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THE EFFECT OF EPIDERMAL GROWTH FACTOR ON RNA, DNA, ACID PHOSPHATASE, AND LACTIC-DEHYDROGENASE IN NEWBORN RAT SKIN

I. Simeonidou[1] and G. Caccianiga[2*]

[1] Department of Parasitology, School of Veterinary Medicine, Aristotle University, Thessaloniki, Greece;
[2] School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.

**Correspondence to*:
Gianluigi Caccianiga,
School of Medicine and Surgery,
University of Milano-Bicocca,
Monza, Italy.
e-mail: gianluigi.caccianiga@unimib.it

ABSTRACT

Epidermal Growth Factor (EGF) works by binding with high affinity to its EGF receptor (EGFR) on the cell membrane. EGF stimulates intracellular calcium levels, and increases glycolysis and DNA and protein synthesis through the action of intrinsic tyrosine kinase activity of the receptor that initiates a signal transduction cascade. Injection of minute amounts of EGF into newborn rats produces hyperplasia of the epidermis with a marked increase in the protein and nucleic acid content per unit, along with phosphatase and lactic dehydrogenase in the injection area. The activity of a number of epidermal enzymes is also increased by EGF. In this study, the biological effect of EGF with a growth-stimulating activity on epidermal cells was investigated.

KEYWORDS: *epidermal growth factor, skin, hyperplasia, DNA, RNA, lactic dehydrogenase, acid phosphatase*

INTRODUCTION

Neutrophins (NTs) are protein molecules that regulate the organism at both the central nervous system (CNS) and peripheral levels (1). NTs include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4).

NT-3 is involved in CNS development by regulating the activity of synapses and the growth of nerve fibers and neurons. NT-3 belongs to the nerve growth factor (NGF) family and is encoded by the NTF3 gene. It has a similar amino acid sequence as NT-4 (2). BDNF is the most abundant NT in the CNS that can cross the blood-brain barrier (BBB) and can be delivered throughout the body. It has long been reported that the growth and differentiation of some cell types are directly influenced and regulated by specific growth factors.

Similar to NGF, Epidermal Growth Factor (EGF) is found in high concentrations in the salivary gland of the male mouse from where it can be extracted, purified, and cloned. EGF was discovered by Stanley Cohen in 1965, when he showed the first biological effect of this protein on newborn mice (3). EGF is a 6.045 Kd protein that is composed of 53 amino acid residues and three intramolecular disulfide bonds, which plays an important role in regulating cellular growth, proliferation, and differentiation. EGF stimulates the intrinsic tyrosine kinase activity through its receptor, initiating a signal transduction cascade with a series of cellular biochemical changes, such as increases in intracellular calcium levels,

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glycolysis, and DNA and protein synthesis, followed by cell proliferation (4). EGF also causes an increase in the expression of some genes, including the one coding for the EGF receptor (EGFR) (5).

EGF is one of the first growth factor protein families to be identified and characterized by its structure. It is a transmembrane protein tyrosine kinase composed of two units that is involved in the development of the epidermis in mammals, including humans (6). EGF plays an important role in regulating cell growth, proliferation, and differentiation, and acts by specifically stimulating the growth of skin epithelia and keratinization processes (7).

It has been reported that EGF injections in newborn animals caused precocious eyelid opening (after 6 days instead of 14 days) due to an enhancement of epidermal growth and keratinization (8). Subsequently, the same authors also demonstrated a direct growth-stimulating effect on epidermal cells (8).

Acute ischemic kidney injury induced in rodents can also cause the release of EGF after 24 hours, due to the serine protease that cleaves the EGF precursor. Furthermore, EGF is present in platelets, macrophages, urine, saliva, milk, and plasma, and is capable of stimulating the metabolic effects of the body. The injection of small quantities of EGF produces the rapid growth of all skin epithelium and marked thickening of the keratin state (9).

In this study, the effect of EGF on the synthesis of nucleic acids, proteins, and some enzymatic activities of the epidermis was studied.

MATERIALS AND METHODS

Newborn rats were injected subcutaneously every day with EGF in aqueous solution, in the amount of 2 μg per gram of body weight for a duration of 5 days. 8 animals from the same litter were used for each experiment; 4 were used as controls and the other 4 were subjected to EGF treatment.

At the end of the treatment, the animals were euthanized, and the skin was immediately removed and stretched on special surfaces that were kept constantly cooled to 3-4° centigrade. Circular areas with a diameter of 2 cm were then sectioned from the skin and stretched using a specially constructed circular blade. Then, the separation of the epidermis from the dermis was achieved by incubating the sections of skin in a diluted solution of 2% trypsin in a phosphate crepe buffer for approximately 30 minutes.

After this treatment, the epidermis was separated from the underlying layer of dermis, carefully washed in physiological solution, and the thin sections were used for the various chemical analyses for the determination of nucleic acids. Each piece of the epidermis was extracted with 5% trichloroacetic acid at zero degrees centigrade and washed three times with alcohol ether mixture, and finally, with ether.

The prepared samples were then left to dry at a temperature of 50°C and then extracted again with 5% TCA (2 mm for each sample) in a thermostatic bath at 90°C for 15 minutes. Ribonucleic acid was determined in 0.2 cm square aliquots of this extract using the orcinol reaction for pentoses according to the method of Volkin and Cohen, measuring the D.O. at 660 mμ in a Beckman DU spectrophotometer. For each experiment, a standard curve was made in parallel with pure RNA (Sigma).

When calculating the results, a correction factor for the color produced by the DNA in the reaction with orcinol was considered, after determining that 7.4 μg of DNA gave a reaction equivalent to that of 1 μg of RNA. For the determination of DNA, the phenylalanine reaction for desose-pentoses was used according to the Disch and Schwarz method, modified by Volkin and Cohen, using aliquots of 1 cm^3 of the TCA extract. The optical density of the samples was read at 540 mμ, in a Beckman DU. For each experiment, a standard curve was determined using a DNA stock solution (Sigma). For the determination of the enzymatic activity, the epidermis sections were homogenized in 0.9% NaCl (0.5ml for each 2cm diameter section of skin). The homogenates were centrifuged for 5 minutes at 3000 RPM, and the supernatant was used for analysis. The enzymes examined included acid phosphatase and lactic dehydrogenase. For the determination of acid phosphatase, the method of Bessey et al. was followed and the method of Kornaberg was followed for lactic dehydrogenase. For the determination of dry weight and total nitrogen, whole sections of the epidermis (2 cm diameter) were used, which were freed from the dermis by trypsinization, according to the Kjeldahl method.

RESULTS AND DISCUSSION

The injection of the growth factor EGF in the doses indicated above produced a rapid response of the skin epithelia as soon as the seventh day of treatment, due to the early eruption of the incisor teeth and the early opening of the eyelid fissures. Both these phenomena were due to the keratinization of the respective lining epithelia. At histological examination, the hyperplasia of the skin in the treated animals appeared evident and marked everywhere, as well as the hyperkeratosis. In the table, the results of RNA and DNA levels of the epidermis of the treated animals and the control

animals are reported. The values are expressed in terms of µg/unit of area, with area as a perfectly circular section 2 cm in diameter (Table I).

Table I. *Stimulation of RNA or DNA and acid phosphatase and lactic dehydrogenase in the newborn rat skin after EGF treatment.*

Control (untreated rats) (RNA µg/unit)	2.1 +/- 1.9
Control (DNA µg/unit)	1.0 +/- 0.8
Control (Acid Phosphatase U/ml/h)	4.5 +/- 2.4
Control Lactic-dehydrogenase (µM/ml/h)	7.3 +/- 4.2
RNA µg/unit of area (3.10 cm2) (EGF treated rats)	620 +/- 50
DNA µg/unit of area (EGF treated rats)	340 +/- 30
Acid Phosphatase U/ml/h (EGF treated rats)	19.5 +/- 2.5
Lactic-dehydrogenase µM/ml/h	32.5 +/- 3.1

As can be seen, treatment with EGF causes a significant increase in both RNA and DNA, and the ratio between the experimental mean value and the average control value is 1.33 for DNA and 1.31 for RNA. The increase in the dry weight appears to be even more significant. The ratio between experimental and control groups appears to be 1.65 while the percentage of nitrogen content remains unchanged.

Finally, the table shows the values of some enzymatic activities. It was observed that in the epidermis of the experimental animals, acid phosphatase and lactic dehydrogenase are constantly and markedly increased. The results of the present work demonstrate that the injection of EGF selectively stimulates the growth of the epidermis, confirming previous morphological observations (10). Therefore, when EGF (2 µg/g) is injected into the rat, there is a keratinization of the skin after 5 days, compared to the control group. The effect of EGF is reflected in a significant increase in the content of proteins and nucleic acids in the skin of the treated animals. This increase appears particularly marked when the values relating to the dry weight per unit area are considered. This is due to the abundant keratinization observed in the animals.

CONCLUSIONS

In conclusion, the subcutaneous injection of small doses of EGF strongly stimulates the processes of synthesis and growth of the epithelial cells of the skin. The mechanism through which this effect occurs remains unclear, and clarification could help contribute to the understanding of processes that regulate cellular growth and differentiation (11).

Conflict of interest
The authors declare that they have no conflict of interest.

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