13
Neurotransmitters

CHEMICAL SYNAPTIC TRANSMISSION can be divided into four steps—(1) synthesis and storage of a transmitter substance, (2) release of the transmitter, (3) interaction of the transmitter with receptors at the postsynaptic membrane, and (4) removal of the transmitter from the synaptic cleft. In the previous chapter we considered steps 2 and 3: the release of transmitters and how they interact with postsynaptic receptors. We now turn to the initial and final steps of chemical synaptic transmission: the synthesis of transmitter molecules and their removal from the synaptic cleft after synaptic action.

A Chemical Messenger Must Meet Four Criteria to Be Considered a Neurotransmitter

Before considering the biochemical processes involved in synaptic transmission, it is important to make clear what is meant by a chemical transmitter. The concept is empirical rather than logical and has changed over the years with increased understanding of synaptic transmission.

The concept that nerve stimulation led to release of chemical signals was elaborated as early as 1905 by the physiologist John Newport Langley, who demonstrated that adrenomedullary extracts elicited tissue responses comparable to sympathetic nerve stimulation. However, Thomas Renton Elliott is generally credited with the first experimental evidence of chemical neurotransmission in his observations that the physiological effects of sympathetic nerve stimulation were due to release of adrenaline. In 1921 Otto Loewi demonstrated the release of acetylcholine (ACh) from vagus nerve terminals in frog hearts. Henry Dale extended Loewi's work on ACh, later sharing the Nobel Prize with Loewi. In 1946 Ulf von Euler reported further work on adrenergic transmission. The terms *cholinergic* and *adrenergic* were introduced to indicate that a neuron makes and releases ACh or norepinephrine (or epinephrine), the two substances first recognized as neurotransmitters.

Since that time many other substances have been identified as transmitters. Furthermore, because of the work of Bernard Katz in the 1950s on quantal release (see Chapter 12), it is usually taken for granted that substances acting as transmitters are stored in vesicles at synapses and released by exocytosis. Nevertheless some substances considered to be neurotransmitters are released into the synaptic cleft directly from the cytoplasm as well as by exocytosis. Thus ideas about neurotransmitters have had to be modified continually to accommodate new information about the cell biology of neurons and the pharmacology of receptors.

As a first approximation, a neurotransmitter can be defined as a substance that is released by a neuron and that affects a specific target in

a specific manner. A target can be either another neuron or an effector organ, such as muscle or gland. As with many other operational concepts in biology, the concept of a transmitter is not precise. Neurotransmitters are protean, resembling other released agents in many regards yet also differing from them depending on the site of action and circumstances. Although the actions of hormones and neurotransmitters are quite similar, neurotransmitters usually act on targets that are close to the site of transmitter release, whereas hormones are released into the bloodstream to act on distant targets.

Neurotransmitters differ from autacoids in that a transmitter typically acts on a target other than the releasing neuron itself, whereas an autacoid acts only on the cell from which it was released. Nevertheless, at some synapses transmitters activate not only receptors in the postsynaptic cell but also autoreceptors on the presynaptic terminal. Autoreceptors usually modulate synaptic transmission that is in progress, for example, by limiting further release of transmitter or inhibiting subsequent transmitter synthesis. Receptors can also exist on presynaptic terminals at axo-axonic synapses, where the presynaptic terminal receives synaptic input from another neuron. These receptors function as heteroreceptors that regulate terminal excitability and transmitter release (see Chapter 12).

Importantly, the interaction of neurotransmitters with receptors is typically transient, lasting from milliseconds to minutes. Nevertheless, neurotransmitter action can result in long-term changes within target cells lasting hours or days. Lastly, increasing evidence suggests that a non-neural cell, the astrocyte, can also synthesize, store, and release neurotransmitters as well as express receptors that modulate astrocyte function.

Despite these difficulties in arriving at a strict definition, a limited number of substances of low molecular weight are generally accepted as neurotransmitters. Even so, it is often difficult to demonstrate that a specific transmitter operates at a particular synapse, particularly given the diffusibility and rapid reuptake or degradation of transmitters at the synaptic cleft. Because of this difficulty, many neurobiologists believe that a substance should not be accepted as a neuro-transmitter unless the following four criteria are met:

1. It is synthesized in the presynaptic neuron.
2. It is present in the presynaptic terminal and is released in amounts sufficient to exert a defined action on the postsynaptic neuron or effector organ.
3. When administered exogenously in reasonable concentrations it mimics the action of the endogenous transmitter (for example, it activates the same ion channels or second-messenger pathway in the postsynaptic cell).
4. A specific mechanism usually exists for removing the substance from the synaptic cleft.

The nervous system makes use of two main classes of chemical substances for signaling: small-molecule transmitters and neuroactive peptides, which are short polymers of amino acids. Both classes of neurotransmitters are contained in vesicles, large and small. Neuropeptides are packaged in large dense-core vesicles (approximately 70–250 nm in diameter), which release their contents by exocytosis, similar to those seen in secretory glands and mast cells. Small-molecule transmitters are packaged in small electron-lucent vesicles (~ 40 nm in diameter), which release their contents through exocytosis at active zones closely associated with specific Ca^{2+} channels (see Chapter 12). Large dense-core vesicles can contain both small-molecule transmitters and neuropeptides.

Both types of vesicles are found in most neurons but in different proportions. Small synaptic vesicles are characteristic of neurons that use ACh, glutamate, γ-aminobutyric acid (GABA), and glycine as transmitters, whereas large dense-core vesicles are typical of neurons that use catecholamines and serotonin as transmitters. The adrenal medulla, once used as a model for studying exocytosis, contains only secretory granules that are similar to large dense-core vesicles. Because dense-core vesicles can contain both small-molecule transmitters and neuropeptides, they are important in co-transmission, which is discussed later in this chapter.

Only a Few Small-Molecule Substances Act as Transmitters

A relatively small number of low-molecular-weight substances are gener-

ally accepted as neurotransmitters, including ACh, amino acids or their amine-containing derivatives, adenosine triphosphate (ATP), and ATP metabolites (Table 13–1). The amine chemical messengers share many biochemical similarities. All are charged small molecules that are formed in relatively short biosynthetic pathways and synthesized either from essential amino acids or from precursors derived from the major carbohydrate substrates of intermediary metabolism. Like other pathways of intermediary metabolism, synthesis of these neurotransmitters is catalyzed by enzymes that, almost without exception, are cytosolic. ATP, which originates in mitochondria, is abundantly present throughout the cell.

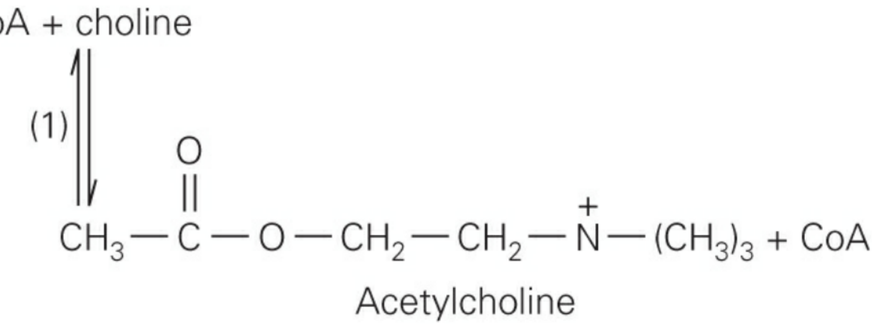
Table 13–1 Small-Molecule Transmitter Substances and Their Precursors

Transmitter	Precursor
Acetylcholine	Choline
Biogenic amines	
Dopamine	Tyrosine
Norepinephrine	Tyrosine
Epinephrine	Tyrosine
Serotonin	Tryptophan
Histamine	Histidine
Melatonin	Serotonin
Amino acids	
Aspartate	Oxaloacetate
γ-Aminobutyric acid	Glutamine
Glutamate	Glutamine
Glycine	Serine
ATP	ADP
Adenosine	ATP
Arachidonic acid	Phospholipids
Carbon monoxide	Heme
Nitric oxide	Arginine

As in any biosynthetic pathway, the overall synthesis of amine transmitters typically is regulated at one rate-limiting enzymatic reaction. The rate-limiting step often is characteristic of one type of neuron and usually is absent in other types of mature neurons.

Acetylcholine

Acetylcholine is the only low-molecular-weight amine transmitter substance that is not an amino acid or derived directly from one. The biosynthetic pathway for ACh has only one enzymatic reaction, catalyzed by choline acetyltransferase (step 1 in the reaction shown below). This transferase is the characteristic and limiting enzyme in ACh biosynthesis. Nervous tissue cannot synthesize choline, which is derived from the diet and delivered to neurons through the blood stream. The co-substrate, acetyl coenzyme A (acetyl CoA), participates in many general metabolic pathways and is not restricted to cholinergic neurons.



Acetylcholine is released at all vertebrate neuromuscular junctions by spinal motor neurons (see Chapter 9). In the autonomic nervous system it is the transmitter for all preganglionic neurons and for parasympathetic postganglionic neurons as well (see Chapter 47). Cholinergic neurons form synapses throughout the brain; those in the nucleus basalis have particularly widespread projections to the cerebral cortex. Acetylcholine (together with a noradrenergic component) is a principle neurotransmitter of the reticular activating system, which modulates arousal, sleep, wakefulness, and other critical aspects of human consciousness.

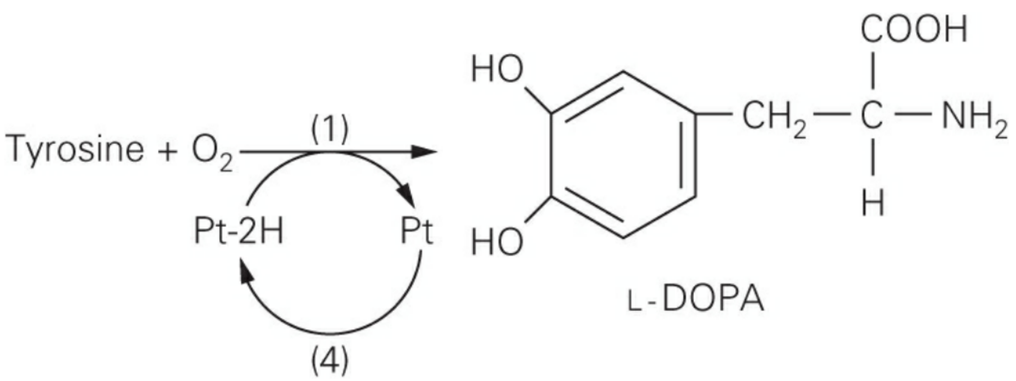
Biogenic Amine Transmitters

The term *biogenic amine*, although chemically imprecise, has been used for decades to designate certain neurotransmitters. This group includes the catecholamines and serotonin. Histamine, an imidazole, is also often referred to as a biogenic amine, although its biochemistry is remote from the catecholamines and the indolamines.

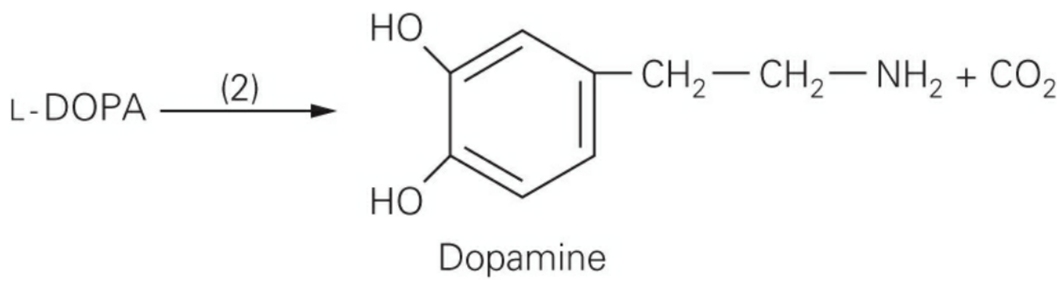
Catecholamine Transmitters

The catecholamine transmitters—dopamine, norepinephrine, and epinephrine—are all synthesized from the essential amino acid tyrosine in a common biosynthetic pathway containing five enzymes: tyrosine hydroxylase, pteridine reductase, aromatic amino acid decarboxylase, dopamine β-hydroxylase, and phenylethanolamine-*N*-methyl transferase. Catecholamines have the catechol nucleus, a 3,4-dihydroxylated benzene ring.

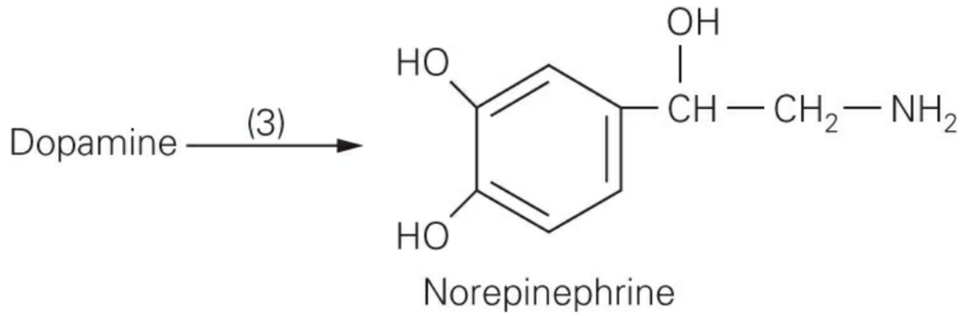
The first enzyme, tyrosine hydroxylase (step 1 below), is an oxidase that converts tyrosine to L-dihydroxyphenylalanine (L-DOPA). This enzyme is rate-limiting for the synthesis of both dopamine and norepinephrine. It is present in all cells producing catecholamines and requires a reduced pteridine cofactor, Pt-2H, which is regenerated from pteridine (Pt) by another enzyme, pteridine reductase, which uses nicotinamide adenine dinucleotide (NADH) (step 4 below). This reductase is not specific to neurons.



L-DOPA is next decarboxylated by aromatic amino acid decarboxylase, also called L-DOPA decarboxylase (step 2 below), to yield dopamine and CO_2:



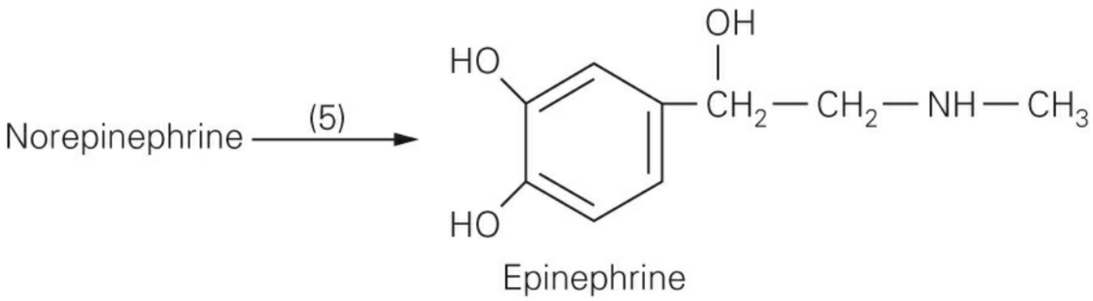
The third enzyme in the sequence, dopamine β-hydroxylase (step 3 below), converts dopamine to norepinephrine. Unlike all other enzymes in the biosynthetic pathways of small-molecule neurotransmitters, dopamine β-hydroxylase is membrane-associated. It is bound tightly to the inner surface of aminergic vesicles as a peripheral protein. Consequently, norepinephrine is the only transmitter synthesized within vesicles.



In the central nervous system norepinephrine is used as a transmitter by neurons with cell bodies in the locus ceruleus, a nucleus of the brain stem with many complex modulatory functions (see Chapter 46). Although these adrenergic neurons are relatively few in number, they project diffusely throughout the cortex, cerebellum, and spinal cord. In the peripheral nervous system norepinephrine is the transmitter of the postganglionic neurons in the sympathetic nervous system (see Chapter 47).

In addition to these four catecholaminergic biosynthetic enzymes, a fifth enzyme, phenylethanolamine-*N*-methyltransferase (step 5 below), methylates norepinephrine to form epinephrine (adrenaline) in the adrenal medulla. This reaction requires *S*-adenosylmethionine as a methyl donor. The transferase is a cytoplasmic enzyme. Thus, for epinephrine to

be formed, its immediate precursor, norepinephrine, must exit from vesicles into the cytoplasm. For epinephrine to be released, it must then be taken up into vesicles. Only a small number of neurons in the brain use epinephrine as transmitter.



Epinephrine

The production of these catecholamine neurotransmitters is controlled by feedback regulation of the first enzyme in the pathway. Not all cells that release catecholamines express all five biosynthetic enzymes, although cells that release epinephrine do. During development the expression of the genes encoding these synthetic enzymes is independently regulated and the particular catecholamine produced by a cell is determined by which enzyme(s) in the step-wise pathway are not expressed. Thus, neurons that release norepinephrine do not express the methyltransferase, and neurons that release dopamine do not express the transferase or dopamine β-hydroxylase.

Of the four major dopaminergic nerve tracts, three arise in the midbrain (see Chapter 46). Dopaminergic neurons in the substantia nigra that project to the striatum are important for the control of movement and are affected in Parkinson disease and other disorders of movement. The mesolimbic and mesocortical tracts are critical for affect, emotion, attention, and motivation and are implicated in schizophrenia and drug addiction (see Chapters 48, 49, and 62). A fourth dopaminergic tract, the tuberoinfundibular pathway, originates in the arcuate nucleus of the hypothalamus and projects to the pituitary gland, where it regulates secretion of hormones (see Chapter 46).

The synthesis of biogenic amines is highly regulated and can be rapidly increased. As a result, the amounts of transmitter available for release can keep up with wide variations in neuronal activity. Opportunities for regulating both the synthesis of catecholamine transmitters and the production of enzymes in the stepwise catecholamine pathway are discussed in Box 13-1.

Trace amines, naturally occurring catecholamine derivatives, may also be transmitters. In invertebrates the tyrosine derivatives tyramine and octopamine play key roles in numerous physiological processes including behavioral regulation. Trace amine receptors also have been identified in mammals, where their function is still a matter of some controversy.

Box 13–1 Catecholamine Production Varies with Neuronal Activity

The production of norepinephrine is able to keep up with wide variations in neuronal activity because it is highly regulated. In autonomic ganglia the amount of norepinephrine is regulated transsynaptically. Activity in the presynaptic neurons, which are both cholinergic and peptidergic, first induces short-term changes in second messengers in the postsynaptic adrenergic cells.

These changes increase the supply of norepinephrine through the cAMP-dependent phosphorylation of tyrosine hydroxylase, the first enzyme in the norepinephrine biosynthetic pathway. Phosphorylation enhances the affinity of the hydroxylase for the pteridine cofactor and diminishes feedback inhibition by end products such as norepinephrine. Phosphorylation of tyrosine hydroxylase lasts only as long as cAMP remains elevated, as the phosphorylated hydroxylase is quickly dephosphorylated by protein phosphatases.

If presynaptic activity is sufficiently prolonged, however, other changes in the production of norepinephrine will occur. Severe stress to an animal results in intense presynaptic activity and persistent firing of the postsynaptic adrenergic neuron, placing a greater demand on transmitter synthesis. To meet this challenge, the tyrosine hydroxylase gene is induced to increase production of the enzyme protein. Elevated amounts of tyrosine hydroxylase are observed in the cell body within hours after stimulation and at nerve endings days later.

This induction of increased levels of tyrosine hydroxylase begins with the persistent release of chemical transmitters from the

presynaptic neurons and prolonged activation of the cAMP pathway in postsynaptic adrenergic cells, which activates the cAMP-dependent protein kinase (PKA). This kinase phosphorylates not only existing tyrosine hydroxylase molecules, but also a transcription factor, cAMP response element binding protein (CREB).

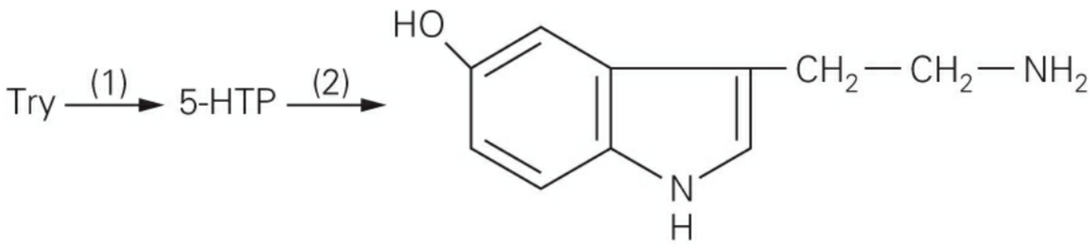
Once phosphorylated, CREB binds a specific DNA enhancer sequence called the cAMP-recognition element (CRE), which lies upstream (5′) of the gene for the hydroxylase. Binding of CREB to CRE facilitates the binding of RNA polymerase to the gene's promoter, increasing tyrosine hydroxylase transcription. Induction of tyrosine hydroxylase was the first known example of a neurotransmitter altering gene expression.

There is a high degree of similarity in amino acid and nucleic acid sequences encoding three of the biosynthetic enzymes: tyrosine hydroxylase, dopamine β-hydroxylase, and phenylethanolamine-*N*-methyltransferase. This similarity suggests that the three enzymes arose from a common ancestral protein.

Moreover, long-term changes in the synthesis of these enzymes are coordinately regulated in adrenergic neurons. At first, this discovery suggested that the genes encoding these enzymes might be located sequentially along the same chromosome and be controlled by the same promoter, like genes in a bacterial operon. But in humans the genes for the biosynthetic enzymes for norepinephrine are not located on the same chromosome. Therefore, coordinate regulation is likely achieved by parallel activation through similar but independent transcription activator systems.

Serotonin

Serotonin (5-hydroxytryptamine or 5-HT) and the essential amino acid tryptophan from which it is derived belong to a group of aromatic compounds called indoles, with a five-member ring containing nitrogen joined to a benzene ring. Two enzymes are needed to synthesize serotonin: tryptophan (Trp) hydroxylase (step 1 in the following reaction), an oxidase similar to tyrosine hydroxylase, and aromatic amino acid decarboxylase, also called 5-hydroxytryptophan (5-HTP) decarboxylase (step 2 in the following reaction).



Serotonin

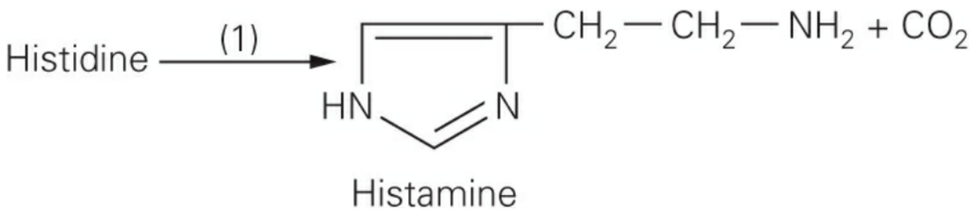
The limiting reaction is catalyzed by the first enzyme in the pathway, tryptophan hydroxylase. Tryptophan hydroxylase is similar to tyrosine hydroxylase not only in catalytic mechanism but also in amino acid sequence. The two enzymes are thought to stem from a common ancestral protein by gene duplication because the two hydroxylases are syntenic, that is, they are encoded by genes close together on the same chromosome (tryptophan hydroxylase, 11p15.3-p14; tyrosine hydroxylase, 11p15.5). The second enzyme in the pathway, 5-hydroxytryptophan decarboxylase, is identical to L-DOPA decarboxylase. Enzymes with similar activity, L-aromatic amino acid decarboxylases, are present in non-nervous tissues as well.

The cell bodies of serotonergic neurons are found in and around the midline raphe nuclei of the brain stem and are involved in regulating attention and other complex cognitive functions (Chapter 46). The projections of these cells (like those of noradrenergic cells in the locus ceruleus) are widely distributed throughout the brain and spinal cord. Serotonin and the catecholamines norepinephrine and dopamine are implicated in depression, a major mood disorder. Antidepressant medications inhibit the uptake of serotonin, norepinephrine, and dopamine, thereby increasing the magnitude and duration of the action of these transmitters, which in turn leads to altered cell signaling and adaptations (see Chapter 63).

Histamine

Histamine, derived from the essential amino acid histi-dine by decar-

boxylation, contains a characteristic five-member ring with two nitrogen atoms. It has long been recognized as an autacoid, active when released from mast cells in the inflammatory reaction and in the control of vasculature, smooth muscle, and exocrine glands (eg, secretion of highly acidic gastric juice). Histamine is a transmitter in both invertebrates and vertebrates. It is concentrated in the hypothalamus, one of the centers for regulating the secretion of hormones (see Chapter 47). The decarboxylase catalyzing its synthesis (step 1 below), although not extensively analyzed, appears to be characteristic of histaminergic neurons.



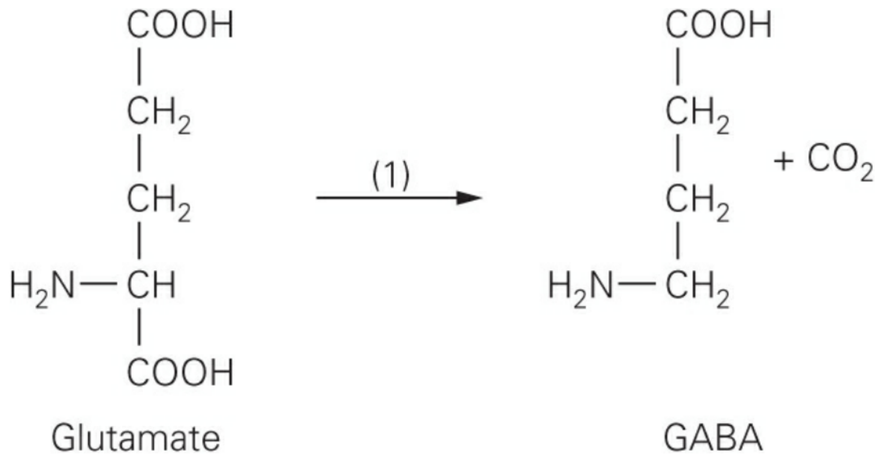
Histamine

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, neither are essential amino acids.

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 below). The glutamate taken up by astrocytes is converted to glutamine by the enzyme glutamine synthase. This glutamine then diffuses back into neurons that use glutamate as a transmitter, where it is hydrolyzed back to glutamate. Phosphate-activated glutaminase (PAG), which is present at high concentrations in these neurons, is responsible for salvaging the molecule for reuse as a transmitter.

Glycine is the major transmitter used by inhibitory interneurons of the spinal cord. It is also an allosteric modulator of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors (see Chapter 10). Glycine is synthesized from serine. Its specific biosynthesis in neurons is not well understood, but its biosynthetic pathway in other tissues is well known. The amino acid GABA is synthesized from glutamate in a reaction catalyzed by glutamic acid decarboxylase (step 1 below):



Glutamate GABA

GABA is present at high concentrations throughout the central nervous system and is also 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 various inhibitory neurons and interneurons, such as the medium spiny neurons of the striatum, striatal interneurons, basket cells of both the cerebellum and the hippocampus, the Purkinje cells of the cerebellum, granule cells of the olfactory bulb, and amacrine cells of the retina.

ATP and Adenosine

ATP and its degradation products (eg, adenosine) act as transmitters at some synapses. Adenosine has an inhibitory effect through a number of adenosine receptors in the central nervous system and caffeine's stimulatory effects depend on inhibition of adenosine binding to its 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 (see Chapter 22).

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 nociceptor dorsal root ganglion cells. ATP released from terminals of the central axons of the dorsal root ganglion cells excites another type of ionotropic purine receptor on neurons in the dorsal horn of the spinal cord.

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, it 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) common to neurons of all types (and found also in glandular tissue, such as the adrenal medulla).

Using the energy generated by the hydrolysis of cytoplasmic ATP, the V-ATPase creates a 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. A number of different vesicular transporters have been identified in mammals that are responsible for concentrating different transmitter molecules in vesicles (Figure 13–1). These proteins span the vesicle membrane 12 times, and are distantly related to a class of bacterial transporters that mediate drug-resistance. (Although related in function, vesicular transporters differ structurally and mechanistically from the transporters in the plasma membrane, which are driven by the Na^+ electrochemical gradient rather than by H^+. See below.)

Transmitter molecules are 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 neurotransmitters up to 100,000-fold relative to their concentration in the cytoplasm. Uptake of transmitters by the transporters is extremely 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 (see Chapter 12).

Although the specificity of transporters is quite marked—the ACh transporter does not transport choline or any other transmitter, and the glutamate transporter hardly carries any aspartate at all—the affinity for their transmitters can be quite low. For example, the Michaelis constant (K_m) for ACh or glutamate transport is approximately 0.3 mM, and for GABA 5 to 10 mM. This low affinity for transmitter presumably does not limit synaptic transmission, however, because the concentrations of these substances in the cytoplasm are normally very high. In contrast, amine transporters have a substantially higher affinity for monoamines (K_m of approximately 1–15 μM), appropriate for the lower cytoplasmic concentration of these substances.

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 both inhibit uptake of amine transmitters by binding to the vesicular monoamine transporter and have played a historic role in development of the biogenic amine hypothesis of depression (see Chapter 63). The psychostimulants amphetamine and 3,4-methyl-

enedioxy-*N*-methylamphetamine (MDMA or ecstasy) deplete vesicles of amine transmitter molecules, most likely by dissipating the pH gradient. These compounds may also compete with the amine transmitters for uptake, and are presumed to interact directly with the transporters, though it is not yet clear whether these drugs and the actual transmitters bind to the same or different sites on the transporters.

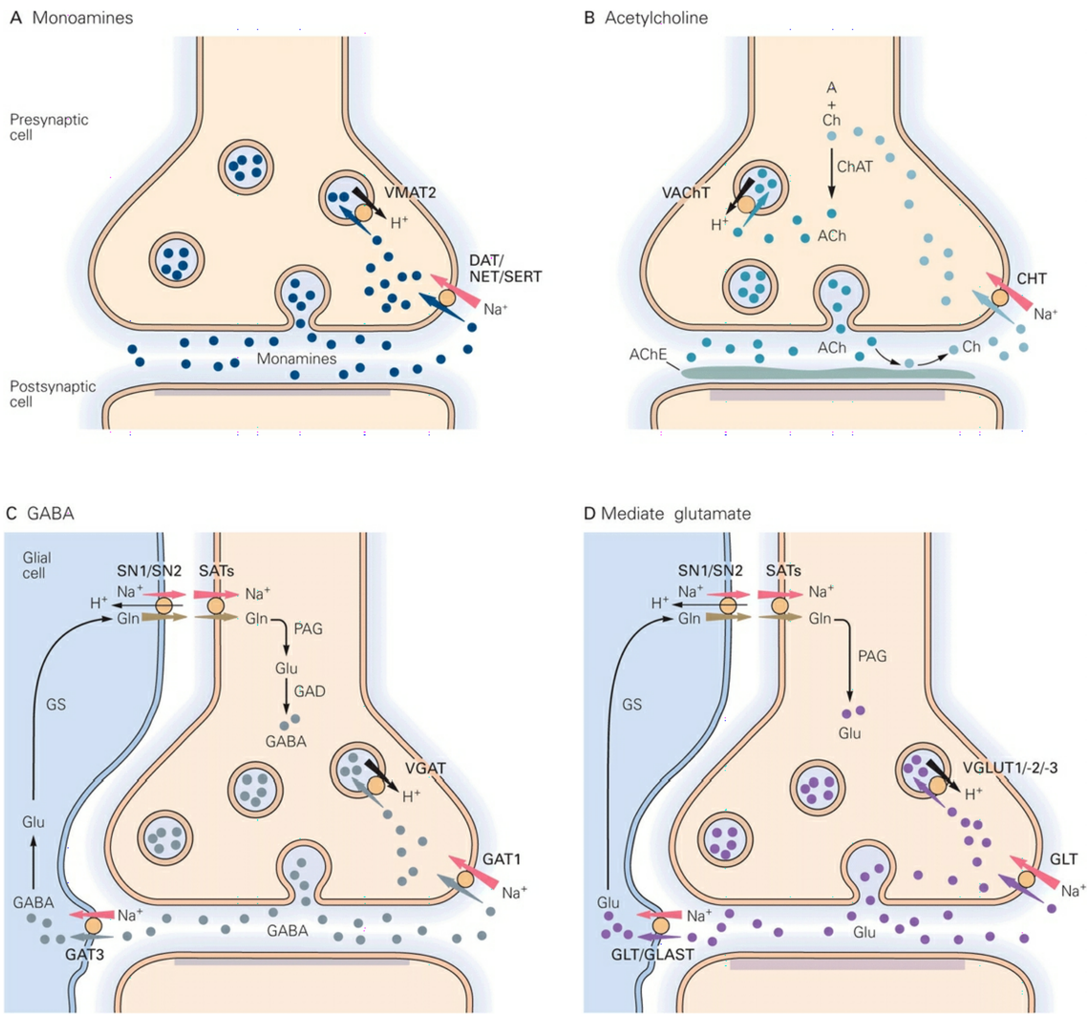


Figure 13-1 Small-molecule transmitters are transported from the cytosol into vesicles or from the synaptic cleft to the cytosol by transporters. Most small-molecule neurotransmitters are released by exocytosis from the nerve terminal and act on specific postsynaptic receptors. The signal is terminated 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 H^+ (**black arrows**) or Na^+ (**red arrows**) electrochemical gradients. (Adapted, with permission, from Chaudhry et al. 2008.)

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. Choline (Ch) is transported back into the nerve terminal (**light blue arrow**) by the choline transporter (CHT), where choline acetyltransferase (ChAT) subsequently catalyzes acetylation of choline to reform 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 glutamate (Glu) is converted by glial glutamine synthetase 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**). 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 (**purple arrow**) at glutamatergic terminals also has been demonstrated for a GLT isoform.

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. Therefore, their release decreases the efficacy of transmission. Several drugs that have been used to treat hypertension, such as phenylethylamines, are taken up into adrenergic terminals and replace norepinephrine in synaptic vesicles. When released, these drugs are not as potent as norepinephrine at postsynaptic adrenergic receptors. Some of these drugs must be actively taken up into neurons by transporters in the external membrane of the cell. These transporter molecules are discussed later in this chapter.

Many Neuroactive Peptides Serve as Transmitters

With the exception of dopamine α-hydroxylase, the enzymes that catalyze the synthesis of the low-molecular-weight neurotransmitters are found in the cytoplasm. These enzymes are synthesized on free polysomes in the cell body 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 nerve terminals where they are released.

In contrast, neuroactive peptides are derived from secretory proteins that are formed in the cell body. Like other secretory proteins, neuroactive peptides or their precursors are first processed in the endoplasmic reticulum and then move to the Golgi apparatus to be processed further. They then leave the Golgi apparatus in secretory granules that are destined to become large dense-core vesicles, which are moved to axon terminals by fast axonal transport.

More than 50 short peptides are pharmacologically active in nerve cells (Table 13–2). Some act as hormones 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 to being hormones in some tissues, they also act as neurotransmitters when released close to a target neuron, where they can cause inhibition or excitation, or both.

Table 13–2 Neuroactive Mammalian Brain Peptides Categorized According to Tissue Localization

Category	Peptide
Hypothalamic releasing hormones	Thyrotropin-releasing hormone Gonadotropin-releasing hormone Somatostatin Corticotropin-releasing hormone Growth hormone-releasing hormone
Neurohypophyseal hormones	Vasopressin Oxytocin
Pituitary peptides	Adrenocorticotropic hormone β-Endorphin α-Melanocyte-stimulating hormone Prolactin Luteinizing hormone Growth hormone Thyrotropin
Pineal hormones	Melatonin
Invertebrate	FMRFamide

peptides	Hydra head activator
	Proctolin
	Small cardiac peptide
	Myomodulins
	Buccalins
	Egg-laying hormone
	Bag cell peptides
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
	Sleep peptide(s)
	Calcitonin
	CGRP
	Neuropeptide Y
	Neuropeptide Yy
	Galanin
	Substance K (neurokinin A)

FMRFamide, Phe-Met-Arg-Phe-amide; CGRP, calcitonin gene-related peptide. (Adapted, with permission, from Krieger 1983.)

Neuroactive peptides have been implicated in modulating sensory perception and emotions. Some peptides, including substance P and 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), and β-endorphin.

Although the diversity of neuroactive peptides is enormous, as a class these chemical messengers share a common cell biology. The most direct way to determine relatedness between peptides is to compare either the amino acid sequences of the peptides or the nucleotide base sequences in the genes that encode them. 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 13–3.

Several different neuroactive peptides can be encoded by a single continuous messenger RNA (mRNA), which is translated into one large polyprotein precursor (Figure 13–2). Polyproteins can serve as a mechanism for amplification by providing more than one copy of the same peptide from the one precursor. As an example, the precursor of glucagon contains two copies of the hormone. Polyproteins generate diversity by producing several distinct peptides cleaved from one precursor, as in the case of the opioid peptides.

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 duct 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 polyproteins to neuroactive peptides takes place within the neuron's major intracellular membrane system and in vesicles. Several peptides are produced from a single polyprotein by limited and specific proteolytic cleavage catalyzed by proteases present 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 site of the peptide bond cleaved is determined by the presence of one or two dibasic amino acid residues (lysine and arginine) in the substrate protein. Cleavage occurs between residue X and two dibasic residues (eg, X-Lys-Lys, -X-Lys-Arg, -X-Arg-Lys, or -X-Arg-Arg). Although cleavage is common at dibasic residues, it can also occur at single basic residues, and polyproteins sometimes are cleaved at other peptide bonds.

Table 13–3 Some Families of Neuroactive Peptides

Family	Peptide members
Opioids	Opiocortin, enkephalins, dynorphin, FMRFamide
Neurohypophyseal hormones	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

FMRFamide, Phe-Met-Arg-Phe-amide.

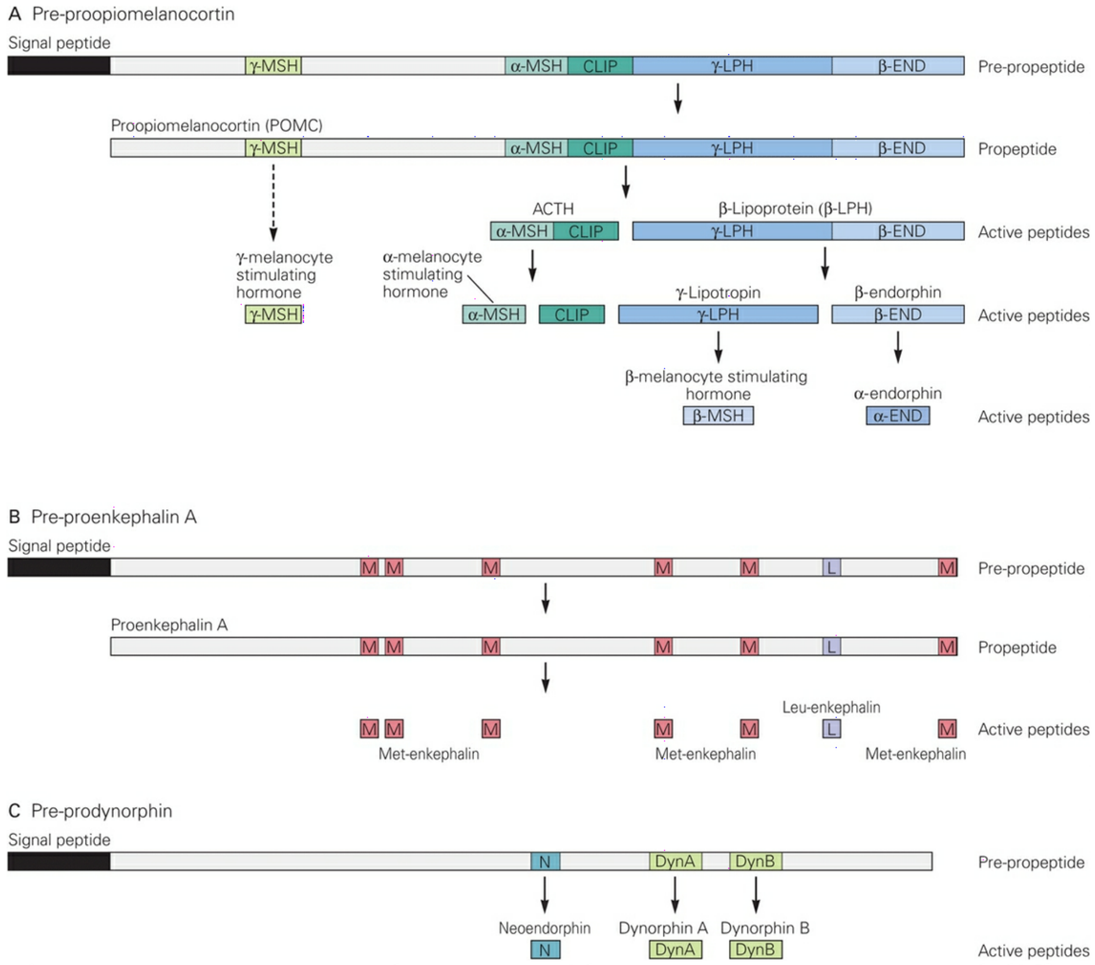


Figure 13-2 Hormone and neuropeptide precursors are processed differentially. In several instances neuropeptides and hormones arise from larger precursor molecules that require multiple rounds of proteinase-mediated cleavage. These precursors are processed differentially to yield their specific peptide 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), which contains an amino-terminal signal sequence that is cleaved to generate a smaller molecule, the propeptide. Differential processing of the three families of peptides gives rise to the opioid peptides—POMC (proopiomelanocortin), enkephalin, and dynorphin.

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 ACTH (adrenocorticotropic hormone) 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 also cleaved into at least three peptides, including α-neoendorphin (N), dynorphin A (Dyn A), and dynorphin B (Dyn B), which 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 polyproteins into 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 carboxypeptidase B (an enzyme that removes an amino acid from the N-terminal end of the peptide if it is basic).

Neurons that produce the same polyprotein may release different neuropeptides as a consequence 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 several other regions of the brain, as well as in the placenta and the 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 differently might contain 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

The metabolism of peptides differs from that of small-molecule transmitters in several important ways: their site of synthesis, the type of vesicle in which they are stored, and the mechanism of exocytotic release. Whereas small-molecule transmitters are chiefly synthesized at axon terminals, neuroactive peptides are made only in the cell body because their synthesis as secretory proteins requires the transfer of the nascent polypeptide chain into the lumen of the endoplasmic reticulum (see Chapter 4).

The large dense-core vesicles in which peptides are stored originate from the trans-Golgi network through a pathway different from that of the synaptic vesicles transporting small-molecule transmitters. Large dense-core vesicles are homologous to the secretory granules of nonneuronal cells and follow the *regulated* secretory pathway. Biogenesis of synaptic vesicles also begins in the trans-Golgi network in the form of precursor vesicles produced in the cell body. These vesicles are then transported down the axon to presynaptic terminals, where the precursor vesicle is thought to first fuse with the presynaptic membrane through the *constitutive* secretory pathway. The precursor vesicle membrane is then retrieved through endocytosis and processed through local endosomes to yield a mature synaptic vesicle capable of participating in the release of neurotransmitter through regulated exocytosis.

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 before release can take place again. There is increasing evidence for local protein synthesis in axons, and this might also be a source of new peptide for release.

The large dense-core vesicles release their contents by an exocytotic mechanism that is not specialized to nerve cells and that does not require active zones; release can thus take place anywhere along the membrane of the axon terminal. As in other examples of regulated secretion, exocytosis of the dense-core secretory 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 results from the large, rapid increase in Ca^{2+} through voltage-gated Ca^{2+} channels tightly clustered at the active zone.

Peptides and Small-Molecule Transmitters Coexist and Can Be Co-released

Neuroactive peptides, small-molecule transmitters, and other neuroactive molecules can coexist in the same dense-core vesicles of a neuron (see Chapter 4). 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 synapse. CGRP activates adenylyl cyclase, raising cyclic adenosine monophosphate (cAMP) levels and cAMP-dependent protein phosphorylation in the target muscles (see Chapter 11). Increased protein phosphorylation results in an increase in the force of contraction. One other 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 effective if there are no receptors nearby.

As mentioned above, 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 13–2).

An interesting example of co-release of two small-molecule transmitters is that of glutamate and dopamine by neurons projecting to the ventral striatum. This co-release may have important implications for modulation of motivated behaviors. It has been established that the vesicular transporter VGlut2 gathers glutamate into vesicles in these dopaminergic terminals, and glutamate is released together with dopamine in response to different patterns of dopaminergic neuron firing. In addition, glutamate uptake also enhances vesicular monoamine storage by increasing the pH gradient that drives vesicular monoamine transport, providing a novel 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, they would prevent new signals from getting through. The synapse would become refractory, mainly because of receptor desensitization resulting from continued exposure to transmitter. Transmitters are removed from the cleft by three mechanisms: diffusion, enzymatic degradation, and reuptake. Diffusion removes some fraction of all chemical messengers.

Enzymatic degradation of transmitter is used only by cholinergic synapses. 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 9-1), 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. The second function is to recapture the choline that otherwise might be lost by diffusion away from the synaptic cleft. Once hydrolyzed by the esterase, the choline lingers in the reservoir provided by the junctional folds and is later taken back up into cholinergic nerve endings by a high-affinity choline transporter. (There is no uptake mechanism for ACh itself.) In addition to acetylcholinesterase, ACh is also degraded by another esterase, butyrylcholinesterase, which can degrade other molecules including cocaine and the paralytic drug succinylcholine. However, the precise functions of butyrylcholinesterase remain to be more fully understood.

Many other enzymatic pathways that degrade released transmitters are not involved in terminating synaptic transmission but are important for controlling the concentration of the transmitter within the neuron or for inactivating transmitter molecules that have diffused away from the synaptic cleft. Many of these degradation enzymes are important clinically—they provide sites for drug action and serve as diagnostic indicators. For example, monoamine oxidase (MAO) inhibitors, which block the degradation of amine transmitters, are used to treat depression and Parkinson disease. Determination of the concentrations of the metabolites of catechol-*O*-methyltransferase (COMT), which is important for degrading biogenic amines and found in the cytoplasm of most cells, provides an index of the efficacy of drugs that affect the synthesis or degradation of the biogenic amines in nervous tissue. COMT is thought to play a particularly important role in regulating cortical dopamine levels due to the low levels of the dopamine uptake transporter.

Box 13–2 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 can be inferred by comparing the distribution of fluorescence under the light microscope with the position of vesicles under the electron microscope.

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 intensifies the electron density of vesicles containing biogenic amines and thus brings out the large number of dense-core vesicles that are characteristic of aminergic neurons.

It is also possible to identify neurons that express the gene for a

particular transmitter enzyme or peptide precursor. Many methods for detecting specific mRNAs depend on the nucleic acid hybridization. One such method is in situ hybridization.

Two single strands of a nucleic acid polymer will pair if their sequence of bases is complementary. With in situ hybridization the strand of noncoding DNA (the negative or antisense strand or its corresponding RNA) is applied to tissue sections under conditions suitable for hybridizing with endogenous (sense) mRNA. If the probes are radiolabeled, autoradiography reveals the locations of neurons that contain the complex formed by the labeled complementary nucleic acid strand and the mRNA.

Hybrid oligonucleotides synthesized with nucleotides containing base analogs tagged chemically, fluorescently, or immunoreactively can be localized cytochemically. Both labels can be used at the same time (Figure 13–3A). More recent modifications of these approaches involve viral or transgenic expression of proteins fused to variants of green fluorescent protein (Figure 13-3B).

Transmitter substances can also be localized by immunocytochemistry. Amino acid transmitters, biogenic amines, and neuropeptides have a primary amino group that becomes covalently fixed within the neurons; this group becomes cross-linked to proteins by aldehydes, the usual fixatives used in microscopy.

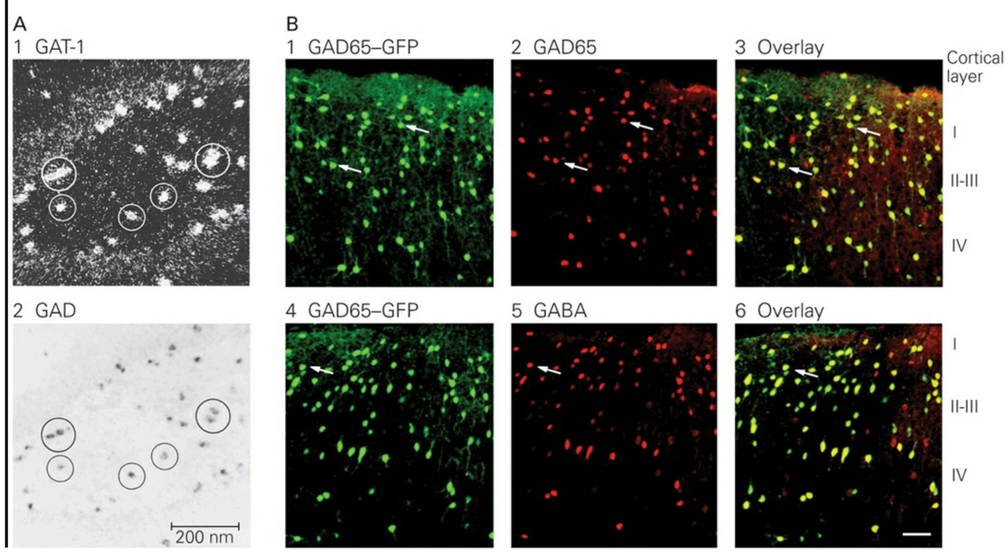


Figure 13-3 Histochemical techniques for visualizing chemical messengers.

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. Neurons expressing both transcripts were labeled by the phosphatase reaction product and by silver grains. **Circles** enclose nerve cell bodies that contain both labels. **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. **Circles** enclose areas containing cells with the greatest reactivity. (Reproduced, with permission, from Sara Augood.)

B. Images of neocortex from a GAD65GFP 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 supragranular layers. Most of the GFP-positive neurons are immunopositive for GAD65 and GABA (arrows). Scale bar = 100 μm. (Adapted, with permission, from López-Bendito et al. 2004.)

For immunohistochemical localization, specific antibodies against the transmitter substances are necessary. Antibodies specific to serotonin, histamine, and many neuroactive peptides can be detected by a second antibody (in a technique called *indirect immunofluorescence*). As an example, if the first antibody is rabbit-derived, the second antibody can be goat antibody raised against rabbit immunoglobulin.

These commercially available secondary antibodies are tagged with fluorescent dyes and used under the fluorescence microscope to locate antigens in regions of individual neurons—cell bodies, axons, and sometimes terminals (Figures 13–3, 13–4).

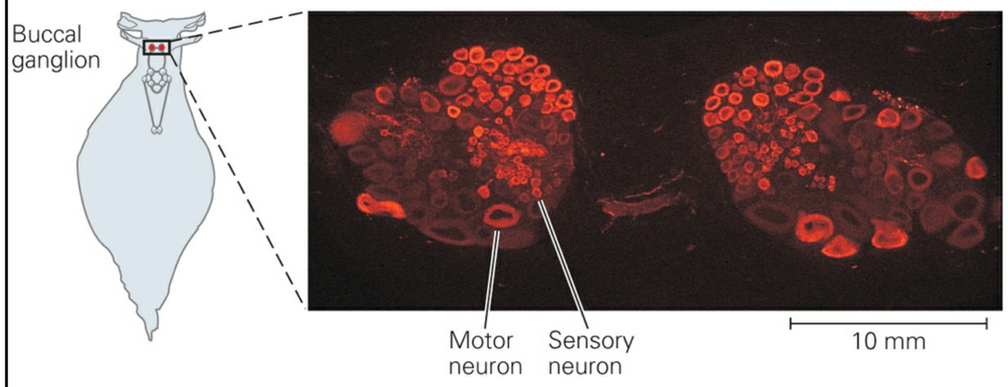


Figure 13-4 An immunochemical technique visualizes a neuropeptide. The buccal ganglion of the marine snail *Aplysia* contains the sensory, motor, and interneurons that control the rhythmic movements of the feeding apparatus of the animal. A cryostat section of the bilaterally symmetrical buccal ganglion is labeled with an antibody raised against FMRFamide. The staining shows immunoreactive FMRFamide in certain sensory neurons (most of the cells with the small cell bodies) as well as certain motor neurons (cells with larger cell bodies). Some of these cells contain one or more other peptides or conventional transmitters such as ACh. (Reproduced, with permission, from Lloyd et al. 1987.)

Ultrastructure localization can be achieved by immunochemical techniques, usually involving a peroxidase-antiperoxidase system. Another method is to use antibodies linked to gold particles, which are electron-dense (Figure 13–5). Spheres of colloidal gold can be generated with precise diameters in the nanometer range, and because they are electron-dense they can be seen in the electron microscope. This technique has the additional useful feature that more than one specific antibody can be examined in the same tissue section if each antibody is linked to gold particles of different sizes.

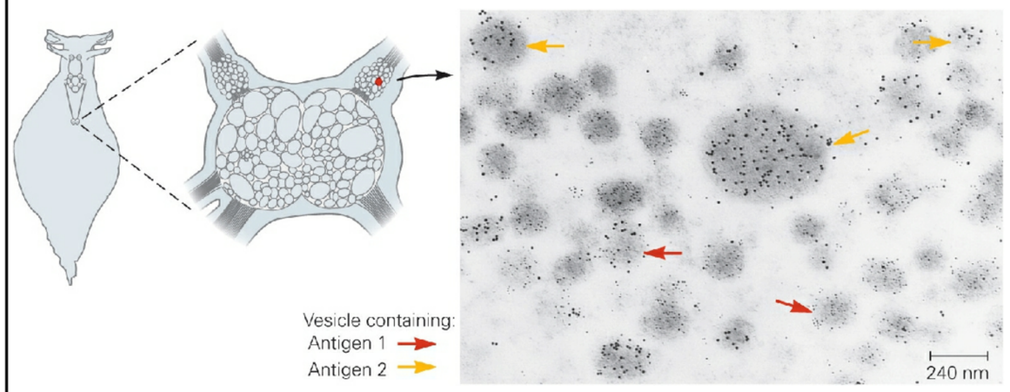


Figure 13-5 Electron-opaque gold particles linked to antibody are used to locate antigens in tissue at the ultrastructural level. The electron micrograph shows a section through the cell body of an *Aplysia* bag cell. Bag cells control reproductive behavior by releasing a group of neuropeptides cleaved from the egg-laying hormone (ELH) precursor. The cells contain several kinds of dense-core vesicles. The cell shown here was treated with two antibodies against different regions of the ELH precursor. One antibody was raised in rabbits and the other in rats. These antibodies were detected with anti-rabbit or anti-rat immunoglobulins (secondary antibodies) raised in goats. Each secondary antibody was coupled to colloidal gold particles of a distinct size. Vesicles identified by antigen 1 are smaller than vesicles identified by antigen 2, indicating that the specific fragments cleaved from the precursor are localized in different vesicles. (Reproduced, with permission, from Fisher et al. 1988.)

The mechanisms for removing neuropeptides from the synaptic cleft are slow diffusion and proteolysis by extracellular peptidases. In contrast, small-molecule transmitters are removed more quickly from the synaptic cleft.

The critical mechanism for inactivation of most neurotransmitters is reuptake at the plasma membrane. This mechanism serves the dual purposes of terminating the synaptic action of the transmitter as well as recapturing transmitter molecules for subsequent reuse. High-affinity uptake, with binding constants of 25 μM or less for the released transmitter, is mediated by transporter molecules in the membranes of nerve terminals and glial cells. Unlike vesicular transporters, which are powered

by the H^+ electrochemical gradient in a countertransport mechanism, plasma membrane transporters are driven by the Na^+ electrochemical gradient through a symport mechanism in which Na^+ ions and transmitter move in the same direction.

Each type of neuron has its own characteristic uptake mechanism. For example, noncholinergic neurons do not take up choline with high affinity. Certain powerful psychotropic drugs can block uptake processes. For example, cocaine blocks the uptake of dopamine, norepinephrine, and serotonin; the tricyclic antidepressants and selective serotonin reuptake inhibitors, such as fluoxetine (Prozac), block uptake of serotonin or norepineprhine. The application of appropriate drugs that block transporter molecules can prolong and enhance synaptic signaling by the biogenic amines and GABA. In some instances drugs act both on transporter molecules on the neuron's surface and on vesicular transporters within the cell. For example, amphetamines must be actively taken up by the dopamine transporter on the external membrane of the neuron before they can operate on the vesicular transporter for amine transmitters.

Transporter molecules for neurotransmitters belong to two distinct groups that are different in both structure and mechanism. High-resolution structures of bacterial homologs from each of these families have been solved recently, which has greatly advanced our understanding of transporter mechanisms. One group, the neurotransmitter sodium symporters (NSS), is a superfamily of transmembrane proteins that thread through the plasmalemma 12 times and includes the transporters of GABA, glycine, norepinephrine, dopamine, serotonin, osmolytes, and amino acids. The other consists of transporters of glutamate; these proteins traverse the plasmalemma eight times and contain two helical hairpins that seem to serve a role in gating access of substrate from each side of the membrane. Each group includes several transporters for each transmitter substance; for example, there are multiple GABA, glycine, and glutamate transporters, each with somewhat different localization, function, and pharmacology.

The two groups can be distinguished functionally. Although both are driven by the electrochemical potential provided by the Na^+ gradient, transport of glutamate requires the countertransport of K^+, whereas transport by the NSS usually requires the co-transport of a Cl^- ion. During transport of glutamate one negatively charged molecule of the transmitter is imported with three Na^+ ions and one proton (symport) in exchange for the export of one K^+. This leads to a net influx of two positive charges for each transport cycle, generating an inward current. As a result of this charge transfer, the negative resting potential of the cell generates a large inward driving force that results in an enormous gradient of glutamate across the cell membrane. In contrast, the NSS proteins transport one to three Na^+ ions and one Cl^- ion together with their substrates. Under most conditions the electrochemical driving force is sufficient for transporters to transport transmitter into the cell, thereby concentrating it inside the cell.

An Overall View

Information carried by a neuron is encoded in electrical signals that travel along its axon to a synapse, where these signals are transformed and carried across the synaptic cleft by one or more chemical messengers.

The two major classes of chemical messengers, small-molecule transmitters and neuroactive peptides, are packaged in vesicles within the presynaptic neuron. After their synthesis in the cytoplasm, small-molecule transmitters are taken up and concentrated in vesicles, where they are protected from degradative enzymes that maintain a constant level of transmitter substance in the cytoplasm. Synaptic vesicles are highly concentrated in nerve endings. Because they are quickly replenished during synaptic activity, much of the small-molecule transmitter in the neuron must be synthesized locally at the terminals.

In contrast, the protein precursors of neuroactive peptides are synthesized only in the cell body; the neuropeptides become packaged in secretory granules and vesicles that are carried from the cell body to the terminals by axoplasmic transport. Unlike the vesicles that contain small-molecule transmitters, these vesicles are not refilled at the terminal.

Given their central importance in brain function, it is not surprising that the enzymes that regulate transmitter biosynthesis are under tight regulatory control. Changes in neuronal activity can produce homeostatic changes in the levels of these enzymes. This regulation can occur both post-translationally in the cytoplasm, as a result of phosphorylation and dephosphorylation reactions, and by transcriptional contol in the

nucleus.

Precise mechanisms for terminating transmitter actions represent a key step in synaptic transmission that is nearly as important as transmitter synthesis and release. Some released transmitter is lost as a result of simple diffusion out of the synaptic cleft. However, for the most part, transmitter actions are terminated by specific molecular reactions. For example, acetylcholine is rapidly hydrolyzed by acetylcholinesterase to choline and acetate, whereas glutamate is taken up into presynaptic terminals and glia by specific transporters that are driven by ion gradients. Some of the most potent psychoactive compounds act by interfering with transmitter reuptake. The psychostimulatory effects of cocaine result from its action to prevent reuptake of catecholamines whereas the blockade of serotonin transporters are responsible for the antidepressant effects of the selective serotonin reuptake inhibitors (SSRIs).

Can we arrive at a comprehensive and precise definition of a neurotransmitter? Probably not, as the definition is empirical. The first step in understanding the molecular strategy of chemical transmission usually involves identifying the contents of synaptic vesicles. Except for those instances in which transmitter is released by transporter molecules or by diffusion through the membrane (in the case of gases and lipid metabolites, see Chapter 11), only molecules suitably packaged in vesicles can be released from a neuron's terminals. But not all molecules released by a neuron are chemical messengers—only those that bind to appropriate receptors and initiate functional changes in that target neuron can usefully be considered neurotransmitters.

Information is transmitted when transmitter molecules bind to receptor proteins in the membrane of another cell, causing them to change shape. Once the molecules of transmitter are bound, the receptor generates electrical or metabolic signals in the postsynaptic cell. The co-release of several neuroactive substances onto appropriate postsynaptic receptors permits great diversity of information to be transferred in a single synaptic action.

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