CHAPTER

20

Peptides

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OUTLINE

NEUROPEPTIDES

Many neuropeptides were originally identified as pituitary or gastrointestinal hormones

Probably the first neuropeptide to be identified was vasopressin, a nine-amino-acid peptide secreted by the nerve endings in the neural lobe of the pituitary. The source of the vasopressin is the magnocellular neurons of the hypothalamus, which send axons to the neurohypophysis; the peptides are then released into the blood in classic neurosecretory fashion. Like vasopressin, a number of gastrointestinal peptides, such as cholecystokinin (CCK), are also found at high concentrations in the nervous system. In the gastrointestinal system, CCK is secreted into the blood by the duodenum and governs the delivery of digestive enzymes and bile acids into the intestine. In contrast to vasopressin and CCK, the hypothalamic releasing factors are peptides released into a special portal blood system

that bathes the anterior pituitary, controlling the secretion of pituitary hormones (see Ch. 55). In this system, 'portal' means two successive capillary beds, one in the hypothalamus and one in the anterior pituitary. Substance P was first purified as a 'sialagogic peptide' causing salivation in a bioassay. Now substance P is recognized as a major bioactive peptide in many neuronal pathways, especially pain signaling. Since there are so many peptides, this chapter focuses on the principles of how neuropeptides are synthesized, stored and released and how they act on the cells they regulate. Comparisons among peptides and smaller, 'fast-acting', 'classical' or 'conventional' neurotransmitters will be emphasized. It is significant to note that the number of neuropeptides far exceeds the number of fast-acting conventional neurotransmitters.

Peptides can be grouped by structural and functional similarity

Although the list of neuropeptides is quite long, as seen in the partial listing in Figure 20-1, new neuropeptides are still being identified. Like GABA and glutamate or dopamine and norepinephrine, which differ by only a single carboxyl or hydroxyl group, yet have very different functions, many neuropeptides with similar structures have very different functions. Vasopressin and oxytocin are the two major neurohypophyseal peptides, and each consists of nine amino acids. These two peptides are identical at seven of those residues and are thought to be the result of gene duplication early in evolution. The actions of the two peptides are distinct: oxytocin causes milk letdown and uterine contraction, while vasopressin causes water retention in the kidney and blood vessel contraction. Likewise, the opiate peptides share a common Tyr–Gly–Gly–Phe–Met/Leu sequence at their NH_2-terminus and are potent endogenous opiates with distinct patterns of selectivity at the various classes of opiate receptor (see Ch. 61). The three glycoprotein hormones from the anterior pituitary, thyroid stimulating hormone (thyrotropin), luteinizing hormone and follicle-stimulating hormone, share a common α subunit but have distinct β subunits, and only the $\alpha\beta$ dimer is biologically active. The tachykinin group includes substance P and various frog skin peptides, all with similar core sequences and –Phe–X–Gly–Leu–Met–NH_2 at the COOH-terminus. The gastrointestinal peptides CCK and gastrin share a common COOH-terminal sequence (Trp–Met–Asp–Phe–NH_2) and are among the few peptides that undergo tyrosine sulfation.

Selected Bioactive Peptides

Hypothalamic releasing factors
- CRH: corticotropin releasing hormone
- GHRH: growth hormone releasing hormone
- GnRH: gonadotropin releasing hormone
- Somatostatin
- TRH: thyrotropin releasing hormone

Pituitary hormones
- ACTH: adrenocorticotropic hormone
- αMSH: α-melanocyte stimulating hormone
- β-endorphin
- GH: growth hormone
- PRL: prolactin
- FSH: follicle stimulating hormone
- LH: luteinizing hormone
- TSH: thyrotropin [thyroid stimulating hormone]

GI and brain peptides
- CCK: cholecystokinin
- Gastrin
- GRP: gastrin releasing peptide
- Motilin
- Neurotensin
- Substance K; substance P (tachykinins)

Circulating
- Angiotensin
- Bradykinin

Frog skin
- Bombesin
- Caerulein
- Ranatensin

Opiate peptides
- β-endorphin
- Dynorphin
- Leu-enkephalin
- Met-enkephalin

Neurohypophyseal peptides
- Oxytocin
- Vasopressin

Neuronal and endocrine
- ANF: atrial natriuretic peptide
- CGRP: calcitonin gene-related peptide
- VIP: vasoactive intestinal peptide

GI and pancreas
- Glucagon
- PP: pancreatic polypeptide
- Ghrelin

Neurons only?
- Galanin
- Neuromedin K
- NPY: neuropeptide Y
- PYY: peptide YY

Endocrine only?
- Calcitonin
- Insulin
- Secretin
- Parathyroid hormone

FIGURE 20-1 **Selected bioactive peptides are grouped by structural similarity or by tissue source.**

The sites of action of CCK and gastrin are distinct: gastrin stimulates gastric acid secretion, while CCK stimulates enzyme and bile acid delivery to the small intestine. Interestingly, the common COOH-terminal tetrapeptide, while inactive in the gastrointestinal tract, is abundant in the cerebral cortex and has important behavioral actions.

The function of peptides as first messengers is evolutionarily very old

In phylogenetic terms, neuropeptides were established very early as molecules affecting intercellular communication. In coelenterates, such as *Hydra*, there are many peptides used in neurotransmission, but many of the 'conventional' neurotransmitter systems, such as acetylcholine, catecholamines and serotonin, covered in previous chapters, are not found (Grimmelikhuijzen et al., 1999). The nerve net is strongly peptidergic in the lowest animal group with a nervous system, the cnidarians, which includes sea anemones, corals, jellyfishes and *Hydra*. Yeast use bioactive peptides such as a- and α-mating factors to communicate.

Various techniques are used to identify additional neuropeptides

Bioassays are the oldest and surest way to identify biologically active peptides; more recently, with the advent of receptor-based assays, peptides have been identified by their ability to bind to a known receptor and thus to displace a ligand or to produce a biological response. The use of a relatively homogeneous tissue source, such as adrenal chromaffin granules, enabled identification of bioactive peptides derived from chromogranin A. Mass spectrometry, with its great sensitivity, is being used to characterize peptides from many sources. As noted above, peptides important in the nervous system were often identified first in some peripheral source, such as CCK in the gut, or even in unusual places, such as frog skin. Since α-amidation of the COOH-terminus of peptides has proven to be a signature of bioactive peptides, assays specific for COOH-terminal α-amides were developed and used to discover neuropeptide Y (See Box) and several other peptides. Molecular biological approaches have also been used to discover many new peptides. Subtractive hybridization and differential display were used to identify both the cocaine- and amphetamine-regulated transcript (CART) and RESP18, a dopamine-regulated transcript. Finally, screens using orphan receptors—G-protein-coupled receptors with no identified ligand—were used to find natural ligands such as orphanin FQ/nociceptin and orexin/hypocretin (Reinscheid et al., 1995; Sakurai et al., 1998). Systematic scans of sequence databases can identify additional family members and potential peptide precursors by taking into account key features of precursor structures.

The neuropeptides exhibit a few key differences from the classical neurotransmitters

First, neuropeptides are present in tissues at much lower concentrations than classical neurotransmitters. Consistent with this, neuropeptides are also active at receptors at correspondingly lower concentrations. For example, the concentration of ACh and NE in synaptic vesicles is in the 100–500 mmol/l range, while the concentration of neuropeptide in a large dense-core vesicle is at most 3–10 mmol/l. Correspondingly, the affinity of ACh for its receptors is in the micromolar to millimolar range, while peptides typically bind to their receptors with affinities in the nanomolar to micromolar range.

Probably the most striking difference between neuropeptides and conventional neurotransmitters is in their biosynthesis (Fig. 20-2). Neuropeptides are derived from larger, inactive precursors that are generally at least 90 amino acid

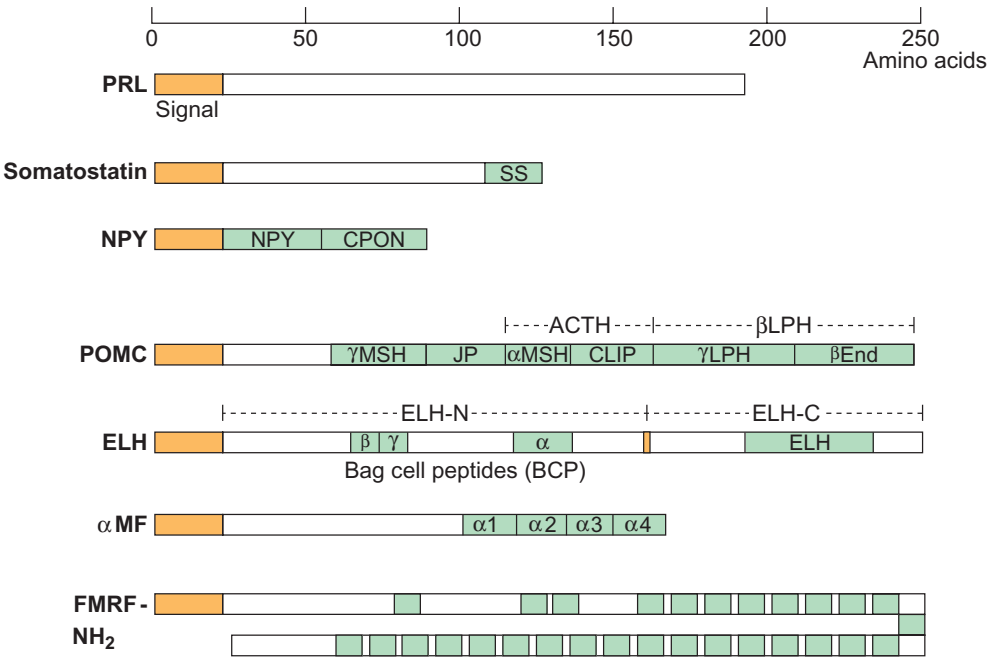

FIGURE 20-2 **Structures of selected bioactive peptide precursors are diagrammed**. The structures of prolactin (*PRL*), somatostatin, neuropeptide Y (*NPY*), pro-opiomelanocortin (*POMC*), egg-laying hormone (*ELH*), yeast α-mating factor (*αMF*) and FMRF-amide precursors (*FMRF-NH$_2$*) are indicated. Signal sequences are shaded and on the left of each precursor. *βEnd*, β-endorphin; *ACTH*, adrenocorticotropic hormone; *CLIP*, corticotropin-like intermediate lobe peptide; *CPON*, C-terminal flanking peptide of NPY; *JP*, joining peptide; *LPH*, lipotropin; *MSH*, melanocyte-stimulating hormone; *SS*, somatostatin.

residues in length (Mains & Eipper, 1990; Zhou et al., 1999; Seidah & Chretien, 1999). The simplest example is prolactin, a pituitary product. The signal sequence for prolactin must be removed and disulfide linkages must form, but no further enzymatic steps are necessary. The next simplest case is somatostatin, in which a single cleavage after signal peptide removal produces the bioactive peptide. Neuropeptide Y (NPY) is derived from proneuropeptide Y after signal peptide removal, cleavage between NPY and the C-terminal flanking peptide of NPY (CPON), and additional modifications, which are discussed below. The pro-opiomelanocortin (POMC) precursor includes several different bioactive peptides, as does the egg-laying hormone (ELH) precursor (Mains & Eipper, 1990). One interesting attribute common to peptide precursors in evolutionarily older species is the existence of multiple copies of the same bioactive peptide in one precursor; this is exemplified by the FMRF-NH_2 (Phe–Met–Arg–Phe–amide) family of peptides, with 59 copies of similar peptides encoded by 22 distinct genes (Li et al., 1999). Even in yeast, a similar process is used, so that four copies of α-mating factor are produced from the α-mating factor precursor. Precursors with multiple copies of bioactive peptide are much less common in evolutionarily more advanced species, although the rat TRH precursor contains five copies of the TRH tripeptide.

The supply of conventional neurotransmitters in small synaptic vesicles is replenished in nerve terminals by local synthesis, and many conventional neurotransmitters are recaptured after secretion. In striking contrast, neuropeptides are initially synthesized in the cell soma, sequestered within the lumen of the secretory pathway and transported down the axon while undergoing cleavages and other processing events. The peptide-containing large dense-core vesicle (LDCV) produced can be used only once. After exocytosis, the membrane components of the LDCV must be reinternalized and either destroyed or transported back to the cell body for reuse. Thus, no recycling of the neuropeptides or their immediate precursors occurs at their site of release or elsewhere.

Release is another area of difference: conventional neurotransmitters are secreted from small synaptic vesicles (SSVs) after cytosolic [Ca^{2+}] transiently reaches concentrations of 50–100 μmol/l, while peptides are released from LDCVs at lower concentrations of cytosolic [Ca^{2+}] (Fig. 20-3). Conventional neurotransmitter release is thought to occur very close to the site of Ca^{2+} entry (see Chs. 7 and 12) while neuropeptides are typically released at a significantly greater distance from the site of Ca^{2+} entry (Fig. 20-4). Furthermore the Ca^{2+} that stimulates exocytosis from LDCVs may come from internal stores or from the extracellular fluid. The location of LDCVs relative to the site of Ca^{2+} influx means that a substantially greater stimulation intensity is necessary for secretion of peptides to occur.

Neuropeptides are often found in neurons with conventional neurotransmitters

As illustrated in Figure 20-3, both conventional neurotransmitters and neuropeptides are found at a majority of the synapses in the nervous system. Neuropeptide expression is extremely plastic, even in the adult. For example, the hypothalamic neurons that express vasopressin and those that synthesize corticotropin-releasing hormone (CRH) are situated close to each other but constitute separate and virtually non-overlapping populations of neurons in the normal animal. However, after glucocorticoid concentrations are lowered by blockade of adrenal cortical function or removal of the adrenal glands, vasopressin neurons begin to express CRH and CRH neurons begin to synthesize vasopressin. This adaptive response can be understood from a teleological point of view by knowing that CRH normally stimulates the adrenocorticotropic hormone (ACTH)-producing cells of the anterior

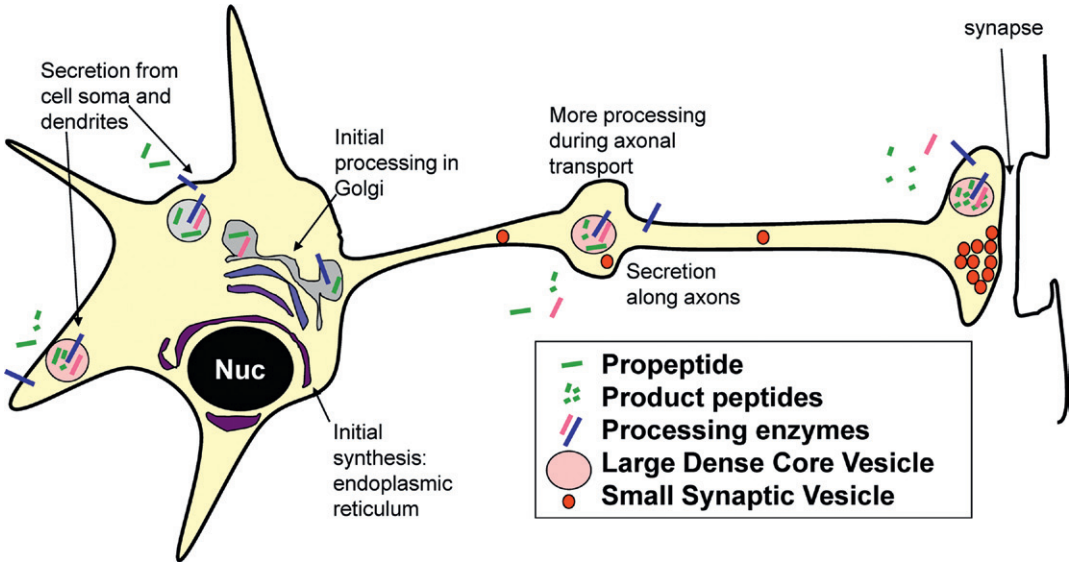

FIGURE 20-3 **Intracellular pathway of bioactive peptide biosynthesis, processing and storage**. Neuropeptide precursors are synthesized on ribosomes at the endoplasmic reticulum and processed through the Golgi. Axonal transport of the large dense-core vesicles and transport into dendrites precedes the actual secretion event, which can occur at multiple sites throughout the soma, axon and dendrites.

pituitary to secrete ACTH and that ACTH stimulates glucocorticoid production in the adrenal cortex. Vasopressin acts synergistically to increase ACTH secretion in times of need, such as following adrenalectomy. In addition to neuropeptides, many LDCVs contain ATP, just as many conventional neurotransmitter vesicles do, so that ATP is released along with neuropeptides. ATP and adenosine can have potent synaptic actions in their own right (see Ch.19 on purinergic signaling).

The biosynthesis of neuropeptides is fundamentally different from that of conventional neurotransmitters

To add to the complexity discussed above, the metabolism of neuropeptide precursors is tissue specific, with a general rule that most precursors are expressed in more than one tissue and that the processing is not identical in different tissues (Fig. 20-5) (Mains & Eipper, 1990; Zhou et al., 1999; Seidah & Chretien, 1999). For example, anterior pituitary corticotropes cleave POMC to ACTH(1–39), a molecule that stimulates adrenal glucocorticoid production. Neurons in the arcuate nucleus cleave ACTH and α-amidate the smaller peptide to create ACTH(1–13)NH_2, which cannot stimulate the adrenal cortex but does have potent behavioral effects in the CNS.

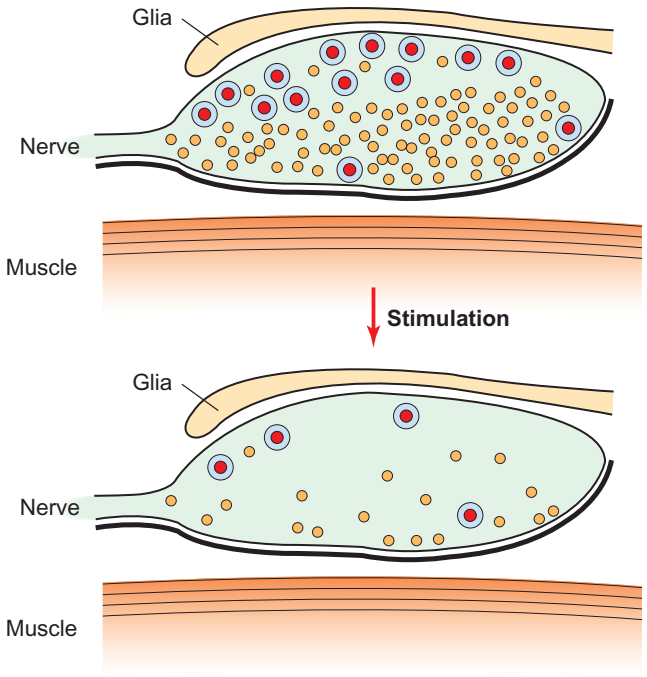

FIGURE 20-4 **Neuropeptides and conventional neurotransmitters are released from different parts of the nerve terminal.** A neuromuscular junction containing both large dense-core vesicles (containing the neuropeptide SCP) and small synaptic vesicles (containing acetylcholine) was stimulated for 30 min at 12 Hz (3.5 s every 7 s). Depletion of the small clear vesicles at the muscle face and of the peptide granules at the nonmuscle face of the nerve terminal was observed. After stimulation, there was an increase in the number of large dense-core vesicles within one vesicle diameter of the membrane. (Adapted from Karhunen et al., 2001.)

Intermediate pituitary melanotropes go one step further and α-*N*-acetylate the amidated peptide to produce α-melanocyte-stimulating hormone (αMSH). The skin-darkening activity of αMSH is especially important in lower vertebrates for which background color adaptation is protective. Similarly, corticotropes produce β-lipotropin (βLPH), which has no activity as an opiate peptide, while melanotropes and CNS neurons cleave βLPH to produce the potent opiate peptide β-endorphin(1–31). In some tissues, the β-endorphin may be shortened at the COOH-terminus, which decreases its opiate activity, or α-*N*- acetylated at its NH_2-terminus, which abolishes opiate activity. The cellular control of these different patterns of processing is beginning to be understood, with the identification of some of the enzymes that mediate these steps.

Other examples of tissue-specific processing include proenkephalin, proglucagon, procholecystokinin and prosomatostatin. Somatostatin neurons in the hypothalamus primarily produce a 14-residue form of the peptide, while somatostatin endocrine cells of the pancreas and intestine produce a 28-residue form derived from the same precursor. Proenkephalin is processed in the adrenal medulla to a set of opiate peptides of 15–35 residues, while proenkephalin in the brain is cleaved primarily to the pentapeptides met-enkephalin and leu-enkephalin. Procholecystokinin in the gut is processed to peptides of approximately 30 residues, which act on the pancreas and gallbladder, while smaller CCK-related peptides with behavioral effects are found in the brain. These smaller CCK-related peptides have no effects when applied to the pancreas or gallbladder. Understanding peptide biosynthesis and metabolism is important, since many smaller peptide fragments are active, frequently exhibiting biological activities distinct from that of the larger parent peptide (Hallberg & Nyberg, 2003).

Many of the enzymes involved in peptide biogenesis have been identified

The most common steps in precursor processing and the enzymes involved are shown in Figure 20-6. The endoproteases involved are prohormone convertases 1 and 2 (PC1 and PC2), the exopeptidase is carboxypeptidase E (CPE), and the α-amidating enzyme is peptidylglycine α-amidating monooxygenase (PAM). The nomenclature for PCs can be confusing and is summarized in Table 20-1. Many steps in

TABLE 20-1 **Prohormone Convertases**

Original name	SPC name	Other names
Furin	SPC1	PACE
PC2	SPC2	
PC1	SPC3	PC3
PACE4	SPC4	
PC4	SPC5	
PC6	SPC6	PC5
PC8	SPC7	PC7, LPC

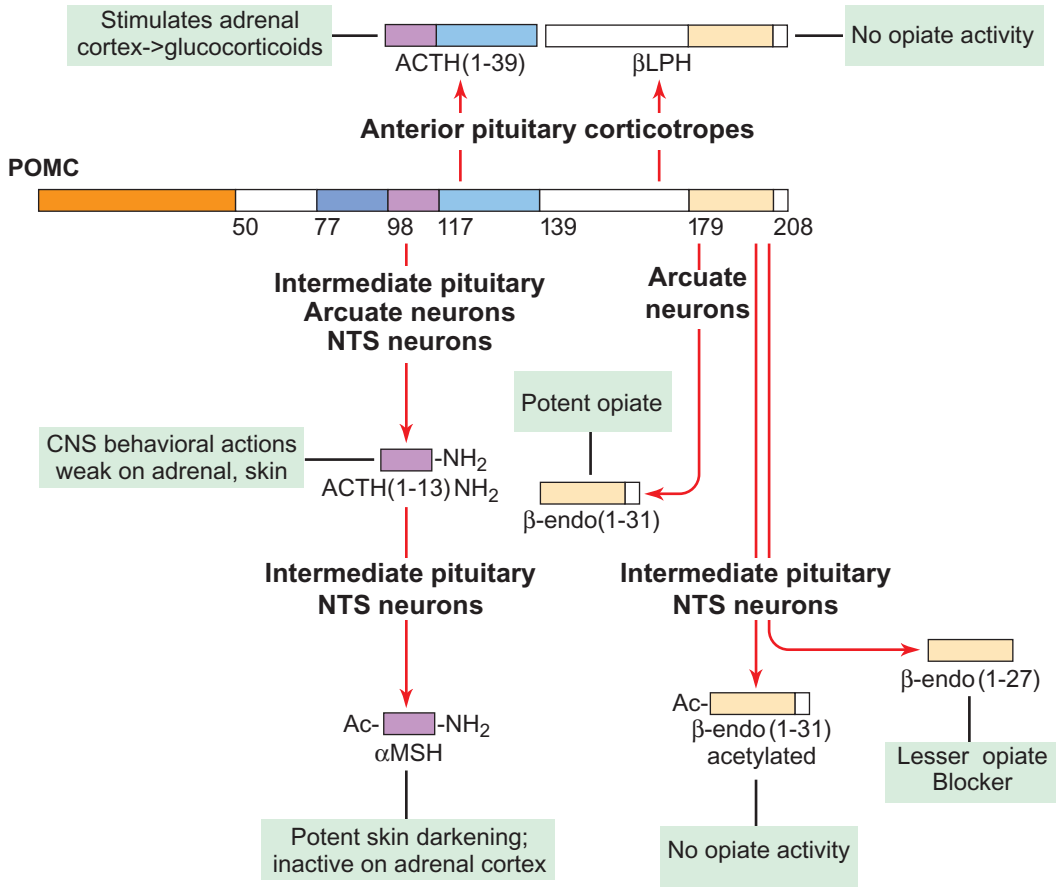

FIGURE 20-5 **Tissue-specific processing of the pro-opiomelanocortin (*POMC*) precursor yields a wide array of bioactive peptide products. Processing of the POMC precursor varies in various tissues**. In anterior pituitary, adrenocorticotropic hormone (*ACTH(1–39)*) and β-lipotropin (β-*LPH*) are the primary products of post-translational processing. Arcuate neurons produce the potent opiate β-endorphin (β-*endo(1–31)*) as well as ACTH(1–13)NH_2. Intermediate pituitary produces α-melanocyte-stimulating hormone (α*MSH*), acetylated β-endo(1–31) and β-endo(1–27). *NTS*, nucleus tractus solitarius.

their biosynthesis, such as signal peptide cleavage, disulfide bond formation, the addition and subsequent modification of N-linked and O-linked oligosaccharides, phosphorylation and sulfation, are not unique to neuropeptides. As shown in Figure 20-3, many of the post-translational steps occur as the maturing neuropeptides travel down the axon toward the synapse in LDCVs. The later steps in neuropeptide biosynthesis (Fig. 20-6) are unique to neurons and endocrine cells.

Key enzymes in neuropeptide biosynthesis include endoproteases, exoproteases and enzymes modifying the ends of the peptides. The discovery and characterization of Kex2p, the endoprotease that cleaves yeast pro-α-mating factor to produce four copies of the pheromone α-mating factor (Fig. 20-2), were key to the discovery of the mammalian prohormone convertases, including furin, PC1/3, PC2, PC4, PC5/6, PC7/8 and PACE4 (Zhou et al., 1999; Seidah et al., 2008; Henrich et al., 2005). The prohormone convertases share homology with bacterial subtilisins at their NH_2-termini, and have an Asp–His–Ser catalytic triad, which consists of the three key amino acids involved in catalysis (denoted D, H and S in Fig. 20-6). All of these enzymes (PCSK1-7) cleave precursors at the consensus site $(K/R)\text{-}(X)_n\text{-}(K/R)\downarrow$, where X is any residue and n = 0, 2, 4 or 6 (Seidah et al., 2008). Eukaryotic enzymes in this family all have an essential 'P' domain COOH-terminal to the subtilisin-related enzymatic domain, followed by a variable region (Zhou et al., 1999). The proregion of each enzyme (Fig. 20-6) must be present during biosynthesis for the protease to fold correctly but must be removed to yield an activated protease. In every case, the proregion is cleaved but remains associated with the enzymatic portion of the molecule, acting as an inhibitor in the heterodimer (Seidah et al., 2008). Full activation occurs when the inhibitory prosegment is cleaved a second time: for PC1/3 and PC2, this occurs in LDCVs, while most of the others are activated in the trans-Golgi or at the cell surface (Seidah et al., 2008). For PC1 and furin, removal of the proregion occurs within a few minutes of biosynthesis while the enzyme is in the endoplasmic reticulum and is most probably an autocatalytic event. For the other prohormone convertases, removal of the proregion occurs much more slowly. The P domains consist of a pair of 4-stranded β sheets, which together bind next to the catalytic domain (Henrich et al., 2005). Expression of active PC2 requires coexpression of the chaperone peptide 7B2 (Fig. 20-6), which blocks proPC2 from assuming a non-activatable

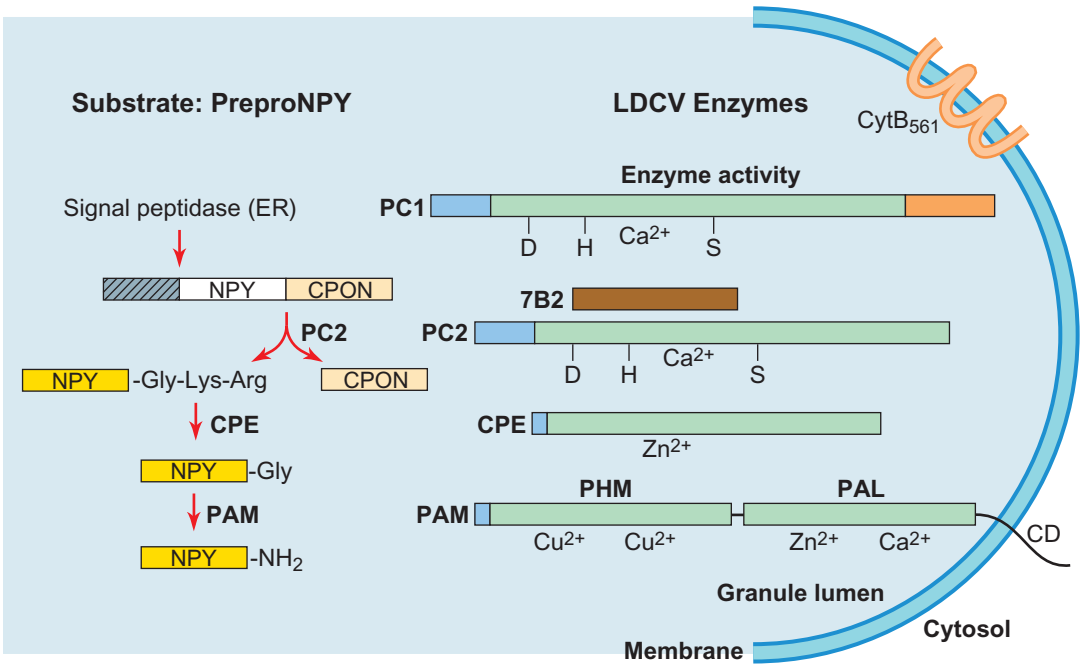

FIGURE 20-6 **Sequential enzymatic steps lead from the peptide precursor to bioactive peptides**. The neuropeptide Y (*NPY*) precursor shown at the left is processed sequentially by the enzymes of the large dense-core vesicles (*LDCV*) shown at the right. *CD*, cytoplasmic domain; *CPE*, carboxypeptidase E; *CPON*, C-terminal flanking peptide of NPY; *ER*, endoplasmic reticulum; *PAL*, peptidyl-α-hydroxyglycine α-amidating lyase; *PAM*, peptidylglycine α-amidating monooxygenase; *PC*, prohormone convertase; *PHM*, peptidylglycine α-hydroxylating monooxygenase.

NEUROPEPTIDE Y

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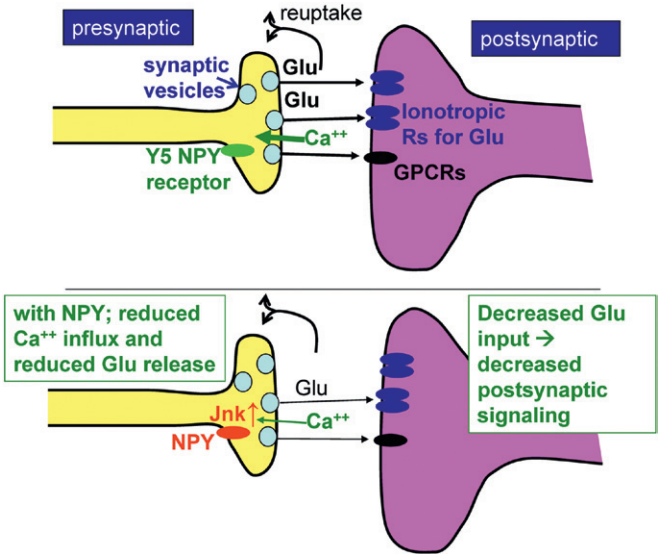
NPY, acting through multiple receptors, plays an essential role in depression, alcoholism and regulation of food intake. Neuropeptide Y, a 36-residue amidated peptide and one of the most abundant peptides in the CNS, is expressed in many brain regions (Colmers et al., 1988). NPY was identified by screening for peptides with a COOH-terminal α-amide group; it was named NPY because it has a Tyrosine residue (Y) at the NH_2-terminal and a Y-amide at the COOH-terminal (Tatemoto, et al., 1982). Receptors for NPY are particularly abundant in the amygdala, hippocampus, hypothalamus, locus coeruleus, striatum, and cortex (Eaton et al., 2007). Humans have five NPY receptors, all of which are GPCRs. Studies with synaptosomes and electrophysiological slice recordings established that NPY, acting through its Y5 receptor, can reduce glutamate release from presynaptic terminals by activating c-Jun Kinase (Jnk) and reducing Ca^{2+} influx into presynaptic terminals (**Figure**) (Colmers et al., 1988; Whittaker et al., 1999; Pellieux et al., 2000). When NPY is increased in the hippocampus of rats, using a viral overexpression system, spatial learning is impaired and long-term potentiation is decreased (Sorensen et al., 2009). In addition, performance in spatial learning tasks is adversely affected. When rats with elevated hippocampal NPY are subjected to electrical stimulation resulting in seizures, seizure sensitivity is normal with elevated NPY, but seizure severity is dramatically reduced (Sorensen et al., 2009).

In human subjects, impaired NPY signaling is believed to be involved in anxiety, depression, alcoholism, schizophrenia, and post traumatic stress disorder (Eaton et al., 2007). Single nucleotide polymorphisms (SNPs) in the human NPY gene which lower NPY peptide levels are associated with depression and alcoholism (Eaton et al., 2007). SNPs in the NPY Y2 and Y5 receptors are strongly associated with alcohol dependence and alcohol withdrawal symptoms, notably seizures (Wetherill et al., 2008; Ciccocioppo et al., 2009). In addition, the NPY receptor SNPs are associated with strong tendencies for comorbidity of alcohol and cocaine dependence. There is decreased NPY in the cerebrospinal fluid of depressed patients and patients with anxiety syndromes (Eaton et al., 2007). As a consequence, orally active, brain penetrant antagonists specific for the NPY Y1 and Y5 receptors are being tested in various psychiatric trials (MacNeil, 2007).

In addition to psychiatric and substance abuse effects, NPY is orexigenic, strongly stimulating food intake (MacNeil, 2007). Some human trials have shown modest, but significant, weight loss with antagonists to the NPY Y5 receptor (MacNeil, 2007; Erondu et al., 2006). Now, combination therapy trials using multiple NPY receptor antagonists along with an endocannabinoid antagonist (endocannabinoids are also orexigenic) are being tested in human subjects (Zhang et al., 2010).

NEUROPEPTIDE Y (*cont'd*)



References

Ciccocioppo, R., Gehlert, D. R., Ryabinin, A., Kaur, S., Cippitelli, A., & Heilig, M. (2009). Stress-related neuropeptides and alcoholism: CRH, NPY, and beyond. *Alcohol*, *43*, 491–498.

Colmers, W. F., Lukowiak, K., & Pittman, Q. J. (1988). Neuropeptide Y action in the rat hippocampal slice: Site and mechanism of presynaptic action. *The Journal of Neuroscience*, *8*, 3827–3837.

Eaton, K., Sallee, F. R., & Sah, R. (2007). Relevance of Neuropeptide Y (NPY) in Psychiatry. *Current Topics in Medicinal Chemistry*, *7*, 1645–1659.

Erondu, N., Gantz, I., Musser, B., Suryawanshi, S., Mallick, M., & Heymsfield, S. B. (2006). Neuropeptide Y5 receptor antagonism does not induce clinically meaningful weight loss in overweight and obese adults. *Cell Metabolism*, *4*, 275–282.

MacNeil, D. J. (2007). NPY Y1 and Y5 Receptor Selective Antagonists as Anti-Obesity Drugs. *Current Topics in Medicinal Chemistry*, *7*, 1721–1733.

Pellieux, C., Sauthier, T., Domenighetti, A., Marsh, D. J., Palmiter, R. D., Brunner, H. R., et al. (2000). Neuropeptide Y (NPY) potentiates phenylephrine-induced mitogen-activated protein kinase activation in primary cardiomyocytes via NPY Y5 receptors. *Proceedings of the National Academy of Sciences U.S.A.*, *97*, 1595–1600.

Sorensen, A. T., Nikitidou, L., Ledri, M., Lin, E. J. D., During, M. J., & Kokaia, M. (2009). Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. *Experimental Neurology*, *215*, 328–333.

Tatemoto, K., Carlquist, M., & Mutt, V. (1982). Neuropeptide Y, a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature*, *296*, 659–660.

Wetherill, L., Schuckit, M. A., Hesselbrock, V., Xuei, X., Liang, T., & Foroud, T. (2008). Neuropeptide Y receptor genes are associated with alcohol dependence, alcohol withdrawal phenotypes, and cocaine dependence. *Alcoholism: Clinical and Experimental Research*, *32*, 2031–2040.

Whittaker, E., Vereker, E., & Lynch, M. A. (1999). Neuropeptide Y inhibits glutamate release and long-term potentiation in rat dentate gyrus. *Brain Research*, *827*, 229–233.

Zhang, L., Lee, N. J., Nguyen, A. D., Enriquez, R. F., Herzog, H., & Sainsbury, A. (2010). Additive actions of the cannabinoid and neuropeptide Y systems on adiposity and lipid oxidation. *Diabetes, Obesity & Metabolism*, *12*, 591–603.

conformation, and prevents expression of PC2 endoproteolytic activity until PC2 has been deposited into secretory granules (Lee & Lindberg, 2008). Pro-SAAS may be a corresponding inhibitor peptide for PC1.

The mammalian endoproteases most clearly involved in neuropeptide processing are PC1 and PC2, Ca^{2+}-dependent proteases found in secretory granules whose expression is limited to neurons and endocrine cells (Fig. 20-6). Several other members of this endoprotease family are more widely expressed, while still others are expressed in restricted locations distinct from neurons and endocrine cells. For example, furin is found in virtually all cells and is localized primarily to the *trans*-Golgi network; furin catalyzes cleavages important in peptide function, such as the initial cleavage of the precursors to ELH, nerve growth factor and parathyroid hormone, as well as cleavage within the insulin receptor precursor to produce the active $\alpha\beta$ dimer form of that receptor. Furin may also be instrumental in the activation of some of the other processing enzymes, such as PC2 and CPE.

PC1 and PC2 cleave at selected pairs of basic amino acids in peptide precursors: Lys–Arg, Arg–Arg, Lys–Lys and Arg–Lys. PC1 may also catalyze cleavages at selected single Arg sites present in some precursors, such as prosomatostatin and procholecystokinin. Cleavages in LDCVs by PCs are tightly controlled, often occurring in a very orderly fashion (Fig. 20-7). The initial cleavages of POMC occur in less than 1 hour (Fig. 20-7, steps 1 and 2, performed by PC1), while other cleavages occur only after several hours (Fig. 20-7, steps 6 and 7, performed by PC2). The endoproteolytic cleavage of propeptides is often the rate-limiting reaction in peptide biosynthetic processing.

The pattern of cleavages catalyzed by PC1, PC2 and furin when expressed in neurons and endocrine cells is much more selective than the pattern of cleavages seen in test tube assays with purified enzymes. For example, although prohormone convertases usually cleave at the COOH-terminus of a pair of basic residues in model peptide substrates, the cleavages in cells can be in the middle of the pairs of basic residues, as in the case of POMC cleavage (cleavage 6; Fig. 20-7), where the basic residues are separated and remain with the two resulting mature peptides (Mains & Eipper, 1990). It is likely that the Ca^{2+} concentration and internal pH of LDCVs are two variables used by neurons and endocrine cells to regulate endoproteolytic activity in LDCVs.

Additional endoproteases may be shown to play a role in neuropeptide biosynthesis. The processing of proANF, which

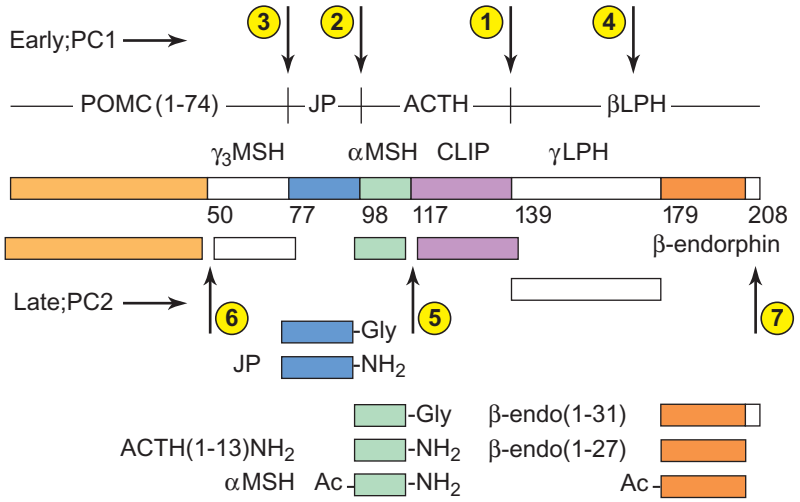


FIGURE 20-7 Processing of the pro-opiomelanocortin (*POMC*) precursor proceeds in an ordered, stepwise fashion. Cleavage of the POMC precursor occurs at seven sites, with some of the reactions being tissue specific. The circled numbers indicate the temporal order of cleavage in tissues where these proteolytic events occur. *ACTH*, adrenocorticotropic hormone; *CLIP*, corticotropin-like intermediate lobe peptide; *JP*, joining peptide; *LPH*, lipotropin; *MSH*, melanocyte-stimulating hormone; *PC*, prohormone convertase.

involves cleavage after a single Arg residue in proANF, cannot involve PC1 or PC2 since there are negligible amounts of these PCs in the heart.

The PCSK family has two more members, Site-1 protease (S1P; also called subtilisin kexin isozyme-1, SKI-1; PCSK8) and PCSK9. These PCs are not directly involved in peptide biosynthesis. PCSK8 has a central role regulating cholesterol biosynthesis, while PCSK9 may act more like a cell surface receptor (Seidah et al., 2008).

CPE is a soluble protein found in virtually all LDCVs in neurons and endocrine cells (Fig. 20-6) (Arolas et al., 2007). It removes basic residues, Lys or Arg, from the COOH termini of peptide intermediates produced by the prohormone convertases, and has a pH optimum of 5.0–5.5, similar to the pH inside LDCVs. It was originally identified by its tissue distribution and substrate specificity, along with its specific inhibition by guanidinoethylmercaptosuccinic acid (GEMSA). CPE is a Co^{2+}- and Zn^{2+}-activated enzyme with a short proregion that is normally removed during maturation of the enzyme; unlike the prohormone convertases, CPE is active with its proregion (which ends –RRRRR) attached. The carboxypeptidase function of peptide processing is not normally rate limiting since peptide intermediates with COOH-terminal basic residues are detected only at extremely low concentrations in tissue or LDCV extracts. The structure of CPE has been modeled, based on the crystal structure of duck carboxypeptidase D domain II (Aloy et al., 2001; Arolas et al., 2007). The carboxypeptidase catalytic core has two domains, one of 300 residues with the one catalytic Zn^{2+} ion, and one with 80 residues. Additional carboxypeptidases were identified in mice lacking CPE, notably CPD, an integral membrane enzyme with two active and one inactive carboxypeptidase domains, plus CPM and CPN (Arolas et al., 2007). The CPE knockout mice have major physiological problems and do accumulate significant amounts of incompletely processed peptides which bear COOH-terminal basic residues, so the bulk of peptide biosynthesis uses CPE (Arolas et al., 2007). Given that cleavage at a pair of basic residues can be in the middle of the pair, there is good reason to think that a basic aminopeptidase will eventually be found in LDCVs.

PAM is a bifunctional enzyme found in nearly all LDCVs (Fig. 20-6) (Prigge et al., 2000). PAM acts on peptide substrates after endoproteolytic cleavage and exopeptidase action, when a COOH-terminal Gly residue is exposed, and converts the peptidyl-Gly into the corresponding peptide-NH_2. About half the known bioactive peptides are α-amidated, and α-amidation is generally crucial to biological potency. The peptidyl-Gly and peptide-COOH forms are usually inactive at physiological concentrations. The first step of the α-amidation reaction is performed by peptidylglycine α-hydroxylating monooxygenase (PHM), which is the NH_2-terminal portion of the bifunctional PAM protein; as with CPE, the pH optima for both enzymatic domains of PAM are around 5.5. PHM binds two Cu^{2+} atoms, which participate in catalysis by undergoing cycles of reduction and oxidation. PHM uses ascorbic acid as the reductant, with one atom of oxygen from O_2 incorporated into the peptide during the hydroxylation step. Thus, PHM is enzymatically very similar to dopamine β-monooxygenase (DBM), which converts dopamine to norepinephrine (see Ch. 14).

The crystal structure of the 315-residue catalytic core of PHM has been solved, revealing two domains of roughly equal size, each with one Cu^{2+} ion. The two copper ions are about 11Å apart, with one Cu^{2+} ion coordinating with three His residues and a second Cu^{2+} ion binding one Met and two His residues (Prigge et al., 2000). The peptide substrate binds close to the Met Cu^{2+} ion (Prigge et al., 2000). The second step of the α-amidation reaction is performed by a second enzymatic domain of PAM, peptidyl-α-hydroxyglycine α-amidating lyase (PAL). The PAL domain constitutes a novel, divalent metal ion-dependent enzyme, whose crystal

structure is a six-bladed β-propeller, containing a catalytic Zn^{2+} ion coordinated with three His residues, and a structural Ca^{2+} ion that interacts with several β strands (Chufan et al., 2010). Neurons primarily express an integral membrane form of the bifunctional PAM protein (Fig. 20-6), while an additional mRNA-splicing event enables some endocrine cells to express soluble versions of the protein, lacking the transmembrane domain. In the integral membrane forms of PAM, the short COOH-terminal domain extends into the cytoplasm and participates in the routing of PAM into LDCVs and its recovery from the cell surface. Recent data demonstrate that the cytoplasmic domain of PAM is liberated in response to secretion and travels to the nucleus, where it regulates expression of selected genes (Rajagopal et al., 2009; Francone et al., 2010). The supply of reduced ascorbate in LDCVs is maintained by cytochrome b_{561}, a heme-containing protein that has six transmembrane domains and shuttles electrons from cytosolic ascorbate to ascorbate in the lumen of the LDCVs (Takigami et al., 2003). Cytochrome b_{561} is also found in catecholamine vesicles, where it performs a similar function for DBM (see Ch.14). Nervous and endocrine tissues have a high-affinity plasma membrane ascorbate uptake system to maintain concentrations of reduced ascorbate about 100-fold above the blood concentration of ascorbate, while many other tissues do not concentrate ascorbate significantly (Takanaga et al., 2004).

Several peptides have NH_2-terminal pyroglutamic acid residues, also termed cyclic glutamic acid (<Glu), which are essential to bioactivity. Prime examples are thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone (GnRH). The enzymes responsible for this modification are two similar glutaminyl cyclases, which convert the original NH_2-terminal Gln into Glu. One glutaminyl cyclase is a soluble, secretory pathway protein of 41 kDa, called QC, and requires divalent metal ion for full activity; the second glutaminyl cyclase (isoQC) is a membrane-associated protein found in the Golgi (Stephan et al., 2009). Interestingly, QC inhibitors are being tested with some success as drugs to reduce amyloid plaque formation in Alzheimer's disease models (Schilling et al., 2008).

A few peptides (e.g. gastrin, cholecystokinin) and receptors (thyrotropin receptor) are subject to sulfation on Tyrosine residues, a modification that occurs in the lumen of the *trans*-Golgi (Westmuckett et al., 2008). Two closely related tyrosine sulfotransferases, TPST1 and TPST2, perform this function using PAPS (3'-phosphoadenosine 5'-phosphosulfate) as the sulfate donor. The double knockout mice cannot produce sulfotyrosine, fail to thrive and are severely hypothyroid, with poor pulmonary function. Tyrosine sulfation is essential for the full gastrointestinal potency of CCK and for thyrotropin receptor function, but has no effect on gastrin activity.

Another important but infrequent modification of peptides is NH_2-terminal α-*N*-acetylation (Figs. 20-5 and 20-7). During POMC processing, α-*N*-acetylation greatly increases the skin-darkening potency of ACTH(1–13)NH_2 while abolishing both the adrenal steroidogenic potency of ACTH and the opiate activity of β-endorphin (Mains & Eipper, 1990). The enzyme(s) responsible for this modification has not yet been purified or cloned.

As an example, Figure 20-7 shows the pattern of processing steps in the POMC system (Mains & Eipper, 1990). The initial endoproteolytic steps (Fig. 20-7, steps 1–4) are mediated by PC1 and occur in all POMC-producing neurons and endocrine cells, usually in the numerical order shown. It is clear that steps 1 and 2 are initiated in the *trans*-Golgi network and continue in LDCVs, while step 4 occurs only in LDCVs. Steps 5–7 occur only in LDCVs and seem to require PC2. In the adult anterior pituitary, corticotropes contain PC1 but not PC2 and perform only cleavages 1–4. However, during early postnatal development, corticotropes also express PC2 and cleavages 5–7 are transiently seen. In the rat, expression of PC2 and cleavage within ACTH (cleavage 5) decline simultaneously a few weeks after birth, at about the time that the adult pattern of ACTH control over adrenal steroidogenesis appears.

Melanotropes and CNS neurons making POMC express both PC1 and PC2 and, thus, the smaller peptide products are seen in these cells. PAM is expressed in all POMC-producing cells, so the α-amidation of joining peptide (JP), a small peptide with no clear biological function, occurs rapidly in all POMC cells (Fig. 20-7). In the melanotropes of the intermediate pituitary and the POMC neurons of the nucleus of the solitary tract, α-*N*-acetylation of ACTH(1–13)NH_2 and β-endorphin occurs. In melanotropes, α-*N*-acetylation of ACTH can occur before cleavage 5. As indicated in Figure 20-5, the particular cleavages made and the modifications made to the NH_2- and COOH-termini of the peptide products determine the mixture of bioactive peptides released.

Neuropeptides are packaged into large, dense-core vesicles

In many cases, the peptide products from the processing of a propeptide are packaged together in an equimolar fashion in LDCVs and the peptides and the soluble processing enzymes (PC1, PHM, CPE) are all released together in response to stimuli (Fig. 20-8) (Mains & Eipper, 1990; Zhou et al., 1999). By comparison, there are also examples where the products of propeptide processing are sorted into different LDCVs or are subject to degradation. In *Aplysia* bag cell neurons, ELH is formed from the COOH terminus of the ELH precursor (Fig. 20-2), while α, β and γ bag cell peptides (BCPs) are formed from the NH_2-terminal portion (Fig. 20-8). The initial cleavage of the pro-ELH precursor occurs in the *trans*-Golgi network and the peptides are then separated into two distinct types of LDCV, which are sent to different parts of the cell (Fig. 20-8). These two sets of peptides mediate a coordinated set of behaviors involved in egg laying. Similarly, in TRH neurons, the NH_2- and COOH-terminal domains of the pro-TRH precursor are separated from each other and stored in distinct LDCVs.

Diversity is generated by families of propeptides, alternative splicing, proteolytic processing and post-translational modifications

The huge number of biologically active peptides is the result of many factors. First, there are several families of genes which clearly evolved from a common ancestor (Fig. 20-1): examples include the three precursors to β-endorphin, dynorphin and the enkephalins; the precursors to gastrin and CCK; the precursors to oxytocin and vasopressin; and the

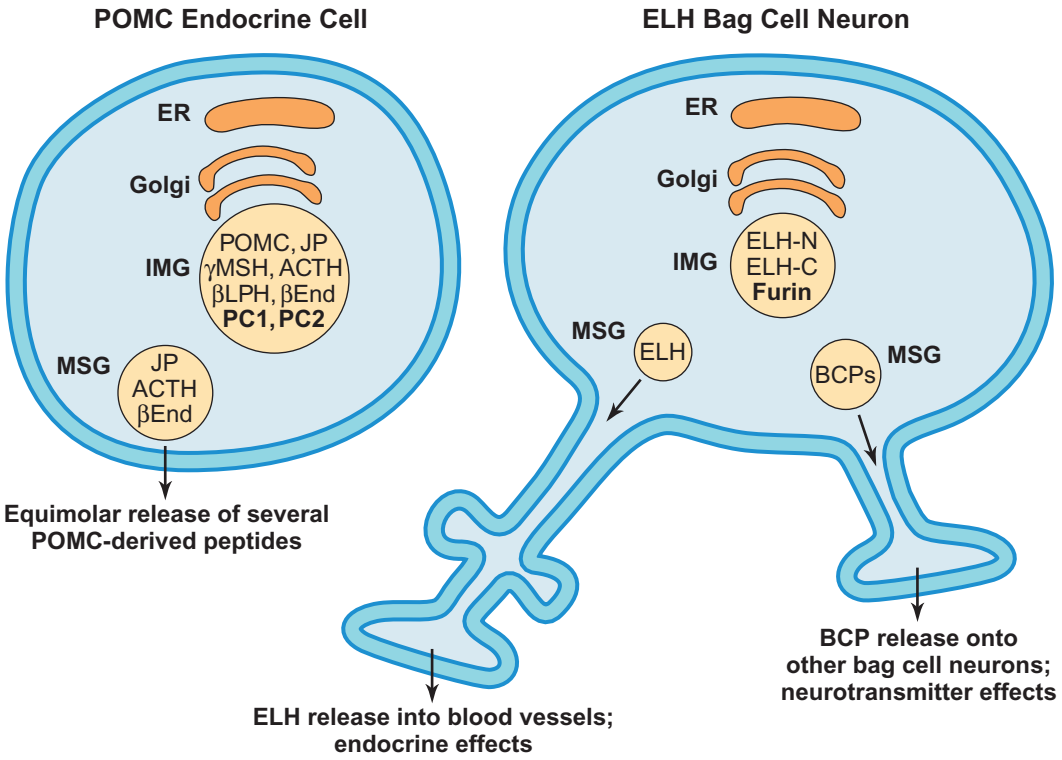


FIGURE 20-8 **Cell-specific packaging of peptides into large dense-core vesicles can lead to very different patterns of peptide secretion**. Sorting of neuropeptides into distinct mature secretory granules (*MSG*) is shown for bag cell neurons but does not occur for endocrine cells. β*End*, β-endorphin; *ACTH*, adrenocorticotropic hormone; *BCP*, bag cell peptide; *ELH*, egg-laying hormone, *POMC*, pro-opiomelanocortin; *ER*, endoplasmic reticulum; *IMG*, immature granules; *JP*, joining peptide; *LPH*, lipotropin; *MSH*, melanocyte-stimulating hormone; *PC*, prohormone convertase.

22 FMRF-NH_2 genes in *C. elegans*. Second, there are several peptide precursors that yield multiple copies of bioactive peptide: examples include the ELH precursor, the α-mating factor precursor, the FMRF-NH_2 precursor, the TRH precursor and the enkephalin precursor. Likewise, several distinct biological activities are found within the POMC and ELH precursors. Third, there is alternative splicing of mRNAs encoding preprohormones, first discovered in the calcitonin and calcitonin gene-related peptide precursors but also seen in the case of the preprotachykinin precursor, which yields substance P, substance K and several other peptides, depending on the splicing pattern (Fig. 20-9) (Helke et al., 1990). Finally, RNA editing can be involved, as in the case of the amphibian bombesin-like peptides, where nucleotides in the mRNA are changed and the final protein is not a direct reflection of the sequence encoded in the gene. RNA editing is also seen in the glutamate and serotonin receptors (Chs. 15 and 17) and will probably be found elsewhere as detection methods become more sophisticated.

NEUROPEPTIDE RECEPTORS

Most neuropeptide receptors are seven-transmembrane-domain, G-protein–coupled receptors

The first neuropeptide receptors characterized were those for substance P and neurotensin, and most have the general architecture shown in Figure 20-10. The basic rules for the function of these seven-transmembrane-domain receptors are the same as the rules established for similar serpentine receptors for conventional small molecule neurotransmitters, such as muscarinic, adrenergic and metabotropic glutamatergic receptors (Chs 12–19). In general, there are more receptors than peptides, and in mammals there are over 100 neuropeptides known; in *Drosophila*, current analyses recognize 22 neuropeptide prohormone precursors and 44 peptide receptor genes (Taghert & Veenstra, 2003). For many peptides, the extracellular domain plays a major role in ligand binding (Fig. 20-10) (Brothers et al., 2003). In addition, the key components of the binding sites for many peptides also include the residues analogous to those important for binding small nonpeptide ligands to their receptors, within the transmembrane domains and in the extracellular loops (Brothers et al., 2003).

The binding specificity of neuropeptide receptors for a given neuropeptide may vary considerably. Five somatostatin receptors have been identified; they bind somatostatin similarly and all inhibit adenylyl cyclase. However, these receptors differ substantially with regard to their interaction with various somatostatin analogs used for therapeutic purposes. For example, the receptor called SSTR2 binds a small peptide analog, octreotide, much more tightly than the other somatostatin receptors. This differential sensitivity is being widely used for therapeutic drug targeting (Yamaguchi et al., 2010). Changing a few amino acids in transmembrane domains VI and VII of SSTR1 to the residues found in SSTR2 (Fig. 20-10)

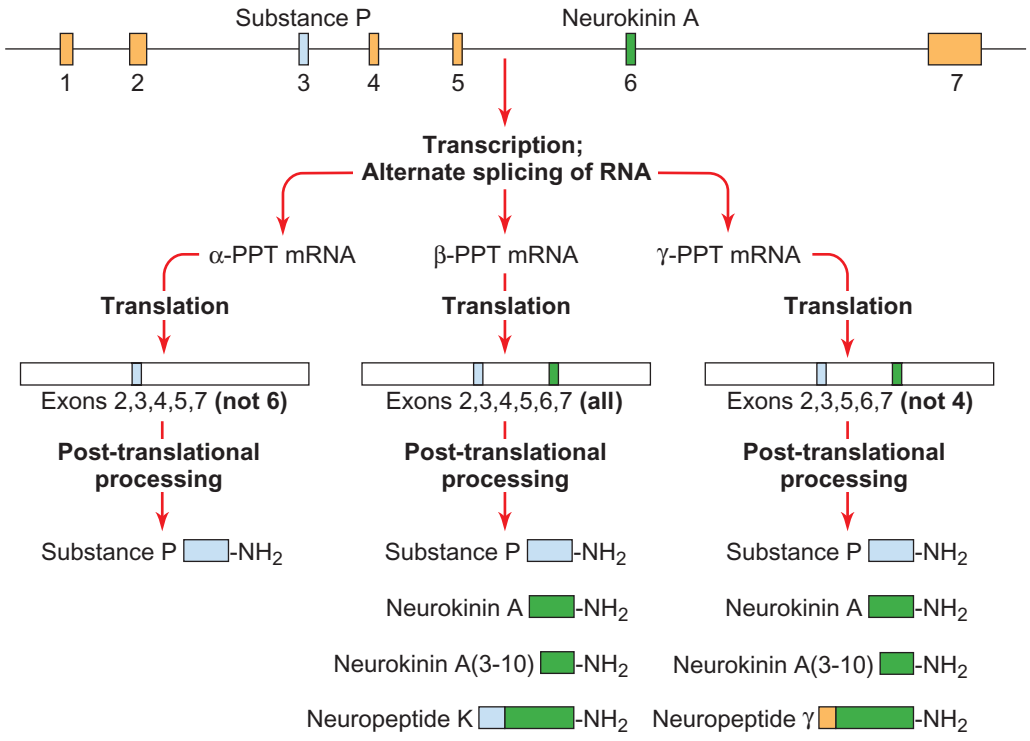


FIGURE 20-9 **Several mechanisms through which the substance P gene gives rise to different bioactive peptides in different neurons.** Alternative splicing of mRNA leads to translation of distinct precursors, and subsequent processing leads to unique mature peptides. *PPT*, pre-protachykinin. (Adapted from Helke et al., 1990.)

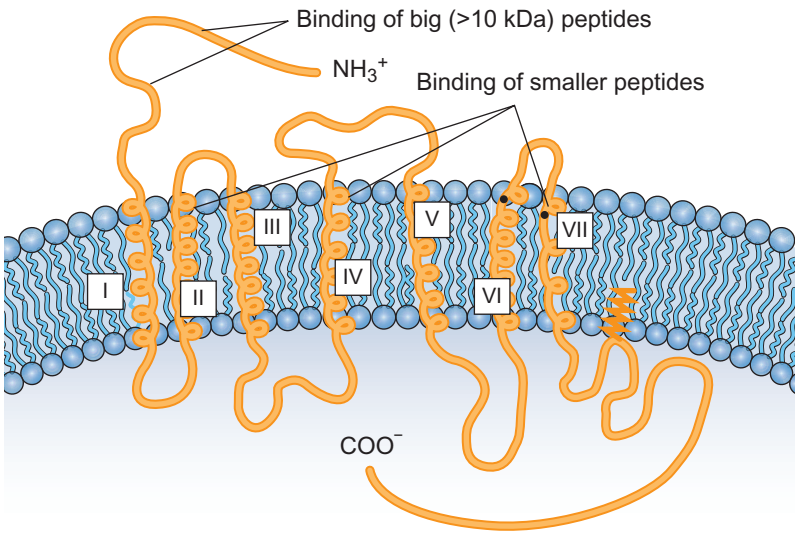


FIGURE 20-10 **Serpentine (seven-transmembrane-domain) receptors for peptides have binding for their peptide ligands within the membrane and in the NH2-terminal loop.**

allows the mutant SSTR1 to bind the peptide analog as well as SSTR2. Similar changes in specificity are seen with several other peptide receptor families (Helke et al., 1990; Brothers et al., 2003; Reubi, 2007).

Neuropeptide receptors are not confined to synaptic regions

Peptidergic neurotransmission often operates on a slower time scale than conventional neurotransmitters, so it is

perhaps not surprising that peptide receptors are often localized at a distance from the synapse. For example, although some substance P terminals contact membranes loaded with substance P receptor, only a small fraction of the substance P receptor-laden membrane is apposed to synaptic terminals. Substance P may diffuse a considerable distance from its release site and still find a receptor with which it can interact (Jimenez-Andrade et al., 2010; Mantyh, 2002).

Expressions of peptide receptors and the corresponding peptides are not well matched

Neuropeptides can act at many sites: they may act directly on a postsynaptic target at the synapse; presynaptically on the terminal that released the peptide (autocrine effects); on an immediately adjacent cell (juxtacrine effects); or on a cell a few cell diameters away from the site of release (paracrine effects). In addition, peptides can exert their actions after traveling through some or all of the vasculature to reach their target (endocrine effects), as in the case of hypothalamic releasing factors and neurohypophyseal hormones.

There is some correspondence of the peptide families and the families of peptide receptors, but it is certainly not simple. For example, there are three related opiate peptide precursors, POMC, proenkephalin and prodynorphin, and three major types of opiate receptor, μ, κ and δ. The best endogenous ligand for the μ receptor is β-endorphin; enkephalins are the best ligands for δ receptors and dynorphins are best for κ receptors. However, the sites at which opiate peptides and opiate receptors are expressed in the brain do not show a simple 1:1 correspondence. Recently, an orphan receptor resembling the opiate receptors was expressed and used to identify its endogenous ligand, which was called orphanin FQ or nociceptin. This preprohormone is most similar to the dynorphin precursor and defines a fourth type of opiate-like peptide containing the sequence Phe–Gly–Gly–Phe instead of the Tyr–Gly–Gly–Phe, which first defined the opiate peptide family. The orphanin FQ peptide is hyperalgesic in that it increases pain sensitivity, an action opposite to that of the opiate peptides. Reduction of the level of the orphanin FQ receptor in brain using antisense oligonucleotides leads to analgesia. There are a number of endogenous peptides with very high affinity for opiate receptors, but whether these peptides are used as intercellular signaling molecules or are created extracellularly is not yet clear (Terskiy et al., 2007; McPherson et al., 2010).

Similarly, five closely related melanocortin receptors that respond to various peptides derived from the POMC precursor have been identified (Fig. 20-7) (Gruber et al., 2009). As expected, the receptor on adrenal cortical cells responds best to ACTH, which normally stimulates adrenal steroidogenesis, and the receptor on melanocytes responds best to αMSH, which causes skin darkening. However, the pattern of melanocortin receptor expression in the brain is not simply explained by the known patterns of peptide expression in the brain or by the known effects of POMC-derived peptides when applied to various brain regions. With this number of peptide receptors, it is obvious that production of final peptide products must be precisely controlled and that different biosynthetic processing pathways can dramatically affect the biological activity observed (Figs 20-5 and 20-7).

The amiloride-sensitive FMRF-amide-gated sodium ion channel is among the few peptide-gated ion channels identified

FMRF-amide induces a fast excitatory depolarizing response in selected neurons due to direct activation of an amiloride-sensitive sodium channel. Using cDNA from the snail *Helix aspersa* and a *Xenopus* oocyte expression system, an amiloride-sensitive, FMRF-amide-activated sodium channel was identified. The acid-sensing ion channels found in sensory neurons are also modulated by FMRF-NH_2 and by neuropeptide FF (Xie et al., 2002). These channels have only two transmembrane domains and as such are similar in structure to the directly gated ion channels activated by some purinergic ligands (Ch.19) and by hydrogen ions.

Neuropeptide receptors are becoming molecular targets for therapeutic drugs

The large number of neuropeptides and even larger number of neuropeptide receptors have raised the hope that neuropeptide receptors could become useful drug targets. Radiolabeled peptides are being used to localize tumors and metastases (Reubi, 2007). Nonpeptidic antagonists acting at specific substance P receptors are being tested as antidepressants and to treat chronic pain (Millan et al., 2010; Sarzi-Puttini et al., 2010). For a number of stress-related psychiatric disorders, CRH is another major target (Kalantaridou et al., 2010; Wang et al., 2010).

Many creatures use peptides to control their environments. Cone snails produce over a thousand different peptide toxins, which are exquisitely targeted to ion channels (Jimenez & Olivera, 2010). Similarly, insects produce peptide toxins to kill each other, with spiders being the champions at this endeavor. The knowledge of which ion channels are affected by these toxins is becoming medically and agriculturally useful—for example, there are distinct calcium channels expressed in different tissues at various developmental times. The natural toxins, which are highly selective among closely related subtypes of ion channel, are being exploited for therapeutic purposes. Similarly, spider toxins are being adapted as insecticides.

NEUROPEPTIDE FUNCTIONS AND REGULATION

The study of peptidergic neurons requires a number of special tools

These tools include methods to detect the neuropeptides both in cells and after release, the enzymes specific to their biosynthesis and their cognate receptors. Since the actions of peptides require secretion, measurements of cell content (e.g., immunostaining) can be deceptive, with a decrease in content reflecting increased release.

Antibody-based detection methods include immunocytochemistry, which gives qualitative data but has very good spatial resolution. Radioimmunoassays provide a quantitative measure of release or content. One of the major limitations of all antibody-based methods is the potential for cross-reactivity among the many peptides. For example, some of the most sensitive 'gastrin' antisera also detect CCK, since the peptides share a common COOH-terminal tetrapeptide sequence. Methods for detection of the mRNAs encoding neuropeptides include Northern blots, which provide quantitative data and information on splice variants, but lack fine anatomical resolution. The more commonly used polymerase chain reaction (PCR), which often is used in a more qualitative manner, provides great sensitivity, and quantitative real-time PCR is becoming much more widely used. Alternatively, *in situ* hybridization preserves anatomical relationships and can be used to obtain both qualitative and quantitative data.

Direct methods for detection of neuropeptides include metabolic labeling with an appropriate radioactive amino acid and chemical isolation of peptides. Direct methods are difficult to apply to peptides or their receptors in the CNS, where the cells expressing the peptide are part of a large population of other neurons or endocrine cells. Reversed-phase high-pressure liquid chromatography or two-dimensional polyacrylamide gel electrophoresis, coupled with mass spectrometric identification, has revolutionized the identification of tiny amounts of peptides and receptors in tissue extracts. Similarly, the ability to mark living neurons producing a specific neuropeptide precursor with a fluorescent tag (e.g., a green fluorescent protein expression driven by the promoter for the peptide precursor) has revolutionized the ability to study the functions of specific peptidergic neurons (Tekinay et al., 2009).

Peptide agonists and antagonists are becoming available for many peptides, and these are essential to a successful dissection of the function of peptidergic synapses, as they have been for conventional neurotransmitters (discussed in Chs. 13–19). These approaches have the advantage that a physiologically important trait, binding of the ligand to the receptor, is being observed; in addition, these approaches can reveal binding to several related receptors, which are then distinguished by antisera, *in situ* hybridization, or drugs specific for receptor subtypes.

Peptides play a role in the plurichemical coding of neuronal signals

Many sets of neurons are chemically coded, giving rise to the concept of plurichemical transmission. For example, from the human lumbar paravertebral ganglion, adrenergic neurons innervating blood vessels in skin and muscle usually contain NPY along with norepinephrine, while adrenergic neurons innervating hair follicles express primarily norepinephrine. The dual transmission onto blood vessels is important since the catecholamine effects undergo rapid tachyphylaxis, while sustained contraction of the blood vessels is mediated by NPY. It is likely that a similar but more complex mixture of phenotypes occurs in the CNS; for example, some CCK-containing neurons also secrete dopamine while others release glutamate (Hokfelt et al., 2002). Peptides are generally released by the same neurons that release fast-acting classical neurotransmitters, and each transmitter has its own unique effects on target tissues.

Neuropeptides make a unique contribution to signaling

The release of neuropeptides generally requires a more intense stimulus than that required for release of fast-acting classical neurotransmitters. The more intense stimulus presumably results in more entry of Ca^{2+} into the presynaptic terminal, allowing Ca^{2} to diffuse from its entry site to the LDCVs (Figs. 20-3 and 20-4). As a result, the contribution of peptides vs. fast-acting conventional neurotransmitters to signaling can vary with the pattern of stimulation. For example, in *Aplysia*, several identified motor neurons devote about 10% of their total protein synthesis to their respective peptide neurotransmitters. Peptide release in each case has been shown to require extracellular Ca^{2}, and release increases with the frequency of action potentials, in the range of frequencies seen in behaving animals. In addition to the peptides, several of the motor neurons secrete ACh. In these motor neurons, peptide release increases more strongly with action potential frequency than does release of ACh, so the ratio of peptide to ACh is dependent on frequency. In addition, there are unique postsynaptic responses, such as elevation of cAMP concentrations in muscles, which can be mimicked only by application of the peptides and not by the conventional neurotransmitters. In other examples, the amount of peptide available for release can be depleted by repeated firing of a terminal since new peptide must arrive by axonal transport, while new conventional neurotransmitters are synthesized or recaptured locally and transported into small synaptic vesicles.

Regulation of neuropeptide expression is exerted at several levels

Control of neuropeptide function is mediated by factors controlling rates of prepropeptide gene transcription, translation, peptide degradation and secretion (Fig. 20-11). On the scale of seconds to minutes, peptide secretion is not always in lockstep with classical transmitter release (example above). Peptides are inactivated by diffusion and by proteolysis, so it would be expected that inhibition of specific extracellular proteases could lead to increased effectiveness of their neuropeptide substrates, as has been shown in the cases of CCK and the enkephalins.

On a time scale of minutes to hours, the transcription of prepropeptide mRNA is regulated. Peptide mRNAs are often immediate early genes, showing extremely rapid responses to stimuli that are not blocked by inhibitors of protein translation. The rate of translation of existing prepropeptide mRNAs can also change dramatically within a few minutes of stimulation. Since processing enzymes must also be synthesized and inserted into the same LDCVs as the peptide precursors, the rate of production of these enzymes might be expected to show changes under physiological conditions. They do show such changes but usually more slowly than the peptide

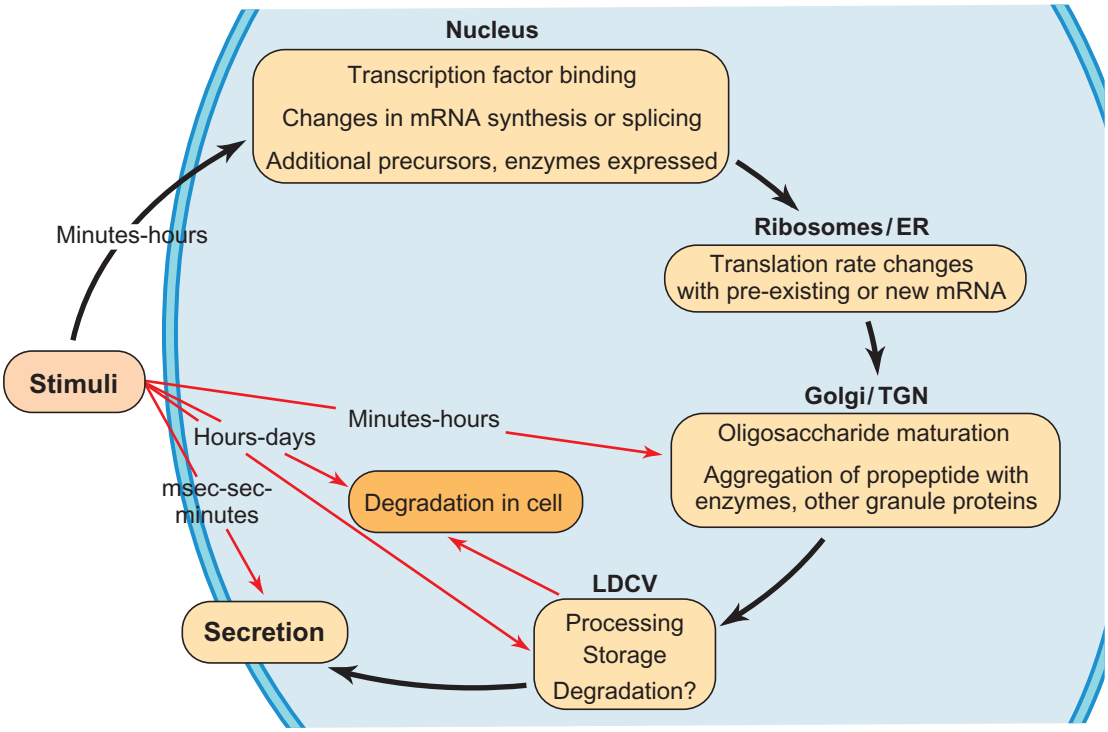


FIGURE 20-11 **Regulation of neuropeptide expression is exerted at several levels**. *ER*, endoplasmic reticulum; *LDCV*, large dense-core vesicle; *TGN*, *trans*-Golgi network.

precursors. As seen above (Figs. 20-5 and 20-8), the peptide products produced are crucially dependent on the mixture of processing enzymes in the LDCVs.

On a scale of hours to days, dramatic changes can be seen in cells producing peptides that are subjected to chronic stimulation or inhibition. POMC production by intermediate pituitary melanotropes is inhibited by treatment of rats with dopaminergic agonists or exposure of frogs to a light background; POMC production is stimulated by treatment of rats with dopaminergic antagonists or exposure of frogs to a dark background. The expression of processing enzymes, the number of LDCVs and the rate of cell division are also responsive to these treatments. Similarly, the processing of different regions of the pro-TRH precursor responds to thyroid hormone status, and it is possible that the routing of ELH and the bag cell peptides is regulated (Fig. 20-8). In response to changes in steroid hormone concentrations that occur during the estrous cycle in female rats, the concentration of CCK mRNA varies more than twofold in a subset of CCK-producing CNS neurons.

PEPTIDERGIC SYSTEMS IN DISEASE

Diabetes insipidus occurs with a loss of vasopressin production in the Brattleboro rat model

In the Brattleboro rat, a single-base deletion in the vasopressin gene alters the COOH terminus of preprovasopressin so that it cannot leave the endoplasmic reticulum; as a result, no neurons make vasopressin. Intriguingly, a frameshift mutation occurs in postmitotic neurons, most often an additional deletion of two nucleotides, so that the neurons somewhat regain the ability to produce vasopressin. In the *hpg* mouse, a spontaneous mutation in the gonadotropin-hormone-releasing hormone gene results in loss of gonadal function in homozygous animals. For both vasopressin and growth hormone, dominant negative mutations occur which block successful expression of the normal gene—these dominant negative phenotypes are presumably due to defects in folding in the endoplasmic reticulum or packaging into large dense-core vesicles.

Mutations and knockouts of peptide-processing enzyme genes cause a myriad of physiological problems

A single-base change in the CPE gene changes Ser^{202} to Pro, yielding a CPE molecule that is malfolded, inactive and incapable of exiting from the endoplasmic reticulum (Arolas et al., 2007). The phenotype observed in these *fat/fat* mice, which includes obesity and late-onset diabetes mellitus, is caused in part by high concentrations of inactive pro-insulin in the blood, with very little mature, functional insulin (Fig. 20-12). In the brains of these *fat/fat* mice, there is an excess of immature, biologically inactive neuropeptide fragments, such as neurotensin–Lys–Arg and thyrotropin-releasing hormone intermediates. It is still unclear how the loss of CPE function causes all the biochemical effects observed, with selective loss of processing of several peptides, while other peptide systems remain relatively quite normal.

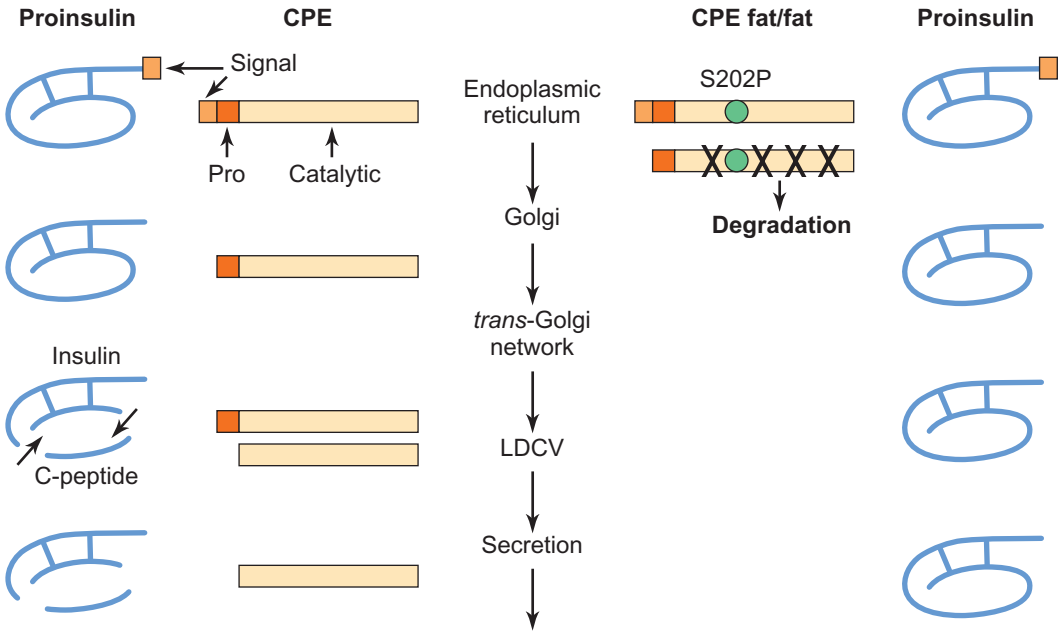


FIGURE 20-12 **The *fat/fat* mutation in carboxypeptidase E (*CPE*) leads to secretion of proinsulin, not mature insulin, and results in diabetes**. The S202P mutation within CPE results in degradation of the enzyme and defective insulin processing in the *fat/fat* heterozygous mouse. *LDCV*, large dense-core vesicle.

The fact that the naturally occurring CPE knockout (*fat/fat*) is viable led to a search for additional CPE-like enzymes, which were indeed found (Arolas et al., 2007). Mouse knockouts for furin and PC7 die in early to mid-gestation, while knockouts for PACE4 and PAM die at or before embryonic day 15 (Czyzyk et al., 2005). Knockouts for PC1, PC2 and the PC2 chaperone-inhibitor 7B2 are viable, although not normal. The PC1 knockout mouse has symptoms due to hyperproinsulinemia (excessive secretion of uncleaved and inactive proinsulin). In contrast, human patients lacking PC1 exhibit early-onset severe obesity, perhaps due to the loss of MSH in the hypothalamus (Czyzyk et al., 2005). The PC2 knockout mouse has deficits related to glucagon and MSH biosynthesis, but is fairly normal. The 7B2 knockout mouse is similar to the PC2 knockout when expressed in C57bl/6 mice; as predicted, 7B2 knockout mice have little or no detectable PC2 activity (Peinado et al., 2005). Mouse strains differ greatly in their endocrine and neurological responses to peptide processing gene ablation; in the 129/SvEv mouse strain, elimination of PC2 or 7B2 yields mice that die shortly after weaning (Lee et al., 2007; Peinado et al., 2005). This may be a reflection of the large differences in the level of expression of certain peptide processing enzymes across mouse strains (Sandberg et al., 2000) and demonstrates how difficult it may be to reveal the key roles of these enzyme in human disease.

Neuropeptides play key roles in appetite regulation and obesity

Many genes for neuropeptides and neuropeptide receptors have been implicated in obesity and cachexia, anorexia and bulimia (Date et al., 2010). For example, NPY administration into the CNS causes overeating and obesity. A second peptide involved in obesity is leptin, a product of adipocytes and the stomach. The leptin gene is defective in the *ob/ob* mouse; in normal mice, leptin binds to its receptor in the hypothalamus, causing a decrease in the synthesis and release of hypothalamic NPY.

Control of feeding behavior involves peripheral peptides (insulin, ghrelin, leptin) plus several peptides in the CNS (orexins/hypocretins, CCK, galanin, MSH, neuropeptide Y, CRH, cocaine-and-amphetamine-regulated transcript (CART)) (Date et al., 2010; Kalantaridou et al., 2010). Some of the same peptides are involved in reward systems crucial to drug addiction. Specific receptor blockers are being tested for many of these peptide-receptor systems, with the hope of very selective actions with minimal side effects. For example, there are two CCK receptor subtypes, CCK-A and CCK-B. Protease inhibitors that slow the degradation and inactivation of endogenous CCK promote satiety via CCK-A receptor. By contrast, the CCK-B receptor is important in mediating anxiety and panic attacks, and CCK antagonists are in clinical use to treat these symptoms.

Enkephalin knockout mice reach adulthood and are healthy

When the proenkephalin gene, one of the three endogenous opiate peptide precursor genes, was eliminated from the mouse genome by genetic engineering, the knockout mice exhibited normal hotplate/tail-flick responses; in normal mice, the tail-flick response (moving the tail away from a hot light) is diminished by prior exposure to another noxious stimulus, an unavoidable electrical foot-shock. This indicates

that enkephalin plays no role in the normal tail-flick response but does play a role in centrally mediated analgesia. Similarly, β-endorphin knockout mice also show a loss in centrally mediated analgesia. Behavioral abnormalities in the enkephalin knockout mice include reduced exploratory activity in an unfamiliar environment and increased aggressiveness in the resident–intruder test. In both enkephalin and β-endorphin knockout mice, there are no compensatory increases in the concentrations of the remaining endogenous opiate peptides.

Neuropeptides are crucial to pain perception

Substance P is released in the spinal cord by a subset of dorsal root ganglion neurons; Substance P is crucial to the sensation of noxious stimuli. It binds to the neurokinin-1 receptor, which gets rapidly internalized and recycled to the plasma membrane of the neurons (Jimenez-Andrade et al., 2010). When substance P receptors are partially ablated, the sensitivity to noxious stimuli decreases. In conditions of chronic pain, the pattern of peptide expression can also change. For example, there is a dramatic increase in the expression of the opiate peptide dynorphin in the spinal cord, while dynorphin is undetectable in the normal animal.

References

Aloy, P., Companys, V., Vendrell, J., Aviles, F. X., Fricker, L. D., Coll, M., et al. (2001). The crystal structure of the inhibitor-complexed carboxypeptidase D domain II and the modeling of regulatory carboxypeptidases. *Journal of Biological Chemistry*, *276*, 16177–16184.

Arolas, J. L., Vendrell, J., Aviles, F. X., & Fricker, L. D. (2007). Metallocarboxypeptidases: Emerging drug targets in biomedicine. *Current Pharmaceutical Design*, *13*, 347–364.

Brothers, S. P., Janovick, J. A., & Conn, P. M. (2003). Unexpected effects of epitope and chimeric tags on gonadotropin-releasing hormone receptors: Implications for understanding the molecular etiology of hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, *88*, 6107–6112.

Chufan, E. E., De, M., Eipper, B. A., Mains, R. E., & Amzel, L. M. (2010). Amidation of bioactive peptides: The structure of the lyase domain of the amidating enzyme. *Structure*, *17*, 965–973.

Czyzyk, T. A., Ning, Y., Hsu, M. S., Peng, B., Mains, R. E., Eipper, B. A., et al. (2005). Deletion of peptide amidation enzymatic activity leads to edema and embryonic lethality in the mouse. *Developmental Biology*, *287*, 301–313.

Date, Y., Mondal, M. S., Kageyama, H., Ghamari-Langroudi, M., Cone, R. D., & Nakazato, M. (2010). Neuropeptide W: An anorectic peptide regulated by leptin and metabolic state. *Endocrinology*, *151*, 2200–2210.

Francone, V. P., Ifrum, M. F., Rajagopal, C., Leddy, C. J., Wang, Y., Carson, J. H., et al. (2010). Signaling from the secretory granule to the nucleus: Uhmk1 and PAM. *Molecular Endocrinology*, *24*, 1543–1558.

Grimmelikhuijzen, C. J. P., Hauser, F., Eriksen, F. F., & Williamson, M. (1999). Invertebrate neurohormones and their receptors. *Results and Problems in Cell Differentiation*, *26*, 339–362.

Gruber, K. A., Fan, W., Akerberg, H., Larhammar, D., Chee, M. J. S., Colmers, W. F., et al. (2009). Neuropeptide Y and gamma-melanocyte stimulating hormone share a common pressor mechanism of action. *Endocrine*, *35*, 312–324.

Hallberg, M., & Nyberg, F. (2003). Neuropeptide conversion to bioactive fragments—an important pathway in neuromodulation. *Current Protein & Peptide Science*, 4, 31–44.

Helke, C. J., Krause, J. E., Mantyh, P. W., Couture, R., & Bannon, M. J. (1990). Diversity in mammalian tachykinin peptidergic neurons: Multiple peptides, receptors, and regulatory mechanisms. *The FASEB Journal*, *4*, 1606–1615.

Henrich, S., Lindberg, I., Bode, W., & Than, M. E. (2005). Proprotein convertase models based on the crystal structures of furin and kexin: Explanation of their specificity. *Journal of Molecular Biology*, *345*, 211–227.

Hokfelt, T., Blacker, D., Broberger, C., Herrera-Marschitz, M., & Snyder, G. (2002). Some aspects on the anatomy and function of central cholecystokinin systems. *Pharmacology and Toxicology*, *91*, 382–386.

Jimenez, E. C., & Olivera, B. M. (2010). Divergent M- and O-superfamily peptides from venom of fish-hunting Conus pariu. *Peptides*, *31*, 1678–1683.

Jimenez-Andrade, J. M., Mantyh, W. G., Bloom, A. P., Xu, H., Vanderah, T. W., & Mantyh, P. W. (2010). A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin: Therapeutic opportunity for treating skeletal pain. *Bone*, *46*, 306–313.

Kalantaridou, S. N., Zoumakis, E., Makrigiannakis, A., Lavasidis, L. G., Vrekoussis, T., & Chrousos, G. P. (2010). Corticotropin-releasing hormone, stress and human reproduction: An update. *Journal of Reproductive Immunology*, *85*, 33–39.

Karhunen, T., Vilim, F. S., Alexeeva, V., Weiss, K. R., & Church, P. J. (2001). Targeting of peptidergic vesicles in cotransmitting terminals. *Journal of Neuroscience*, *21*, RC127.

Lee, S. N., & Lindberg, I. (2008). 7B2 Prevents unfolding and aggregation of prohormone convertase 2. *Endocrinology*, *149*, 4116–4127.

Lee, S. N., Peng, B., Desjardins, R., Pintar, J. E., Day, R., & Lindberg, I. (2007). Strain-specific steroidal control of pituitary function. *Journal of Endocrinology*, *192*, 515–525.

Li, C., Kim, K., & Nelson, L. S. (1999). FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. *Brain Research*, *848*, 26–34.

Mains, R. E., & Eipper, B. A. (1990). The tissue-specific processing of pro-ACTH/endorphin. *Trends in Endocrinology and Metabolism*, *1*, 388–394.

Mantyh, P. W. (2002). Neurobiology of substance P and the NK1 receptor. *Journal of Clinical Psychiatry*, *63*(Suppl. 11), 6–10.

McPherson, J., Rivero, G., Baptist, M., Llorente, J., Henderson, G., & Kelly, E. (2010). Mu-opioid receptors: Correlation of agonist efficacy for signalling with ability to activate internalization. *Molecular Pharmacology* (PMID: 20647394).

Millan, M. J., Dekeyne, A., Gobert, A., Mannoury la Cour, C., Olivier, B., & de Nanteuil, G. (2010). S41744, a dual neurokinin (NK)1 receptor antagonist and serotonin (5-HT) reuptake inhibitor with potential antidepressant properties: A comparison to aprepitant (MK869) and paroxetine. *European Neuropsychopharmacology*, *20*, 599–621.

Peinado, J. R., Laurent, V., Lee, S. N., Peng, B. W., Pintar, J. E., Steiner, D. F., et al. (2005). Strain-dependent influences on the hypothalamo-pituitary-adrenal axis profoundly affect the 7B2 and PC2 null phenotypes. *Endocrinology*, *146*, 3438–3444.

Prigge, S. T., Mains, R. E., Eipper, B. A., & Amzel, L. M. (2000). New insights into copper monooxygenases and peptide amidation: Structure, mechanism and function. *Cellular and Molecular Life Sciences*, *57*, 1236–1259.

Rajagopal, C., Stone, K. L., Francone, V. P., Mains, R. E., & Eipper, B. A. (2009). Secretory Granule to the Nucleus: Role of a multiply phosphorylated intrinsically unstructured domain. *Journal of Biological Chemistry*, *284*, 25723–25734.

Reinscheid, R. K., Nothacker, H. P., Bourson, A., Ardati, A., & Civelli, O. (1995). Orphanin FQ: A neuropeptide that activates an opioidlike G protein-coupled receptor. *Science*, *270*, 792–794.

Reubi, J. C. (2007). Peptide receptor expression in GEP-NET. *Virchows Archiv*, *451*, S47–S50.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., & Yanagisawa, M. (1998). Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, *92*, 573–585.

Sandberg, R., Yasuda, R., Pankratz, D. G., Carter, T. A., Lockhart, D. J., & Barlow, C. (2000). Regional and strain-specific gene expression mapping in the adult mouse brain. *Proceedings of the National Academy of Sciences U.S.A.*, *97*, 11038–11043.

Sarzi-Puttini, P., Atzeni, F., & Cazzola, M. (2010). Neuroendocrine therapy of fibromyalgia syndrome: An update. *Annals of the New York Academy of Sciences*, *1193*, 91–97.

Schilling, S., Zeitschel, U., Hoffmann, T., Heiser, U., Cynis, H., Demuth, H. U., et al. (2008). Glutaminyl cyclase inhibition attenuates pyroglutamate A-beta and Alzheimer's disease–like pathology. *Nature Medicine*, *14*, 1106–1111.

Seidah, N. G., & Chretien, M. (1999). Proprotein and prohormone convertases: A family of subtilases generating diverse bioactive polypeptides. *Brain Research*, *848*, 45–62.

Seidah, N. G., Mayer, G., Zaid, A., Rousselet, E., Nassoury, N., Poirier, S., et al. (2008). The activation and physiological functions of the proprotein convertases. *The International Journal of Biochemistry & Cell Biology*, *40*, 1111–1125.

Stephan, A., Wermann, M., von Bohlen, A., Koch, B., Cynis, H., Demuth, H. U., et al. (2009). Mammalian glutaminyl cyclases and their isoenzymes have identical enzymatic characteristics. *The FEBS Journal*, *276*, 6522–6536.

Taghert, P. H., & Veenstra, J. A. (2003). Drosophila neuropeptide signaling. *Advances in Genetics*, *49*, 1–65.

Takanaga, H., Mackenzie, B., & Hediger, M. A. (2004). Sodium-dependent ascorbic acid transporter family SLC23. *Pflugers Archiv*, *447*, 677–682.

Takigami, T., Takeuchi, F., Nakagawa, M., Hase, T., & Tsubaki, M. (2003). Stopped-flow analyses on the reaction of ascorbate with cytochrome b561 purified from bovine chromaffin vesicle membranes. *Biochemistry*, *42*, 8110–8118.

Tekinay, A. B., Nong, Y., Miwa, J. M., Lieberam, I., Ibanez-Tallon, I., Greengard, P., et al. (2009). A role for LYNX2 in anxiety–related behavior. *Proceedings of the National Academy of Sciences U.S.A.*, *106*, 4477–4482.

Terskiy, A., Wannemacher, K. M., Prem, N., Yadav, P. N., Tsai, M., Tian, B., et al. (2007). Search of the human proteome for endomorphin-1 and endomorphin-2 precursor proteins. *Life Sciences*, *81*, 1593–1601.

Wang, S. S., Yan, X. B., Hofman, M. A., Swaab, D. F., & Zhou, J. N. (2010). Increased expression level of corticotropin-releasing hormone in the amygdala and in the hypothalamus in rats exposed to chronic unpredictable mild stress. *Neuroscience Bulletin*, *26*, 297–303.

Westmuckett, A. D., Hoffhines, A. J., Borghei, A., & Moore, K. L. (2008). Early postnatal pulmonary failure and primary hypothyroidism in mice with combined TPST-1 and TPST-2 deficiency. *General and Comparative Endocrinology*, *156*, 145–153.

Xie, J., Price, M. P., Berger, A. L., & Welsh, M. J. (2002). DRASIC contributes to pH-gated currents in large dorsal root ganglion sensory neurons by forming heteromultimeric channels. *Journal of Neurophysiology*, *87*, 2835–2843.

Yamaguchi, M., Yamada, Y., Hosokawa, Y., Iwamoto, R., Hoshida, Y., & Matsuzawa, Y. (2010). Long-term suppressive effect of octreotide on progression of metastatic gastrinoma with multiple endocrine neoplasia type 1: Seven-year follow-up. *Internal Medicine*, *49*, 1557–1563.

Zhou, A., Webb, G., Zhu, X., & Steiner, D. F. (1999). Proteolytic processing in the secretory pathway. *Journal of Biological Chemistry*, *274*, 20745–20748.