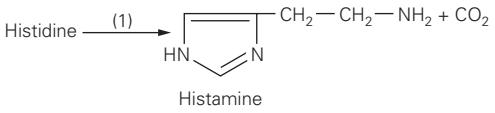


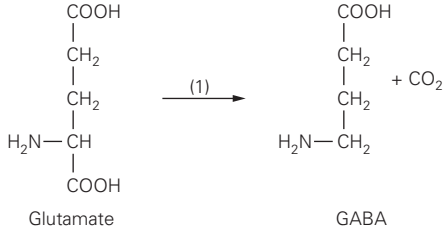
As described in the next section, the biogenic amines are loaded into synaptic and secretory vesicles by two transporters, VMAT1, mostly in peripheral cells, and VMAT2, mostly in the central nervous system. As the transporters are not selective for a given biogenic amine, a mixture of transmitters can be present. Some neurons co-release dopamine with norepinephrine, whereas secretory vesicles from the adrenal medulla can co-release epinephrine and norepinephrine.

Amino Acid Transmitters

In contrast to acetylcholine and the biogenic amines, which are not intermediates in general metabolic pathways and are produced only in certain neurons, the amino acids glutamate and glycine are not only neurotransmitters but also universal cellular constituents. Because they can be synthesized in neurons and other cells, neither is an essential amino acid.

Glutamate, the neurotransmitter most frequently used at excitatory synapses throughout the central nervous system, is produced from α-ketoglutarate, an intermediate in the tricarboxylic acid cycle of intermediary metabolism. After it is released, glutamate is taken up from the synaptic cleft by specific transporters in the membrane of both neurons and glia (see later). The glutamate taken up by astrocytes is converted to glutamine by the enzyme glutamine synthase. This glutamine is transported back into neurons that use glutamate as a transmitter, where it is hydrolyzed to glutamate by the enzyme glutaminase. Cytoplasmic glutamate is then loaded into synaptic vesicles by the vesicular glutamate transporter, VGLUT.

Glycine is the major transmitter used by inhibitory interneurons of the spinal cord. It is also a necessary cofactor for activation of the *N*-methyl-D-aspartate (NMDA) glutamate receptors (Chapter 13). Glycine is synthesized from serine by the mitochondrial form of the serine hydroxymethyltransferase. The amino acid GABA is synthesized from glutamate in a reaction catalyzed by glutamic acid decarboxylase (step 1 below):



GABA is present at high concentrations throughout the central nervous system and is detectable in other tissues. It is used as a transmitter by an important class of inhibitory interneurons in the spinal cord. In the brain, GABA is the major transmitter of a wide array of inhibitory neurons and interneurons. Both GABA and glycine are loaded into synaptic vesicles by the same transporter, VGAT, and thus can be co-released from the same vesicles.

ATP and Adenosine

ATP and its degradation products (eg, adenosine) act as transmitters at some synapses by binding to several classes of G protein–coupled receptors (the P1 and P2Y receptors). ATP can also produce excitatory actions by binding to ionotropic P2X receptors. Caffeine's stimulatory effects depend on its inhibition of adenosine binding to the P1 receptors. Adenine and guanine and their sugar-containing derivatives are called purines; the evidence for transmission at purinergic receptors is especially strong for autonomic neurons that innervate the vas deferens, bladder, and muscle fibers of the heart; for nerve plexuses on smooth muscle in the gut; and for some neurons in the brain. Purinergic transmission is particularly important for nerves mediating pain (Chapter 20).

ATP released by tissue damage acts to transmit pain sensation through one type of ionotropic purine receptor present on the terminals of peripheral axons of dorsal root ganglion cells that act as nociceptors. ATP released from terminals of the central axons of these dorsal root ganglion cells excites another type of ionotropic purine receptor on neurons in the dorsal horn of the spinal cord. ATP and other nucleotides also act at the family of P2Y G protein–coupled receptors to modulate various downstream signaling pathways.

Small-Molecule Transmitters Are Actively Taken Up Into Vesicles

Common amino acids act as transmitters in some neurons but not in others, indicating that the presence of a substance in a neuron, even in substantial amounts, is not in itself sufficient evidence that the substance is used as a transmitter. For example, at the neuromuscular junction of the lobster (and other arthropods), GABA is inhibitory and glutamate is excitatory. The concentration of GABA is approximately 20 times greater in inhibitory cells than in excitatory cells,

supporting the idea that GABA is the inhibitory transmitter at the lobster neuromuscular junction. In contrast, the concentration of the excitatory transmitter glutamate is similar in both excitatory and inhibitory cells. Glutamate therefore must be compartmentalized within these neurons; that is, *transmitter* glutamate must be kept separate from *metabolic* glutamate. In fact, transmitter glutamate is compartmentalized in synaptic vesicles.

Although the presence of a specific set of biosynthetic enzymes can determine whether a small molecule can be used as a transmitter, the presence of the enzymes does not mean that the molecule will be used. Before a substance can be released as a transmitter, it usually must first be concentrated in synaptic vesicles. Transmitter concentrations within vesicles are high, on the order of several hundred millimolar. Neurotransmitter substances are concentrated in vesicles by transporters that are specific to each type of neuron and energized by a vacuolar-type H^+-ATPase (V-ATPase) that is found not only in synaptic and secretory vesicles but also in all organelles in the secretory pathway, including endosomes and lysosomes.

Using the energy generated by the hydrolysis of cytoplasmic ATP, the V-ATPase creates an H^+ electrochemical gradient by promoting the influx of protons into the vesicle. Transporters use this proton gradient to drive transmitter molecules into the vesicles against their concentration gradient through a proton-antiport mechanism. A number of different vesicular transporters in mammals are responsible for concentrating different transmitter molecules in vesicles (Figure 16–1). These proteins span the vesicle membrane 12 times and are distantly related to a class of bacterial transporters that mediate drug resistance. (Vesicular transporters differ structurally and mechanistically from the transporters in the plasma membrane, as discussed later.)

Transmitter molecules are classically modeled to be taken up into a vesicle by vesicular transporters in exchange for the transport of two protons out of the vesicle. Because the maintenance of the pH gradient requires the hydrolysis of ATP, the uptake of transmitter into vesicles is energy-dependent. Vesicular transporters can concentrate some neurotransmitters such as dopamine up to 100,000-fold relative to their concentration in the cytoplasm. Uptake of transmitters by the transporters is rapid, enabling vesicles to be quickly refilled after they release their transmitter and are retrieved by endocytosis; this is important for maintaining the supply of releasable vesicles during periods of rapid nerve firing (Chapter 15).

The specificity of transporters for substrate is quite variable. The vesicular ACh transporter (VAChT) does not transport choline or other transmitters. Likewise, the vesicular glutamate transporters, for which there are three types (VGLUT1, 2, and 3) that are differentially expressed in the CNS, carry negligible amounts of the other acidic amino acid, aspartate. However, VMAT2 can transport all of the biogenic amines as well as drugs including amphetamine and even some neurotoxic compounds such as *N*-methyl-4-phenylpyridinium (MPP^+). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a contaminant of a synthetic opiate drug of abuse, is metabolized to MPP^+ by the enzyme monoamine oxidase (MAO) type B. In fact, VMAT1 was cloned by Robert Edwards and colleagues based on the ability of the transporter to protect cells from the neurotoxic effects of MPP^+; cells expressing VMAT were able to sequester the toxin in vesicle-like compartments, thereby lowering its cytoplasmic concentration and promoting cell survival. By expressing genes obtained from a cDNA library from adrenal pheochromocytoma cells in a mammalian cell line sensitive to MPP^+, Edwards was able to identify cells that expressed VMAT1 based on their selective survival. VMAT2 was subsequently identified by homology cloning, as well as directly by a number of other groups.

Transporters and V-ATPases are present in the membranes of both small synaptic vesicles and large dense-core vesicles. Vesicular transporters are the targets of several important pharmacological agents. Reserpine and tetrabenazine inhibit uptake of amine transmitters by binding to the vesicular monoamine transporter. The psychostimulants amphetamine, methamphetamine, and 3,4-methylenedioxy-*N*-methylamphetamine (MDMA or ecstasy) act to deplete vesicles of amine transmitter molecules, but also cause their efflux from the cytoplasm into the extracellular space via the plasma membrane biogenic amine transporters (see below). These compounds accumulate inside vesicles through proton-antiport–driven transport mediated by VMAT, which diminishes the proton gradient necessary for loading amine transmitters into vesicles.

Drugs that are sufficiently similar to the normal transmitter substance can act as *false transmitters.* These are packaged in vesicles and released by exocytosis as if they were true transmitters, but they often bind only weakly or not at all to the postsynaptic receptor for the natural transmitter, and thus their release decreases the efficacy of transmission. Several drugs historically used to treat hypertension, such as α-methyldopa and guanethidine, are taken up into adrenergic synapses

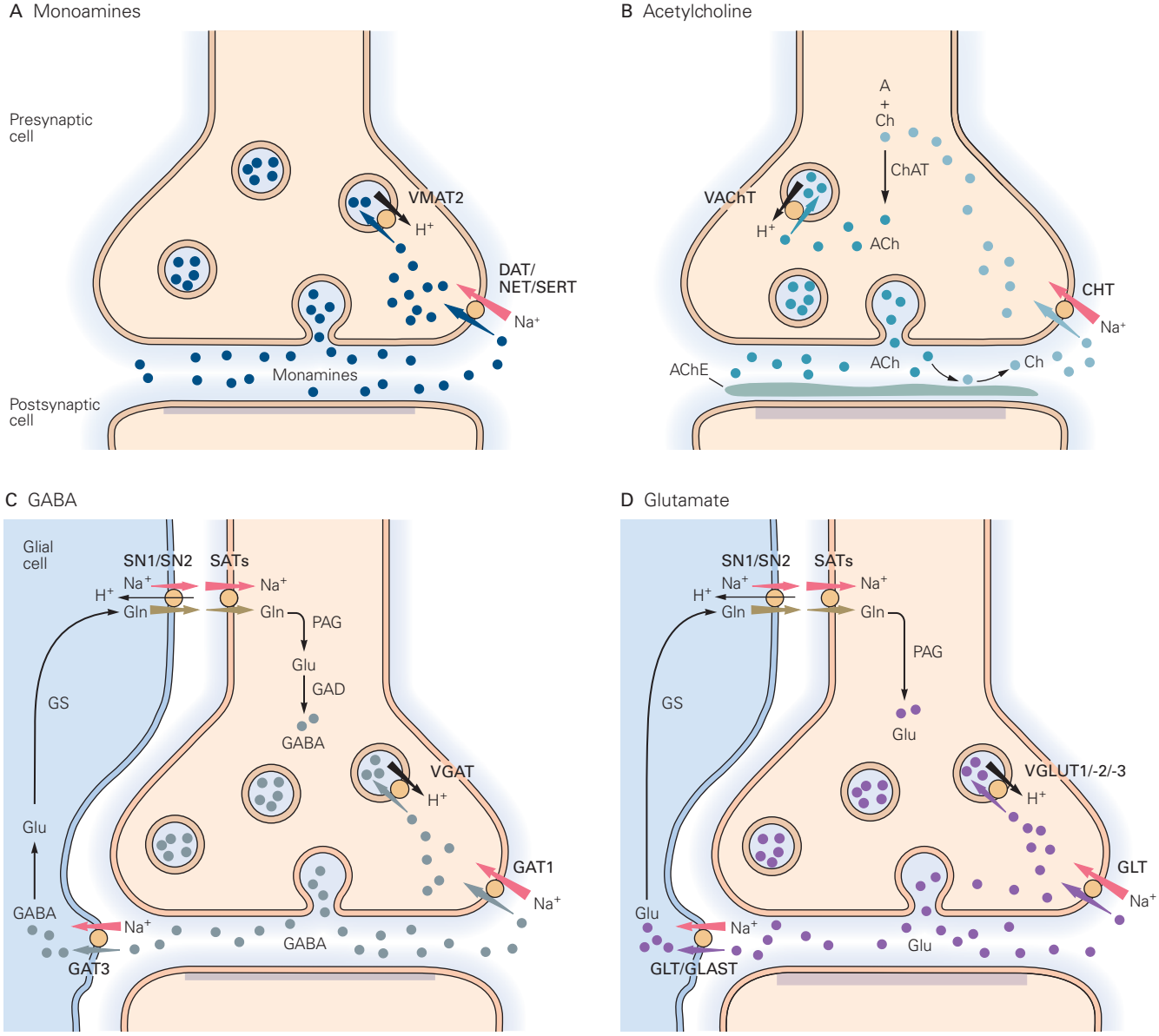


Figure 16–1 **Small-molecule transmitters are transported from the cytosol into vesicles or from the synaptic cleft to the cytosol by transporters.** Most small-molecule neurotransmitters are released by exocytosis from the nerve terminal and act on specific postsynaptic receptors. The signal is terminated and transmitter recycled by specific transporter proteins located at the nerve terminal or in surrounding glial cells. Transport by these proteins (**orange circles**) is driven by the electrochemical gradients of H^+ (**black arrows**) or Na^+ (**red arrows**). (Adapted, with permission, from Chaudhry et al. 2008. Copyright © 2008 Springer-Verlag.)

A. Three distinct transporters mediate reuptake of monoamines across the plasma membrane. The dopamine transporter (**DAT**), norepinephrine transporter (**NET**), and serotonin transporter (**SERT**) are responsible for the reuptake (**dark blue arrows**) of their cognate transmitters. The vesicular monoamine transporter (**VMAT2**) transports all three monoamines into synaptic vesicles for subsequent exocytotic release.

B. Cholinergic signaling is terminated by metabolism of acetylcholine (**ACh**) to the inactive choline and acetate by acetylcholinesterase (**AChE**), which is located in the synaptic cleft (**green bar**). Choline (**Ch**) is transported by the choline transporter (**CHT**) back into the nerve terminal (**light blue arrow**) where choline acetyltransferase (**ChAT**) subsequently catalyzes acetylation of choline to reconstitute ACh. The ACh is transported into the vesicle by the vesicular ACh transporter (**VAChT**).

C. At GABAergic and glycinergic nerve terminals, the GABA transporter (**GAT1**) and glycine transporter (GLYT2, not shown) mediate reuptake of GABA and glycine (**gray arrow**), respectively. GABA may also be taken up by surrounding glial cells (eg, by GAT3). In the glial cells, GABA is first converted to glutamate (**Glu**) by GAD. Glu is then is converted by glial glutamine synthetase (**GS**) to glutamine (**Gln**). Glutamine is transported back to the nerve terminal by the concerted action of the system N transporter (**SN1/SN2**) and system A transporter (**SAT**) (**brown arrows**). In the nerve terminal, phosphate-activated glutaminase (**PAG**) converts glutamine to glutamate, which is converted to GABA by glutamate decarboxylase (**GAD**). VGAT then transports GABA into vesicles. The glial transporter GLYT1 (not shown) also contributes to the clearance of glycine.

D. After release from excitatory neuronal terminals, the majority of glutamate is taken up by surrounding glial cells (eg, by **GLT** and **GLAST**) for conversion to glutamine, which is subsequently transported back to the nerve terminals by SN1/SN2 and a type of SAT (**SATx**) (**brown arrows**). Reuptake of glutamate at glutamatergic terminals also has been demonstrated for a GLT isoform (**purple arrows**). Glutamate is transported into vesicles by **VGLUT**.

(and converted into α-methyldopamine in the case of α-methyldopa) and replace norepinephrine in synaptic vesicles. When released, these drugs fail to stimulate postsynaptic adrenergic receptors, thereby relaxing vascular smooth muscle by inhibiting adrenergic tone. Tyramine, which is found in high quantities in dietary red wine and cheese, also acts as a false transmitter; however, it also can act as a stimulant by releasing biogenic amines through a mechanism akin to amphetamine. Another false transmitter, 5-hydroxydopamine, can produce an electron-dense reaction product and has been used to identify synaptic vesicles that acquire biogenic amines.

More recently, several fluorescent false neurotransmitters have been designed, enabling researchers to use imaging methods to monitor the uptake and release of neurotransmitter derivatives during synaptic activity in rodent and fly brain (see Figure 16–5 in Box 16–2).

An unexpected finding is that dopamine can be released from dendrites as well as from axons, despite the lack of synaptic vesicles in dendrites. Organelles that express VMAT2 seem likely to be the source of the release, albeit with different requirements for intracellular Ca^{2+} than classical neurotransmission at presynaptic terminals. For technical reasons, this phenomenon has been studied mostly in dendrites of dopaminergic neurons of the substantia nigra: dopamine can be measured directly by electrochemical techniques, and the dendrites are well separated from the cell bodies. However, it is possible that dendritic neurotransmitter release occurs more widely throughout the nervous system.

Many Neuroactive Peptides Serve as Transmitters

The enzymes that catalyze the synthesis of the low-molecular-weight neurotransmitters, with the exception of dopamine β-hydroxylase, are found in the cytoplasm. These enzymes are synthesized on free polysomes in the cell body and probably in dendrites and are distributed throughout the neuron by axoplasmic flow. Thus, small-molecule transmitter substances can be formed in all parts of the neuron; most importantly, they can be synthesized at axonal presynaptic sites from which they are released.

In contrast, neuroactive peptides are derived from secretory proteins that are formed in the cell body. More than 50 short peptides are produced by neurons or neuroendocrine cells and exert physiological actions (Table 16–2). Some act as hormones

Table 16–2 Neuroactive Mammalian Peptides

Category	Peptide
Hypothalamic neuropeptides	Thyrotropin-releasing hormone Gonadotropin-releasing hormone Corticotropin-releasing factor (CRF) Growth hormone–releasing hormone Melanocyte-stimulating hormone Melanocyte-inhibiting factor Somatostatin β-Endorphin Dynorphin Galanin Neuropeptide Y Orexin Oxytocin Vasopressin
Neurohypophyseal neuropeptides	Oxytocin Vasopressin
Pituitary peptides	Adrenocorticotropic hormone β-Endorphin α-MSH Prolactin Luteinizing hormone Growth hormone Thyrotropin
Pineal hormones	Melatonin
Basal ganglia	Substance P Enkephalin Dynorphin Neuropeptide Y Neurotensin Cholecystokinin Glucagon-like peptide-1 Cocaine- and amphetamine-regulated transcript (CART)
Gastrointestinal peptides	Vasoactive intestinal polypeptide Cholecystokinin Gastrin Substance P Neurotensin Methionine-enkephalin Leucine-enkephalin Insulin Glucagon Bombesin Secretin Somatostatin Thyrotropin-releasing hormone Motilin
Heart	Atrial natriuretic peptide
Other	Angiotensin II Bradykinin Calcitonin Calcitonin gene-related peptide (CGRP) Galanin Leptin Sleep peptide(s) Substance K (neurokinin A)

on targets outside the brain (eg, angiotensin and gastrin) or are products of neuroendocrine secretion (eg, oxytocin, vasopressin, somatostatin, luteinizing hormone, and thyrotropin-releasing hormone). In addition, many neuropeptides act as neurotransmitters when released close to a target neuron, where they can cause inhibition, excitation, or both.

Neuroactive peptides have been implicated in modulating sensory perception and affect. Some peptides, including substance P and the enkephalins, are preferentially located in regions of the central nervous system involved in the perception of pain. Other neuropeptides regulate complex responses to stress; these peptides include γ-melanocyte-stimulating hormone, corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH), dynorphin, and β-endorphin.

Although the diversity of neuroactive peptides is enormous, as a class these chemical messengers share a common cell biology. A striking generality is that neuroactive peptides are grouped in families with members that have similar sequences of amino acid residues. At least 10 have been identified; the seven main families are listed in Table 16–3.

Several different neuroactive peptides can be encoded by a single continuous messenger RNA (mRNA), which is translated into one large polyprotein precursor (Figure 16–2). Polyproteins can serve as a mechanism for amplification by providing more than one copy of the same peptide from the one precursor. For example, the precursor of glucagon contains two copies of the hormone. Polyproteins also generate diversity by producing several distinct peptides cleaved from one precursor, as in the case of the opioid peptides. The opioid peptides are derived from polyproteins encoded by three distinct genes. These peptides are endogenous ligands for a family of G protein–coupled receptors. In addition to endogenous agonists, the mu opioid receptor also binds drugs with analgesic and addictive properties, such as morphine and synthetic derivatives, including heroin and oxycodone.

The processing of more than one functional peptide from a single polyprotein is not unique to neuroactive peptides. The mechanism was first described for proteins encoded by small RNA viruses. Several viral polypeptides are produced from the same viral polyprotein, and all contribute to the generation of new virus particles. As with the virus, where the different proteins obviously serve a common biological purpose (formation of new viruses), a neuronal polypeptide will in many instances yield peptides that work together to serve a common physiological goal. Sometimes the biological functions appear to be more complex, as peptides with related or antagonistic activities can be generated from the same precursor.

A particularly striking example of this form of synergy is the group of peptides formed from the precursor of egg-laying hormone (ELH), a set of neuropeptides that govern diverse reproductive behaviors in the marine mollusk *Aplysia*. Egg-laying hormone can act as a hormone causing the contraction of oviduct muscles; it can also act as a neurotransmitter to alter the firing of several neurons involved in producing behaviors, as do the other peptides cut from the polyprotein.

The processing of neuroactive peptides takes place within the neuron's intracellular membrane system and in vesicles. Several peptides are produced from a single polyprotein by limited and specific proteolytic cleavage, catalyzed by proteases within these internal membrane systems. Some of these enzymes are serine proteases, a class that also includes the pancreatic enzymes trypsin and chymotrypsin. As with trypsin, the cleavage site of the peptide bond is determined by basic amino acid residues (lysine and arginine) in the substrate protein. Although cleavage is most common at dibasic residues, it can also occur at single basic residues, and polyproteins sometimes are cleaved at other peptide bonds.

Table 16–3 The Main Families of Neuroactive Peptides

Family	Peptide members
Opioids	Opiocortin, enkephalins, dynorphin, FMRFamide (Phe-Met-Arg-Phe-amide)
Neurohypophyseal neuropeptides	Vasopressin, oxytocin, neurophysins
Tachykinins	Substance P, physalaemin, kassinin, uperolein, eledoisin, bombesin, substance K
Secretins	Secretin, glucagon, vasoactive intestinal peptide, gastric inhibitory peptide, growth hormone–releasing factor, peptide histidine isoleucine amide
Insulins	Insulin, insulin-like growth factors I and II
Somatostatins	Somatostatins, pancreatic polypeptide
Gastrins	Gastrin, cholecystokinin

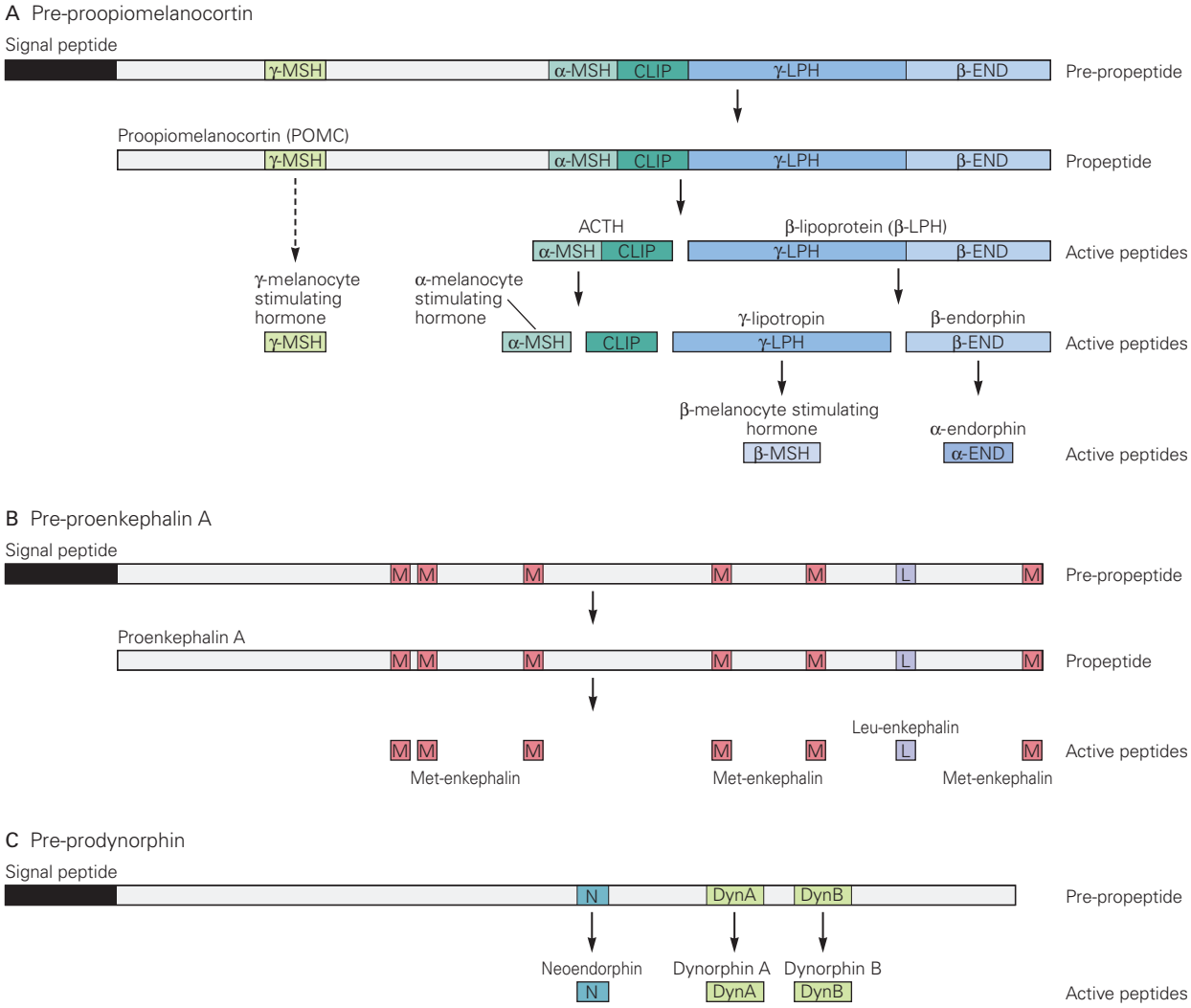


Figure 16–2 Hormone and neuropeptide precursors are processed differentially: The opioid family of neuropeptides. The opioid neuropeptides are derived from larger precursor molecules that require multiple rounds of protease-mediated cleavage. These precursors are processed differentially to yield their specific peptide products. Transport of these precursors through the membrane of the endoplasmic reticulum is initiated by a hydrophobic signal sequence. Internal cleavages often occur at basic residues within the polypeptide. Moreover, these precursors have key cysteine residues and sugar moieties that play roles in their processing and function. Generally, the first iteration of processing begins with the newly synthesized polyprotein precursor (known as the pre-propeptide form). Cleavage of an amino-terminal signal sequence generates a smaller molecule, the propeptide. Three major opioid peptide precursor proteins are encoded by three genes: *proopiomelanocortin* (***POMC***), *proenkephalin* (***PENK***), and *prodynorphin* (***PDYN***) (not shown). Differential processing of the three resultant pre-propeptides gives rise to the major opioid peptides—endorphins, enkephalins, and dynorphins.

A. The POMC precursor is processed differently in different lobes of the pituitary gland, resulting in α-melanocyte-stimulating hormone (**α-MSH**) and **γ-MSH**, corticotropin-like intermediate lobe peptide (**CLIP**), and β-lipotropin (**β-LPH**). β-LPH is cleaved to yield **γ-LPH** and β-endorphin (**β-END**), which themselves yield β-melanocyte-stimulating hormone (**β-MSH**) and α-endorphin (**α-END**), respectively. The endoproteolytic cleavages within adrenocorticotropic hormone (**ACTH**) and β-LPH take place in the intermediate lobe but not the anterior lobe.

B. Similar principles are evident in the processing of the enkephalin precursor, which gives rise to six Met-enkephalin peptides and one Leu-enkephalin peptide.

C. The dynorphin precursor is cleaved into at least three peptides that are related to Leu-enkephalin since the amino-terminal sequences of all three peptides contain the sequence of Leu-enkephalin.

Other types of peptidases also catalyze the limited proteolysis required for processing neuroactive peptides. Among these are thiol endopeptidases (with catalytic mechanisms like that of pepsin), amino peptidases (which remove the N-terminal amino acid of the peptide), and carboxy-peptidase B (an enzyme that removes an amino acid from the C-terminal end of the peptide if it is basic).

Different neurons that produce the same polyprotein may release different neuropeptides because of differences in the way the polyprotein is processed. An example is proopiomelanocortin (POMC), one of the three branches of the opioid family. POMC is found in neurons of the anterior and intermediate lobes of the pituitary, in the hypothalamus, and in several other regions of the brain, as well as in the placenta and gut. The same mRNA for POMC is found in all of these tissues, but different peptides are produced from POMC in different tissues in a regulated manner. One possibility is that two neurons that process the same polyprotein might differently express proteases with different specificities within the lumina of the endoplasmic reticulum, Golgi apparatus, or vesicles. Alternatively, the two neurons might contain the same processing proteases, but each cell might glycosylate the common polyprotein at different sites, thereby protecting different regions of the polypeptide from cleavage.

Peptides and Small-Molecule Transmitters Differ in Several Ways

Large dense-core vesicles are homologous to the secretory granules of nonneuronal cells. These vesicles are formed in the trans-Golgi network, where they are loaded with neuropeptides and other proteins that enable formation of the dense core. The dense-core vesicles are then transported from the soma to presynaptic sites in axons. In addition to containing neuropeptides, these vesicles often contain small molecule transmitters due to their expression of vesicular transporters. After large dense-core vesicles release their contents through exocytosis, the membrane is not recycled to form new large dense core vesicles. Rather the vesicles must be replaced by transport from the soma. In contrast, mature small synaptic vesicles are not synthesized in the soma. Rather, their protein components are delivered to release sites by transport of large dense-core precursor vesicles. To form a mature small synaptic vesicle, the precursor vesicles must first fuse with the plasma membrane. Following endocytosis, mature synaptic vesicles are then produced by local processing. Once their contents are released by exocytosis, synaptic vesicles can be rapidly recycled to maintain their local concentration during periods of sustained neural firing.

Although both types of vesicles contain many similar proteins, dense-core vesicles lack several proteins needed for release at the active zones. The membranes from dense-core vesicles are used only once; new dense-core vesicles must be synthesized in the cell body and transported to the axonal terminals by anterograde transport. Moreover, no uptake mechanisms exist for neuropeptides. Thus, once a peptide is released, a new supply must arrive from the cell body. Although there is evidence for local protein synthesis in some axons, it has not been shown that this provides new peptides for release.

The large dense-core vesicles release their contents by an exocytotic mechanism that is not specialized to nerve cells and does not require active zones; release can thus take place anywhere along the membrane of the axon that has the appropriate fusion machinery. As in other examples of regulated secretion, exocytosis of the dense-core vesicles depends on a general elevation of intracellular Ca^{2+} through voltage-gated Ca^{2+} channels that are not localized to the site of release. As a result, this form of exocytosis is slow and requires high stimulation frequencies to raise Ca^{2+} to levels sufficient to trigger release. This is in contrast to the rapid exocytosis of synaptic vesicles following a single action potential, which initiates the large, rapid increase in Ca^{2+} through voltage-gated Ca^{2+} channels tightly clustered at the active zone (Chapter 15).

Peptides and Small-Molecule Transmitters Can Be Co-released

Neuroactive peptides, small-molecule transmitters, and other neuroactive molecules coexist in the same dense-core vesicles of some neurons (Chapters 7 and 15). In mature neurons, the combination usually consists of one of the small-molecule transmitters and one or more peptides derived from a polyprotein. For example, ACh and vasoactive intestinal peptide (VIP) can be released together and work synergistically on the same target cells.

Another example is calcitonin gene–related peptide (CGRP), which in most spinal motor neurons is packaged together with ACh, the transmitter used at

the neuromuscular junction. CGRP activates adenylyl cyclase, raising cyclic adenosine monophosphate (cAMP) levels and cAMP-dependent protein phosphorylation in the target muscles (Chapter 14). Increased protein phosphorylation results in an increase in the force of contraction. Another example is the co-release of glutamate and dynorphin in neurons of the hippocampus, where glutamate is excitatory and dynorphin inhibitory. Because postsynaptic target cells have receptors for both chemical messengers, all of these examples of co-release are also examples of cotransmission.

As already described, the dense-core vesicles that release peptides differ from the small clear vesicles that release only small-molecule transmitters. The peptide-containing vesicles may or may not contain small-molecule transmitter, but both types of vesicles contain ATP. As a result, ATP is released by exocytosis of both large dense-core vesicles and synaptic vesicles. Moreover, it appears that ATP may be stored and released in a number of distinct ways: (1) ATP is co-stored and co-released with transmitters, (2) ATP release is simultaneous but independent of transmitter release, and (3) ATP is released alone. Co-release of ATP (which after release can be degraded to adenosine) may be an important illustration that coexistence and co-release do not necessarily signify cotransmission. ATP, like many other substances, can be released from neurons but still not be involved in signaling if there are no receptors nearby.

As mentioned earlier, one criterion for judging whether a particular substance is used as a transmitter is that the substance is present in high concentrations in a neuron. Identification of transmitters in specific neurons has been important in understanding synaptic transmission, and a variety of histochemical methods are used to detect chemical messengers in neurons (Box 16–2).

The glutamate synaptic vesicle transporters VGLUT2 and VGLUT3 are expressed in neurons that release other classes of neurotransmitter, particularly cholinergic, serotonergic, and catecholaminergic neurons. An interesting example of co-release of two small-molecule transmitters is that of glutamate and dopamine by neurons projecting to the ventral striatum, cortex, and elsewhere. This co-release may have important implications for modulation of motivated behaviors and for establishing the patterns of axonal projections. In some cases, glutamate is released together with dopamine in response to different patterns of dopaminergic neuron firing. While there is a controversy regarding whether the same synaptic vesicles can accumulate both neurotransmitters, in isolated synaptic vesicles, glutamate uptake enhances vesicular monoamine storage by increasing the pH gradient that drives vesicular monoamine transport, providing a presynaptic mechanism to regulate quantal size.

Removal of Transmitter From the Synaptic Cleft Terminates Synaptic Transmission

Timely removal of transmitters from the synaptic cleft is critical to synaptic transmission. If transmitter molecules released in one synaptic action were allowed to remain in the cleft after release, this would impede the normal spatial and temporal dynamics of signaling, initially boosting the signal but preventing new signals from getting through. The synapse would ultimately become refractory, mainly because of receptor desensitization resulting from continued exposure to transmitter.

Transmitter substances are removed from the cleft by three mechanisms: diffusion, enzymatic degradation, and reuptake. Diffusion removes some fraction of all chemical messengers, but in brain regions with very high innervation and thus a high requirement for neurotransmitter release, diffusion can play a relatively small role in tapering signaling. In contrast, in regions of low innervation, diffusion is a major means by which signaling is decreased.

At cholinergic synapses, the dominant means of clearing ACh is enzymatic degradation of the transmitter by acetylcholinesterase. At the neuromuscular junction, the active zone of the presynaptic nerve terminal is situated just above the junctional folds of the muscle membrane. The ACh receptors are located at the surface of the muscle facing the release sites and do not extend deep into the folds (see Figure 12–1), whereas acetylcholinesterase is anchored to the basement membrane within the folds. This anatomical arrangement of transmitter, receptor, and degradative enzyme serves two functions.

First, on release, ACh reacts with its receptor; after dissociation from the receptor, the ACh diffuses into the cleft and is hydrolyzed to choline and acetate by acetylcholinesterase. As a result, the transmitter molecules are used only once. Thus, one function of the esterase is to punctuate the synaptic message. Second, the choline that otherwise might be lost by diffusion away from the synaptic cleft is recaptured. Once hydrolyzed by the esterase, the choline lingers in the reservoir provided by the junctional folds and is taken back up into cholinergic nerve endings by a high-affinity choline transporter. (Unlike the biogenic amines, there is no uptake mechanism for ACh itself at the plasma membrane.) In addition to acetylcholinesterase, ACh is also degraded by another esterase,

Box 16–2 Detection of Chemical Messengers and Their Processing Enzymes Within Neurons

Powerful histochemical techniques are available for detecting both small-molecule transmitter substances and neuroactive peptides in histological sections of nervous tissue.

Catecholamines and serotonin, when reacted with formaldehyde vapor, form fluorescent derivatives. In an early example of transmitter histochemistry, the Swedish neuroanatomists Bengt Falck and Nils Hillarp found that the reaction can be used to locate transmitters with fluorescence microscopy under properly controlled conditions.

Because individual vesicles are too small to be resolved by the light microscope, the exact position of the vesicles containing the transmitter was inferred by comparing the fluorescence under the light microscope with the position of vesicles under the electron microscope. A number of fluorescent false transmitters, particularly those that mimic catecholamines, are substrates for plasma membrane and/or vesicular transporters, enabling their use to label vesicles and assess their turnover in living tissue. In addition, a variety of genetically expressed neurotransmitter reporters based on green fluorescent protein can be used to detect extracellular levels of neurotransmitters.

Histochemical analysis can be extended to the ultrastructure of neurons under special conditions. Fixation of nervous tissue in the presence of potassium permanganate, chromate, or silver salts, or the dopamine analog

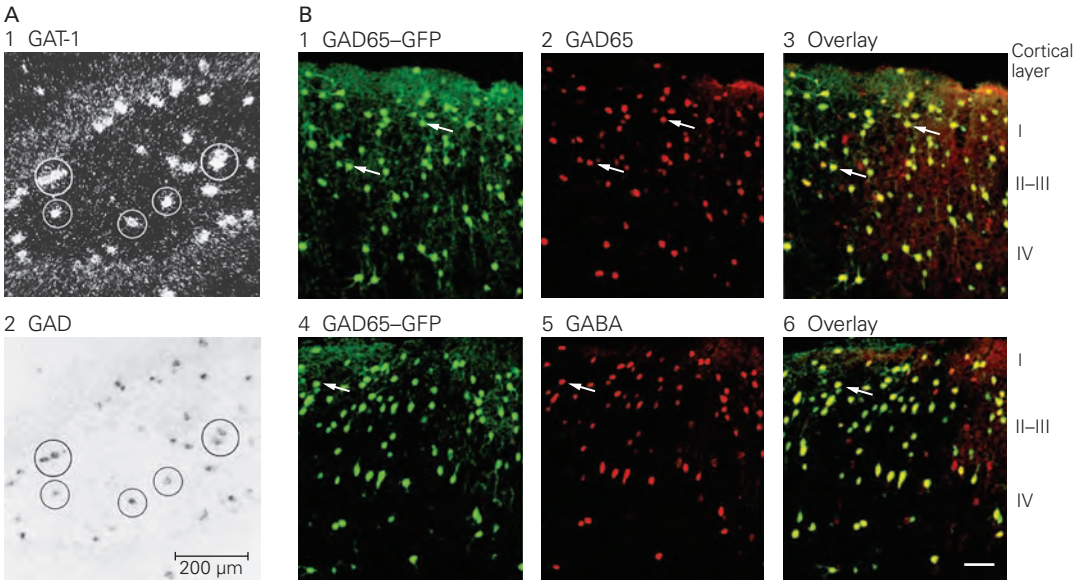


Figure 16–3 Techniques for visualizing chemical messengers.

A. A light-microscope section of the hippocampus of a rat. **1.** In situ hybridization using a probe for the mRNA encoding GAT-1, a GABA transporter. The probe was end-labeled with α-^{35}S-dATP and visualized by clusters of silver grains in the overlying autoradiographic photographic emulsion. **2.** In situ hybridization of the mRNA for glutamic acid decarboxylase (**GAD**), the specific biosynthetic enzyme for GABA, was carried out with an oligonucleotide probe linked to the enzyme alkaline phosphatase. The GAD probe was visualized by accumulation of colored alkaline phosphatase reaction product in the cytoplasm. Neurons expressing both GAT-1 and GAD transcripts were labeled by silver grains and the phosphatase reaction, respectively, and are indicated by circles enclosing cells bodies that contain both labels. (Used with permission of Sarah Augood.)

B. Images of neocortex from a GAD65-GFP transgenic mouse in which green fluorescent protein (**GFP**) is expressed under the control of the GAD65 promotor. GFP is co-localized with GAD65 (1–3) and GABA (4–6) (both detected by indirect immunofluorescence) in neurons in the different layers. Most of the GFP-positive neurons are immunopositive for GAD65 and GABA (**arrows** show selected examples). Scale bar = 100 µm. (Adapted, with permission, from López-Bendito et al. 2004. Copyright © 2004 Oxford University Press.)