a small range during normal neuronal activity, an indication of the paramount importance of such functional processes for the maintenance of membrane potential, generation of action potential, synaptic transmission, electrogenic transport of neurotransmitters and neuronal plasticity, but also for regulating osmotic balance, cell volume, proper pH and ionic microenvironment for the normal functioning of macromolecules including enzymes, Na^+-coupled transport of nutrients into cells (Chao and Xia, 2010). Since a plethora of neuronal functions is critically dependent on the maintenance of Na^+, K^+, Ca^{2+} and Cl^- transmembrane distribution, deregulated ionic balances as well as loss of ion gradients are considered to be key events in brain pathological conditions (Yu and Choi, 2000).

K^+ homeostasis and neurodegeneration

One of the first consequence following an impairment of energy metabolism in the brain is a change in transmembrane ion gradients, thus overcoming the homeostatic mechanisms that maintain constant ionic concentrations in the cerebrospinal fluid and neurons. Hypoxia, ischemia, oxygen-glucose deprivation (OGD), and other neurodegenerative triggers can promote sustained changes in ionic concentrations; these are mostly characterized by enhanced K^+ efflux and Na^+-, Ca^{2+}- and Cl^- influx. Among these ion species, K^+ seems to play a primary role in the regulation of neuronal cell death/survival. Indeed, while a growing number of evidence demonstrates that low intracellular K^+ levels ([K+]i) lead to apoptosis in neurons, a decreased expression/function of some subclasses of K^+ channels, by causing neuronal hyper-excitability, appears sufficient to promote apoptotic neuronal death (Shah and Aizenman, 2014). In this view, K^+ channels-activating drugs, by reducing neuronal excitability and subsequent energy loss, might represent a useful tool to prevent neuronal cell death.

K^+ Efflux and Apoptotic Cell Death

Apoptotic cell death contributes significantly to the neuronal loss observed in a number of neurological disorders, including Alzheimer's disease and stroke (Thompson, 1995). DNA fragmentation, mitochondrial damage, and caspase activation are the main features of apoptotic cell

death; irrespective of stimulus type, apoptotic cells also display a reduced cell volume, termed apoptotic volume decrease (AVD), and decreased intracellular ionic strength (Bortner et al., 1997). The loss of cellular K^+, originally considered as a side-effect of the apoptotic process, has progressively gained a pathogenetic role in this process because of the following landmark observations (brilliantly reviewed in Shah and Aizenman, 2014): 1. Key apoptotic enzymes such as caspase-3 and nucleases are inhibited by physiological intracellular concentrations of K^+ ions. Although this observation was first made in lymphocytes (Hughes et al., 1997), in neurons exposed to serum deprivation, low $[K^+]_i$ promoted the DNA binding activity of p53 and Forkhead, proapoptotic transcriptional factors, whereas it inhibited that of cAMP-responsive element-binding protein, an anti-apoptotic transcriptional factor (Yang et al., 2006). This evidence strongly indicates that reduced intracellular K^+ concentrations provide a permissive environment for apoptotic signalling cascades; 2. Apoptotic stimuli enhance the loss of intracellular K^+. Reduced K^+ concentrations are observed in cortical neurons following serum deprivation (Yu et al., 1997). Among the potential mechanisms contributing to the observed intracellular K^+ loss during apoptotic stimuli (such as those occurring during and after cerebral hypoxia/ischemia), a reduced recovery of extracellular K^+ mainly mediated by the Na^+-K^+ ATPase, or an increased leakage of K^+ due to an increased activity of various classes of K^+ channels (e.g. voltage-gated K^+ channels, ATP-sensitive K^+ channels, Ca^{2+}-activated K^+ channels and Na^+-activated K^+ channels), as well as of ionotropic glutamate receptor channels, have been proposed; and 3. K^+ ionophores (mainly valinomycin) promote apoptotic signalling and cell death. As a corollary of this, an increase in the extracellular K^+ concentrations, by decreasing the transmembrane K^+ gradient and blocking K^+ efflux, opposes apoptotic signalling and promotes cell survival. This phenomenon has been well described in cerebellar granule neurons, which are commonly cultured in the presence of elevated (15-25 mM) extracellular concentrations of K^+; reducing this values to 5 mM promotes apoptosis, as testified by vacuole formation, nuclear pyknosis, volume shrinkage, and neurite retraction. Increasing extracellular K^+ also protects neurons from apoptosis induced by oxidants, staurosporine, glutamate, ceramide, neurotoxic amyloid-β (Aβ) peptides, and serum deprivation. In keeping with a pro-apoptotic role for intracellular K^+ loss is the observation that several K^+ channel blockers attenuate apoptotic signalling cascades and cell death in neurons (Leung, 2010). Moreover, several lines of evidence suggest that neuroprotection induced by high extracellular K^+ and by K^+ channel blockers such as tetraethylammonium (TEA) is not a consequence of an enhanced activity of voltage-gated Ca^{2+} channel caused by membrane depolarization, as Ca^{2+} channel blockers do not abrogate their neuroprotective effects.

Several studies have been carried out to discover the molecular identity of the K^+ channel subtypes responsible for the enhanced K^+ efflux accompanying neuronal apoptosis. Although it appears that A-type voltage-gated K^+ channels, Ca^{2+}-activated K^+ channels, K_{ATP} channels, Na^+-dependent K^+ channels, and two-pores TASK channels are involved in the modulation of damage of specific neuronal populations during exposure to distinct neurotoxic stimuli, the contribution of TEA-sensitive delayed rectifier-type voltage-gated K^+ channels of the $K_v2.1$ subfamily has by far received the largest attention (reviewed in Misonou and Trimmer, 2009). $K_v2.1$, the predominant mediator of delayed rectifying K^+ currents in neurons, has been identified as the critical contributor to the intracellular K^+ loss associated with apoptosis in cortical, hippocampal, and cerebellar granule neurons. Activation of $K_v2.1$ currents has been demonstrated during neuronal treatment with oxidants, such as DTDP, which induces an intracellular release of zinc (Zn^{2+}) from metal-binding proteins, enhancing plasma membrane delivery of $K_v2.1$ channels, and amplifying $K_v2.1$ K^+ currents, thus producing a "pro-apoptotic" intracellular environment (Sensi et al., 2011). These pro-apoptotic $K_v2.1$ channels appear to be expressed in highly-clustered structures in the plasma membrane and to be activated at rather positive values of membrane potential. In contrast, neuronal activity or sublethal ischemia stimulates $K_v2.1$ channel dephosphorylation-dependent declustering, which, along with hyperpolarizing voltage-gated activation, induces neuronal tolerance to ischemic or

epileptic challenge. Intriguingly, the toxic nitrogen radical species peroxynitrite mainly released from activated microglial cells, is also known to promote intracellular Zn^{2+} release leading to a remarkable augmentation of $K_v2.1$ current density (Knoch et al., 2008).

Recent research has suggested that changes in K^+ channel activity may play a major pathogenetic role in Alzheimer's disease (AD). In fact, β-amyloid fragments (Aβ), generated upon processing of the integral membrane amyloid precursor protein (Suh and Checler, 2002), alter the properties of K^+currents in mammalian neurons (Jalonen et al., 1997; Yu et al., 1998; Jhamandas et al., 2001; Ramsden et al., 2001; Pannaccione et al., 2005). We have more recently demonstrated that the Aβ peptide up-regulated the expression of $K_v3.4$ channel subunit and of the accessory subunit MIRP2. Thus, the increase in $K_v3.4$ functional activity appears to be involved in Aβ neurotoxicity (Pannaccione et al., 2007).

Neuroprotective Roles of K^+ channels

As reported, one of the earliest events occurring in neurons exposed to a wide variety of neurodegenerative stimuli is the increased expression and function of various classes of K^+ channels. However, whether this response is neurotoxic due to the enhanced loss of intracellular K^+, or rather neuroprotective because of a decreased cellular excitability thereby limiting energetic expenses, is still matter of debate. In fact, in addition to results showing that $K_v2.1$ channels critically contribute to oxidant injury-induced neuronal apoptosis, emerging evidence indicate that the observed functional changes (channel dephosphorylation, hyperpolarizing shift in the voltage-dependence, and declustering) may reduce neuronal excitotoxicity in the context of an injurious stimulus. The hyperpolarizing shift in $K_v2.1$ channel voltage-gated activation is thought to reduce excitability and, consequently, excitotoxicity in neurons facing an ischemic or epileptic challenge. Sublethal chemical ischemia, which renders rat cortical neurons tolerant to subsequent NMDA receptor-mediated excitotoxicity, induces $K_v2.1$ channel dephosphorylation and declustering, and produces a hyperpolarized shift in voltage-gated activation, implicating these channel modifications in promoting neuroprotection (Shah and Aizenmann, 2014). It should be mentioned that several recent studies have suggested that $K_v2.1$ role in neuronal function might be unrelated to its ion-conducting ability; in fact, it has been proposed that $K_v2.1$ channel clusters may serve as voltage sensors of neuronal activity that convey changes in membrane potential to cytosolic signaling pathways, and that $K_v2.1$ membrane clusters (the majority of which appear to be non-conducting in cultured hippocampal neurons) represent sites of depolarization-driven vesicle trafficking and neurotransmitter release (O'Connell et al., 2010; Fox et al., 2013), an observation also confirmed in neuroendocrine cells (Singer-Lahat et al., 2008). Other K_v channels which are involved in reducing neuronal excitability and cell death in the context of ischemic injury have also been identified. Among these, the resistance to anoxic cell death in large aspiny neurons in the striatum seems to be accounted for by the large expression of K_v1-mediated delayed rectifying K^+ currents (Deng et al., 2005). Similarly, in the same neurons, but not in medium spiny neurons which are more vulnerable to ischemic neuronal damage, an ischemic injury-promoted rise in A-type K^+ currents leading to a decreased excitability and excitotoxic cell death has also been reported (Deng et al., 2011).

In addition to classical voltage-gated channels, neuroprotective roles for other K^+ channels have been firmly established. In particular, recent findings suggested small conductance Ca^{2+}-activated channels (SK/KCNN1-3/KCa2 channels) as regulators of microglial activation, thus linking neuroinflammation with neurodegeneration. SK/KCa2 channels, by regulating Ca^{2+} homeostasis, may elicit a dual mechanism of action with protective properties in neurons and inhibition of inflammatory responses in microglia (Dolga and Culmsee, 2012). On the other hand, large conductance Ca^{2+}-activated K^+ channels (Slo1/BK channels), which are particularly abundant in axons and nerve terminals (Knaus et al., 1996; Misonou et al., 2006), where they stabilize the neuronal membrane potential and regulate excitatory neurotransmitter release (Raffaelli et al., 2004; Martire et al., 2010), have been extensively investigated. Activation of BK channels in ischemic cells could block Ca^{2+} entry from a number of potential sources and minimize neuronal depolarization. Opener-dependent

increases in channel function during ischemia may limit accumulation of potentially pathologic levels of Ca^{2+}, reduce neurotransmitter release and energy expenditure and significantly attenuate infarct growth. As a matter of fact, BK openers such as BMS-204352 have been successfully used in preclinical ischemia models, in which they showed significant reduction of brain damage (Gribkoff et al., 2001; Hewewasam et al., 2002), and attenuated cerebral edema and neurologic motor impairment in rats after traumatic brain injury (Cheney et al., 2001); however, despite its good tolerability profile, BMS-204352 failed to show superior efficacy in acute stroke patients when compared to placebo in a wide phase III study (Jensen, 2002). In organotypic hippocampal slice cultures (OHSCs), the pharmacological blockade of BK channels during OGD enhanced cell damage because of a large and prolonged increase in $[Ca^{2+}]$ i (Rundén-Pran et al., 2002). Moreover, middle cerebral artery occlusion (MCAO) produced larger infarct volume and more severe neurological deficits in homozygous mice lacking BK channel alpha subunit when compared to wild-type mice; similarly, NMDA intracerebral injections caused larger neurotoxicity in homozygous mice compared to wild-type (Liao et al., 2010). These findings strongly support the idea that BK channels provide powerful neuroprotection against Ca^{2+} overload induced by several insults.

Neuroprotective actions of the adipokine leptin have been also shown to involve the activation of BK channels. In fact, leptin, in addition to a firmly established role in promoting appetite suppression and energy expenditure, is also known to exert neuroprotective effects in several in vitro and in vivo models of neurotoxicity (Signore et al., 2008), including oxygen-glucose deprivation, hypoxia, ischemia, neurotrophic factor withdrawal, and excitotoxic or oxidative stimuli in neuronal populations from distinct brain areas (Dicou et al., 2001; Zhang et al., 2007; Weng et al., 2007; Guo et al., 2008; Valerio et al., 2009; Gavello et al., 2012). Among the molecular mechanisms responsible for the effects of leptin, neuronal silencing via K^+ channels opening appears to play a major role; in particular, BK channels have been shown to mediate at least in part leptin effects on neuronal excitability and viability following neurotoxic stimuli. Indeed, in a recent study, we have shown that leptin is endowed with significant neuroprotective effects in both rat and mouse cortical neurons exposed to NMDA; the pharmacological blockade of BK channels, or the lack of one ($Slo1^{+/-}$ mice) or both ($Slo1^{-/-}$ mice) Slo1 alleles fully counteracted leptin-mediated neuroprotection. Furthermore, $[Ca^{2+}]_i$ monitoring in single mouse cortical neurons revealed that acute leptin application prompted an oscillatory behavior in $[Ca^{2+}]$i. These results reveal that the activation of BK channels is an obligatory step for leptin-induced neuroprotection, highlighting leptin-based intervention via BK channel activation as a potential strategy to treat neurodegenerative diseases (Mancini et al., 2014).

Mitochondrial K^+ channels and neuronal preconditioning

Mitochondria are ubiquitous and dynamic organelles responsible for many crucial cellular processes in eukaryotic organisms. Being responsible for the production of over 90% of cellular ATP, the regulation of intracellular Ca^{2+} and redox signaling, mitochondria are considered the gatekeepers of life and death (Correia et al., 2010). The inner membrane of mitochondria only shows a low diffusive permeability to most cations (including K^+ ions), because ion leaks would short-circuit the protonmotive force and ATP would not be synthesized. Nevertheless, cations leaks occur at significant rates in respiring mitochondria, and they are physiologically important. Inward K^+ leak causes matrix swelling, and inward proton leak dissipates energy and contributes to the basal metabolic rate. The mitochondrial K^+ cycle consists of several components. The electron transport system ejects protons, leading to generation of a protonmotive force, and to a membrane potential ($\Delta\Psi$) of about 190 mV. This $\Delta\Psi$ drives K^+ by diffusion ("K^+ leak") and through K^+-selective channels; K^+ influx into the matrix is accompanied by an osmotic-guided entry of H_2O, thus generating matrix swelling. It has been proposed that the mitochondrial K^+ cycle plays a crucial role in the regulation of the mitochondrial volume, to prevent excessive swelling or contraction of the matrix, as well as in the regulation of reactive oxygen species (ROS) production (Garlid and Paucek, 2003).

The term "preconditioning" identifies the ability of a previous transient, sublethal insult to protect against a subsequent potentially lethal stimulus. Recent studies have established that mitochondrial-centered mechanisms are important mediators in promoting development of the preconditioning response. Among the mitochondrial mechanisms involved in neuronal preconditioning, K^+ channels located on the inner mitochondrial membrane have received considerable attention in the last 15 years. Several different K^+ channels have been identified in the inner mitochondrial membrane and their activation may initiate neuronal preconditioning. Activation of these channels allows K^+ ions to flow into mitochondria and results in depolarization. The two most likely targets of preconditioning are the ATP-sensitive K^+ ($mitoK_{ATP}$) and the large conductance Ca^{2+} activated K^+ (mitoBKCa) channels. While there is extensive evidence for the existence and importance of the $mitoK_{ATP}$ channels in neuronal preconditioning, there is speculation that the mitoKCa channels, if present, are not involved in neuronal preconditioning (Busija et al., 2008).

$MitoK_{ATP}$ channels have been first identified in brain mitochondria in 2001 (Bajgar et al., 2001). Similarly to their plasma membrane counterparts, $mitoK_{ATP}$ channels consist of two subunits, called $mitoK_{IR}$ (K_{IR} 6.2) and mitoSUR (receptor of sulfonylureas), both possible pharmacological targets (Garlid et al., 2003). Functional studies demonstrated that oxidative stress, antihypertensive drugs (diazoxide and, although much less potently, cromakalin), and the general anesthetic isoflurane (Nakae et al., 2003) activate $mitoK_{ATP}$ channels, whereas these channels are inhibited by the selective blocker 5-hydroxydecanoic (5-HD) (Liu et al., 2002) and by sulphonylureas oral hypoglycemic agents such as glyburide (Jabůrek et al., 1998). $MitoK_{ATP}$ channels can be activated by many signaling pathways; in particular, it has been proposed that $mitoK_{ATP}$ channels are activated by phosphorylation by one of the pKC isoforms (protein kinase C epsilon; εPKC); mitochondrial εPKC levels significantly increase in rat hippocampus, suggesting an anti-apoptotic function of this kinase, protecting mitochondrial functions, through a mechanism involving $mitoK_{ATP}$ channel activation (Raval et al., 2007). Activation of $mitoK_{ATP}$ channels, decreasing mitochondrial $\Delta\Psi$, would increase the activity of the electron transport chain and ATP synthesis. In fact, activation of $mitoK_{ATP}$ channels has a protective effect against cerebral damage by reducing the accumulation of mitochondrial Ca^{2+} and preventing the formation of the mitochondrial permeability transition pore (MPTP; Fig. 2), an early event in programmed cell death (Wu et al., 2006); moreover, $mitoK_{ATP}$ activation has been also shown to increase the levels of Bcl2 and to inhibit the association of Bax with mitochondria in neurons exposed to an apoptotic insult, suggesting that $mitoK_{ATP}$ activation may stabilize mitochondrial function by differentially modulating proapoptotic and antiapoptotic proteins (Liu et al., 2002). However, the exact sequence of events by which $mitoK_{ATP}$ become activated during ischemic preconditioning and the molecular events which, following their activation, mediate the increased resistance of neuronal cells to neurotoxic triggers are yet unknown.

On the other hand, mitoBKCa channels were initially described in the human glioma cell line LN229 by electrophysiological experiments (Siemen et al., 1999), and later also in heart (Xu et al., 2002) and brain (Douglas et al., 2006) mitochondria. These channels are activated by Ca^{2+} (hence their name) and by NS1619 (at mM concentrations), whereas, similarly to plasma membrane BKCa channels, they are blocked by charibdotoxin (ChTx), iberiotoxin (IbTx), and paxilline (at nM concentrations). mitoBKCa appear to be a molecular link between the signals mediated by cellular/mitochondrial Ca^{2+} and that dependent on the mitochondrial membrane potential: in fact, changes in intramitochondrial Ca^{2+} levels directly affect the permeability of the inner mitochondrial membrane to K^+, which results in a Ca^{2+}-dependent modulation of the membrane potential and, as a result, of the oxidative phosphorylation. However, the lack of selective modulators for mitoBKCa channels is a serious limitation when interpreting the pharmacological preconditioning obtained in neurons with NS1619, as none of these effects could be antagonized with any of the traditional BKCa channel blockers (Busija et al., 2008).

Molecular pathophysiology of K_v7 channels

Among K_v channels, the K_v7 family encompasses five members (from $K_v7.1$ to $K_v7.5$), each showing

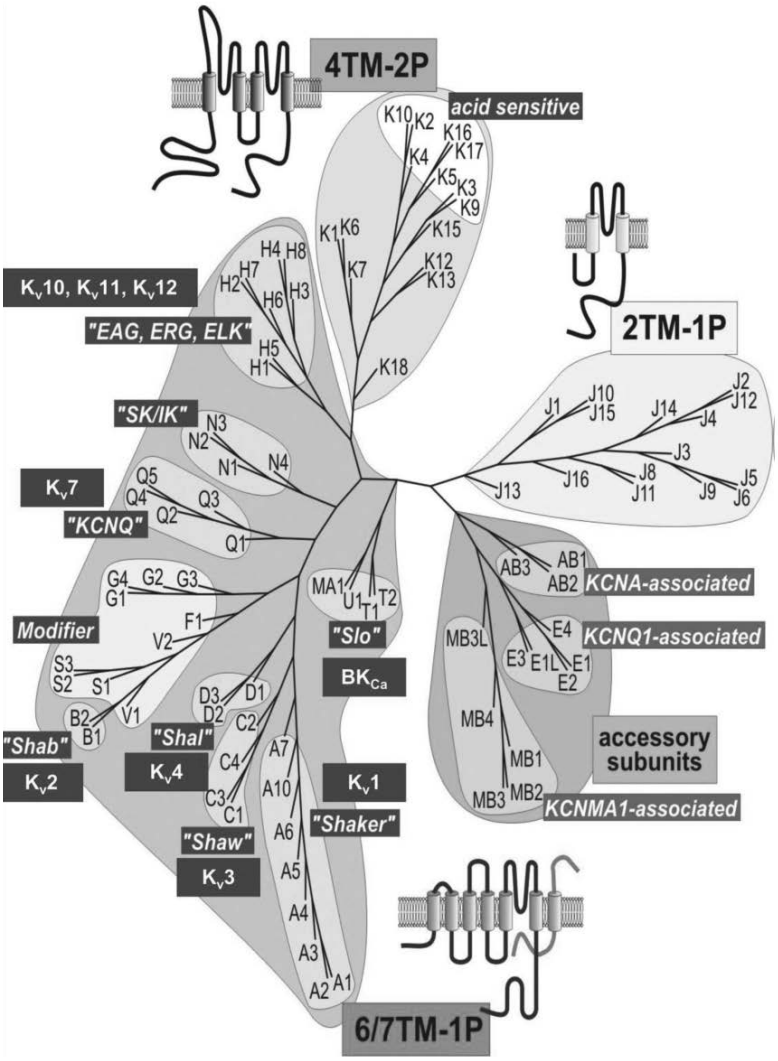

Fig. 1. *"KCN" K^+ channel families. Phylogenetic tree of human K^+ channels using the "KCN" nomenclature of the "Human Genome Organization." For simplicity, the letter code for K^+ channels (KCN) has been omitted (e.g., KCNA1 is depicted as A1). The tree has been constructed using "UPGMA" (http://bibiserv.techfak.uni-bielefeld.de/dialign/) based on DIALIGN fragment weight scores on full length protein sequences. In case of splice variants, transcript variant 1 (according to NCBI) has been used for the alignment. Modified from Heitzmann and Warth (2008).*

a different tissue distribution and physiological role (Soldovieri et al., 2011) (Fig. 3). Indeed, $K_v7.1$ is manly expressed in the heart, pancreas, thyroid gland, brain, gastrointestinal tract, portal vein, and the inner ear. In cardiac myocytes, in association with the accessory subunit KCNE1, $K_v7.1$ underlies I_{Ks}, a slow K^+-selective current involved in the late phase of action potential repolarization. $K_v7.2$, $K_v7.3$, $K_v7.4$, and $K_v7.5$ show prevalently neuronal localization; homo- or heterotetrameric assembly of $K_v7.2$ and $K_v7.3$ subunits, with possible additional contribution from $K_v7.4$ and $K_v7.5$ subunits at specific neuronal sites, represents the molecular basis of the M-current (I_{KM}), a slowly activating and deactivating K^+ current highly regulated by Gq/11-coupled receptors (Delmas and Brown, 2005). I_{KM} regulates membrane excitability in the sub-threshold range for action potential generation, acting as a brake for neuronal firing; indeed, reduction

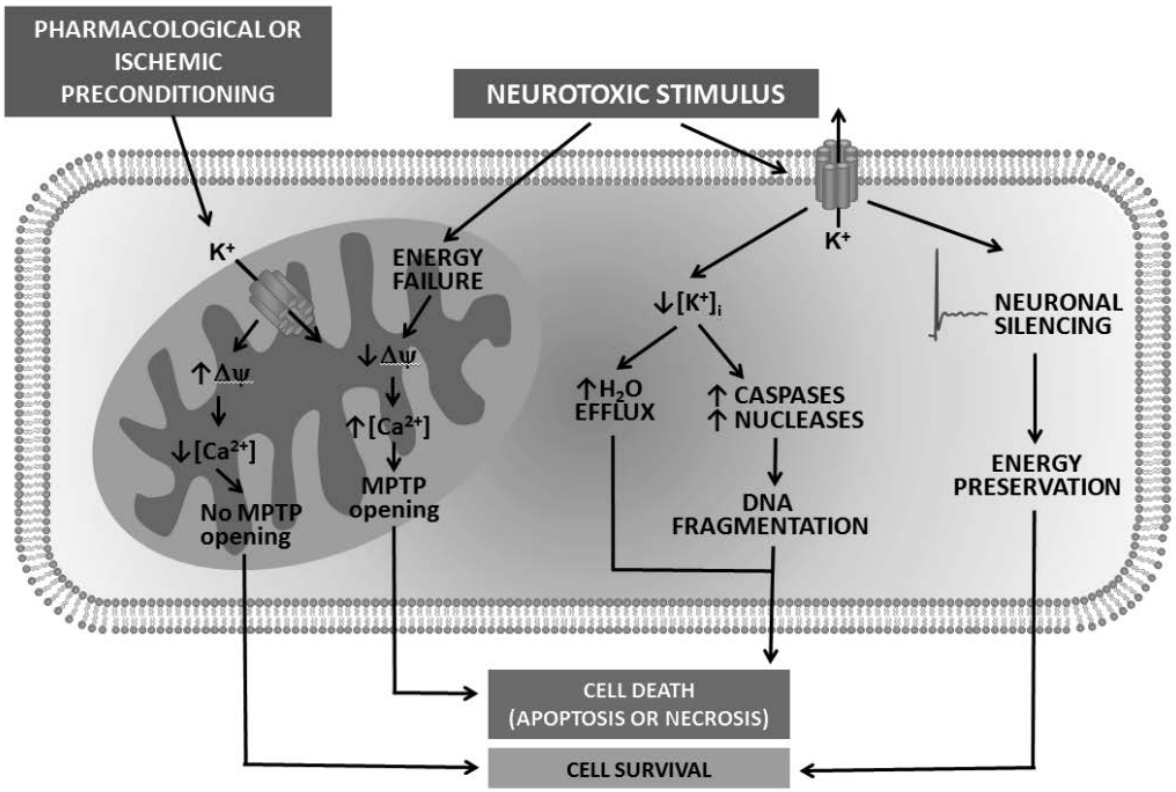

Fig. 2. *Potential mechanisms responsible for the neuroprotective and neurogenerative responses involving plasma membrane and mitochondrial K^+ channels.*

	$K_v7.1$	$K_v7.2$	$K_v7.3$	$K_v7.4$	$K_v7.5$
Tissue distribution	Heart, marginal cells of the stria vascularis in the inner ear, small intestine, pancreas, thyroid gland, forebrain neuronal networks and brainstem nuclei, lung, gastrointestinal tract, ovaries, smooth and skeletal muscle	Hippocampus, neocortex, striatum, globus pallidus, cerebellum, PNS	Hippocampus, neocortex, striatum, globus pallidus, cerebellum, amygdala, thalamus, PNS	in cochlear and vestibular organs of the inner hear, central auditory pathways , smooth muscle and skeletal muscle	Hippocampus, caudate putamen, piriform and entorhinal cortex, auditory system, PNS, smooth and skeletal muscle
Human channelo-phaties	Long QT Syndrome (LQT) Short QT Syndrome (SQT)	Benign Familial Neonatal Seizures (BFNS) Epileptic Encephalopathy	Benign Familial Neonatal Seizures (BFNS)	Nonsyndromic hearing loss (DFNA2)	
Disease target	Arrhythmias	epilepsy, neurophatic pain, migraine, tinnitus, anxiety, attention-deficit hyperactivity disorders, mania, bipolar disease, and schizophrenia	epilepsy, neurophatic pain, migraine, tinnitus, anxiety, attention-deficit hyperactivity disorders, mania, bipolar disease, and schizophrenia	Deafness, Hypertension, cerebral vasospasm, bronchodilation	Hypertension, cerebral vasospasm, bronchodilation

Fig. 3. *Pathophysiological roles of the five members of the KCNQ (K_v7) family of voltage-gated K^+ channels. Modified from Soldovieri et al. (2011).*

of this current is often sufficient to increase neuronal excitability. $K_v7.4$ subunits are mainly expressed in cochlear and vestibular organs of the inner hear, as well as in central auditory pathways (Kubisch et al., 1999); more recent work has revealed expression of $K_v7.4$ subunits also in skeletal muscle (Iannotti et al., 2010), as well as in visceral and vascular smooth muscle (Greenwood and Ohya, 2009). $K_v7.5$ expression, in addition to the brain, has been also detected in human adult skeletal muscle (Lerche et al., 2000; Schroeder et al., 2000), and, together with $K_v7.1$ and $K_v7.4$, in vascular smooth muscle cells (Yeung et al., 2007).

The pathophysiological importance of these channels is emphasized by the fact that mutations in four of the five K_v7 genes are associated to different hereditary channelophaties in humans. In particular, mutations in $K_v7.1$ have been found in families affected by arrythmogenic diseases such as dominant (the Romano-Ward syndrome) and recessive (the Jervell and Lange-Nielsen syndrome) chromosome 11-linked form of the Long QT syndrome (Wang et al., 1996), and the short QT syndrome (Bellocq et al., 2004). More recently, single nucleotide polymorphisms in $K_v7.1$ have been suggested to confer susceptibility to type 2 diabetes (Unoki et al., 2008). Mutations in $K_v7.4$ underlie a rare form of deafness (DFNA2), characterized by symmetric, predominantly high-frequency sensorineural hearing loss that is slowly progressive across all frequencies (Kubisch et al., 1999).

Mutations in $K_v7.2$ (KCNQ2) and $K_v7.3$ (KCNQ3) genes, encoding for voltage-gated K^+ channel subunits underlying most of the neuronal I_{KM} (Wang et al., 1998), are responsible for early-onset epileptic diseases with a widely-diverging phenotypic presentation. Earlier studies revealed that $K_v7.2$ (Biervert et al., 1998; Singh et al., 1998) or, more rarely, $K_v7.3$ (Charlier et al., 1998) gene defects are responsible for Benign Familial Neonatal Seizures (BFNS), an autosomal-dominant epilepsy of newborns. BFNS-affected patients suffer from recurrent seizures that begin in the very first days of life and remit after a few weeks or months, with mostly normal interictal EEG, neuroimaging, and psychomotor development (Bellini et al., 2010). BFNS is characterized by the occurrence of focal, multifocal or generalized unprovoked tonic-clonic convulsions starting around day 3 of post-natal life and spontaneously disappearing after few weeks or months (Plouin, 1994). Although neurocognitive development is normal in most BFNS-affected individuals, follow-up studies have revealed that seizures or other neurological or neuropsychiatric abnormalities can occur in up to 15% of the patients (Dedek et al., 2001; Coppola et al., 2003; Wuttke et al., 2007). BFNS-causing mutations are 10 times more likely to be found in $K_v7.2$ than in $K_v7.3$; all $K_v7.3$ mutations described to date are missense, whereas $K_v7.2$ mutations consist of truncations, splice site defects, or missense, non-sense and frame-shift mutations, as well as sub-microscopic deletions or duplications (Heron et al., 2007; Soldovieri et al., 2007). $K_v7.2$ mutations have been also detected in sporadic cases of benign neonatal seizures (Ishii et al., 2009; Sadewa et al., 2008; Miceli et al., 2009). Following several reports questioning the benignity of the clinical course in BFNS patients (Steinlein et al., 2007), de novo missense $K_v7.2$ mutations have been more recently found in neonates affected with pharmacoresistant seizures, distinct EEG and neuroradiological features, and various degrees of developmental delay, defining a "$K_v7.2$ encephalopathy" (Weckhuysen et al., 2012). De novo missense $K_v7.2$ mutations have been also shown as one of the most common cause of early-onset EEs, including the Ohtahara syndrome (Saitsu et al., 2012; Kato et al., 2013), the most severe and earliest developing age-related EE, and it is now believed that they account for at least 15-25% of sporadic cases of neonatal-onset epileptic encephalopathy.

K_v7 channels in neurodegeneration

The role of K_v7 channels in the regulation of cell death/survival, or protection from damage in neurons, in particular in the hippocampus, has been investigated in various experimental studies. Such studies do not clearly establish a neuroprotective or neurodegenerative role for K_v7 channels, possibly because of the different experimental models used. Earlier reports showed that two M-type K^+ channel blockers, linopirdine and its analog XE991, promoted survival of rat sympathetic neurons deprived of nerve growth factor (Xia et al., 2002). More recently, the same Authors demonstrated that $K_v7.2/3$ channel openers N-ethylmaleimide (NEM) and the non-opioid analgesic drug flupirtine caused dose-dependent K^+ efflux, intracellular K^+ depletion,

caspase-3 activation, and cell death in hippocampal cultures, whereas little cell death was induced by NEM in cortical cultures. This differential vulnerability between hippocampal and cortical cultures appeared to be related to I_{KM} expression, whose size was larger in cultured hippocampal neurons when compared to cortical neurons. NEM-induced cell death was antagonized by the K_v7 channel inhibitor XE991, or by elevated extracellular K^+ concentration. NEM-induced cell death appeared to be of the apoptotic type, as revealed by the occurrence of mitochondria membrane depolarization, cytochrome c release, formation of apoptosome complex, and apoptosis-inducing factor (AIF) translocation into the nucleus; all these events were attenuated by blocking $K_v7.2/3$ channels (Zhou et al, 2011). By contrast, Gamper and collaborators (2006) demonstrated that reactive oxygen species (ROS), which are increased during ischemic damage and in other neurodegenerative diseases, activate three of five K_v7 channels ($K_v7.2$, $K_v7.4$, and $K_v7.5$); consistent with the role of I_{KM} in neuronal excitability, oxidation-induced enhancement of I_{KM} produced a hyperpolarization and a dramatic reduction of action potential firing frequency in rat sympathetic neurons. The same Authors also showed that blockade of I_{KM} with XE991 dramatically increased OGD-induced neuronal death in organotypic hippocampal slices (OHSCs), but did not affect survival of normoxic slices; moreover, the I_{KM} enhancer retigabine decreased OGD-induced neuronal death. These data suggest that M channels participate in cytoprotective neuronal silencing in an oxidative stress-related model of neurodegeneration. Other studies have pointed out the neuroprotective effects of I_{KM}-activators (retigabine and flupirtine) in different experimental models, such as OHSC exposed to N-methyl-D-aspartate (NMDA), oxygen and glucose deprivation followed by reoxygenation (OGD), or serum withdrawal (SW) (Boscia et al, 2006). Region-specific vulnerability of hippocampal subfields occurred with each of these injury models. Specifically, CA1 was the most susceptible region to both NMDA and OGD-induced neurodegeneration, whereas selective cell death in the dentate gyrus (DG) occurred upon OHSCs exposure to SW. However, in these models the neuroprotective effects of I_{KM} activators seem to be due to an anti-oxidant action more than a direct activation of I_{KM}. According to this study, neuroprotective effects of flupirtine and retigabine are more noticeable in some hippocampal areas such as dentate gyrus, a brain region showing remodelling after chronic epileptogenic stimuli.

Flupirtine- and retigabine-induced neuroprotection has also been observed in two "in vivo" models of ischemia, namely the four vessels occlusion (4-VO) rat (Block et al, 1997) and a mouse model of cerebral photo-induced thrombosis (Bierbower et al, 2011); in both animal models, these drugs reduced infarct area and/or the subsequent related cognitive impairment. In support of a neuroprotective role of K_v7-mediated neuronal silencing during neuronal degeneration, is also a recent study investigating the molecular basis for the degeneration of hair cells and spiral ganglion neurons (SGNs) in DFNA2, an autosomal dominant version of progressive hearing loss, caused by mutations in K_v7 currents channels (Lv et al., 2010). In this study, it was found that a long-term inhibition of K_v7 currents caused a sustained increase in $[Ca^{2+}]_i$ leading to significant SGN apoptotic death.

In support of a protective role of neuronal silencing during neurodegenerative conditions is also the observation that drugs developed for the treatment of epilepsy are endowed with significant neuroprotective effects. The rationale underlying this approach is that pharmacological targets of AEDs may have a role in determining neuronal damage after an injury. For instance, excitotoxicity is a common event after both brain ischemia and epilepsy (Costa et al., 2006). For these reasons, by decreasing excitatory transmission or enhancing inhibitory neurotransmitter release, AEDs might counteract abnormal brain excitability and represent a valid tool against the activation of deleterious mechanisms leading to neuronal cell death. Indeed, it has been recently demonstrated that tiagabine and vigabatrin (GABAergic AEDs) produce neuroprotection against in vitro ischemia (Costa et al., 2004). Similarly, other AEDs such as lamotrigine and remacemide (Calabresi et al., 2003; Willmore, 2005) or carbamazepine, valproic acid, topiramate and levetiracetam (Willmore, 2005; Costa et al., 2006) exert neuroprotection against in vitro ischemia.

CONCLUSIONS

The critical role played by K^+-selective channels in the control of neuronal excitability is well known;

however, their participation in cell survival or death has received much less attention when compared to Ca^{2+} channels or ionotropic glutamate receptors. This is perhaps due to the conflicting evidence showing on one side that a loss of intracellular K^+ causes the activation of death-triggering phenomena, whereas on the other hand that their activation leads to neuronal silencing and cellular protection; some of the mechanisms mentioned in the text are schematically represented in Fig. 2. A better understanding of these complex roles, herein briefly reviewed, is likely to improve the treatment of a variety of neurodegenerative diseases, ranging from acute hypoxia-ischemia to slowly-progressing Alzheimer disease. In this perspective, the development of novel pharmacological tools provided of a higher degree of selectivity for specific classes of K^+ channels involved in the various molecular steps of neuroprotective responses is likely to be a major leap forward.

ACKNOWLEDGEMENTS

We are deeply indebted to all the collaborators contributing to the work performed in our laboratory cited in the present review. Studies in the Author's laboratory have been supported by grants from: Telethon (GGP07125), the Fondazione San Paolo – IMI (Project Neuroscience), Regione Molise (Convenzione AIFA/Regione Molise), the Science and Technology Council of the Province of Avellino, and PRIN 2009.

REFERENCES

Bajar R, Seetharaman S, Kowaltowski AJ, Garlid KD, Paucek P (2001). Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. J Biol Chem 276: 33369-33374.

Bellini G, Miceli F, Soldovieri MV, Miraglia del Giudice E, Coppola G, Taglialatela M (2010) KCNQ2-Related Disorders. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. GeneReviews® [Internet]. University of Washington, Seattle (WA).

Bellocq C, van Ginneken AC, Bezzina CR, Alders M et al. and Wilde AA (2004) Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. Circulation 109: 2394-2397.

Besancon E, Guo S, Lok J, Tymianski M, Lo EH (2008) Beyond NMDA and AMPA glutamate receptors: emerging mechanisms for ionic imbalance and cell death in stroke. Trends Pharmacol Sci 29: 268-275.

Biasini E, Unterberger U, Solomon IH, Massignan T et al. and Harris DA (2013) A mutant prion protein sensitizes neurons to glutamate-induced excitotoxicity. J Neurosci 33: 2408-2418.

Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK (1998) A potassium channel mutation in neonatal human epilepsy. Science 279: 403-406.

Bierbower SM, Watts LT, Shapiro MS (2011) M-type K^+ channels: in vivo neuroprotective role during cerebrovascular stroke. 55th annual meeting of Biophysical Society, Baltimore, March 5-9, 2011.

Block F, Pergande G, Schwarz M (1997) Flupirtine reduces functional deficits and neuronal damage after global ischemia in rats. Brain Res 754: 279-284.

Bortner CD, Hughes FM, Cidlowski JA (1997) A primary role for K^+ and Na^+ efflux in the activation of apoptosis. J Biol Chem 272: 32436-32442.

Boscia F, Annunziato L, Taglialatela M (2006) Retigabine and flupirtine exert neuroprotective actions in organotypic hippocampal cultures. Neuropharmacology 51: 283-294.

Busija DW, Gaspar T, Domoki F, Katakam PV, Bari F (2008) Mitochondrial-Mediated Suppression of ROS Production Upon Exposure of Neurons to Lethal Stress: Mitochondrial Targeted Preconditioning. Adv Drug Deliv Rev 60: 1471–1477.

Calabresi P, Marti M, Picconi B, Saulle E et al. and Bernardi G (2003) Lamotrigine and remacemide protect striatal neurons against in vitro ischemia: an electrophysiological study. Exp Neurol 182: 461-469.

Chao D, Xia Y (2010) Ionic storm in hypoxic/ischemic stress: can opioid receptors subside it? Prog Neurobiol 90: 439-470.

Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M (1998) A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat Genet 18: 53-55.

Cheney JA, Weisser JD, Bareyre FM, Laurer HL et

al. and McIntosh TK (2001) The maxi-K channel opener BMS-204352 attenuates regional cerebral edema and neurologic motor impairment after experimental brain injury. J Cereb Blood Flow Metab 21: 396-403.

Coppola G, Castaldo P, Miraglia del Giudice E, Bellini et al. and Tagliatatela M (2003). A novel KCNQ2 K^+ channel mutation in benign neonatal convulsions and centrotemporal spikes. Neurology 61: 131-134.

Correia SC, Santos RX, Perry G, Zhu X, Moreira PI, Smithd MA (2010) Mitochondria: The Missing Link Between Preconditioning and Neuroprotection. J Alzheimers Dis 20(Suppl 2): S475–S485

Costa C, Leone G, Saulle E, Pisani F, Bernardi G, Calabresi P (2004). Coactivation of GABA(A) and GABA(B) receptor results in neuroprotection during in vitro ischemia. Stroke 35: 596-600.

Costa C, Martella G, Picconi B, Prosperetti C et al. and Calabresi P (2006) Multiple mechanisms underlying the neuroprotective effects of antiepileptic drugs against in vitro ischemia. Stroke 37: 1319-1326.

Dedek K, Kunath B, Kananura C, Reuner U, Jentsch TJ, Steinlein OK (2001) Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K^+ channel. Proc Natl Acad Sci USA 98: 12272-12277.

Delmas P, Brown DA (2005) Pathways modulating neural KCNQ/M (K_v7) potassium channels. Nat Rev Neurosci 6: 850-862.

Deng P, Pang Z-P, Zhang Y, Xu Z (2005) Increase of delayed rectifier potassium currents in large aspiny neurons in the neostriatum following transient forebrain ischemia. Neuroscience 131: 135-146.

Deng P, Pang Z-P, Lei Z, Shikano S et al. and Xu ZC (2011) Upregulation of A-type potassium currents protects neurons against cerebral ischemia. J Cereb Blood Flow Metab 31: 1823-1835.

Dicou E, Attoub S, Gressens P (2001) Neuroprotective effects of leptin in vivo and in vitro. Neuroreport 12: 3947-3951.

Dolga AM, Culmsee C (2012) Protective roles for potassium SK/K(Ca)2 channels in microglia and neurons. Front Pharmacol 3: 196.

Douglas RM, Lai JC, Bian S, Cummins L, Moczydlowski E, Haddad GG (2006) The calcium-sensitive large-conductance potassium channel (BK/MAXI K) is present in the inner mitochondrial membrane of rat brain. Neuroscience 139:1249-1261

Fox PD, Loftus RJ, Tamkun MM (2013) Regulation of Kv2.1 K^+ conductance by cell surface channel density. J Neurosci 33: 1259-1270.

Fraser SP, Suh YH, Djamgoz MB (1997) Ionic effects of the Alzheimer's disease beta-amyloid precursor protein and its metabolic fragments. Trends Neurosci 20: 67-72.

Gamper N, Zaika O, Li Y, Martin P et al. and Shapiro MS (2006) Oxidative modification of M-type K^+ channels as a mechanism of cytoprotective neuronal silencing. EMBO J 25: 4996-5004.

Garlid KD, Paucek P (2003). Mithocondrial potassium trasport: the K^+ cycle. Biochim Biophys Acta 1606: 23-41.

Gavello D, Rojo-Ruiz J, Marcantoni A, Franchino C, Carbone E, Carabelli V (2012). Leptin counteracts the hypoxia-induced inhibition of spontaneously firing hippocampal neurons: a microelectrode array study. PLoS One 7: e41530.

Greenwood IA, Ohya S (2009) New tricks for old dogs: KCNQ expression and role in smooth muscle. Br J Pharmacol 156: 1196-1203.

Gribkoff VK, Starrett JE Jr, Dworetzky SI, Hewawasam P et al. and Yeola SW (2001) Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels. Nat Med 7: 471-477.

Guo Z, Jiang H, Xu X, Duan M, Mattson MP (2008) Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. J Biol Chem 283: 1754-1763.

Heitzmann D, Warth R (2008) Physiology and pathophysiology of potassium channels in gastrointestinal epithelia. Physiol Rev 88:1119-1182.

Heron SE, Cox K, Grinton BE, Zuberi SM et al. and Mulley JC (2007) Deletions or duplications in KCNQ2 can cause Benign Familial Neonatal Seizures. J Med Genet 44: 791-796.

Hewawasam P, Fan W, Knipe J, Moon SL, Boissard CG, Gribkoff VK, Starrett JE (2002) The synthesis and structure-activity relationships of 4-aryl-3-aminoquinolin-2-ones: a new class of calcium-dependent, large conductance, potassium (maxi-K) channel openers targeted for post-stroke neuroprotection. Bioorg Med Chem Lett 12: 1779-1783.

Huang H, Gao TM, Gong LW, Zhuang ZY, Li X

(2001) Potassium channel blocker TEA prevents CA1 hippocampal injury following transient forebrain ischemia in adult rats. Neurosci Lett 305: 83-86.

Hughes FM Jr, Bortner CD, Purdy GD, Cidlowski JA (1997) Intracellular K^+ suppresses the activation of apoptosis in lymphocytes. J Biol Chem 272: 30567-30576.

Iannotti FA, Panza E, Barrese V, Viggiano D, Soldovieri MV, Taglialatela M (2010) Expression, localization, and pharmacological role of K_v7 potassium channels in skeletal muscle proliferation, differentiation, and survival after myotoxic insults. J Pharmacol Exp Ther 332: 811-820.

Ishii A, Fukuma G, Uehara A, Miyajima T et al. and Hirose S (2009) A de novo KCNQ2 mutation detected in non-familial benign neonatal convulsions. Brain Dev 31: 27-33.

Jabůrek M1, Yarov-Yarovoy V, Paucek P, Garlid KD (1998) State-dependent inhibition of the mitochondrial KATP channel by glyburide and 5-hydroxydecanoate. J Biol Chem. 273:13578-13582.

Jalonen TO, Charniga CJ, Wielt DB (1997) beta-amyloid peptide-induced morphological changes coincide with increased K and Cl channel activity in rat cortical astrocytes. Brain Res 746: 85-97.

Jensen BS (2002) BMS-204352: a potassium channel opener developed for the treatment of stroke. CNS Drug Rev 8: 353-360.

Jhamandas JH, Cho C, Jassar B, Harris K, MacTavish D, Easaw J (2001) Cellular mechanisms for amyloid beta-protein activation of rat cholinergic basal forebrain neurons. J Neurophysiol 86:1312-1320.

Jorgensen PL, Hakansson KO, Karlish SJ (2003). Structure and mechanism of Na^+/K^+-ATPase: functional sites and their interactions. Annu Rev Physiol 65: 817-849.

Karki P, Seong C, Kim JE, Hur K et al. and Park IS (2007) Intracellular K^+ inhibits apoptosis by suppressing the Apaf-1 apoptosome formation and subsequent downstream pathways but not cytochrome c release. Cell Death Differ 14: 2068-2075.

Kato M, Yamagata T, Kubota M, Arai H et al. and Saitsu H (2013) Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. Epilepsia 54: 1282-1287.

Knaus HG, Schwarzer C, Koch RO, Eberhart A et al. and Sperk G (1996) Distribution of high-conductance Ca^{2+}-activated K^+ channels in rat brain: targeting to axons and nerve terminals. J Neurosci 16: 955-963.

Knoch ME, Hartnett KA, Hara H, Kandler K, Aizenman E (2008) Microglia induce neurotoxicity via intraneuronal Zn2+ release and a K^+ current surge. Glia 56: 89-96.

Kubisch C, Schroeder BC, Friedrich T, Lütjohann B et al. and Jentsch TJ (1999) KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell 96: 437-446.

Lerche C, Scherer CR, Seebohm G, Derst C, Wei AD, Busch AE, Steinmeyer K (2000) Molecular cloning and functional expression of KCNQ5, a potassium channel subunit that may contribute to neuronal M-current diversity. J Biol Chem 275: 22395-22400.

Leung YM (2010). Voltage-gated K^+ channel modulators as neuroprotective agents. Life Sciences 86: 775-780.

Liao Y, Kristiansen AM, Oksvold CP, Tuvnes SA, Gu N, Rundén-Pran E, Ruth P, Sausbier M, Storm JF (2010) Neuronal Ca^{2+}-Activated K^+ Channels Limit Brain Infarction and Promote Survival. PLoS ONE 5: e15601.

Liu D, Lu C, Wan R, Auyeung WW, and Mattson MP (2002). Activation of mitochondrial ATP-dependent potassium channels protects neurons against ischemia-induced death by a mechanism involving suppression of Bax translocation and cytochrome c release. J Cereb Blood Flow Metab 22: 431-443.

Liu D, Slevin JR, Lu C, Chan SL, Hansson M, Elmer E, Mattson MP (2003) Involvement of mitochondrial K^+ release and cellular efflux in ischemic and apoptotic neuronal death. J Neurochem 86: 966-979.

Lv P, Wei D, Yamoah EN (2010) K_v7-type channel currents in spiral ganglion neurons: involvement in sensorineural hearing loss. J Biol Chem 285: 34699-34707.

Mancini M, Soldovieri MV, Gessner G, Wissuwa B et al. and Taglialatela M (2014) Critical role of large-conductance calcium- and voltage-activated potassium channels in leptin-induced neuroprotection of N-Methyl-D-Aspartate-exposed cortical neurons. Pharmacol Res 87: 80-86.

Martire M, Barrese V, D'Amico M, Iannotti FA et al. and Taglialatela M (2010) Pre-synaptic BK channels selectively control glutamate versus GABA release from

cortical and hippocampal nerve terminals. J Neurochem 115: 411-422.

Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. Eur J Pharmacol 698: 6-18.

Miceli F, Soldovieri MV, Lugli L, Bellini G et al. and Taglialatela M (2009). Neutralization of a unique, negatively-charged residue in the voltage sensor of $K_v7.2$ subunits in a sporadic case of benign familial neonatal seizures. Neurobiol Dis 34: 501-510.

Misonou H, Trimmer JS (2009) The diverse roles of K^+ channels in brain ischemia. Annunziato L (Ed.) New strategies for stroke intervention. Humana Press pp. 211-224.

Misonou H, Menegola M, Buchwalder L, Park EW et al. and Trimmer JS (2006) Immunolocalization of the Ca^{2+}-activated K^+ channel Slo1 in axons and nerve terminals of mammalian brain and cultured neurons. J Comp Neurol 496: 289-302.

Nakae Y, Kwok WM, Bosnjak ZJ, Jiang MT (2003) Isoflurane activates rat mitochondrial ATP-sensitive K^+ channels reconstituted in lipid bilayers. Am J Physiol Heart Circ Physiol. 284:H1865-H1871.

Nistico R, Piccirilli S, Sebastianelli L, Nistico G, Bernardi G, Mercuri NB (2007) The blockade of K^+-ATP channels has neuroprotective effects in an in vitro model of brain ischemia. Int Rev Neurobiol 82: 383-395.

O'Connell KM, Loftus R, Tamkun MM (2010) Localization-dependent activity of the Kv2.1 delayed-rectifier K^+ channel. Proc Natl Acad Sci USA 107: 12351-12356.

Pannaccione A, Secondo A, Scorziello A, Calì G, Tagliatatela M, Annunziato L (2005) NF-kB activation by reactive oxygen species mediates voltage-gated K^+ current enhancement by neurotoxic beta-amyloid peptides in NGF-differentiated PC-12 cells and hippocampal neurons. J Neurochem 94: 572-586.

Pannaccione A, Boscia F, Scorziello A, Adornetto A et al., and Annunziato L (2007) Up-regulation and increased activity of Kv3.4 channels and their accessory subunit minK-related peptide 2 induced by amyloid peptide are involved in apoptotic neuronal death. Mol Pharmacol 72: 665-673.

Panayiotidis MI, Bortner CD, Cidlowski JA (2006) On the mechanism of ionic regulation of apoptosis: would the Na^+/K^+-ATPase please stand up? Acta Physiol (Oxf) 187: 205-215.

Plouin P (1994) Benign Familial Neonatal Convulsions. In: Idiopathic generalized epilepsies: clinical, experimental and genetic aspects, edited by Malafosse A, Hirsch E, Marescaux C, Broglin D, and Bernasconi R. John Libbey, London: 39-44.

Raffaelli G, Saviane C, Mohajerani MH, Pedarzani P, Cherubini E (2004) BK potassium channels control transmitter release at CA3-CA3 synapses in the rat hippocampus. J Physiol 557: 147-157.

Ramsden M, Plant LD, Webster NJ, Vaughan PF, Henderson Z, Pearson HA (2001) Differential effects of unaggregated and aggregated amyloid beta protein (1-40) on K^+ channel currents in primary cultures of rat cerebellar granule and cortical neurones. J Neurochem 79: 699-712.

Raval AP, Dave KR, DeFazio RA, and Perez-Pinzon MA (2007). epsilonPKC phosphorylates the mitochondrial K^+ (ATP) channel during induction of ischemic preconditioning in the rat hippocampus. Brain Res 1184:345-353.

Rundén-Pran E, Haug FM, Storm JF and Ottersen OP (2002) BK channel activity determines the extent of cell degeneration after oxygen and glucose deprivation: a study in organotypical hippocampal slice cultures. Neuroscience 212:277-288.

Sadewa AH, Sasongko TH, Gunadi, Lee MJ et al. and Nishio H (2008) Germ-line mutation of KCNQ2, p.R213W, in a Japanese family with Benign Familial Neonatal Convulsion. Pediatr Int 50: 167-171.

Saitsu H, Kato M, Koide A, Goto T et al. and Matsumoto N (2012) Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. Ann Neurol 72: 298-300.

Schroeder BC, Hechenberger M, Weinreich F, Kubisch C, Jentsch TJ (2000) KCNQ5, a novel potassium channel broadly expressed in brain, mediates M-type currents. J Biol Chem 275: 24089-24095.

Sensi SL, Paoletti P, Koh JY, Aizenman E, Bush AI, Hershfinkel M (2011) The neurophysiology and pathology of brain zinc. J Neurosci 31: 16076-16085.

Shah NH, Aizenman E (2014) Voltage-gated potassium channels at the crossroads of neuronal function, ischemic tolerance, and neurodegeneration. Transl Stroke Res 5: 38-58.

Siemen D, Loupatatzis C, Borecky J, Gulbins E, Lang

F (1999) Ca^{2+}-activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. Biochem Biophys Res Commun 257:549-554.

Signore AP, Zhang F, Weng Z, Gao Y, Chen J (2008) Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials. J Neurochem 106: 1977-1990.

Singer-Lahat D, Chikvashvili D, Lotan I (2008) Direct interaction of endogenous Kv channels with syntaxin enhances exocytosis by neuroendocrine cells. PLoS One 3: e1381.

Singh NA, Charlier C, Stauffer D, DuPont BR et al. and Leppert M (1998) A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat Genet 18: 25-29.

Soldovieri MV, Miceli F, Taglialatela M (2011) Driving with no brakes: molecular pathophysiology of K_v7 potassium channels. Physiology (Bethesda) 26: 365-376.

Soldovieri MV, Cilio MR, Miceli F, Bellini G et al. and Taglialatela M (2007) Atypical gating of M-type potassium channels conferred by mutations in uncharged residues in the S4 region of KCNQ2 causing Benign Familial Neonatal Convulsions. J Neurosci 27: 4919-4928.

Steinlein OK, Conrad C, Weidner B (2007) Benign Familial Neonatal Convulsions: always benign? Epilepsy Res 73: 245-249.

Suh YH, Checler F (2002) Amyloid precursor protein, presenilins, and alphasynuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. Pharmacol Rev 54: 469-525.

Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267: 1456-1462.

Valerio A, Dossena M, Bertolotti P, Boroni F et al. and Nisoli E (2009) Leptin is induced in the ischemic cerebral cortex and exerts neuroprotection through NF-kappaB/c-Rel-dependent transcription. Stroke 40: 610-617.

Unoki H, Takahashi A, Kawaguchi T, Hara K, et al. and Maeda S (2008) SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 40: 1098-1102.

Wang HS, Pan Z, Shi W, Brown BS et al. and McKinnon D (1998) KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science 282: 1890-1893.

Wang Q, Curran ME, Splawski I, Burn TC et al. and Keating MT. (1996) Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 12: 17-23.

Weckhuysen S, Mandelstam S, Suls A, Audenaert D et al. and de Jonghe P (2012) KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol 71: 15-25.

Weng Z, Signore AP, Gao Y, Wang S et al. and Chen J (2007) Leptin protects against 6-hydroxydopamine-induced dopaminergic cell death via mitogen-activated protein kinase signaling. J Biol Chem 282: 34479-34491.

Willmore LJ (2005) Antiepileptic drugs and neuroprotection: current status and future roles. Epilepsy Behav 7 Suppl 3: S25-28.

Wu L, Shen F, Lin L, Zhang X, Bruce IC, and Xia Q (2006) The neuroprotection conferred by activating the mitochondrial ATP-sensitive K^+ channel is mediated by inhibiting the mitochondrial permeability transition pore. Neurosci Lett 402:184-189.

Wulff H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. Nat Rev Drug Discov 8: 982-1001.

Wuttke TV, Jurkat-Rott K, Paulus W, Garncarek M, Lehmann-Horn F, Lerche H (2007) Peripheral nerve hyperexcitability due to dominant-negative KCNQ2 mutations. Neurology 69: 2045-2053.

Xia S, Lampe PA, Deshmukh M, Yang A et al. and Yu SP (2002) Multiple channel interactions explain the protection of sympathetic neurons from apoptosis induced by nerve growth factor deprivation. J Neurosci 22: 114-122.

Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, Sidor A, O'Rourke B (2002) Cytoprotective role of Ca^{2+}-activated K^+ channels in the cardiac inner mitochondrial membrane. Science 298:1029-1033

Yang Q, Yan D, Wang Y (2006) K^+ regulates DNA binding of transcription factors to control gene expression related to neuronal apoptosis. Neuroreport 17: 1199-1204.

Yeung SY, Pucovský V, Moffatt JD, Saldanha L, Schwake M, Ohya S, Greenwood IA (2007) Molecular expression and pharmacological identification of a role for K_v7 channels in murine vascular reactivity. Br J Pharmacol 151: 758-770.

Yu SP, Yeh CH, Sensi SL, Gwag BJ et al. and Choi DW (1997) Mediation of neuronal apoptosis by enhancement

of outward potassium current. Science 278: 114-117.

Yu SP, Farhangrazi ZS, Ying HS, Yeh CH, Choi DW (1998) Enhancement of outward potassium current may participate in beta-amyloid peptide-induced cortical neuronal death. Neurobiol Dis 5: 81-88.

Yu SP, Choi DW (2000) Ions, cell volume, and apoptosis. Proc Natl Acad Sci USA 97: 9360-9362.

Zhang F, Wang S, Signore AP, Chen J (2007) Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. Stroke 38: 2329-2336.

Zhao YM, Sun LN, Zhou HY, Wang XL (2006) Voltage-dependent potassium channels are involved in glutamate-induced apoptosis of rat hippocampal neurons. Neurosci Lett 398: 22-27.

Zhou X, Wei J, Song M, Francis K, Yu SP (2011) Novel role of KCNQ2/3 channels in regulating neuronal cell viability. Cell Death Differ 18: 493-505.