

ORIGIN, STRUCTURE AND PHYSIOLOGICAL ROLE OF THE EPIDERMAL GROWTH FACTOR: A REVIEW

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Summary

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Colostrum and milk contain a large number of peptide substances that are known to possess a biological activity, including a growth promoter one. The amounts of peptide growth factors secreted in milk, vary according to the individual animal species and the period of lactation. The epidermal growth factor (EGF) promotes the growth of the mammary gland during the mammogenesis, regulates the differentiation of functional epithelial cells in the alimentary system for the postnatal period of newborns and promotes the somatic growth in both animals and men. Some peptides from the group of EGF-like ligands are involved in the proliferation and differentiation of cell structures of the nervous system. In the light of their physiological importance for the normal development of newborns in the postnatal period, the detailed study on the mechanisms of growth factors would contribute to the optimization of the technologies in intensive animal rearing systems.

Key words: epidermal growth factor (EGF), epidermal growth factor receptor (EGFR), milk, small intestine, weaning age

INTRODUCTION

During the last years, the interest of researchers is focused on a heterogeneous group of proteins and peptides, detected in animal and human colostrum and milk that has a marked positive effect on newborns' growth. This group bears the general name of growth factors, the most important among which are as follows: epidermal growth factor (EGF), somatotropin (growth hormone, GH), keratinocyte growth factor (KGF), insulin-like growth factor 1 (IGF-1), erythropoietin (Epo), glucagon-like polypeptide (GLP), transforming growth factor (TGF) and hepatocyte growth factor (HGF) (Cummins & Thompson, 2002).

The examination of the mechanisms of their action provides new opportunities in animal husbandry and reveals broad perspectives for application in medicine, due to their marked effect on tissue differentiation in alimentary and immune systems (Donovan & Odle, 1994; Playford *et al.*, 2000; Pouliot & Gauthier, 2006).

The amount of EGF in milk, including colostrum, varies according to the period of lactation and the animal species (Table 1).

In men, EGF is released in the milk secretion before the birth and is detected in amounts of 25–40 ng/mL (colostrum) and 5–12 ng/mL (milk) (Donovan & Odle,

Table 1. EGF content (ng/mL) in the colostrum and the milk of some animal species and in men

| Species | Colostrum | Milk | Reference |
|---------|-----------|---------|-------------------------------|
| Man | 25–40 | 5–12 | Cummins & Thompson, 2002 |
| Cattle | 4–325 | 1–150 | Pouliot & Gauthier, 2006 |
| Pigs | ≈1500 | 150–250 | Jaeger <i>et al.</i> , 1987 |
| Rats | 2–3 | 6–12 | Raaberg <i>et al.</i> , 1990 |
| Mice | 30–100 | 65–260 | Grueters <i>et al.</i> , 1985 |
| Sheep | 2±0.3 | <0.8 | Gow <i>et al.</i> , 1991 |

1994; Dehnhard *et al.*, 2000). The mice and pigs, EGF contents in colostrum and milk are higher than in men whereas in rats, EGF is lower in the colostrum period and achieves a peak by the 19th day of lactation (Xu, 1996; Cummins & Thompson, 2002).

Despite the available literature data about EGF content in colostrum and milk in cattle and goats, the reported values are contradictory. In pasteurized cow milk, a concentration of 2.5 ng/mL is reported (Read *et al.*, 1985). Some authors have isolated a protein fraction with higher molecular weight than that of EGF from the colostrum and milk of these species. According to them, this was betacellulin (21–22 kDa), and it, but not EGF, was the leading growth factor in the milk of cows and goats (Dunbar *et al.*, 1999; Dehnhard *et al.*, 2000).

The data about the content and the levels of EGF during the different periods of lactation in rabbits are few and incomplete. In the scientific literature, there is only scarce information about EGF presence in the milk secretion in this animal species (Ellis & Picciano, 1992). Our unpublished data revealed that the EGF concentrations in the milk of lactating rabbits were the highest by the end of lactation.

The purpose of the present review was to follow out the origin, the molecular structure and the physiological role of the

epidermal growth factor in different animal species and men, as well as to discuss some of its applications in medicine.

SITES OF SYSTEMIC EGF SYNTHESIS

EGF is isolated from numerous tissues and body fluids in men and animals. It is present at the highest concentrations in the amniotic fluid, the saliva, colostrum, milk and urine, but is also detected in mucous secretions from the respiratory and alimentary tracts (Brazzel *et al.*, 1991; Kelly *et al.*, 1997; Dehnhard *et al.*, 2000). The data about its blood plasma concentration are rather contradictory. Some authors reported plasma EGF levels in mice, rats and men of about 1 ng/L (Scheving *et al.*, 1989; Brazzel *et al.*, 1991; Grosvenor *et al.*, 1993). According to others, the presence of EGF in the milk and its absence in plasma or blood suggest a paracrine mode of action of this factor, that is synthesized locally, for instance in the udder (Donovan & Odle, 1994; Dehnhard *et al.*, 2000; Wiley *et al.*, 2003). In kidneys and the mammary gland, EGF is part of the big molecule of membrane-bound precursor, located extracellularly. In the sublingual gland of rodents, however, this precursor is entirely stored under the form of secretory granules in the cellular endoplasmatic reticulum and the process of formation of

EGF-like growth factors from it occurs intracellularly. Therefore, the pathways of EGF synthesis in the extracellular pool should be completely different. In the first instance, the precursor should be degraded by extracellular proteases whereas in the second one, EGF is released from secretory granules (Grosvenor *et al.*, 1993; Donovan & Odle, 1994; Marechal, 1999).

Despite the fact that EGF synthesis sites are not strictly defined in the different animal species and men, it could be assumed that other sites of synthesis that are not adequately studied (physiological fluids, tissues and organs) could also exist. This hypothesis of ours is based upon the contradictory and conflicting data as well as the species-related peculiarities, described in the available literature.

MOLECULAR STRUCTURE. EGF-LIKE FAMILY OF LIGANDS

In 1960, Stanley Cohen isolated a thermostable polypeptide from murine sublingual salivary glands that is responsible for the early development and the growth of incisors in newborns (Cohen, 1962). Later, having studied its primary structure, it was found out that it was a single chain of 53 amino acid residues (Carpenter & Cohen, 1979). Only three amino acids involved in protein biosynthesis (lysine, alanine and phenylalanine) are not included in its structure. The molecular weight of this polypeptide is 6045 Da and it is called epidermal growth factor (mEGF-mouse) because of its potent stimulating effect on epidermal proliferation and keratinization (Savage *et al.*, 1972; Carpenter & Cohen, 1979; Brazzel *et al.*, 1991; Lu *et al.*, 2001). The human epidermal growth factor (hEGF, urogastron) was isolated and identified by Gregory (1975). It possess a

molecular weight and amino acid sequence, identical to those of mEGF with the exception of the 16th amino acid, equal biological activity and potential for binding to the same membrane receptors (Brazzel *et al.*, 1991).

Human EGF is synthesized under the form of a soluble membrane-bound precursor containing 1207 amino acids, with molecular weight of about 140 kDa. This large molecule is a substrate of extracellular proteases that split its chain into peptides of various sizes (Grosvenor *et al.*, 1993; Marechal, 1999). The structure and the functional traits of proteolysis products are very similar to these of the EGF and make up the family of EGF-like ligands (Apella *et al.*, 1988; Marechal, 1999; Harris *et al.*, 2003). It comprises:

- heparin binding EGF-like growth factor (HB-EGF);
- transforming growth factor- α (TGF- α);
- amphiregulin (AR);
- epiregulin (EPR);
- betacellulin (BTC);
- neuregulins – new differentiation factor (NDF) or NRG-1; heregulin (HRG); acetylcholine receptor-inducing activity (ARIA) and glial growth factor (GGF), regulating the proliferation and differentiation of Schwann cells and oligodendrocytes in the nervous system.

The degree of homology among the different EGF ligands is the lowest outside the cysteine fragments forming the disulfide bridges and in some glycine residues, needed for the formation of the specific EGF domain (Xian, 2007).

TYPES OF RECEPTORS, INTERACTING WITH EGF-LIKE LIGANDS

EGF belongs to the group of peptide growth factors including also insulin, in-

sulin-like and platelet growth factors. Their receptors possess a tyrosine kinase activity, are located on cell's surface and are key regulators not only of normal pathways, but are essential for the development of numerous tumour growths (Pawson, 1995). The uncontrolled activity of these enzymes resulting from mutation or enhanced cell expression, could lead to various forms of neoplastic formations. More than 70% of known oncogenes and proto-oncogenes, involved in the pathogenesis of malignant growths, encode the synthesis of enzymes with protein kinase activity (Weiss & Schlessinger, 1998). In the human genome, about 29 genes coding 58 proteins with tyrosine kinase activity, part of the structure of these receptors, are identified (Robinson, 2001).

Depending on the type of the bound ligand, the receptors are grouped in 17 classes. Most of them are active as independent subunits and others perform their action by grouping in polymeric complexes. The extracellular region consists of a big protein domain that binds the respective ligand as growth factor or hormone. The intracellular C-terminus is responsible for the kinase activity of these receptors (Harris *et al.*, 2003; Zhang *et al.*, 2006).

The EGF-receptor (EGFR; ErbB-1; a.k.a. HER1 in men) consists of a single polypeptide chain (170 kDa, 1186 amino acid residues) and a significant number of N-bound oligosaccharides. The extracellular domain has a very high affinity to EGF-like ligands. Chemically, this part of the receptor contains 10–11 N-bound oligosaccharide residues, usually with a high content of cytokine residues (about 10%), that are able to form more than 25 disulfide bridges. It is believed that these regions participate in ligands binding (Carpenter & Cohen, 1990; Jorissen *et al.*,

2000; Lu *et al.*, 2001; Xian, 2007).

In men, three receptors with a structure, homologous to that of ErbB-1 are identified: ErbB-2/HER2-new (Schechter *et al.*, 1985), ErbB-3/HER3 (Kraus *et al.*, 1989) and ErbB-4/HER4 (Plowman *et al.*, 1993). There is about 40–50% similarity between the EGF-receptor and its three homologues, that varies in the different domains being the most constant at the tyrosine kinase residues – about 80% (Pelles & Yarden, 1993).

As a result of the binding of the receptor to the ligand at a 1:1 ratio (one molecule ligand to one molecule receptor), homo- or heterodimerization of receptors is occurring. A new complex, consisting of 2 ligand molecules and 2 molecules receptor is formed. Both intracellular domains of receptors are coming closer at a degree enough for auto- or transphosphorylation and thus, intracellular tyrosine kinase is activated with subsequent initiation of multiple cascades, one of them being the activation of Ras-Raf-mitogen-activated protein kinase (MAPK) (Popov, 1992; Dehnhard *et al.*, 2000; Jorissen *et al.*, 2000; Ogiso *et al.*, 2002; Goodsell, 2003; Xian, 2007). MAPK acts in several directions: it phosphorylates the ribosome protein S6, altering mRNK translation, passes from the cytoplasm into the nucleus where alters the transcription of genes involved in the duration of cell's life, affects the chromatin structure and changes the activity of transcription factors (Avruch *et al.*, 2001; Orton *et al.*, 2005).

PHYSIOLOGICAL ROLE OF EGF

In the middle of the 90-ties of the last century, the role of EGF in the regulation of growth, the maturation, function and maintenance of the epithelial tissue (par-

ticularly that of the mammary gland and the gastrointestinal tract) and the nervous system, is definitely understood. The physiological roles of EGF are as follows:

- maintains of intestinal maturation and function in neonates – supports the functional maturation of intestine for absorption of nutrients, its growth and development;
- regulates the nature of body's structure elements: promotes the epithelial tissue growth, decreases fat content and the muscle mass;
- regulates the supply of nutrients in milk – promotes the growth and the development of the mammary gland, regulates the lactation (quantity and quality of milk);
- reduces the plasma concentrations of proteins, responsible for the binding and transportation of insulin-like growth factor-1 (IGFBP-3), reduces also plasma IGF-1 levels;

In supraphysiological concentrations, EGF causes retarded growth via delayed body development in the postnatal period, disproportionate growth etc. (Xian, 2007).

In the alimentary tract, apart from the salivary glands, EGF is directly secreted in intestinal lumen from cells of different origin: the cells of Bruner's glands, Panet and goblet cells (Scheving *et al.*, 1989; Kelly *et al.*, 1997). It is relatively stable in acid medium and passes through the stomach of suckling animals almost intact. Its destruction in the stomach of rats during the suckling period is insignificant, but after the weaning, the EGF breakdown in the small intestine lumen occurs 12 times more intensively (Britton *et al.*, 1988). Furthermore, it is resistant to the action of pancreatic proteolytic enzymes as well (Hardin *et al.*, 1999).

EGF stimulates gastrin secretion and is a potent, specific and direct inhibitor of

H⁺ secretion from the parietal cells of the fundic glands, without influencing the alkaline secretion from the duodenum and the pancreas (Konturek *et al.*, 1984; Ford *et al.*, 1997).

It enhances the proliferation of the goblet cells in rabbits, that results in increased barrier function of the intestinal mucosa, and reduction of cases of bacterial translocation that are in the background of gastrointestinal disorders in the weaning period (Okuyama *et al.*, 1998; Go *et al.*, 1994).

Together with somatotropin, EGF increases the transport of glutamine in enterocytes from the small intestinal lumen in rabbits and men (Salloum *et al.*, 1993; Ray *et al.*, 2003; Wolfgang *et al.*, 2003). Being a primary energy source for enterocytes, glutamine is metabolized to α -ketoglutarate, and then is completely oxidized in the Krebs' cycle, yielding 30 mols ATP from 1 mol glutamine (Souba *et al.*, 1985; Yang *et al.*, 2000). The transport protein for this amino acid is stored in the vesicles of the brush border of enterocytes in the jejunum (Salloum *et al.*, 1993). EGF activates the intracellular protein kinase-C and MAPK. This results in increased mRNK levels, involved in the synthesis of the transport protein and consequently, the number of transport molecules increases. Thus, the transportation of glutamine is enhanced without influencing the affinity of the carrier molecule to the transported molecule (Salloum *et al.*, 1993; Wolfgang *et al.*, 2003). The increased transport of glutamine is necessitated not only in higher energy demands, but also from its role of precursor in the biosynthesis of proteins and nucleotides in cells of the small intestine (Salloum *et al.*, 1993; Reeds *et al.*, 2000; Yang *et al.*, 2000).

EGF stimulates the transport of nutrients by increasing the activity of Na⁺/K⁺-ATP-ase in the basolateral surface of enterocytes. Some authors designate EGF as a potent stimulator of glucose absorption in small intestine (Bird *et al.*, 1996). The transporter molecules involved in glucose transport, are: SGLT1 (sodium/glucose co-transporter, member 1, involved in active glucose transport) – located on the microvilli membrane in the brush border of the apical part of enterocytes and GLUT2 (glucose transporter, type 2, involved in passive glucose transport) – in the basal membrane of enterocytes. EGF contributes to the inclusion of transporters in these membranes and thus, exerts its stimulating effect upon glucose transport in small intestine. The degree of increase in the glucose transport under the effect of EGF is dose-dependent and is not influenced by the route of its application (enteral or parenteral) (Schwartz & Storozuk, 1988), but it is found out that with advancing of age, EGF diminishes the small intestinal glucose absorption (Bird *et al.*, 1994).

At the same time, EGF increases the activity of several enzymes (γ -glutamyl transferase and α -glucosidase), located in the enterocytic brush border (microvilli), that presumes its intricate role in increasing the transport of nutrients at the level of enterocytes (Goodlad *et al.*, 1991; Hardin *et al.*, 1999). It was found out, that in 3-day-old pigs, EGF increases the activity of sucrase and maltase by the time when the newly formed enterocytes begin to accumulate these enzymes in the brush border zone (James *et al.*, 1987). After intraperitoneal application for 15 days in suckling rabbits, EGF increased the mass of the pancreas, its protein content and amylase activity (O'Loughlin *et al.*, 1985).

The EGF is directly related to the augmentation intensity of the absorption surface of the small intestine in two ways. From one side, it promotes the growth of intestinal mucous structures and the migration of enterocytes from the crypts to the villi' surface (Donovan & Odle, 1994; Hardin *et al.*, 1999; Cummins & Thompson, 2002), and from the other, it participates directly in the elongation of functional microvilli in the apical part of the plasmatic membrane, resulting in rapid increase in the surface area of the enterocytic brush border (Hardin *et al.*, 1999).

Some authors have named the continuously secreting EGF in small intestine lumen "luminal surveillance peptide" that is essential in stimulation of the recovery after an injury (Playford, 1995).

APPLICATION OF EGF AND EGF-LIKE LIGANDS IN MEDICINE

During the last 15–20 years, the interest of numerous researchers has been focused on the application of EGF in medicine in the treatment of a number of illnesses.

Due to the fact, that EGF and EGF-like ligands bind the same receptors in target cells, the fields where scientists are looking for application of these substances in medicine and that are about to be approved, are the following:

- *skin diseases* – in wound healing (Rayner *et al.*, 2000), psoriasis (Poulin *et al.*, 2005);
- *alimentary tract diseases* – notably, colon carcinoma, ulcerous colitis and proctitis (Farrell, 2003); small intestine inflammatory diseases, postoperative therapy following massive small intestine resection in combination with somatotrophic hormone (Playford *et al.*, 2004; Avissar *et al.*, 2005), in children

with congenital atrophy of enterocytic microvilli (Acosta & Lizama, 1998);

- *bone disorders* (osteoporosis – combined with IGF-1, IGF-2, amphiregulin) (Xian, 2007);
- as *cytoprotectors*, taking into account that on its own, EGF is not initiating malignant transformations of cells and that in *in vitro* studies, growth factors are shown to reduce the toxic effects of some antitumour preparations (Sonis *et al.*, 1992; Acosta & Lizama, 1998; Taylor *et al.*, 2001).

The parenteral application of EGF in pharmacological doses has some effects that could be extremely useful for prevention or therapy in numerous pathologies of the internal organs. It was established that in patients with active gastric and duodenal ulcers, the concentration of endogenous EGF in the gastric secretion was considerably lower (Acosta & Lizama, 1998). On the other side, it was reported that the increased EGF secretion and expression of EGF-receptors in the stomach was a part of an adaptive cytoprotective mechanism directed against various irritating and necrotizing agents (Brzozowski *et al.*, 1996). Therefore, endogenous EGF seems to be important for the recovery of rats with indomethacin-induced gastric ulcer, because the removal of their sublingual glands delays the recovery and the restoration of the epithelium (Skov-Olsen *et al.*, 1986).

Numerous studies have evidenced that when the stomach was intact, EGF had no effect upon its acid secretion. In a study of histamine-induced gastric hyperacidity in monkeys however, it was shown that the intravenous administration of h-EGF and its derivative h-EGF 1-48 at a dose of 1 nmol/kg, inhibited significantly the gastric acid secretion, whose peak after application was achieved by the 90th (for EGF)

and the 60th min (for h-EGF 1-48), respectively (Guglietta & Lesch, 1993).

Regardless of the achieved success in the treatment of various disorders through application of EGF and EGF-like ligands, it should be acknowledged that many investigators are still continuing to quest expanding the spectrum of medical pathologies, where growth factors could be applied.

In conclusion it could be assumed, that at this time, there are no data about the application of EGF in the available veterinary medical literature. The studies on this factor are focused on investigations of its effects in laboratory animals and assay of its blood plasma and milk concentrations as well as on its influence on the postnatal development of neonates. Furthermore, the problem with the physiological effects of EGF in pets (dogs, cats, etc.) is not sufficiently studied that provides a field for future investigations. Extensive studies on the physiological role of EGF could be also performed in various domestic animal species (sheep, goats, swine), where the available information is not adequate.

In the light of the physiological importance of growth factors for the normal development of individuals during the postnatal period, detailed investigation on their mechanisms of action would contribute to the optimization of technologies in intensive animal rearing systems.

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