

# Neuropeptidases

## Key References

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## Overview

The term 'neuropeptidases' refers to those enzymes that participate in the inactivation of synaptically released neuropeptides and therefore serve to turn off the generated peptide signal. In general, these enzymes are integral plasma membrane proteinases located as ectoenzymes. Some of the enzymes listed in the accompanying Tables are able to hydrolyze neuropeptides, but are present as cytosolic or secreted enzymes, or not present as ectoenzymes. Their physiological roles are therefore not fully established.

To date, only a relatively small group of CNS peptidases have been characterized as neuropeptide-inactivating ectoenzymes. The majority are zinc metalloenzymes for which specific and potent inhibitors have generally been developed. Some of these are found in abundance elsewhere, especially the renal and intestinal brush border membrane, but others appear to be more specific to the nervous system, e.g. pyroglutamyl aminopeptidase II. Neprilysin (NEP) is the prototype neuropeptidase originally discovered in the CNS as an enkephalin-degrading activity and subsequently as a substance P-hydrolyzing enzyme. It is appropriately located on neuronal membranes especially in the striatonigral pathway. Thus, it can act in an analogous manner to that of acetylcholinesterase at cholinergic terminals. Like several other neuropeptidases, it is also present in cells of the immune system where it may hydrolyze immunoregulatory peptides. *In vivo*, NEP is a broadly specific enzyme hydrolyzing a wide range of susceptible peptide substrates (enkephalins, substance P, atrial natriuretic peptide) as is its close homolog NEP II or secreted endopeptidase (SEP). Some other neuropeptidases appear to be much more sub-

strate-specific. For example, pyroglutamyl amino-peptidase II appears to hydrolyze thyrotropin releasing hormone (TRH) exclusively. The unusual peptidase, glutamate carboxypeptidase II (GCP II) specifically inactivates the peptide neurotransmitter, N-acetylaspartylglutamate (NAAG) and GCP II inhibition can protect against some forms of neuronal death and may have applications in treatment of neuropathy.

Studies of changes in levels of neuropeptides in neurological disease have been limited in extent and no consistent pattern yet emerges. Likewise, factors regulating the expression of neuropeptidases both in the normal and the pathological state in the nervous system are little explored. Inhibitors of neuropeptidases are useful both as pharmacological tools in studies of neuropeptide physiology and also as potential therapeutic agents. Selective inhibitors of these enzymes have, to date, been obtained from natural products (e.g. phosphoramidon as NEP and endothelin converting enzyme 2 inhibitor) or designed (e.g. thiorphan selective for NEP) by analogy with similar enzymes from bacterial or other sources (e.g. thermolysin). Dual peptidase inhibitors (e.g. of NEP and angiotensin converting enzyme (ACE)) are increasingly finding favor as potential new therapeutics. Design of ACE, NEP and neurolysin inhibitors, in particular, will be aided by their recently solved three-dimensional structures.

A particular role for several neuropeptidases (especially NEP) has recently emerged in the turnover of the amyloidogenic A $\beta$ -peptide in Alzheimer's disease and age-dependent loss of NEP in the brain may contribute to the pathology. Strategies to up-regulate these CNS peptidases may

therefore prove beneficial but raises some concerns with regard to potential side effects of their chronic inhibition.

We can expect a rapid expansion of peptidase discovery and characterization in the coming years. For example, genome sequencing studies reveal that there are probably 8-10 NEP-like enzymes in the human genome and 24 in *Drosophila*. The table below describes the best characterized neuropeptidases to date. This includes a novel homolog of angiotensin converting enzyme (ACE2), not inhibited by classical ACE inhibitors, which appears to counterbalance the actions of ACE through its ability to convert angiotensin II to angiotensin-(1-7). Like several ectopeptidases, ACE2 serves serendipitously as a viral receptor, in this case for the severe acute respiratory syndrome (SARS) virus.

# Neuropeptidases

## Endopeptidases

<b>NAME</b>	Neprilysin (EC 3.4.24.11)	Thimet oligopeptidase (EC 3.4.24.15)	Neurolysin (EC 3.4.24.16)	Prolyl oligopeptidase (EC 3.4.21.26)
<b>ALTERNATE NAME</b>	Endopeptidase-24.11, Neutral endopeptidase, Enkephalinase, CALLA, CD10	Endopeptidase-24.15, Endo-oligopeptidase A	Endopeptidase-24.16, NT degrading endopeptidase, Oligopeptidase M	Post-proline cleaving enzyme, TRH deamidating enzyme
<b>TYPICAL SUBSTRATES <i>In vitro</i></b>	Leu- ( <b>L9133</b> ) and Met-Enkephalin ( <b>M6638</b> ), ANP ( <b>A1663</b> (h), <b>A8208</b> (r)), ET-1 ( <b>E7764</b> ), ET-2 ( <b>E9012</b> ), ET-3 ( <b>E9137</b> ), CCK ( <b>C2175</b> ), NT ( <b>N6383</b> ), SS ( <b>S9129</b> , <b>S1763</b> ), SP ( <b>S6883</b> ), NKA ( <b>N4267</b> ), NKB ( <b>N4143</b> ), Amyloid $\beta$ -peptide	AI ( <b>A9650</b> (h), <b>A2928</b> (s)), All ( <b>A9525</b> (h)), BK ( <b>B3259</b> ), LHRH ( <b>L7134</b> (h), <b>L4897</b> (s)), NT ( <b>N6383</b> ), SS ( <b>S9129</b> , <b>S1763</b> ), Nociceptin/Orphanin FQ ( <b>O4011</b> )	AI ( <b>A9650</b> (h), <b>A2928</b> (s)), All ( <b>A9525</b> (h)), BK ( <b>B3259</b> ), NT ( <b>N6383</b> ), SP ( <b>S6883</b> ), SS ( <b>S9129</b> , <b>S1763</b> )	AI ( <b>A9650</b> (h), <b>A2928</b> (s)), All ( <b>A9525</b> (h)), BK ( <b>B3259</b> ), LHRH ( <b>L7134</b> (h), <b>L4897</b> (s)), NT ( <b>N6383</b> ), SP ( <b>S6883</b> ), TRH ( <b>P1319</b> , <b>P2161</b> ), vasopressin ( <b>V0377</b> ), oxytocin ( <b>O3251</b> , <b>O4375</b> )
<b>PROTEINASE CLASS</b>	Metallo (Zn <sup>2+</sup> )	Metallo (Zn <sup>2+</sup> )	Metallo (Zn <sup>2+</sup> )	Serine
<b>TYPICAL INHIBITORS</b>	Phosphoramidon ( <b>R7385</b> ), Thiorphan ( <b>T6031</b> )	CPP-Ala-Ala-Tyr-pAB, CPE-Ala-Ala-Phe-pAB, Phosphodiepryl 21	Phosphodiepryl 03, Pro-Ile, Phosphodiepryl 22, Phosphodiepryl 33	Cbz-Pro-Prolinal
<b>SUBCELLULAR LOCALIZATION</b>	Plasma membrane	Soluble and (membrane ?) (nuclear ?)	Soluble, plasma membrane (mitochondrial)	Soluble
<b>TISSUE DISTRIBUTION</b>	Kidney, intestine, lung, reproductive tissue, brain	Ubiquitous, esp. testis	Kidney, brain, liver	Widely distributed
<b>PHYSIOLOGICAL EFFECTS</b>	Blood pressure, pain regulation, anti-inflammatory, antidiarrhoeic	Vascular physiology, reproductive physiology, immune surveillance	Modulation of neurotensin physiology	Learning and memory peptide digestion
<b>DISEASE RELEVANCE</b>	Cardiovascular, cancer (esp. prostate), Alzheimer's disease	Hypertension	Not known	Cognitive disorders, neurological disease

## Aminopeptidases

<b>NAME</b>	Aminopeptidase N ( <b>L6007</b> , <b>L9776</b> , <b>L5006</b> ) (EC 3.4.11.2)	Aminopeptidase A (EC 3.4.11.7)	Aminopeptidase B (EC 3.4.11.6)	Aminopeptidase P (EC 3.4.11.9)
<b>ALTERNATE NAME</b>	Aminopeptidase M; CD13 membrane alanyl aminopeptidase	Aspartate or glutamyl aminopeptidase, angiotensinase A BPI/6C3 antigen	Aminopeptidase MI? arylamidase II, arginine- aminopeptidase	X-Pro aminopeptidase, Aminopeptidase P2 ?
<b>TYPICAL SUBSTRATES <i>In vitro</i></b>	Leu- ( <b>L9133</b> ) and Met-Enkephalin ( <b>M6638</b> ), $\gamma$ - ( <b>E6386</b> ) and $\beta$ -endorphin ( <b>E6261</b> (h), <b>E0637</b> (b), <b>E1142</b> (r)) AllI	AI ( <b>A9650</b> (h), <b>A2928</b> (s)), All ( <b>A9525</b> (h)), CCK8	Leu- ( <b>L9133</b> ) and Met-Enkephalin ( <b>M6638</b> ), BK ( <b>B3259</b> ), LTA4, SRIF ( <b>S9129</b> )	BK ( <b>B3259</b> ), SP ( <b>S6883</b> ), PYY ( <b>P1306</b> (h), <b>P5801</b> (p)), enterostatin ( <b>E2643</b> (h)) NPY ( <b>N5017</b> (h), <b>N3266</b> (p), <b>N6269</b> (sh))
<b>PROTEINASE CLASS</b>	Metallo (Zn <sup>2+</sup> )	Metallo (Zn <sup>2+</sup> )	Metallo (Zn <sup>2+</sup> ), Thiol Cl <sup>-</sup> activated	Metallo (Zn <sup>2+</sup> )

## FOOTNOTES

## Neuropeptidases

<b>TYPICAL INHIBITORS</b>	Amastatin ( <b>A1276</b> ), Bestatin ( <b>B8385</b> ), Actinonin ( <b>A6671</b> ), PC18	Amastatin ( <b>A1276</b> ), EC33	Arphamenine A ( <b>A2302</b> ), Arphamenine B ( <b>A2177</b> ), bestatin ( <b>B8385</b> ), lysine thiol	Apstatin ( <b>A1395</b> )
<b>SUBCELLULAR LOCALIZATION</b>	Plasma membrane	Plasma membrane	Soluble, secreted, Golgi	Plasma membrane, GPI-anchored, cytosolic
<b>TISSUE DISTRIBUTION</b>	Abundant in kidney, intestine, liver, placenta, brain, lung, hematopoietic (myeloid) cells, vascular and endothelial cells	Lung, kidney, placenta, intestine, brain hematopoietic cells, endothelial and epithelial cells	Widely distributed, esp. kidney, intestine, lung, heart, brain, pituitary pancreas, testis	Kidney, liver, intestine, lung serum, vascular endothelial cells, hematopoietic cells
<b>PHYSIOLOGICAL EFFECTS</b>	Pain regulation, T cell development, angiogenesis, viral receptor (coronavirus 229E) glutathione metabolism	Blood pressure regulation tumor progression/metastasis ?	Inflammation	Peptide digestion, blood pressure regulation
<b>DISEASE RELEVANCE</b>	Cancer, metastasis, autoimmune disease	Hypertension, cancer	Inflammatory disorders	Cardiovascular disease

### Aminopeptidases (continued)

<b>NAME</b>	Dipeptidylpeptidase IV ( <b>D7052</b> ) (EC 3.4.14.5)	Pyroglutamyl aminopeptidase II (EC 3.4.19.6)	Tripeptidyl peptidase III
<b>ALTERNATE NAME</b>	Post-proline dipeptidyl aminopeptidase, CD26, gp110	TRH degrading ectoenzyme	CCK degrading enzyme
<b>TYPICAL SUBSTRATES</b> <i>In vitro</i>	SP ( <b>S6883</b> ), PYY ( <b>P1306</b> (h), <b>P5801</b> (p)), NPY ( <b>N5017</b> (h), <b>N3266</b> (p), <b>N6269</b> (sh)), Enterostatin ( <b>E2643</b> (h)), glucagon-like peptide-1 ( <b>G3265</b> (h)), glucagons ( <b>G1774</b> )	TRH ( <b>P1319</b> , <b>P2161</b> )	CCK ( <b>C2175</b> )
<b>PROTEINASE CLASS</b>	Serine	Metallo (Zn <sup>2+</sup> )	Serine
<b>TYPICAL INHIBITORS</b>	Diprotin A ( <b>I9759</b> ), Diprotin B	Glp-Asn-Pro-Tyr-Trp-AMC	Butabindide
<b>SUBCELLULAR LOCALIZATION</b>	Plasma membrane	Plasma membrane	Plasma membrane cytosol
<b>TISSUE DISTRIBUTION</b>	Kidney, small intestine, liver, placenta, hematopoietic cells	Brain, pituitary, retina, lung, liver	Liver, brain, gut, erythrocytes
<b>PHYSIOLOGICAL EFFECTS</b>	Digestion, regulation of proliferation and differentiation of lymphocytes, regulation of glucose metabolism	Regulation of pituitary hormone secretion	Modulation of cholecystokinin physiology, protein degradation
<b>DISEASE RELEVANCE</b>	Diabetes, CNS trauma, epilepsy, spinocerebellar degeneration	Neuroendocrine disorders	Obesity ?

## FOOTNOTES

# Neuropeptidases

## Carboxypeptidases

<b>NAME</b>	NAALA dipeptidase (EC 3.4.17.21)	Carboxypeptidase H (EC 3.4.17.10)	Carboxypeptidase N (EC 3.4.17.3)	Carboxypeptidase P ( <b>C5396</b> ) (EC 3.4.16.2)	Peptidyl dipeptidase A ( <b>A2580, A6778</b> ) (EC 3.4.15.1)	Angiotensin converting enzyme-2
<b>ALTERNATE NAME</b>	NAAG hydrolase, prostate-specific membrane antigen (PSMA), glutamate carboxypeptidase II	Carboxypeptidase E, enkephalin convertase	Kininase I, arginine carboxypeptidase	Prolylcarboxypeptidase, angiotensinase C, Pro-X carboxypeptidase	Angiotensin I-converting enzyme; kininase II	ACE2 (ACEH)
<b>TYPICAL SUBSTRATES IN VITRO</b>	NAAG ( <b>A5930</b> )	Enkephalin hexapeptides, dynorphin (1-13) ( <b>D7017</b> ), BK ( <b>B3259</b> ), atriopeptin II ( <b>A9035</b> )	Enkephalin hexapeptides, dynorphin (1-13) ( <b>D7017</b> ), BK ( <b>B3259</b> ), atriopeptin II ( <b>A9035</b> )	All ( <b>A9525</b> (h)), [des-Arg <sup>9</sup> ]-bradykinin enterostatin ( <b>E2643</b> (h))	AI ( <b>A9650</b> (h), <b>A2928</b> (s)), BK ( <b>B3259</b> ), CCK ( <b>C2175</b> ), gastrin ( <b>G9020</b> (h), <b>G1276</b> (r)), Leu- ( <b>L9133</b> ) and Met-Enkephalin NT ( <b>N6383</b> ) ( <b>M6638</b> ), Ac-Ser-Asp-Lys-Pro (hemoregulatory peptide), NT ( <b>N6383</b> ), LH-RH ( <b>L8008, L4897</b> ), substance P ( <b>S6883</b> )	AI ( <b>A9650</b> (h), <b>A2928</b> (s)), All ( <b>A9525</b> (h)), [des-Arg <sup>9</sup> ]-bradykinin
<b>PROTEINASE CLASS</b>	Metallo	Metallo	Metallo (Zn <sup>2+</sup> )	Serine	Metallo (Zn <sup>2+</sup> )	Metallo (Zn <sup>2+</sup> )
<b>TYPICAL INHIBITORS</b>	Quisqualate ( <b>Q2128</b> )	GEMSA ( <b>50975</b> ), MGTA	MGTA	Z-Pro-Prolinal	Captopril ( <b>C4042</b> ) Lisinopril ( <b>L6292</b> ), Enalaprilat ( <b>616958</b> )	EDTA ( <b>ED255</b> ) MLN-4760
<b>SUBCELLULAR LOCALIZATION</b>	Plasma membrane	Membrane, secretory vesicles	Soluble	Lysosomal	Plasma membrane, soluble (plasma)	Plasma membrane
<b>TISSUE DISTRIBUTION</b>	Brain, intestine, prostate, kidney	Brain, pituitary, pancreas, neuroendocrine tissues	Liver, blood, (stomach, kidney, lung)	Brain, heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, leukocytes vascular endothelial cells	Lung, kidney, testis, intestine, brain, endothelial cells, blood	Kidney, heart, testis, intestine
<b>PHYSIOLOGICAL FACTORS</b>	Regulation of glutamate metabolism	Neuroendocrine physiology, pancreatic physiology muscle strength/coordination	Protection against anaphylaxis	Inflammation, vasoregulation	Cardiovascular regulation, fertility, water and salt balance, hematopoietic stem, cell proliferation	Cardiac physiology, receptor for SARS virus, fat uptake/metabolism (?)
<b>DISEASE RELEVANCE</b>	Neuroprotection, schizophrenia, prostate cancer (marker)	Obesity, diabetes	Angioedema	Septic shock	Hypertension, congestive diabetes, nephropathy	Cardiovascular disease, obesity, diabetes, SARS

### Abbreviations

**AI:** Angiotensin I  
**AI1:** Angiotensin II  
**AI11:** Angiotensin III  
**AMC:** 7-Amido-4-methyl coumarin  
**ANP:** Atrial natriuretic peptide  
**BK:** Bradykinin  
**CALLA:** Common acute lymphoblastic leukemia antigen  
**CCK:** Cholecystokinin  
**CD:** Cluster differentiation antigen  
**CPE:** Carboxy-phenyl ethyl  
**CPP:** Carboxy-phenyl propyl

**EDTA:** Ethylenediaminetetraacetic acid  
**EC33:** (S)-3-Amino-4-mercaptobutyl sulfonic acid  
**ET-1:** Endothelin-1  
**ET-2:** Endothelin-2  
**ET-3:** Endothelin-3  
**GEMSA:** Guanidinoethylmercaptosuccinic acid  
**GlP:** Pyroglutamyl  
**GPI:** Glycosylphosphatidylinositol  
**LHRH:** Luteinizing hormone-releasing hormone  
**MGTA:** 2-Mercaptomethyl-3-guanidinoethylthiopropionic acid

**MLN:** (S,S)-2-[1-Carboxy-2-[3-(3,5-dichlorobenzyl)-3H-imidazol-4-yl]-ethylamino]-4-methylpentanoic acid)  
**NAAG:** N-Acetyl-L-aspartyl-L-glutamate  
**NAALA:** N-Acetylated a-linked acidic dipeptidase  
**NKA:** Neurokinin A  
**NKB:** Neurokinin B  
**NPY:** Neuropeptide Y  
**NT:** Neurotensin  
**PC18:** 2-Amino-4-methylsulfonyl butane thiol  
**PYY:** Peptide YY  
**SARS:** Severe acute respiratory syndrome

**SP:** Substance P  
**SRIF:** Somatostatin  
**SS:** Somatostatin  
**TRH:** Thyrotropin releasing hormone  
**b:** bovine  
**h:** human  
**p:** porcine  
**r:** rat  
**s:** salmon  
**sh:** sheep