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Enkephalinase (EC 3.4.24.11) — Neutral Endopeptidase (NEP) — Characterization, Distribution and Role in the Human Gastrointestinal Tract

Enkefalinaza (EC 3.4.24.11) — neutralna endopeptydaza — charakterystyka, występowanie i rola w przewodzie pokarmowym człowieka

Enkephalinase, designated also as neutral endopeptidase (NEP) or endopeptidase 24.11 is one of numerous peptidases present in mucosal epithelial cells of gastrointestinal tract.

ENZYME STRUCTURE AND CELLULAR LOCALISATION

Enkephalinase is a membrane-bound metalloendopeptidase containing in its molecular structure one atom of zinc (6). It displays homology with aminopeptidases A, B and E also that with bacterial thermolysin (31). The enzyme posseses a single trans-membrane spanning domaine near the N-terminal of the molecule and an extracellular domain that comprises most of the protein mass (7). Human enkephalinase displays high homology with animal enkephalinase; its molecular cloning and amino acid sequence was established (23). Neutral endopeptidase consists of 750 amino acids, containing a short cytoplasmic domain (27 amino acids), a single membrane-spaining segment with 23 amino acids and the large extracellular domain. The cellular topography of the enzyme is very asymmetric, resembling the topography of two other brush-border peptidases: sucrase-isomaltase and gamma-glutamylo-transpeptidase (7). Enkephalinase is expressed in the apical part of the cell and it is not present in its baso-lateral part (16).

Enkephalinase, similarly to other metallopeptidases, possesses the maximal activity in neutral *pH* and it is blocked by phosphoramidon (15), but its most selective inhibitors are: thiorphan, acetorphan and sinorphan (28).

ENZYME SUBSTRATE SPECIFICITY

The first substrates characterized for enkephalinase were endogenous opioid pentapeptides: Met-enkephaline and Leu-enkephaline, two neurotransmitters present in central nervous system — CNS (21). The enzyme has been designated "enkephalinase" thanks to its capacity to cleave the amine bound Gly^3-Phe^4 of

enkephalins. However, at the present moment, there are many peptide compounds, acting as neurotransmitters, neurohormones or paracrinic modulators, being the substrates for enzyme action to hydrolize the amine bounds of hydrophobic residue, independent of their different chemical structure (22). Initially, the Enzyme Commission proposed for enkephalinase the name "neutral proteinase" of kidney brush-border, but it was not appropriate because the enzyme is widely distributed in many other organs and systems. "Neutral endopeptidase" (NEP) or "endopeptidase 24.11" (EC 3.4.24.11) seems to be the best name, but "enkephalinase" is still the most popular in all of bibliography (24).

Enkephalinase does not display narrow substrate specificity for opioid peptides, but it can cleave other peptides, such as substance P (37), bradykinin (36), neurokinin A (25), neurotensin (25), luliberin (36), angiotensin I and angiotensin II (9), but in opposition to angiotensin converting enzyme (ACE) it does not convert angiotensin I to angiotensin II but it hydrolizes both of them to other products (3). The enkephalinase capacity to cleave chemotactic peptide and to hydrolize the thymic humoral factor gamma 2 was proved (15).

Recently demonstrated enkephalinase ability to inactivation of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) is very important in the clinical practice. This discovery can find the application in the therapy of hypertonic disease and in the regulation of electrolytes and water balance (32).

Endothelins — the peptides produced in endothelial cells and possessing very potent vasoconstrictor activity are also the enkephalinase's substrates (30).

Among gastrointestinal peptides regulating secretion or motility of the digestive system there are numerous compounds cleaved by enkephalinase: gastrin releasing peptide, also designated as bombesine (5), vasoactive intestinal polypeptide — VIP (14), gastrin or cholecystokinin (4).

DISTRIBUTION OF ENKEPHALINASE

Enkephalinase in as enzyme widely distributed in the human and animal tissues. It was originally purified from rabbit kidney, where it is extremely abundant (18).

The central nervous system (CNS) is also a very rich source of this enzyme. Using the methods with ^{125}I- labelled monoclonal antibodies or with ^{3}H- labelled specific inhibitors, or fluorescent histochemical localization, the enzyme has been localized to neural cell bodies, cell processes or terminal synapses in the brain and medulla (20). The antinociceptive responses after enkephalinase inhibitors administration proved its participation in the mechanisms of analgesia (12, 21).

Enkephalinase activity has been described in the airways epithelium (20), synovial fibroblasts (17), in placenta and pregnant uterus (27), in human plasma, neutrophils and leukemic lymphocytes and also in progenitors of B and T cells (2).

ENKEPHALINASE IN THE GASTROINTESTINAL TRACT

The digestive tract is also a very rich source of enkephalinase. It is present in the salivary glands, stomach, pancreas and intestinal brush-border of the rat (29), guinea pig (26), dog, pig (11) and human (13).

The enkephalinase studies in human are relatively recent during the last fifteen years. However, little is known about the distribution of enkephalinase in human peripheral organs, results published by certain authors being very fragmentary and isolated (20).

In the light of all this information, this is still very difficult and complicated problem to determine the role of neutral endopeptidase in the digestive tube, where its activity is relatively strong, although varies considerably from one part to the other.

In our own experimental results, carried on the isolated epithelial cells from different parts of human digestive tract, using the labelled ^{3}H-D-Ala^{2}, Leu^{5}--enkephalin as substrate and specific enkephalinase inhibitor L-Thiorphan (20), the highest enzymatic activity was observed in the duodenal and intestinal epithelium, with gradual decrease into lower parts of the gastrointestinal tube (33). Enkephalinase activity in the stomach and colon was similarly low. Similar enkephalinase activity distribution in rat digestive tract was described by Pollard et al. (29).

This enzymatic activity distribution, with the highest values in the gastrointestinal tract segments considered as the most potent in proteolytic action suggests, that enkephalinase could constitute the alternative intestinal pathway of extracellular hydrolysis of proteins and peptides instead of the pancreatic insufficiency, according to the model proposed in 1988 by Difu Guan et al. (13) for other similar neutral intestinal proteases.

Studies on the enkephalinase expression during ontogeny of digestive tract demonstrated its presence during the early gestation period in human and different experimental animals (19). Enkephalinase activity in the rat ileum in terminal gestation stage, at the 19th day, was almost twice as that observed in the adult rat (34). In suckling animals this activity is still increasing (19). This fact can also confirm the hypothesis about enkephalinase involvement in terminal process of hydrolysis of smaller peptides, in this situation coming from partially digested milk proteins (13, 19).

Besides, the enkephalinase role in digestive system can be involved in its indirect effect on physiological regulation of the secretory and motoric functions

by degradation of numerous biologically active peptides and peptide hormones, as cholecystokinin, bombezine, VIP or enkephalins. Distribution of the opioid receptors in the gastrointestinal tract corresponds to the enkephalinase activity distribution (10).

The earliest studies on the role of enkephalins in central nervous system showed their involvement in the antinociceptive responses. Enkephalinase doses decrease or abolish this analgetic effect of enkephalins (12, 21). Subsequently it was proved that long term opiates administration increases activity of the enzyme degrading enkephalins and this finding has decided the application of enkephalinase inhibitors in the analgetic therapy (12).

The peripheral effect of enkephalins action, also known very well, is their antidiarrheal response. It results in the decrease of myoelectric activity of ileal smooth muscle, which increases the ileal transit and the absorption of Na^+ and Cl^- ions in the gut (1). The prevention of endogenous opioids degradation at peripheral sites by enkephalinase inhibition may be very effective pharmacological application.

Enkephalinase can also hydrolize kinins, especially bradykinine and kallidine — particularly strongly acting on the mammalian gastrointestinal tract and simulating intestinal muscular contraction and secretion of sodium and chloride ions (36). A spectacular example of this action is an inhibitory effect of enkephalinase on substance P-induced contraction in gut smooth muscle [8].

Enkephalinase does not display narrow substrate specificity, so there are still many various possibilities of interactions in the frame of degradation of regulating gastrointestinal peptides, unknown so far in the digestive system.

REFERENCES

1. Binder H. J. et al.: Role of opiate receptors in regulation of enkephalin stimulation of active sodium and chloride absorption. Am. J. Physiol. **247**, G432, 1984.
2. Beaumont J. et al.: Neutral endopeptidase 24.11 and angiotensin converting enzyme like activity in CALLA positive and CALLA negative lymphoid cells. Biochem. Biophys. Res. Com. **160**, 1323, 1989.
3. Bunnett N. W. et al.: Catabolism gastrin releasing peptide and substance P by gastric membrane-bound peptidases. Peptides **6**, 277, 1985.
4. Bunnett N. W. et al.: Metabolism of gastrin and cholecystokinin by endopeptidase 24.11 from the pig stomach. Am. J. Physiol. **255**, G676, 1988.
5. Bunnett N. W. et al.: Isolation of endopeptidase 24.11 (EC 3.4.24.11 „Enkephalinase") from the pig stomach. Gastroenterology **95**, 952, 1988.
6. Danielsen E. M. et al.: A neutral endopeptidase in the microvillar membrane of pig intestine. Biochem. J. **191**, 645, 1980.
7. Devault A. et al.: Amino acid sequence of rabbit kidney neutral endopeptidase 24.11 (enkephalinase) deduced from a complementary DNA. EMBO Journal **6**, 1317, 1987.
8. Djokic T. D. et al.: Neutral endopeptidase inhibitors potentiate substance P-induced concentration in gut smooth muscle. Am. J. Physiol. **256**, G39, 1989.

9. Erdos E. G., Skidgel R. A.: Renal metabolism of angiotensin I and II. Kidney Int. Suppl. **30**, S24, 1990.
10. Ferri G-L. et al.: VIP, substance P and met-enkephalin-immunoreactive invertation of the human gastroduodenal mucosa and Brunner's gland. Gut **25**, 948, 1948.
11. Gee N. S. et al.: An immunoradiometric assay for endopeptidase 24.11 shows it to be a widely distributed enzyme in pig tissues. Biochem. J. **228**, 119, 1985.
12. George S. R. et al.: Endogenous opioids are involved in the genetically determined high preference for ethanol consumption. Alcohol Clin. Exp. Res. **15**, 668, 1991.
13. Guan Difu et al.: Protein digestion in human and rat small intestine: role of new neutral endopeptidases. Am. J. Physiol. **255**, G212, 1988.
14. Hachisu M. et al.: Enzymatic degradation of helodermin and vasoactive intestinal polypeptide. J. Pharmacobiodyn. **14**, 126, 1991.
15. Indig F. E. et al.: Hydrolysis of thymis humoral factor gamma 2 by neutral endopeptidase (EC 3.4.24.11). Biochem. J. **231**, 357, 1991.
16. Jalal F. et al.: Neutral endopeptidase, a major brush-border protein of the kidney proximal nephron, is directly targeted to the apical domain when expressed in Madin-Darby canine kidney cells. J. Biol. Chem. **266**, 19826, 1991.
17. Johnson A. R. et al.: Neutral endopeptidase from nuchal ligament of fetal calves. J. Cell. Biochem. **43**, 243, 1990.
18. Kerr M. A., Kenny A. J.: The purification and specificity of a neutral endopeptidase from rabbit kidney brush-border. Biochem. J. **137**, 477, 1974.
19. Lecavallier H. et al.: Presence of endopeptidase 24.11 during the ontogeny of the small intestine in different species. Biol. Neonate **56**, 342, 1989.
20. Llorens C. et al.: Dipeptidyl carboxypeptidase (enkephalinase) activity: selective radioassay, properties and regional distribution in human brain. J. Neurochem. **39**, 1081, 1982.
21. Malfroy B. et al.: High-affinity enkephalin-degrading peptidase in brain is increased after morphine. Nature **276**, 523, 1978.
22. Malfroy B., Burnier J.: New substrates for enkephalinase (neutral endopeptidase) based on fluorescent energy transfer. Biochem. Biophys. Res. Com. **143**, 58, 1987.
23. Malfroy B. et al.: Molecular cloning and amino acid sequence of human enkephalinase neutral endopeptidase). FEBS Letters **229**, 206, 1988.
24. Matsas R. et al.: Substance P and (leu)enkephalin are hydrolized by an enzyme in pig caudate synaptic membranes that is identical with the endopeptidase of kidney microvilli. Proc. Natl. Acad. Sci. USA **80**, 3111, 1983.
25. Nau R. et al.: Proteolytic inactivation of substance P and neurokinin A in the longitudinal muscle layer of guinea pig small intestine. J. Neurochem. **47**, 856, 1986.
26. Nishimura E. et al.: Autoradiographic localisation of opioid receptors in the gastrointestinal tract of the rat and guinea pig. Gastroenterology **81**, 1084, 1986.
27. Ottlecz A. et al.: Neutral metalloendopeptidase associated with the smooth muscle cells of pregnant rat uterus. J. Cell Biochem. **45**, 401, 1991.
28. Pollard H. et al.: Characterisation of two probes for the localisation of enkephalinase in rat brain: (3H) Thiorphan and a^{125} I-labelled monoclonal antibody. Eur. J. Pharmacol. **133**, 155, 1987.
29. Pollard H. et al.: Immunoautoradiographic localisation of enkephalinase (EC 3.4.24.11) in rat gastrointestinal tract. Neuropeptides **19**, 169, 1991.
30. Pollock D., Opgenorth T. J.: Evidence for metalloprotease involvement in the *in vivo* effects of big endothelin 1. Am. J. Physiol. **261**, R257, 1991.
31. Rocques B. P. et al.: Complete differentiation between enkephalinase and angiotensin-converting enzyme inhibition by retro-thiorphan. PNAS, USA **80**, 3178, 1983.

32. Shepperson N. B. et al.: Inhibition of neutral endopeptidase (EC 3.4.24.11) leads to an atrial natriuretic factor-mediated natriuretic, diuretic and antihypertensive responses in rodents. Clin. Biol. **80**, 265, 1991.
33. Skrzydło-Radomańska B. et al.: Activity of enkephalinase in different parts of human digestive system. Acta Physiol. Pol. 41 Suppl. **34**, 265, 1990.
34. Skrzydło-Radomańska B. et al.: Enkephalinase activity in the intestinal epithelial cells of the fetus of 19 days and their immortalized and transformed counterparts the SLC-cells lines. J. Physiol. Pharmacol. **44**, 65, 1993.
35. Spillantini M. G. et al.: Characterization of endopeptidase 3.4.24.11 ("enkephalinase") activity in human plasma and cerebral fluid. Biochem. Pharmacol. **39**, 1353, 1990.
36. Stephenson S. L., Kenny A. J.: The metabolism of neuropeptides. Hydrolysis of peptides by the phosphoramidonintensitive rat kidney enzyme "endopeptidase-2" by rat microvillar membranes. Biochem. J. **255**, 45, 1988.
37. Tramontana M. et al.: The effect of thiorphan on relase of sensory neuropeptides from guinea-pig cerebral venous sinuses. Pharmacol. Res. **23**, 285, 1991.

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STRESZCZENIE

Enkefalinaza, zwana także neutralną endopeptydazą (NEP), jest enzymem związanym z błoną komórkową, szeroko rozpowszechnionym w mózgu i tkankach obwodowych: nerkach, drogach oddechowych, we krwi i w przewodzie pokarmowym człowieka oraz różnych gatunków zwierząt. Największą aktywność enkefalinazy w obrębie przewodu pokarmowego stwierdzono w dwunastnicy i jelicie cienkim. Bierze ona udział w końcowych procesach hydrolizy białek oraz w degradacji biologicznie aktywnych peptydów regulujących funkcje układu trawienia.