CHAPTER

7

Pharmacology and Biochemistry of Synaptic Transmission

Classical Transmitters

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The study of the nervous system 100 years ago was a period of claim and counterclaim, confusion, and recrimination—not unlike politics today or, for that matter, science. The reason for this rowdy transition to the twentieth century was a body of accumulating data that argued against the prevailing wisdom. In the second half of the nineteenth century most held that the brain is a large continuous network, with each of its cells in physical contact. The remarkably sharp eye and interpretative powers of the Spaniard Santiago Ramón y Cajal revealed a very different picture, in which neurons are independent structures, not in physical contact with one another, and thus the basic unit of the brain (see Shepherd, 1991, and Chapter 1). Although it would be the middle of the twentieth century before electron microscopic data provided the final confirmation of Cajal's hypothesis, his view of neurons as the independent units of the nervous system gained widespread acceptance much earlier. In turn, this acceptance brought about a new debate: how do neurons that are not in physical contact communicate? The answer to this seemingly simple question continues to occupy neuroscience today and is evolving continuously. In this chapter we discuss briefly several means through which cells communicate with each other and then discuss in considerable detail the major means of communication, chemical synaptic transmission. Another means of communication, electrical transmission through gap junctions, is discussed in Chapter 9.

As discussed in Chapter 1, neurons vary widely in form and function but share certain structural characteristics. From the cell body emanates processes (axons and dendrites) representing polarized compartments of the cell. Axons can be short or long and remain local or project to distant areas. In contrast, dendrites are local. The general concept arose that axons transmit information, which is conveyed to the dendrites or soma of follower cells. Cajal hypothesized that there is a critical gap between the part of the neuron sending information (e.g., the axon) and the cell receiving this information. This gap was termed the synapse by Sherrington (see Shepard, 1991). Thus, there were presynaptic neurons that were separated from their follower (postsynaptic) neurons by the synapse. This general conceptual framework remains in place today, although there are many exceptions to specific aspects, including dendrites that release transmitters and axons that receive inputs from other neurons.

DIVERSE MODES OF NEURONAL COMMUNICATION

The controversy surrounding the nature of neuronal communication—chemical or electrical—was in full force for the first half of the twentieth century, even though evidence was marshaled in support of the chemical mode of communication midway through the nineteenth century (see Valenstein, 2005). Taking advantage of two excitable cells that were easily accessible, the axon of neurons and muscle cells, a series of studies pointed to communication between these two distinct cells. In 1849, Claude Bernard noted that

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curare, the active constituent of a poison applied to arrows in South America, blocks nerve-to-muscle neurotransmission (Bernard, 1849). This effect was subsequently shown to be due to binding of curare to postsynaptic receptors for the neurotransmitter acetylcholine (ACh), thus disrupting neuromuscular transmission. In a seminal series of studies using the isolated frog heart, Otto Loewi (1921) demonstrated chemical transmission by showing that ACh was released on nerve stimulation and activated a target muscle.

Although it is now clear that the major means through which neurons convey information to other cells is through chemical communication, neurons also use several other processes for intercellular communication. These include electrical synaptic transmission (see Chapter 9), ephaptic interactions, and autocrine, paracrine, and long-range signaling, to which molecules produced by both neural and nonneural cells contribute. The nonsynaptic mode of intercellular communication with the longest range (distance) is humoral (hormonal signaling). For example, some peripheral hormones can enter the brain to modulate neuronal activity. The neuronal targets of these hormones have specific receptors that respond to the humoral signal, which may evoke either a short-term response (acutely change neuronal activity) or long-lasting changes in gene expression by targeting nuclear hormone receptors.

This chapter focuses on molecules, termed neurotransmitters, which convey information from one (presynaptic) neuron to another (postsynaptic) neuron. However, molecules produced by neurons can also be used in intercellular communication that does not require synaptic specializations. These molecules range from neuroactive steroids and lipids that resemble the active constituents of marijuana to gases. Such molecules may activate receptors on the same cell that releases them (see Fig. 7.1), or regulate nearby but not adjacent cells through paracrine signaling (Fig. 7.1). Moreover, the direction of information flow is not fixed: the gaseous transmitters and some other compounds may act as retrograde neurotransmitters, with a "post"-synaptic neuron releasing a substance to signal a "pre"-synaptic element. Soluble factors can act on high- or low-affinity receptors and can act either locally or over some distance. The role of such molecules is thought to be primarily in modulating neural activity, although they may also provide trophic support ("guidance cues") for neurons that are elaborating their processes (axons) as they route to make connections. A more extended discussion of the "unconventional" transmitters as well as peptide transmitters and growth factors can be found in the next chapter.

The most intimate nonsynaptic mode of intercellular communication is the ephapse, in which electrical impulses in one cell or extracellular ion accumulation in the vicinity of one cell can directly affect the activity of an adjacent cell. In this case (see Fig. 7.1), the only morphological requirement is closely apposed membranes. Ephaptic interactions are either transient or sustained, and can be induced by various treatments.

CHEMICAL TRANSMISSION

Chemically mediated transmission is the major means by which a signal is communicated from one nerve cell to another and is the mode of neuronal communication on which this and the next chapter focus. A relatively simple definition of a neurotransmitter is an endogenous substance that is released from neurons, acts on receptors typically located on the membranes of postsynaptic cells, and produces a functional change in the properties of the target cell. However, the devil is in the details, and in order for a molecule to be designated a neurotransmitter certain criteria must be met. There is a relatively small number of compounds that meet all these criteria. These "classic" transmitters (which include acetylcholine, dopamine, serotonin, norepinephrine, glutamate, and γ-aminobutyric acid) were all identified by the mid-twentieth century. Since then it has become apparent that there is a much larger number of chemical messengers that broadly qualify as intercellular transmitters but do not meet one or more of the classic criteria.

The Criteria for Definition as a Neurotransmitter

The first criterion for defining a compound as a neurotransmitter is that it must be synthesized by and released from neurons. This usually means that the presynaptic neuron should contain a transmitter and the appropriate enzymes required for synthesis of that neurotransmitter. However, synthesis in the nerve terminal is not an absolute requirement. For example, peptide transmitters are synthesized in the cell body and transported to their release sites in axon terminals (see Chapter 8).

Second, the substance should be released from nerve terminals in a chemically or pharmacologically identifiable form—i.e., one should be able to isolate the transmitter and characterize its chemical structure.

Third, a neurotransmitter should reproduce at the postsynaptic cell the same effects that are observed after stimulation of the presynaptic neuron. Moreover,

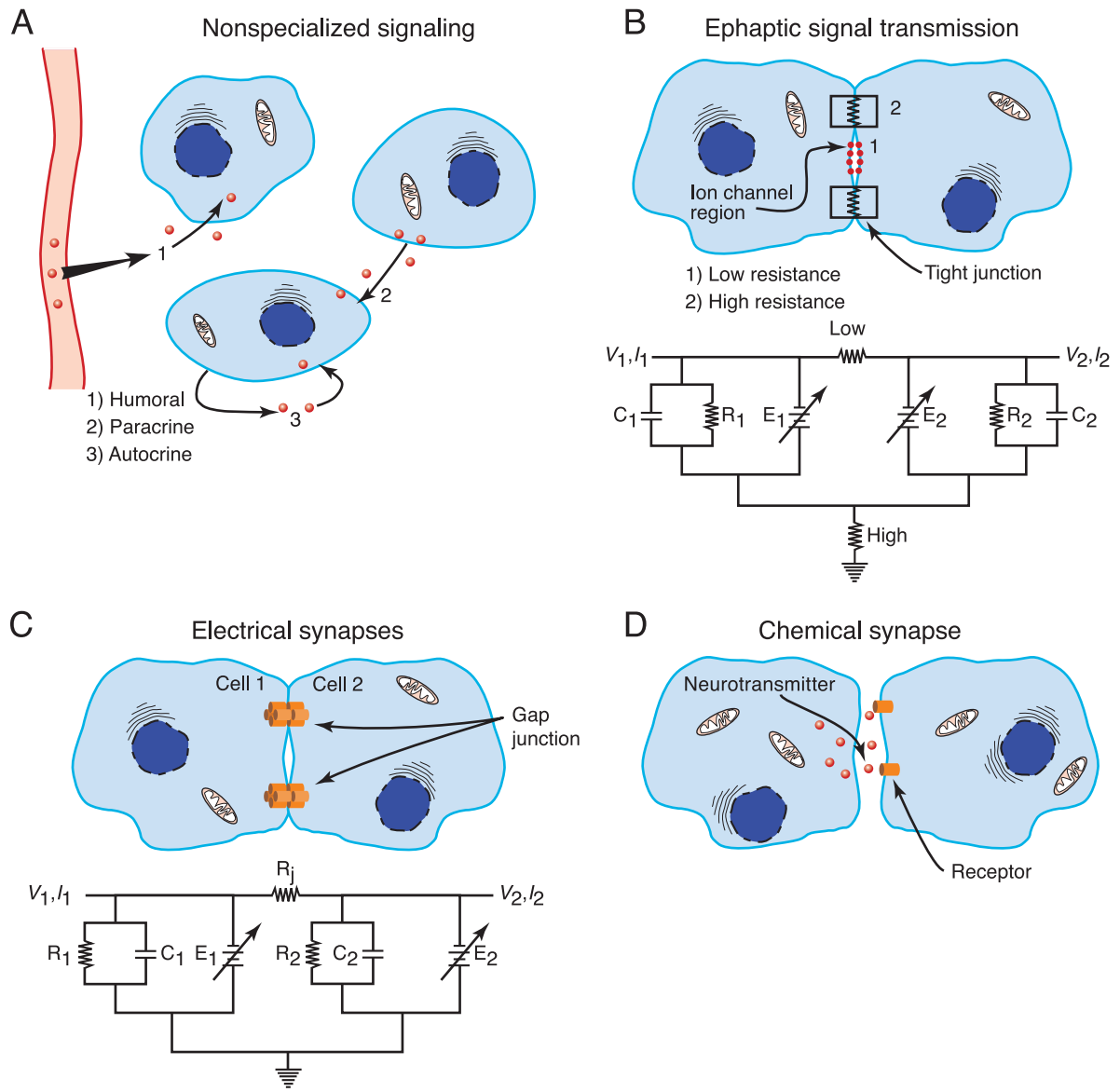

FIGURE 7.1 **The multiple modes of intercellular signaling.** (A) Substances (red) produced outside the nervous system (1) or by cells within the CNS (2, 3) can affect neuronal activity, acting through (1) humoral, (2) paracrine, and (3) autocrine mechanisms. (B) Ephaptic transmission. Two apposed cell membranes showing regions of low (1, red label indicating channels) and high (2, sawtooths, representing tight junctions) resistances. The electrical equivalent circuit is shown below. Communication between these cells is permitted through membrane regions of low resistance. The presence of tight junctions (2) between apposed cells favors an increase in current density by preventing current flow into the bulk extracellular space. Charges accumulate in the narrow intercellular space and affect capacitance and resistive components of the cell membranes. (C) Electrical synapses. Gap junction channels provide low-resistance pathways between adjacent cells, allowing direct communication between the cytoplasms of both cells. In contrast with the ephaptic mode of transmission, current flows directly from cell to cell and not through the extracellular space. The electrical equivalent circuit is shown below, differing from that of the ephapse in (B) primarily in the absence of a resistance to ground. (D) Chemical synapses. Neurotransmitters released from the presynaptic terminal (on the left) diffuse across the cleft to bind to postsynaptic receptors, thereby opening ion channels or otherwise modifying intracellular signaling, thereby changing conductance of the postsynaptic membrane and producing currents that excite or inhibit the cell.

the concentrations of the applied transmitter should be similar to those measured after stimulation-elicited release of the neurotransmitter.

Fourth, the effects of a putative neurotransmitter should be blocked by known competitive antagonists in a dose-dependent manner. In addition, treatments that disrupt the synthesis of the candidate transmitter should block the effects of presynaptic stimulation.

Fifth, there should be appropriate active mechanisms to terminate the action of the putative neurotransmitter. Such mechanisms can include enzymatic degradation and reuptake of the substance into the

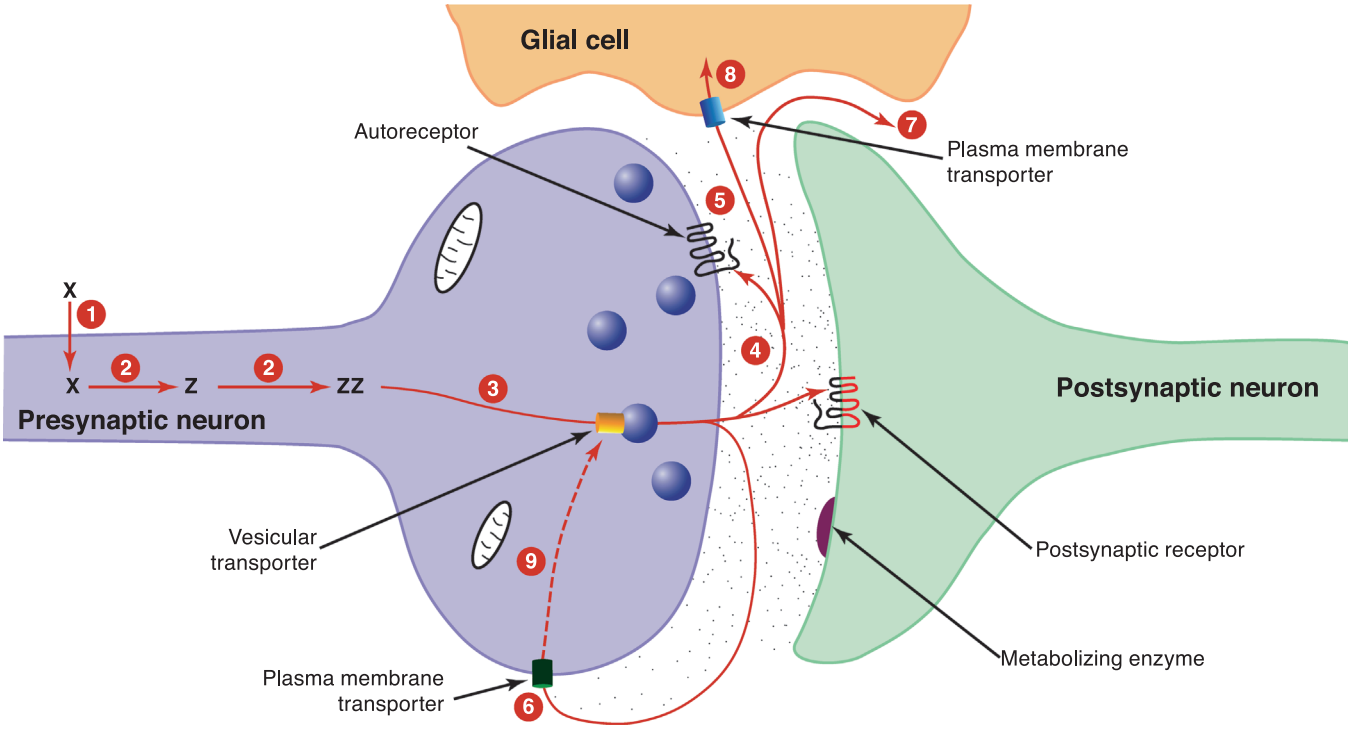

FIGURE 7.2 **Schematic representation of the life cycle of a classic neurotransmitter.** After accumulation of a precursor amino acid (X) into the neuron (step 1), the amino acid precursor is sequentially metabolized (step 2) to yield the mature transmitter (ZZ). The transmitter is then accumulated into vesicles by the vesicular transporter (step 3), where it is poised for release and protected from degradation. The released transmitter can interact with postsynaptic receptors (step 4) or autoreceptors (step 5) that regulate transmitter release, synthesis, or firing rate. Transmitter actions are terminated by means of a high-affinity membrane transporter (step 6) that is usually associated with the neuron that released the transmitter. Alternatively, the actions of the transmitter may be terminated by means of diffusion (step 7) or by accumulation into glia through a membrane transporter (step 8). When the transmitter is taken up by the neuron, it is subject to metabolic inactivation (step 9).

presynaptic neuron or glial cells through specific transporter molecules.

The Five Steps of Chemical Neurotransmission: Synthesis, Storage, Release, Receptor Binding, and Transmitter Inactivation

The general mechanisms of chemical synaptic transmission are depicted in Fig. 7.2. Synaptic transmission consists of a number of steps; each of these steps is a potential site of drug action.

1. *Biosynthesis of the neurotransmitter in the presynaptic neuron*. In order for the transmitter to be synthesized, precursors of the transmitter should be present in appropriate sites in the neuron. Moreover, 1) the enzymes that catalyze the conversion of precursor(s) into transmitter should be present in an active form, and 2) these enzymes and their cofactors should be present in the appropriate compartment in the neuron. The synthetic pathways for neurotransmitter formation have long been targeted as an important site of action for clinically useful drugs. An example is α-methyl-*p*-tyrosine, a drug used in the treatment of an adrenal gland tumor (pheochromocytoma) that causes very high blood pressure and increases the risk of strokes. This tumor releases massive amounts of norepinephrine, a neurotransmitter that acts to constrict blood vessels and increase cardiac output and thereby elevates blood pressure. α-Methyl-*p*-tyrosine blocks the synthesis of catecholamines such as norepinephrine, thereby lowering blood pressure.
2. *Storage of the neurotransmitter or its precursor or both in the presynaptic nerve terminal*. Classic and peptide transmitters are stored in synaptic vesicles, where they are sequestered and protected from enzymatic degradation and are readily available for release. In the case of so-called "classic" neurotransmitters (such as acetylcholine, biogenic amines, and amino acids), the synaptic vesicles are small (~50 nm in diameter). In contrast, neuropeptide transmitters in the brain are stored in large dense-core vesicles (~100 nm in diameter), which usually release their contents in response to high firing rates or burst firing of neurons. Because most neurotransmitters are synthesized in the cytosol of neurons, an active

process must accumulate the transmitter into the vesicle. The proteins responsible for this action are the vesicular transporters.

3. *Release of the neurotransmitter into the synaptic cleft*. Transmitter-containing vesicles fuse with the cellular membrane and release of the transmitter occurs. Neurons use two pathways to secrete proteins. The release of most neurotransmitters occurs by a *regulated* pathway controlled by extracellular signals. The mechanisms involved in neurotransmitter release are discussed more fully in Chapter 15. Membrane components, viral proteins, and extracellular matrix molecules are secreted through the *constitutive* pathway, which is not triggered by extracellular stimulation. Some unconventional transmitters (e.g., growth factors) are in part synthesized and released in the constitutive pathway.
4. *Binding and recognition of the neurotransmitter by target receptors*. Transmitters that are released interact with receptors located on the target (postsynaptic) cell. These receptors fall into two broad classes. The first are metabotropic receptors, which are coupled to intracellular G proteins as effectors (see Chapters 4 and 10). The other group of receptors, termed *ionotropic receptors*, forms ion channels that are either ligand- or voltage-gated, and through which ions such as sodium and calcium enter the cell. We usually think of neurotransmitter receptors as being localized to the postsynaptic neuron. However, receptors are also found on presynaptic neurons, which can respond to release of the transmitter from that cell (autoreceptors) or to a transmitter release from a different cell (heteroceptor). Autoreceptors can be thought of as a homeostatic feedback system that modulates the function of presynaptic neurons, regulating transmitter release or synthesis or the firing rate of neurons. A more thorough discussion of neurotransmitter receptors may be found in Chapter 10.
5. *Inactivation and termination of the action of the released transmitter*. Continuous activation of a neuron by a presynaptic input does not convey meaningful dynamic information to the recipient neuron. Moreover, such sustained signaling often has adverse effects; examples include tetanus in muscles or seizure discharges in neurons. There are multiple processes to terminate the action of neurotransmitters, both active and passive. The active mechanisms include reuptake of the transmitter through specific membrane transporter proteins. Most commonly found on the presynaptic neuron, at or close to the synapse, there can be multiple transporters for a given neurotransmitter. In addition, some transporter proteins are also present on postsynaptic neurons or astrocytes, a type of glial cell (see Chapter 1). A second active means of terminating the actions of a neurotransmitter is by enzymatically degrading the released transmitter to an inactive substance. In addition, glial cells can accumulate certain released transmitters. Diffusion of the transmitter away from the transmitter receptors in the synapse is a passive mechanism that can stop neuronal signaling.

The five steps just described form a logical scaffold for understanding chemical neurotransmission. However, there are particular and peculiar intricacies for each of the steps and for each of the many neurotransmitters. Catecholamines are a structurally defined group of neurotransmitters that have been extensively studied, and which this chapter will focus on as illustrative examples. This discussion is followed by shorter discussions of four other classical neurotransmitters. These include the indoleamine serotonin (5-hydroxytryptamine), acetylcholine, and the amino acids GABA (γ-aminobutyric acid) and glutamate. The key differences between classical and other neurotransmitters or chemical messengers, among which are peptide neurotransmitters and unconventional transmitters such as nitric oxide and growth factor, will be discussed in the next chapter.

CLASSICAL NEUROTRANSMITTERS

The adjective *classical* is used to distinguish acetylcholine, the catecholamines, serotonin, and the amino acid transmitters from other neurotransmitters. Although the designation is somewhat arbitrary, classical transmitters can be differentiated from others on several grounds. As discussed previously and in Chapter 15, the vesicles storing classical neurotransmitters are smaller than those containing peptides. In addition, classical transmitters or their metabolic products are subject to active reuptake by the presynaptic cell and can be thought of as homeostatically regulating and conserving their products; there is no comparable energy-dependent high-affinity reuptake process for nonclassical transmitters. Finally, most classic transmitters are enzymatically synthesized in the nerve terminal. In contrast, peptides and some unconventional transmitters are synthesized in the soma from a large precursor protein and then transported to the nerve terminal.

The Catecholamine Neurotransmitters

The term *catecholamine* generally refers to organic compounds with a catechol nucleus (a benzene ring

with two adjacent hydroxyl substitutions) and an amine group. In practice, however, the term is used to describe the endogenous compounds dopamine (dihydroxyphenylethylamine), norepinephrine, and epinephrine. These three neurotransmitters are formed by successive enzymatic steps, each requiring a distinct enzyme (see Fig. 7.3). The localization of particular synthesizing enzymes to different cells results in distinct dopamine-, norepinephrine-, and epinephrine-containing neurons in the brain. The catecholamines also have hormonal functions in the peripheral nervous system.

In the peripheral nervous system, dopamine is present mainly as a precursor for norepinephrine but also has important biological activity in the kidney. Norepinephrine is the sympathetic nervous system neurotransmitter in mammals; in contrast, epinephrine is the sympathetic transmitter in frogs. Despite this species difference in the functions of a given neurotransmitter, the biochemical aspects of neurotransmission as a general rule are remarkably constant across vertebrate species and, indeed, invertebrates.

Catecholamine Biosynthesis

The amino acids phenylalanine and tyrosine are precursors for catecholamines. These amino acids are found in high concentrations in both the plasma and brain. In mammals, tyrosine can be derived from dietary phenylalanine by an enzyme, phenylalanine hydroxylase, which is mainly found in the liver. Phenylketonuria, a disorder caused by insufficient amounts of phenylalanine hydroxylase, results in very high plasma and brain levels of phenylalanine. Unless dietary phenylalanine intake is restricted intellectual impairment results (see Box 7.1).

Catecholamine synthesis is usually considered to begin with tyrosine, which represents a branch point for many important biosynthetic processes in animal tissues. The sequence of enzymatic steps required to synthesize catecholamines from tyrosine was first postulated by Blaschko in 1939 and confirmed by Nagatsu and coworkers in 1964, when they demonstrated that the enzyme tyrosine hydroxylase (TH) converts the amino acid L-tyrosine into 3,4-dihydroxyphenylalanine (L-DOPA). All of the enzymes in the catecholamine biosynthetic pathway have been purified to homogeneity, which has allowed for detailed analyses of the kinetics, substrate specificity, and cofactor requirements of these enzymes and aided in the development of useful inhibitors of the enzymes. Moreover, the development of antibodies against the purified enzymes has permitted the precise localization of the enzymes by immunohistochemical techniques.

The hydroxylation of L-tyrosine by TH results in the formation of the dopamine precursor L-DOPA, which is almost immediately metabolized to dopamine by L-aromatic amino acid decarboxylase (AADC). This decarboxylation step is so rapid that one cannot routinely measure L-DOPA in brain without first inhibiting AADC. In dopamine-containing neurons of the brain the decarboxylation of L-DOPA to dopamine is the final step in transmitter synthesis. However, in neurons that use norepinephrine (also known as noradrenaline) or epinephrine (adrenaline) as transmitters, the enzyme dopamine beta-hydroxylase (DBH) is present; this enzyme oxidizes dopamine to yield norepinephrine. Finally, in neurons in which epinephrine is the transmitter, a third enzyme, phenylethanolamine *N*-methyltransferase (PNMT), converts norepinephrine into epinephrine. Thus, a neuron that uses epinephrine as its transmitter has four enzymes (TH, AADC, DBH, and PNMT) that sequentially metabolize tyrosine to epinephrine. Noradrenergic neurons express only the enzymes TH, AADC, and DBH, and thus norepinephrine cannot be further metabolized to epinephrine. Similarly, because dopamine neurons lack DBH and PNMT, the catecholamine end product in these neurons is the transmitter dopamine. The enzymes and cofactors taking part in catecholamine synthesis are illustrated in Fig. 7.4.

TYROSINE HYDROXYLASE

In human beings, a single tyrosine hydroxylase gene gives rise to four TH mRNAs through alternative splicing (Lewis et al., 1993). In contrast, in most primates two TH mRNA isoforms are present. Still different are the rat and mouse, which possess but a single TH mRNA. It has been speculated that the different forms of TH in humans are associated with differences in activity of the enzyme, but conclusive data on the functional significance of the various isoforms are lacking.

Tyrosine hydroxylase function is determined by two factors: changes in enzyme activity (the rate at which the enzyme converts a precursor to its product) and changes in the amount of enzyme protein present. A major determinant of TH activity is phosphorylation of the enzyme (Fig. 7.4), which occurs at four different serine sites at the amino terminus of the protein (Haycock and Haycock, 1991). These four serine residues are differently phosphorylated by various kinases. A second means of regulating the activity of the enzyme is through end-product inhibition: catecholamines can inhibit the activity of TH through competition for a required pterin cofactor for the enzyme (see Iversen et al., 2009).

An increased neuronal demand for catecholamine synthesis can be met by inducing new TH protein or by activating (by phosphorylation) the enzyme. The degree to which increases in catecholamine synthesis

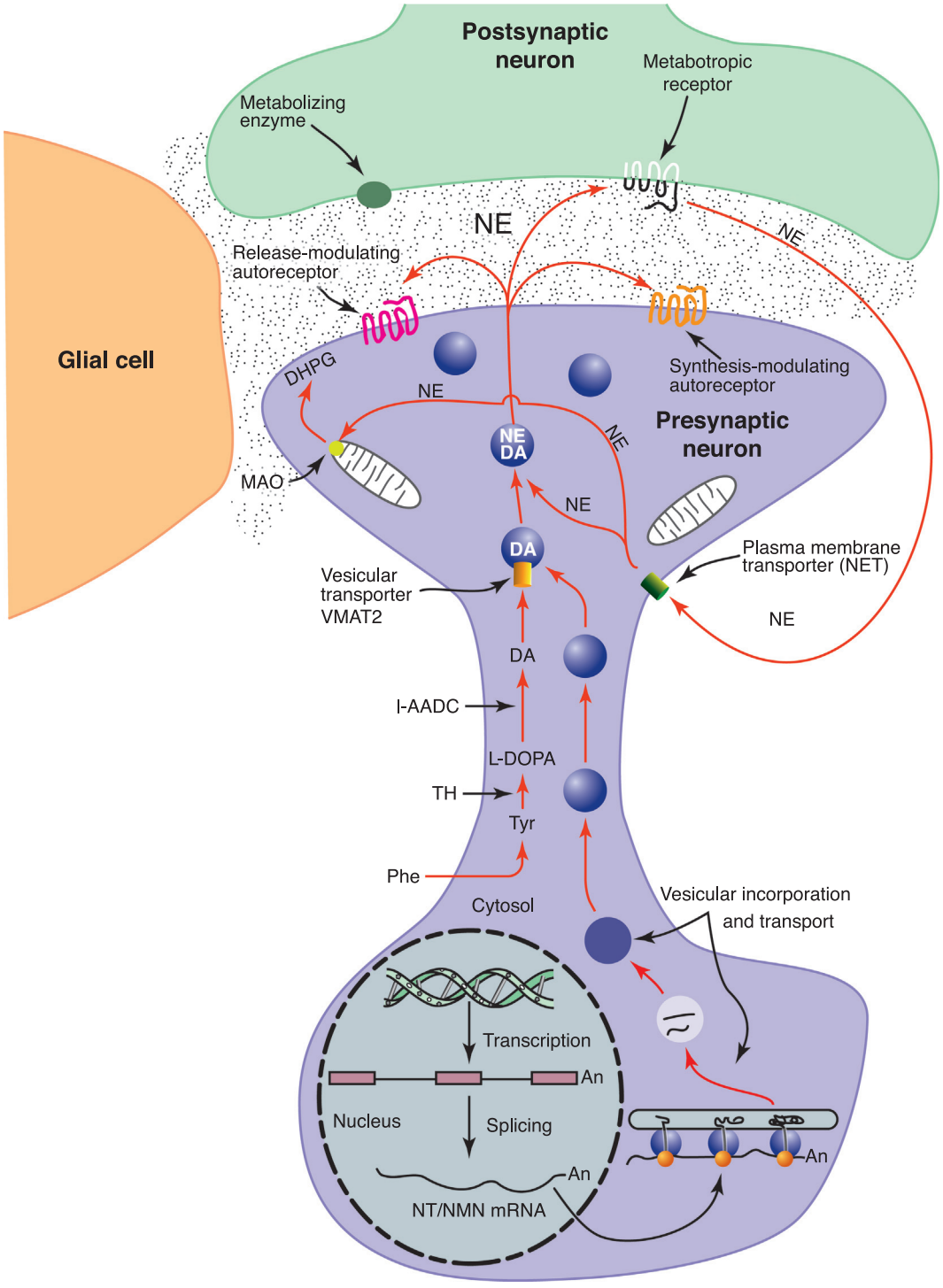

FIGURE 7.3 **Characteristics of a norepinephrine (NE)-containing catecholamine neuron.** Tyrosine is sequentially metabolized by tyrosine hydroxylase (TH) and L-aromatic amino acid decarboxylase (l-AADC) to dopamine (DA). The DA is then accumulated by the vesicular monoamine transporter. In dopaminergic neurons this is the final step. However, in this noradrenergic neuron, the DA is metabolized to NE by dopamine-β-hydroxylase (DBH), which is present in the vesicle. Once NE is released, it can interact with postsynaptic noradrenergic receptors or different types of presynaptic noradrenergic autoreceptors. The accumulation of NE by the high-affinity membrane norepinephrine transporter (NET) terminates the extracellular actions of NE. Once accumulated by the neuron, the NE can be metabolized to inactive species (for example, DHPG) by key degradative enzymes, such as monoamine oxidase (MAO), or taken back up by the vesicular transporter.

BOX 7.1

PKU AND METABOLISM

Classic phenylketonuria (PKU) is a genetic disease caused by mutations in the enzyme phenylalanine hydroxylase (PAH), resulting in loss of the enzyme's ability to hydroxylate phenylalanine (Phe) to tyrosine. PAH plays an integrated dual role in the metabolism of humans and other mammals. First, it provides an endogenous supply of tyrosine, thereby making consumption of this amino acid unnecessary for normal growth. Second, the reaction catalyzed by PAH is an essential step in the complete oxidation of Phe. When PAH levels are low or absent, as in PKU, blood levels of Phe are typically 20- to 50-fold higher than normal. A tiny fraction of the elevated Phe is converted to the phenylketone phenylpyruvic acid, which is excreted in the urine, hence the name of the disease.

The increased concentration of Phe seen in PKU spares the body, but spoils—indeed, devastates—the developing brain, and typically leads to severe mental retardation unless steps are taken to limit dietary Phe intake. The majority of untreated PKU patients suffer severe intellectual impairment (IQ $<$ 20). They also have a somewhat higher incidence of seizures and tend to have fair skin and hair. The latter effect is due to the inhibition of melanin formation by the excess Phe.

PKU is inherited as an autosomal recessive trait. The vast majority of PKU babies are conceived when both parents are heterozygotes, each one harboring one normal gene and one "PKU" gene. Thus, on average, one-fourth of the children born to such parents have PKU, one-fourth are normal, and half are heterozygotes. The incidence of heterozygosity for PKU is about 1 in 55.

The mechanism by which hyperphenylalaninemia damages the developing brain is unknown but probably involves competition by the high Phe levels with brain uptake of other essential amino acids.

Phenylalanine hydroxylase functions *in vivo* as part of a complex multicomponent system consisting of two other enzymes, dihydropteridine reductase and pterin 4α-carbinolamine dehydratase, and a nonprotein coenzyme, tetrahydrobiopterin (BH4). During the hydroxylation reaction, BH4 is stoichiometically oxidized; i.e., for every molecule of Phe converted to tyrosine by PAH, a molecule of BH4 is oxidized. The function of the two ancillary enzymes is to regenerate BH4.

With the realization that the hydroxylating system consists of four essential components, it was predicted that there might be variant forms of PKU caused by lack of one of the other components of the hydroxylating system. During the last 20 years, patients with these predicted variants have been described. Defects in the reductase or in one of the several enzymes essential for the synthesis of BH4 (but not the one due to defects in the dehydratase) were originally called "lethal" or "malignant" PKU. In all probability, these variants are deadly because BH4 and the reductase are also essential for the functions of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), thus leading to deficits in catecholamine and serotonin systems. These patients therefore suffer from three different metabolic lesions. Fortunately, these variants are extremely rare, accounting for between 1 and 2% of all PKU patients, and can be treated (with varying degrees of success) by feeding them the compounds beyond the metabolic blocks in TH and TPH (i.e., L-DOPA and 5-hydroxytryptophan) and, when needed, large doses of BH4.

If one can dare say that there is anything fortunate about this dreadful disease, it is that it is extremely rare, with an average incidence of about 1/12,000. The frequency, however, varies widely among different ethnic groups, being only 1/200,000 in Japan but as high as 1/5000 in Ireland.

One auspicious feature of the disease is that the affected infants are essentially normal at birth, which raised the hope that some way might be found to prevent the intellectual deterioration of PKU. This hope was realized about 50 years ago with the introduction of a low-Phe diet (not a no-Phe diet!), which has proven to be largely if not totally effective in preventing brain damage, at least as reflected by the normal IQ of PKU patients who are started on the diet shortly after birth. The low-Phe diet is a heavy burden for both patients and their families, and it was once hoped that the diet could be discontinued after 6 or 7 years. It now appears that a longer period is beneficial. The goal of the diet is to keep blood Phe levels from rising no more than five- to six-fold normal levels.

Women with PKU who are contemplating having children raise dietary issues as well. If women with PKU went off the diet at some earlier time, they must resume it before they become pregnant, or the fetus risks *in utero* damage caused by the mother's high levels of Phe, a condition called *maternal PKU*.

Since the *sine qua non* of successful dietary treatment of PKU is to start the diet as soon as possible after birth, its success was closely tied to the development of a cheap and rapid test for PKU. The Guthrie test, which was introduced in 1961, gives a semiquantitative measure of Phe levels in a drop of blood and has been widely used for screening newborns; the blood drop can

BOX 7.1 (*cont'd*)

be collected on a piece of filter paper and then mailed to a suitable laboratory.

PKU is noteworthy in that the brain is the only organ that is damaged by mutations in an enzyme that is found only in the liver in humans. This provides a valuable lesson: metabolically, no organ in the body is an island unto itself.

Seymour Kaufman

Note: Box 7.1 is a U.S. government work in the public domain.

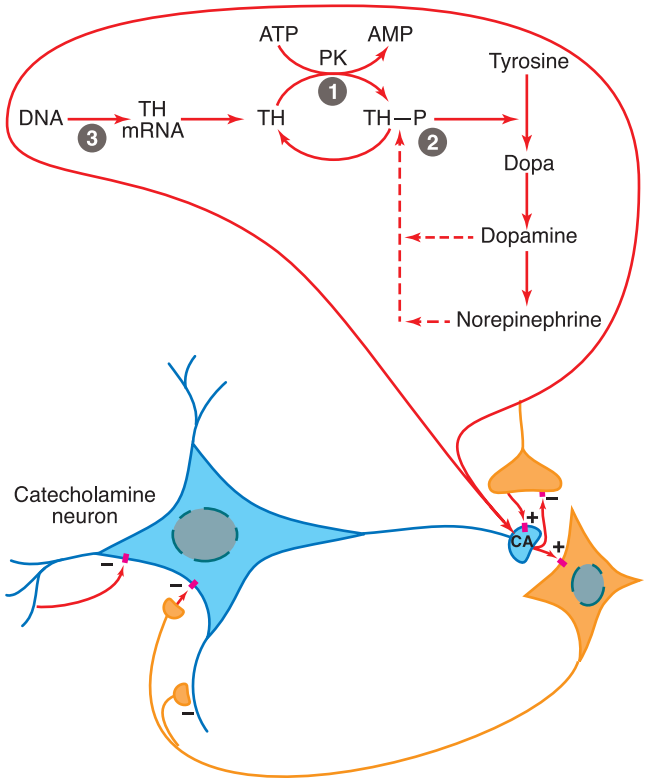

FIGURE 7.4 **Schematic for the regulation of TH enzymatic activity.** The numbered sites depict the three major types of regulation from TH phosphorylation (1), accomplished by the action of specific protein kinases (PK), to end-product inhibition (2) to changes in TH gene transcription (3) and the subsequent increase in protein.

depend on *de novo* synthesis of new enzyme protein or changes in enzymatic activity differs in various catecholamine neurons. For example, increased synthetic demand in brainstem noradrenergic neurons is accomplished primarily by increasing TH gene expression, with the resultant increase in TH protein. In contrast, the same conditions and treatments that increase TH gene expression in brainstem noradrenergic neurons fail to increase TH mRNA levels in midbrain dopamine neurons. In these dopamine cells synthesis is regulated primarily by altering the activity of TH enzyme.

The synthesis of catecholamines starts with the entry of tyrosine into the brain. This process is an energy-dependent one in which tyrosine competes with large neutral amino acids as a substrate for a transporter. Because brain levels of tyrosine are high enough to saturate TH, under basal conditions synthesis of catecholamines is not increased by administration of tyrosine. An exception to this occurs in catecholamine neurons that have a high basal firing rate, such as dopamine neurons that innervate the prefrontal cortex, in which tyrosine can regulate catecholamine synthesis, particularly under pathological conditions (Tam et al., 1990).

Because TH is normally saturated by tyrosine, TH is the rate-limiting step for catecholamine synthesis under basal conditions. However, under conditions of neuronal activation, the enzyme responsible for norepinephrine synthesis, DBH, becomes rate limiting (Scatton et al., 1984), and thus under certain conditions tyrosine availability may regulate catecholamine synthesis.

Tyrosine hydroxylase is a mixed-function oxidase that has moderate substrate specificity, hydroxylating phenylalanine as well as tyrosine. TH function requires a biopterin cofactor and iron (Fe^{2+}). Tetrahydrobiopterin (BH4) is an essential cofactor for TH as well as several other enzymes (Thorny et al., 2000). Because the levels of the reduced BH4 cofactor are not saturated under basal conditions, endogenous BH4 levels are important in regulating TH activity. The rate-limiting step in the synthesis of the BH4 cofactor is GTP cyclohydrolase (Ichinose et al., 1994). Thus, the activity of this enzyme is critical in regulating tyrosine hydroxylase and other pteridine-dependent enzymes. BH4 is also of clinical significance: a mutation in the gene encoding GTP cyclohydrolase causes a movement disorder called dopa-responsive dystonia.

L-AROMATIC AMINO ACID DECARBOXYLASE

The L-DOPA formed by tyrosine hydroxylation is quickly decarboxylated by L-aromatic amino acid carboxylase (also known in the brain as "dopa decarboxylase") to the neurotransmitter dopamine. AADC has low substrate specificity and decarboxylates tryptophan as well as tyrosine. Because AADC is present in both catecholaminergic and serotonergic neurons, it

plays an important role in the synthesis of both types of transmitters. In dopaminergic neurons, AADC is the final enzyme of the synthetic pathway.

Dopamine does not cross the blood–brain barrier. In contrast, its precursor, L-DOPA, readily enters the brain. Accordingly, L-DOPA has achieved fame as a treatment for Parkinson's disease, which is due to loss of dopamine in the striatum. L-DOPA treatment of Parkinson's disease patients increases brain dopamine concentrations and thus provides symptomatic relief. Although L-DOPA readily enters the brain, there are decarboxylating enzymes in the liver and capillary endothelial cells that readily degrade the dopamine precursor, preventing appreciable amounts of L-DOPA from reaching the brain. L-DOPA is therefore administered in combination with a peripheral decarboxylase inhibitor that does *not* readily enter the brain. The administration of this peripheral decarboxylase inhibitor protects L-DOPA from being metabolized before it enters the brain, and thereby sharply increases central dopamine concentrations.

The AADC gene in mammalian organisms has promotor sequences that lead to one AADC transcript being expressed in the brain and another in peripheral tissues. AADC mRNA is expressed in all catecholamine- and indoleamine-containing neurons in the CNS.

AADC requires pyridoxal 5-phosphate as a cofactor. The regulation of AADC has not been as intensively studied as that of TH, but data suggest that AADC is regulated primarily through induction of new protein rather than changes in activity.

DOPAMINE β-HYDROXYLASE

Noradrenergic and adrenergic neurons contain the enzyme dopamine β-hydroxylase (DBH), a mixed-function oxidase that converts dopamine into norepinephrine. In noradrenergic neurons, this is the final step in catecholamine synthesis. Humans appear to possess two different DBH mRNAs that are generated from a single gene.

DBH is a copper-containing glycoprotein that requires ascorbic acid as the electron source during the hydroxylation of dopamine. Dicarboxylic acids such as fusaric acid are not absolute requirements but stimulate the enzymatic conversion of dopamine into norepinephrine. DBH does not have a high degree of substrate specificity and *in vitro* oxidizes almost any phenylethylamine to its corresponding phenylethanolamine. For example, in addition to forming norepinephrine from dopamine, DBH converts tyramine into octopamine and α-methyldopamine into α-methylnorepinephrine. Interestingly, many of the resultant structurally similar metabolites can replace norepinephrine at the noradrenergic nerve ending, acting as false neurotransmitters. More recently receptors for the trace amines such as octopamine have been cloned and characterized (Zucchi et al., 2006). These receptors are expressed in both brain and gut, and may in part be responsible for the side effects of certain antidepressant medications and the therapeutic effects of other drugs, and have recently been suggested to be critical for the effects of methamphetamine.

The regulation of DBH is less completely understood than that of TH. It appears that under conditions that increase the activity of noradrenergic neurons not only does TH activity increase, but so does that of DBH, which actually becomes rate limiting at high firing rates of noradrenergic neurons. Because DBH is not found in the cytosol, but is localized to the vesicle, when noradrenergic neurons are activated DBH becomes saturated and more dopamine accumulates in the vesicle. Depolarization can thereby cause dopamine as well as norepinephrine release when DBH is saturated—i.e., the noradrenergic neuron can be a Trojan horse for dopamine.

PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE

Phenylethanolamine *N*-methyltransferase (PNMT) is abundant in the adrenal medulla, where it methylates norepinephrine to form epinephrine, the major adrenal catecholamine. A single PNMT gene with three exons has been cloned. The transcript is present in the adrenal medulla and in the brainstem, where epinephrine is synthesized in two nuclei.

PNMT requires *S*-adenosylmethionine as the donor for methylation of the amine nitrogen of norepinephrine. The enzyme has modest substrate specificity and will transfer methyl groups to the nitrogen atom on a variety of beta-hydroxylated amines. However, adrenal and brain PNMT are distinct from nonspecific *N*-methyltransferases found in the lung, which can methylate indoleamines such as serotonin.

The regulation of PNMT activity in the brain has not been extensively studied. In the adrenal gland, glucocorticoids regulate activity of the enzyme, and activity is increased in response to nerve growth factor.

Storage of Catecholamines and their Enzymes

VESICULAR STORAGE

It has been known since the 1960s that much of the norepinephrine in sympathetic nerve endings and in adrenal chromaffin cells is present in specialized subcellular particles termed *granules*. Similarly, most of the norepinephrine and other catecholamines in the brain are stored in vesicles.

Vesicular storage of transmitters serves as a depot from which the transmitter can be released by appropriate physiological stimuli. Catecholamines are stored

in small vesicles located near the synapse, where they are ready for fusion with the cellular membrane and subsequent exocytosis. In addition to being the substrate of release, the accumulation of catecholamines into the highly acidic vesicle prevents oxidation of the amine and offers protection from metabolic inactivation by intraneuronal enzymes or attack by toxins that have gained entry to the neuron. Thus, the vesicle is an environment in which catecholamines can be stored and protected from degradation.

The norepinephrine-synthesizing enzyme DBH differs from the other catecholamine-synthesizing enzymes by being present in the vesicles rather than the cytosol. This means that only after dopamine is accumulated into the vesicles by the vesicular monoamine transporter (VMAT) is dopamine metabolized to norepinephrine. As noted earlier, because DBH is a vesicular enzyme and is the rate-limiting step in norepinephrine synthesis when noradrenergic neurons are strongly activated, noradrenergic axons can also release dopamine.

VESICULAR MONOAMINE TRANSPORTERS

The ability of vesicles to take up catecholamines and other amines depends on the presence of the vesicular monoamine transporter (VMAT; Weihe and Eiden, 2000; Blakely and Edwards, 2012). The VMAT is distinct from the neuronal membrane transporter (which is discussed in detail below) both in substrate affinity and localization. Two vesicular monoamine transporter genes have been cloned. One is found in the adrenal cells that synthesize and release monoamines. The other is present in catecholamine and serotonin neurons. VMAT2, the isoform found in the brain, shows modest substrate specificity and transports catecholamines and serotonin, as well as histamine, into vesicles. VMATs are Mg^{2+}-dependent and inhibited by reserpine, a drug that disrupts vesicular storage of monoamines (Blakely and Edwards, 2012).

Reserpine has been used in India for centuries as a folk medicine to treat hypertension and psychoses. The discovery that reserpine depletes vesicular stores of monoamines was critical to the understanding of the mechanism through which reserpine alleviates psychotic symptoms, and shed light on how certain toxins can cause a Parkinson's disease-like syndrome. The use of reserpine in the treatment of hypertension and psychoses was reported in international journals in the early 1930s, but the therapeutic actions of reserpine were not widely appreciated in Western medicine until a generation later. At that time, Bernard Brodie and coworkers discovered that reserpine depleted brain stores of serotonin. Contemporaneously, it became known that the hallucinogen lysergic acid diethylamide (LSD) is structurally related to the neurotransmitter serotonin. This observation led to the proposal that the antipsychotic actions of reserpine were due to its ability to deplete brain stores of serotonin. However, it was soon realized that reserpine depletes both serotonin and catecholamines in the brain, and thus the antipsychotic effects of reserpine might be due to either serotonin or catecholamine depletion (or both).

To determine if serotonin or catecholamine transmitters were more important, in the early 1950s Arvid Carlsson and colleagues (see Carlsson, 1972) administered catecholamine and serotonin precursors to reserpine-treated rats to replenish monoamine levels, and then examined locomotor activity, which is decreased by reserpine treatment. Motor function was restored by administration of the dopamine precursor L-DOPA but not by the serotonin precursor 5-hydroxytryptophan.

At that time dopamine was considered to be only a precursor to norepinephrine, and an independent role for dopamine was not recognized. Carlsson found that despite the ability of L-DOPA to restore locomotor activity in reserpine-treated rats, L-DOPA did not restore brain concentrations of norepinephrine and epinephrine in these animals. However, Carlsson demonstrated that L-DOPA treatment of reserpinized animals increased brain dopamine levels, suggesting that dopamine was a transmitter in its own right. Moreover, the ability of L-DOPA to replenish dopamine was interpreted to suggest that the primary mechanism through which reserpine exerts antipsychotic effects is by disrupting dopamine transmission. These and subsequent studies led to the hypothesis that dysfunction of central dopamine systems underlies schizophrenia. This hypothesis soon became the dominant view guiding schizophrenia research. All known antipsychotic drugs used to treat schizophrenia prevent the action of dopamine at one type of dopamine receptor. However, the newer generation of antipsychotic drugs block both certain dopamine and serotonin receptors.

Vesicles store transmitter that can be poised for release, with different populations of vesicles being near to the synaptic membrane and another population of vesicles found at a greater distance from the membrane. Some of the latter vesicles may not normally release transmitter. Studies examining the effects of virally transfecting and overexpressing VMAT2 in small synaptic vesicles in cultured midbrain dopamine neurons observed both increases in the amount of transmitter released from a vesicle as well as increased frequency of transmitter release events (Pothos et al., 2000). These data are consistent with the recruitment of vesicles that do not normally release dopamine.

The cloning of the VMATs revealed a significant homology of these vesicular transporters to a group of bacterial antibiotic drug-resistance transporters, suggesting a role for VMAT in detoxification. This indeed

is the case. VMAT allows the vesicles to sequester toxins and thereby reduce toxicity. This is best exemplified by studies in mice that lack the VMAT2 gene (VMAT2 knockouts) and heterozygous mice bearing one copy of VMAT2 (see Edwards, 1993). Heterozygous VMAT2 mice were more susceptible to the parkinsonian toxin MPTP, which kills dopamine neurons in the midbrain. The interpretation of this observation was that the decrease in VMAT in the heterozygous mice led to less uptake of the toxin by the vesicle. In turn, this resulted in more of the toxin in the cytosol of the dopamine neuron, ultimately disrupting mitochondrial function and causing dopamine cell death.

Release of Catecholamines

The release of catecholamines from vesicles typically occurs by the same Ca^{2+}-dependent process (exocytosis) that has been described for other transmitters (see Chapter 15 for a more extended discussion). This most often is at the synaptic cleft, where a presynaptic axon terminal is apposed to a specialized postsynaptic area. However, catecholamine axons may also release their transmitter from varicosities (swellings) along the axon.

Catecholamine release has also been observed to occur through at least two other mechanisms. First, catecholamines can be released by a reversal of the catecholamine transporters inserted into a neuron's membrane. Normally these transporters accumulate the transmitter released into the synapse to terminate the action of the transmitter. However, the transporter may paradoxically expel transmitter from the cytosol to the extracellular space. For example, administration of amphetamine will increase levels of dopamine in the synapse by reversing the normal direction of transporter action, so that the transporter extrudes dopamine from the neuron instead of accumulating it. Second, dopamine (and other catecholamines) can be released from dendrites through a process that is independent of calcium.

REGULATION OF CATECHOLAMINE SYNTHESIS AND RELEASE BY AUTORECEPTORS

The enzymes that control catecholamine synthesis can be regulated at the transcriptional level and by post-translational modifications that alter enzymatic activity. In addition, both the synthesis and release of catecholamines can be regulated by interaction of the released catecholamine transmitter with specific autoreceptors that are located on the nerve terminal. Autoreceptors that respond to the released transmitter also regulate the firing rate of catecholamine neurons.

Dopamine autoreceptors are perhaps the best characterized of the catecholamine autoreceptors. Autoreceptors exist on most parts of the neuron, including the soma, the dendrites, and nerve terminals. They can be defined functionally by the events that they regulate. Thus, there are functionally distinct synthesis-, release-, and impulse-modulating dopamine autoreceptors (see Iversen et al., 2009). All three types of dopamine autoreceptors belong to the D2 family of dopamine receptors, which includes three different receptors (D_2, D_3, and D_4). Although it is clear that there are D_2 autoreceptors, the presence of D_3 autoreceptors has been a topic of debate. However, even assuming that there are both D_3 and D_2 autoreceptors, in at least two cases the same receptor must play functionally different autoreceptor roles by effecting different intracellular transduction cascades.

Release-modulating autoreceptors appear to be a common regulatory feature on catecholamine neurons and other neurons that use classic transmitters. Dopamine that is released from the neuron interacts with the autoreceptor and dampens further release of the transmitter. This can be thought of as a homeostatic feedback mechanism.

Autoreceptors also directly regulate the synthesis of dopamine. Again, dopamine that is released from the neuron acts homeostatically at synthesis-modulating autoreceptors to control synthesis: agonists that mimic the actions of dopamine decrease synthesis, whereas dopamine antagonists that block the receptor increase dopamine synthesis. Somewhat surprisingly, synthesis-modulating autoreceptors are not found on all dopamine neurons. Certain midbrain dopamine neurons that send their axons to the prefrontal cortex lack synthesis-modulating autoreceptors, as do neurons in the hypothalamus that release dopamine into the pituitary stalk. Because release-modulating autoreceptors may indirectly regulate synthesis, the presence of synthesis-modulating autoreceptors may not be necessary in certain neurons.

The third type of autoreceptor modulates the firing rate of neurons, and is referred to as an impulse-modulating autoreceptor; it is localized to the dendritic and cell body regions of the neuron. As noted earlier, because the release of dopamine can alter dopamine synthesis, impulse-modulating autoreceptors can also be expected to change dopamine synthesis. Thus, all three types of dopamine autoreceptors may regulate synthesis. This interdependence of regulatory processes over catecholamine neurons appears to be characteristic of monoamine neurons.

We have discussed dopamine autoreceptors at some length. However, there are also autoreceptors on norepinephrine neurons. Although it is clear that norepinephrine autoreceptors regulate release of norepinephrine, the direct regulation of norepinephrine synthesis by synthesis-modulating autoreceptors is not well established. We do know, however, that there are two

norepinephrine autoreceptors. One of these, an α_2 receptor, inhibits norepinephrine release, but a second norepinephrine receptor actually facilitates release.

Inactivation of Catecholamine Neurotransmission

Continuous stimulation of a postsynaptic neuron by a transmitter is not desirable for two reasons. The first is that the normal activity of neurons is not continuous, but fluctuates, with neurons sometimes firing and sometimes being quiet. Accordingly, continuous stimulation does not convey dynamic information to the postsynaptic neurons from the presynaptic neuron. This can be most easily understood with respect to receptors that form ion channels, in which continued action of a neurotransmitter would lead to inappropriate ion concentrations across the membrane and thus disrupt neurotransmission. A second reason that continuous stimulation is not desirable is that such constant stimulation is often pathological, resulting in damage to and toxic loss of postsynaptic neurons.

There are several different mechanisms for terminating the actions of a catecholamine. Perhaps the simplest is for the transmitter to diffuse away from the synapse, drifting to sites where receptors are sparse or not present, or being diluted to concentrations so low that signaling to receptors does not occur. The major mode on terminating the actions of classical transmitters is by uptake of the released transmitter, such as dopamine or norepinephrine, by neuronal membrane-associated transporter proteins. In turn, the transmitter that has been taken up into the presynaptic terminal may be taken up a second time into storage vesicles by VMAT, from where they can be reused. A third mechanism of inactivating the actions of transmitters is by the actions of specific enzymes.

ENZYMATIC INACTIVATION OF CATECHOLAMINES

Enzymatic inactivation was originally thought to be the major means by which catecholamines are inactivated in the CNS, but it now appears that the metabolism of catecholamines to compounds that lack receptor activity play a secondary role. Nonetheless, enzymatic inactivation remains important for two reasons. The first is that certain drug treatments for neuropsychiatric disorders are based on manipulation of the key enzymes that degrade catecholamines (see Box 7.2). Second, enzymatic inactivation is the major mode of terminating the action of circulating catecholamines in the bloodstream, such as epinephrine released from the adrenal.

Two major enzymes take part in catecholamine catabolism: monamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT). Either enzyme can act independently or on the products of the other, leading to catecholamine metabolites that are deaminated, *O*-methylated, or both. COMT is a relatively nonspecific enzyme that transfers methyl groups *S*-adenosylmethionine to the *m*-hydroxyl group of catechols. COMT is found in both peripheral tissues and central nervous system and is the major means of inactivating circulating catecholamines that are released from the adrenal gland.

Two forms of MAO have been identified on the basis of substrate specificities and selective enzyme inhibitors. MAO_A has a high affinity for norepinephrine and serotonin and is selectively inhibited by clorgyline. In contrast, MAO_B has a higher affinity for *o*-phenylethylamines and is selectively inhibited by the monoamine oxidase inhibitor (MAOI) deprenyl. Both MAO_A and MAO_B are associated with the outer mitochondrial membrane. The MAOs oxidatively deaminate catecholamines and their *O*-methylated derivatives to form inactive and unstable aldehyde derivatives. These aldehydes can be further catabolized by dehydrogenases and reductases to form corresponding acids and alcohols.

NEURONAL CATECHOLAMINE TRANSPORTERS

The reuptake of a released neurotransmitter is the major mode of terminating the synaptic actions of classical transmitters in the brain. In addition, the accumulation of the transmitter by membrane transporters also allows intracellular enzymes that degrade the transmitter to act, thus bolstering the actions of extracellular enzymes.

Neuronal reuptake of catecholamines, and indeed of all transmitters for which an active reuptake process has been identified, has several characteristics (Kristensen et al., 2011). The reuptake process is energy-dependent and saturable, and depends on Na^+ co-transport as well as extracellular Cl^-. Because reuptake depends on coupling to the Na^+ gradient across the neuronal membrane, toxins that inhibit Na^+, K^+-ATPase inhibit reuptake. However, under certain conditions, the coupling of transporter function to Na^+ flow may lead to local changes in the membrane Na^+ gradient and thereby paradoxically extrude ("release") the transmitter, as noted earlier.

The membrane catecholamine transporters are not Mg^{2+}-dependent and are not inhibited by reserpine. These characteristics distinguish the neuronal membrane transporters from the vesicular monoamine transporters. The catecholamine transporters are localized to neurons. Although there appears to be an uptake process that accumulates catecholamines in glial cells, the process is not a high-affinity one and the functional significance of the glial uptake of catecholamines remains unclear.

Two distinct mammalian catecholamine transporter proteins, the dopamine transporter (DAT) and norepinephrine transporter (NET), have been cloned and characterized pharmacologically. The two transporters

BOX 7.2

MAO AND COMT INHIBITORS IN THE TREATMENT OF NEUROPSYCHIATRIC DISORDERS

Depression

One hypothesis concerning the pathophysiology of depression posits a decrease in noradrenergic tone in the brain. MAO_A inhibitors such as tranylcypromine effectively increase norepinephrine levels (as well as dopamine and serotonin concentrations) and were once a mainstay of the treatment of depression. More recently the use of MAO inhibitors in depression has been largely supplanted by the introduction of drugs that increase extracellular norepinephrine levels by blocking reuptake of the transmitter (tricyclic antidepressants) and other agents to increase serotonin or dopamine levels by blocking SERT or DAT [*fluoxetine* (Prozac) and *bupropion* (Welbutrin), respectively].

The treatment of depression with MAO_A inhibitors, although still useful for certain patients who do not respond to other antidepressants, is marred by a large number of side effects. Among the most serious side effects is hypertensive crisis. Patients who are treated with MAO_A inhibitors and eat foods that contain large amounts of tyramine (such as aged cheeses) cannot metabolize the ingested tyramine. Because tyramine releases catecholamines from nerve endings and relatively small amounts of tyramine increase blood pressure significantly, a marked increase in blood pressure and a high risk for stroke may develop.

Parkinson's Disease

Deprenyl, a specific inhibitor of MAO_B, has been used as an initial treatment for Parkinson's disease (PD). The use of deprenyl in the treatment of PD and the rationale for its use were based on data from studies of a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The systemic administration of MPTP to humans and other primates results in a relatively specific degeneration of midbrain dopamine neurons and a marked parkinsonian syndrome. MPTP toxicity was first noted in a group of opiate addicts. In an attempt to synthesize a designer drug that was a meperidine (Demerol) derivative, the structurally related MPTP was inadvertently produced. Addicts who injected this drug developed a severe parkinsonian syndrome. Subsequent animal studies showed that MPTP itself is not toxic, but that the active metabolite of MPTP, MPP^+, is highly toxic. The formation of MPP^+ from MPTP is catalyzed by MAO_B, and animal studies soon revealed that treatment with MAO inhibition by deprenyl could prevent MPTP toxicity.

The realization that MPTP administration rather faithfully reproduces the cardinal signs and symptoms of Parkinson disease reawakened interest in environmental toxins as a cause of PD. This interest led to the idea that treatment with deprenyl might be useful in slowing the progression of PD, putatively caused by an environmental toxin. The first evaluation of clinical trials of newly diagnosed PD patients indicated that daily administration of deprenyl increased the amount of time required before patients needed other drugs for the relief of symptoms; however, when the MAO_B inhibitor was withdrawn, patients treated with deprenyl regressed and appeared no better than untreated subjects. It now appears that the actions of deprenyl are due at least in part to the symptomatic improvement that results from increasing dopamine levels by inhibiting degradation of the transmitter rather than to slowing of the progression of PD. Moreover, low levels of methamphetamine are generated by the metabolism of deprenyl; because methamphetamine potently releases dopamine from nerve terminals, this would result in a symptomatic improvement.

Catechol *O*-methyltransferase, which together with MAO is responsible for the enzymatic degradation of catecholamines, is also a target in the treatment of PD. Two inhibitors of COMT, one that is active peripherally and another that is active both peripherally and centrally, are used to prevent the enzymatic inactivation of L-DOPA. By inhibiting COMT these drugs prolong the therapeutic action of L-DOPA and smooth out the characteristic fluctuations in therapeutic response to dopa.

Schizophrenia

Changes in catecholamine function have been a subject of intense scrutiny in schizophrenia, with much attention focusing on a loss of dopaminergic tone in the prefrontal cortex. One allelic variant of the COMT gene substitutes a single methionine for a valine, and results in a much reduced activity of the enzyme. Recent data have examined COMT alleles for full versus low COMT activity in normal subjects and schizophrenics. Individuals bearing the allele that confers lower COMT activity have improved performance on cognitive tasks that involve the prefrontal cortex; the performance of schizophrenic persons on these tasks is impaired. There is a significant increase in transmission of the COMT allele conferring high enzyme activity to schizophrenic subjects, and it has been proposed that COMT activity may confer increased risk of developing schizophrenia.

share significant sequence homology and are members of a class of transporter proteins (including serotonin and amino acid transmitter transporters) with 12 transmembrane domains. Neither transporter is very specific, with each accumulating both dopamine and norepinephrine. In fact, NET has a higher affinity for dopamine than for norepinephrine. A specific transporter for epinephrine-containing neurons has been identified in the frog but not in mammalian species.

The regional distribution of DAT and NET largely follows the expected localization to dopamine and norepinephrine neurons, respectively. However, DAT is not expressed in all dopamine cells. The tuberoinfundibular dopamine neurons, which are hypothalamic cells that release dopamine into the pituitary portal blood system, lack demonstrable DAT mRNA and protein. Because dopamine that is released from tuberoinfundibular neurons is carried away in the vasculature, the existence of a transporter protein on these dopamine neurons would be superfluous. However, DAT is also not expressed or expressed at very low levels on some forebrain dopamine axons, particularly those that innervate the prefrontal cortex.

Although studies defining the cellular localization of the two catecholamine transporters did not uncover many surprises, studies of the subcellular localization of the transporters did yield an unexpected finding. The use of antibodies generated against DAT revealed that the transporter is typically expressed *outside* of the synapse, in the extra- or peri-synaptic region of the axon terminal. This finding suggests that the transporter may be used to inactivate (accumulate) dopamine that has escaped from the synaptic cleft and, thus, that diffusion is the initial process by which dopamine is removed from the synapse. This observation is consistent with recent studies indicating that perisynaptic concentrations of dopamine can reach 1.0 mM or more, a value roughly comparable to the affinity of the cloned DAT for DA. Receptors for dopamine and many other transmitters are also found extrasynaptically (indeed, along the length of axons); this observation, coupled with the presence of catecholamine transporters in extrasynaptic regions, suggests that extrasynaptic (also referred to as "paracrine" or volume) neurotransmission (see Agnati et al., 2010) is of considerable importance for catecholaminergic signaling.

How neurotransmitter transporter proteins are regulated has been a major area of research in recent years. Chronic treatment with inhibitors of catecholamine reuptake alters the number of transporter sites, but the precise regulatory mechanisms remain unclear. There are phosphorylation sites on DAT and NET, and thus changes in neurotransmitter release may alter function through interaction with autoreceptors and subsequent activation of serine–threonine kinases. The mechanisms that regulate expression of DAT and other monoamine transporters to the synaptic membrane is a topic of considerable interest to those interested in the therapeutic and adverse effects of psychostimulant drugs, most of which block DAT (Schmitt and Reith, 2010).

The DAT knockout mouse has been particularly useful in clarifying the role of DAT in dopaminergic neurons and, by extension, the role of other monoamine transmitter transporters. The constitutive loss of the DA transporter results in a large number of changes in dopaminergic function, ranging from an increase in extracellular DA levels and delayed clearance of released DA to a decrease in tissue concentrations of DA in the face of increased DA synthesis (Gainetdinov, 2008). In addition, there is a loss of autoreceptor-mediated tone, including deficits in release-, synthesis-, and impulse-modulating autoreceptor function (Jones et al., 1999). The alterations in DA knockout mice have been suggested to reflect a disinhibition of tyrosine hydroxylase due to a lack of intraneuronal DA to provide feedback inhibition of the enzyme and a markedly increased rate of DA turnover, such that synthesis and release of the neurons are accelerated. Interestingly, many of these biochemical deficits seen in the DAT knockout mice resemble the normal "physiological" functions of dopamine neurons that innervate the prefrontal cortex (see Roth and Elsworth, 1995).

Psychostimulants, such as cocaine and amphetamine, exert their physiological and behavioral effects by increasing extracellular levels of catecholamines; cocaine also increases extracellular serotonin levels. The mechanism through which psychostimulants increase catecholamine levels is by blocking DAT and NET. In particular, cocaine shows a very high affinity for the dopamine transporter; amphetamine is a less potent inhibitor of reuptake but also induces release of catecholamines from the cytoplasm. Paradoxically, studies in mice with targeted null mutations of the DAT have found that these mice self-administer cocaine. This may have been due to the involvement of transmitters other than dopamine such as serotonin, in the actions of cocaine, particularly serotonin.

The NET is also a target of clinically important drugs. The tricyclic antidepressants potently inhibit norepinephrine reuptake, with significantly weaker effects on the dopamine and serotonin transporters. In addition, there are newer antidepressants that inhibit NET rather selectively. Mice lacking NET behave like antidepressant-treated wildtype mice; interestingly, NET knockout mice are hyper-responsive to psychostimulant-elicited locomotor stimulation (Xu et al., 2000).

Serotonin

Scientists were aware in the nineteenth century that a substance in the blood induces powerful contractions of smooth muscle organs. However, more than a century passed until Page and his collaborators in 1948 succeeded in isolating the compound (which they proposed to be a possible cause of high blood pressure) from platelets. At the same time, Italian researchers characterized a substance present in high concentrations in intestinal mucosa that caused contractions of gastrointestinal smooth muscle. The material isolated from blood platelets was given the name *serotonin*, and the substance isolated from the intestinal tract was called *enteramine*. Subsequently, both materials were purified and shown to be identical: 5-hydroxytryptamine (5-HT), which is usually referred to as serotonin. The laboratory synthesis of serotonin soon allowed direct comparison of serotonin with the purified compound isolated from platelets, which conclusively demonstrated that serotonin possessed all the biological features of the natural substance.

Serotonin is found in neurons as well as several types of peripheral cells, including platelets, mast cells, and enterochromaffin cells. In fact, the brain accounts for only about 1% of body stores of serotonin.

Although the purification and identification of serotonin were based on studies of blood pressure regulation, the possible relation of serotonin to psychiatric disorders propelled research on the central effects of serotonin. The observation that the indole structure of serotonin was similar to that of the psychedelic agent LSD and a number of other psychotropic compounds led to theories linking abnormalities of serotonin function to various psychiatric disorders, including schizophrenia and depression. Fifty years later that role of serotonergic signaling in psychiatric disorders remains a major focus of research.

The basic principles of the biochemical neuropharmacology of synaptic transmission as revealed by studies of catecholamines also apply to neurons that use serotonin as a transmitter. We will therefore outline the nature of chemical transmission in serotonergic neurons, focusing on differences that are unique to serotonergic neurons.

Synthesis of Serotonin

The outline of serotonin biosynthesis is very similar to that of catecholamine transmitters: an amino acid gains entry to the central nervous system and is metabolized in specific neurons via a series of enzymatic steps to yield serotonin.

Once tryptophan enters the serotonergic neuron it is hydroxylated by tryptophan hydroxylase, the rate-limiting step in serotonin synthesis (see Fig. 7.5). The resultant serotonin precursor, 5-hydroxytryptophan (5-HTP), is subsequently decarboxylated by aromatic amino acid decarboxylase. Thus, only two critical enzymes (tryptophan hydroxylase and AADC) are involved in the synthesis of serotonin (Iversen et al., 2009; Hensler, 2012).

TRYPTOPHAN HYDROXYLASE

The rate-limiting step in serotonin synthesis is tryptophan hydroxylase. However, in contrast to the catecholamines, the availability of the precursor amino acid tryptophan plays an important role in regulating the synthesis of serotonin.

Because serotonin cannot cross the blood–brain barrier, brain cells must synthesize the amine. Tryptophan is present in high concentrations in the plasma, and changes in dietary sources of tryptophan can substantially alter brain levels of serotonin. An active uptake process facilitates entry of tryptophan into the brain. However, other large neutral aromatic amino acids compete for this transport process. Accordingly, brain levels of tryptophan are determined by plasma concentrations of competing neutral amino acids as well as the plasma levels of tryptophan itself.

There are two genes that encode for two different tryptophan hydroxylase proteins, TPH1 and TPH2. The latter is the major form found in brain. In contrast, TPH1 is mainly present in peripheral tissues but is found in lower levels in the brain. In addition, there are differences in the two forms of tryptophan hydroxylase. For example, expression of TPH2 but not TPH1 is regulated by glucocorticoids (Clark et al., 2008). There are a number of polymorphisms in the TPH2 gene that are associated with psychiatric disorders, including depression and bipolar disorder.

In serotonergic neurons, the synthesis of serotonin from tryptophan is increased in a frequency-dependent manner in response to electrical stimulation of serotonergic cells. Tryptophan hydroxylase requires both molecular oxygen and a reduced pterin cofactor.

L-Aromatic Amino Acid Decarboxylase

Aromatic amino acid decarboxylase metabolizes the serotonin precursor 5-HTP to the transmitter serotonin. This is the same enzyme found in catecholaminergic neurons. Just as in catecholamine cells, in which the precursor L-DOPA is almost instantaneously converted into dopamine by AADC, the precursor 5-HTP is so rapidly decarboxylated in serotonergic cells that central concentrations of 5-HTP under basal conditions are negligible. Because AADC is not saturated with 5-HTP under physiological conditions, it is possible to increase the content of serotonin in brain in two ways: by administering

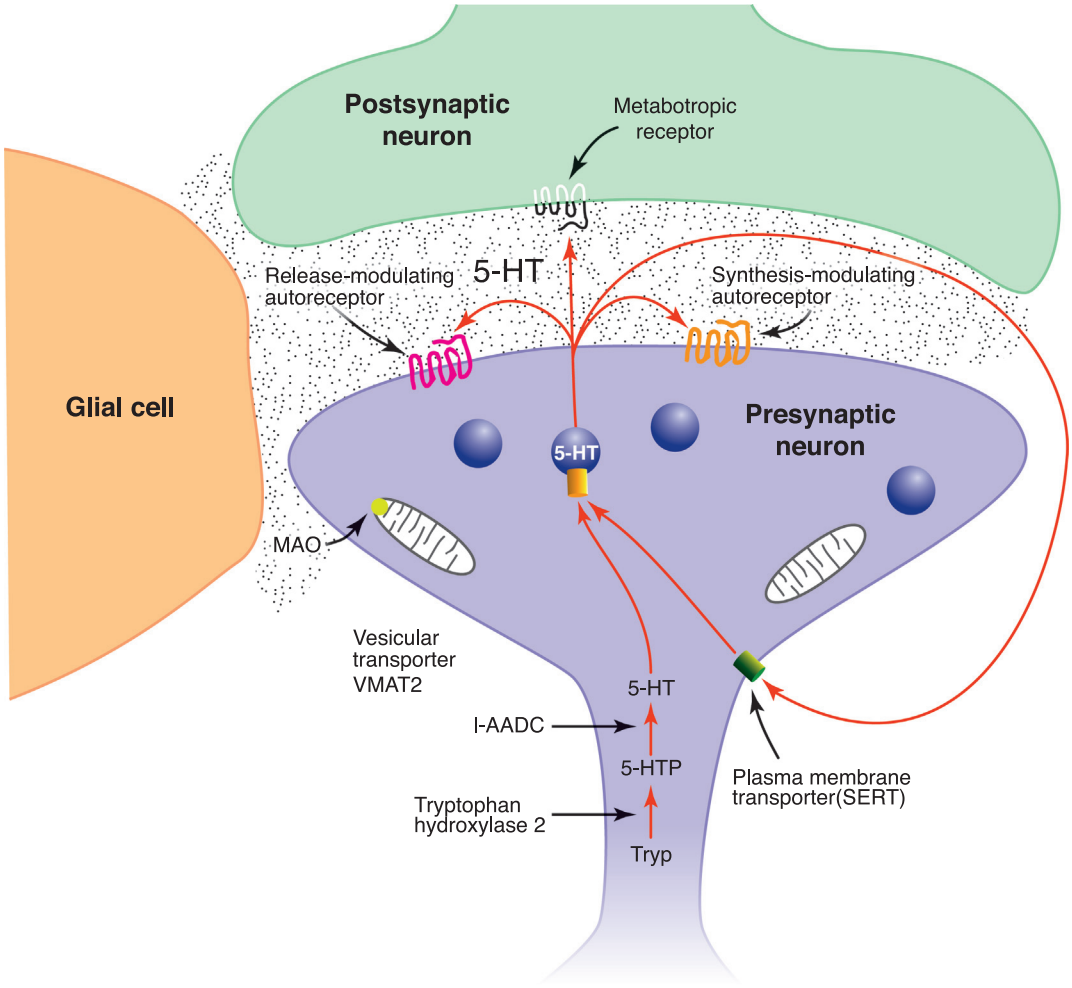

FIGURE 7.5 **Depiction of a serotonergic neuron.** Tryptophan (Tryp) in the neuron is sequentially metabolized by tryptophan hydroxylase and L-AADC to yield serotonin (5-HT). The serotonin is accumulated by the vesicular monoamine transporter. When released, the serotonin can interact with both postsynaptic receptors and presynaptic autoreceptors. 5-HT is taken up by the high-affinity serotonin transporter (SERT), and once inside the neuron it can be reaccumulated by vesicular transporter or metabolically inactivated by MAO and other enzymes.

5-HTP (just as administration of L-DOPA increases dopamine) and by increasing dietary intake of tryptophan.

ALTERNATIVE TRYPTOPHAN METABOLIC PATHWAYS

Although serotonin is generally thought of as the final product of tryptophan synthesis, in one area of the brain serotonin can be further metabolized. In the pineal gland, serotonin is metabolized to the hormone melatonin (5-methoxy-*N*-acetyltryptamine), which plays important roles in both sleep and sexual behavior. The production of melatonin from serotonin requires two enzymatic steps: *N*-acetylation of serotonin to form *N*-acetylserotonin, followed by its rapid methylation by 5-hydroxyindole-*O*-methyltransferase to melatonin.

In peripheral tissues, most tryptophan is not metabolized to serotonin but is instead metabolized in a separate series of reactions called the *kynurenine pathway*. The same kynurenine shunt is present in the brain, and leads to the accumulation of neuroactive substances that may be of clinical importance. The two major tryptophan metabolites that are generated by the kynurenine shunt are quinolinic acid and kynurenic acid. Quinolinic acid is a potent agonist at *N*-methyl-D-aspartate (NMDA) receptors and causes neurotoxicity and convulsions. Kynurenine is an antagonist at the same NMDA receptors. It appears that the ratio of these tryptophan metabolites may be of importance in stroke, in which cell death is in part mediated by NMDA receptors, and recent data suggest that the products of the kynurenine shunt may be important in schizophrenia (Schwarcz, 2004; Wonodi and Schwarcz, 2010).

Storage and Release of Serotonin

VESICULAR ACCUMULATION AND STORAGE OF SEROTONIN

Neuronal serotonin is stored primarily in vesicles and is released by an exocytotic mechanism. VMAT2, the same transporter that accumulates dopamine in catecholamine neurons, also transports serotonin into vesicles in serotonergic neurons. Since catecholamines and serotonin share a common vesicular transporter, it is not surprising that reserpine, which depletes vesicular stores of catecholamines, also depletes serotonin from serotonin neurons.

REGULATION OF SEROTONIN SYNTHESIS AND RELEASE

Although there are many similarities between catecholamine and serotonergic neurons, there are also some important regulatory differences. Because serotonin neurons are sensitive to changes in plasma levels of the precursor amino acid tryptophan, and thus dietary changes can regulate serotonin levels in the brain. In addition, it appears that increases in intracellular serotonin levels do not significantly alter serotonin synthesis *in vivo*; In contrast, in catecholaminergic neurons transmitter synthesis is influenced by end-product inhibition.

Short-term requirements for increases in serotonin synthesis appear to be accomplished by phosphorylation of tryptophan hydroxylase, which changes its kinetic properties without necessitating the synthesis of additional enzyme. In contrast, long-term increases in serotonin transmission are accomplished by the synthesis of (new) tryptophan hydroxylase protein.

SEROTONIN AUTORECEPTORS REGULATE SEROTONIN RELEASE AND SYNTHESIS

As is the case in catecholamine neurons, there are functionally dissociable somatodendritic and terminal autoreceptors on serotonin neurons. The impulse-modulating autoreceptor located on somatodendritic areas of serotonin neurons is a 5-HT_{1A} receptor; this receptor is also found as a heteroceptor on nonserotonergic neurons. The 5-HT_{1D} receptor (the human ortholog of the rodent 5-HT_{1B} receptor) is the release-modulating autoreceptor on the axon terminals of serotonergic neurons. Available drugs do not completely discriminate between the 5-HT_{1D} and 5-HT_{1B} receptors, hampering research and leading to difficulties in generalizing data from the rat or mouse to other species, including humans.

Inactivation of Released Serotonin

As in the case of the catecholamine neurotransmitters, transporter-mediated reuptake is the major means of terminating the action of serotonin. Serotonin is accumulated into neurons from which it was released by a specific serotonin transporter. In addition, the same enzymes that are involved in enzymatic inactivation of catecholamines are also found in serotonin neurons, where they can metabolize serotonin to an inactive compound.

REUPTAKE

Serotonin is inactivated primarily by the reuptake of the transmitter by a plasma membrane serotonin transporter. The serotonin transporter (SERT) belongs to the same family of 12-transmembrane-domain transporters as the catecholamine transporters. The SERT shares with other transporter family members an absolute requirement for Na^+ co-transport.

The SERT is an important clinical target for therapeutic drugs. Just as the norepinephrine transporter is the target of tricyclic antidepressant drugs, SERT is the target of a different class of antidepressants, the *selective serotonin reuptake inhibitors* (SSRIs); these drugs have become the initial choice among medications used in the treatment of major depression. Mixed SERT–NET inhibitors are also used to treat depression. The ability of antidepressant drugs to alter monoamine inactivation by blocking serotonin and norepinephrine transporters or by disrupting enzymatic inactivation of the monoamines has led to the dominant theories of the pathogenesis of depression, which suggests a critical modulatory role for both norepinephrine and serotonin (Heninger et al., 1996). Some recent data have suggested that dopamine transporter antagonists may also have some antidepressant actions.

As noted previously, cocaine and other psychostimulants block the dopamine transporter and thereby sharply increase extracellular dopamine levels. However, cocaine also increases extracellular serotonin levels by blocking SERT. Interestingly, even though the dopamine transporter is a major target of cocaine, DAT knockout mice continue to self-administer cocaine, as do SERT knockout mice. However, in mice bearing double DAT–SERT knockouts, cocaine self-administration is reduced (Sora et al., 2000), suggesting that both transporters must be targeted for the rewarding effects of psychostimulants to be manifested.

ENZYMATIC DEGRADATION

The primary catabolic pathway for serotonin is oxidative deamination by monoamine oxidase. The resultant product, 5-hydroxyindole acid aldehyde, is further oxidized to 5-hydroxyindoleacetic acid (5-HIAA), or can be reduced to 5-hydroxytryptophol. In the brain and cerebrospinal fluid 5-HIAA is the primary metabolite of serotonin. Monoamine oxidase

inhibitors increase serotonin levels and have been used extensively as antidepressants. MAOIs are effective antidepressants but are not used as the first line of drugs to treat depression because of the potential for serious side effects.

γ-Aminobutyric Acid: The Major Inhibitory Neurotransmitter

A number of amino acids fulfill most of the criteria for neurotransmitters. The three best studied of these are GABA, the major inhibitory transmitter in brain; glutamate, which is the major excitatory transmitter in brain; and glycine, another inhibitory amino acid (Olsen and Li, 2012). The broad principles outlined in the discussion of catecholamine neurotransmitters are also applicable to the amino acid transmitters, although certain aspects of the synthesis of amino acid transmitters are less completely understood compared with the catecholamines.

A major difference between the biogenic amines transmitters and the amino acid transmitters is that the latter are derived from intermediary glucose metabolism. This dual role for the amino acid transmitters dictates that there must be mechanisms to segregate the transmitter and general metabolic pools of the amino acid transmitters. A second difference between amino acid and biogenic amine transmitters is that amino acid transmitters released from neurons are readily taken up by glial cells as well as neurons. We review GABA as a prototypic amino acid transmitter, focusing on differences between the catecholamine transmitters and GABA.

GABA was discovered in 1950 by Eugene Roberts, who subsequently found that GABA had a neurotransmitter role (see Roberts, 1986). GABA is ubiquitous in the CNS, as might be expected for a transmitter derived from the metabolism of glucose. Although the presence of GABA as a transmitter in neurons is widespread, it nonetheless is found in distinct populations of neurons. Although originally thought to be a transmitter in local circuit interneurons but not long-axoned projection neurons, it is now clear that there are many types of GABAergic projection neurons.

GABA Biosynthesis

Several aspects of the synthesis of GABA differ from that of the monoamines (see Fig. 7.6). These differences are due to precursors of GABA being part of cellular intermediary metabolism rather than dedicated solely to a neurotransmitter synthetic pool.

THE GABA SHUNT AND GABA TRANSAMINASE

GABA is ultimately derived from glucose metabolism. α-Ketoglutarate formed by the Krebs (tricarboxylic acid) cycle is transaminated to the amino acid glutamate by the enzyme GABA α-oxoglutarate transaminase (GABA-T). In those cells in which GABA is used as a transmitter, the presence of the enzyme glutamic acid decarboxylase (GAD) permits the formation of GABA from glutamate derived from α-ketoglutarate.

One unusual feature of the GABA synthetic pathway is that intraneuronal GABA is inactivated by the actions of GABA-T, which appears to be associated with mitochondria (Fig. 7.6). Thus, GABA-T is both a key synthetic enzyme and a degradative enzyme. GABA-T metabolizes GABA to succinic semialdehyde, but only if α-ketoglutarate is present to receive the amino group that is removed from GABA. This unusual GABA shunt serves to maintain supplies of GABA.

GLUTAMIC ACID DECARBOXYLASE

The critical biosynthetic enzyme for GABA is glutamic acid decarboxylase (GAD). GAD is localized exclusively in the central nervous system to neurons that use GABA as a transmitter.

There are two isoforms of GAD, which are encoded by two distinct genes (Erlander and Tobin, 1991). These two isoforms, designated GAD65 and GAD67 in accord with their molecular weights, exhibit somewhat different intracellular distributions, suggesting that the two GAD forms may be regulated in different ways (Soghomonian and Martin, 1988). GAD requires a pyridoxal phosphate cofactor for activity. GAD65 and GAD67 differ significantly in their affinity for this cofactor: GAD65 has a relatively high affinity for the cofactor, while the larger GAD isoform does not. The affinity of GAD65 for the cofactor results in the ability of GAD65 enzyme activity to be efficiently and quickly regulated. In contrast, the activity of GAD67 is mainly determined by induction of new enzyme protein rather than through post-translational mechanisms.

A major question concerning amino acid transmitters is how the transmitter pools are kept distinct from the general metabolic pools in which the amino acids serve. GAD is necessary for synthesis of the transmitter GABA, and thus GAD is a marker of GABAergic neurons. GAD is a cytosolic enzyme, but GABA-T, which converts α-ketoglutarate into the GAD substrate glutamate, is present in mitochondria. Thus, the metabolic pool is present in the mitochondria, but glutamate destined for the transmitter pool must be exported from the mitochondria to the cytosolic compartment. This export process is poorly understood.

Glutamate is not only a precursor to the formation of GABA but also is the major excitatory neurotransmitter. GAD is not found in neurons that use glutamate as a transmitter, and thus glutamatergic neurons

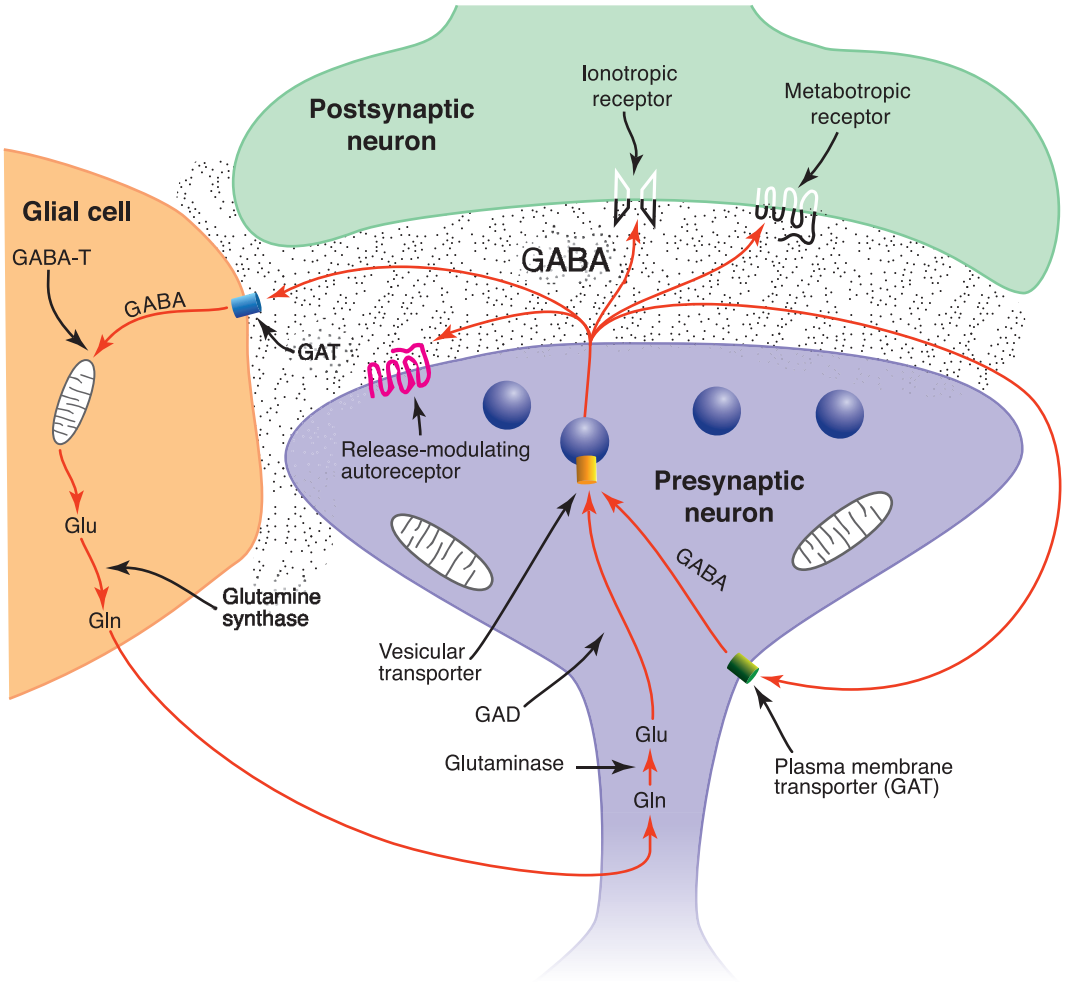

FIGURE 7.6 **Schematic depiction of the life cycle of a GABAergic neuron.** α-Ketoglutarate formed in the Krebs cycle is transaminated to glutamate (Glu) by GABA transaminase (GABA-T). The transmitter GABA is formed from the Glu by glutamic acid decarboxylase (GAD). GABA that is released into the extracellular space of the synapse is taken up by high-affinity GABA transporters (GAT) found on neurons and glia. Gln, glutamine.

do not use GABA as a transmitter. What prevents GABA neurons from using the precursor glutamate as a transmitter are vesicular transporters specific for GABA but not glutamate (Takamori et al., 2000). Because both GABA and glutamate cause very rapid changes in postsynaptic neurons, one hyperpolarizing neurons and the other depolarizing them, it is not surprising (and probably often fortunate) that the two amino acid transmitter pools are generally not colocalized. However, anatomical studies have indicated the presence of isolated populations of neurons in which GABA and glutamate are colocalized (Quaglino et al., 1999), and both the vesicular GABA transporter and the vesicular glutamate transporter VGluT3 have been reported to be colocalized in some GABA neurons (Stensrud et al., 2013). These data suggest that an inhibitory neuron also can be excitatory! The functional significance of this arrangement remains unclear.

Storage and Release of GABA

VESICULAR INHIBITORY AMINO ACID TRANSPORTER

A vesicular transporter in GABAergic cells accumulates GABA. The transporter was cloned on the basis of homology to unc-47 in the worm *Caenorhabditis elegans* (McIntire et al., 1997). The strategy of using data from the worm to direct studies in mammalian species has been very useful in identifying a variety of mammalian transmitter-related genes. The vesicular GABA transporter differs from the catecholamine vesicular

transporters VMAT1/2 by belonging to a class of proteins that have 10 rather than 12 transmembrane domains. The vesicular GABA transporter shares with the VMATs, however, a lack of substrate specificity and will transport the inhibitory transmitter glycine as well as GABA. Consistent with this pharmacology, the vesicular GABA transporter is found in glycine- as well as GABA-containing neurons. These data have led to the suggestion that the vesicular GABA transporter can be more accurately referred to as a vesicular inhibitory amino acid transporter (Weihe and Eiden, 2000). Interestingly, there have been reports of rare GABA neurons that lack the transporter, raising the specter of another (related) transporter in these neurons or, alternatively, some unique functional attribute of these cells.

REGULATION OF GABA RELEASE BY AUTORECEPTORS

The major postsynaptic GABA receptor is the $GABA_A$ receptor, which contains the chloride ion channel (see Chapter 10). This multimeric receptor complex is formed by a number of different subunit proteins. Pharmacological studies indicate that autoreceptor-mediated regulation of GABA neurons takes place predominantly through $GABA_B$ receptors located on GABAergic nerve terminals. Immunohistochemical studies have revealed that both $GABA_B$ and $GABA_A$ receptors are present on postsynaptic non-GABAergic neurons. It is possible that these $GABA_A$ postsynaptic receptors respond to GABA released from a neuron that is presynaptic to another GABA neuron expressing the $GABA_A$ site. Because an anatomical arrangement of one GABA neuron terminating on another GABA cell would have the same functional consequence as an autoreceptor (decreasing subsequent transmitter release), it has been difficult to distinguish between true autoreceptors and heteroceptors *in vivo*.

Inactivation of Released GABA

Uptake of several transmitters by glial cells as well as neurons has been reported. The dual glial–neuronal reuptake is common in neurons using amino acid transmitters, probably because amino acids have important roles as both transmitters and constituents of intermediary cellular metabolism. However, the ability of glia to avidly accumulate GABA and other amino acids distinguishes amino acid transmitters from other classic transmitters.

GABA TRANSPORTER PROTEINS

Reuptake is the primary mode of inactivation of GABA that is released from neurons. There are four GABA transporter (GAT) proteins expressed in the CNS, providing a diverse means of regulating GABA neurons (see Cherubini and Conti, 2001). In addition, a betaine transporter that accumulates GABA has been cloned. Two types of GABA transporters were long known as being neuronal and glial and were defined on the basis of pharmacological specificity. However, the cloning of GABA transporters, which belong to the same family of transporter genes that includes the catecholamine transporters, revealed an unexpected finding. *In situ* hybridization and immunohistochemical studies showed that one of the GATs found in brain, which on pharmacological grounds was defined as a "glial" transporter, is present in both neurons and glia. Moreover, the other GATs appear to be expressed in both neurons and glia.

The presence of multiple GABA transporter proteins for the same transmitter, localized to a single type of cell (e.g., neurons), differs from the situation for catecholamine transmitters, in which a single membrane-associated transporter protein with relatively poor substrate specificity is found in a neuron. An obvious question arises: Why are there multiple transporters for GABA? GATs are expressed in both GABAergic neurons and non-GABAergic cells (presumably cells that receive a GABA innervation). Although it appears that more than one type of GAT can be present in a glial cell, such colocalization may not be the case in neurons. It is also possible that different transporters are targeted to different parts of neurons. For example, one GAT might be present in dendrites and another expressed in axons, with corresponding different functional requirements. Another possibility is that the GATs may act as co-transporters for other amino acids: transporters for β-alanine and taurine have not been cloned, but these amino acids are accumulated by GATs. Finally, it is possible that one or more of these transporters frequently works in the outward direction, serving as a paradoxical mechanism for the release of GABA.

ENZYMATIC INACTIVATION OF GABA

As is the case in catecholamine neurons, GABA can be enzymatically inactivated. The enzyme GABA-transaminase (GABA-T) is used to degrade GABA but, in contrast to enzymes involved in catecholamine inactivation, plays another role in synthesizing GABA. Although GABA-T is present in high concentration in GABAergic neurons, it is also found in non-GABAergic neurons as well as a number of peripheral tissues. Electron microscope data suggest that GABA-T is associated with mitochondria. However, pharmacological studies of subcellular fractions of cells suggest that the activity of GABA-T associated with synaptosomes (pinched-off axon terminals as well as the postsynaptic membrane) associated with mitochondria is less than that seen in membrane fractions that lack mitochondria, suggesting that GABA may be metabolized either extraneuronally or in postsynaptic neurons.

Glutamate and Aspartate: The Excitatory Amino Acid Transmitters

Excitatory amino acid transmitters account for most of the fast synaptic transmission that occurs in the mammalian CNS. Glutamate and aspartate are the major excitatory amino acid neurotransmitters, but several related amino acids, such as homocysteic acid and *N*-acetylaspartylglutamate, may also have neurotransmitter roles. The excitatory amino acids, like the inhibitory amino acid transmitter GABA, participate in intermediary metabolism as well as cellular communication; the same problem encountered in our discussion of dissociating the neurotransmitter from metabolic roles of GABA also holds for excitatory amino acids. The intertwining of the transmitter roles of amino acids and intermediary metabolism has historically made it difficult for glutamate to fulfill all the criteria necessary to designate glutamate and aspartate as neurotransmitters. Nonetheless, it is now widely accepted that glutamate and aspartate do indeed function as excitatory transmitters in the nervous system. We briefly consider glutamate biosynthesis and regulation, focusing on the differences between excitatory and inhibitory amino acid transmitters. Many of the general principles addressed in the section on GABA are applicable to glutamate and therefore not discussed in detail.

Biosynthesis of Glutamate

Although glutamic acid is present in very high concentrations in the central nervous system, brain glutamate and aspartate levels are derived solely by local synthesis from glucose because neither amino acid crosses the blood–brain barrier. Two processes contribute to the synthesis of glutamate in the nerve terminal. As mentioned previously in our discussion of GABA, glutamate is formed from glucose through the Krebs cycle and transamination of α-ketoglutarate. In addition, glutamate can be formed directly from glutamine (see Fig. 7.7). Because glutamine is synthesized in glial cells, there is an unusual interaction of glia and neurons in determining the degree of glutamate that is available as a transmitter. The glutamine that is formed in glia is transported into nerve terminals and then locally converted by glutaminase into glutamate (Hassel and Dingledine, 2012). Thus, the synthesis of glutamate depends critically on the enzyme glutaminase. A phosphate-activated glutaminase (PAG) has been suggested to be the specific form of the enzyme responsible for the synthesis of the transmitter pool of glutamate. However, a different form of PAG is found in relatively high concentrations in peripheral tissues such as the liver (Conti and Minelli, 1994). PAG is localized to mitochondria; as discussed in the section on GABA, the process by which glutamate is exported to allow vesicular storage of the transmitter remains poorly understood.

Storage and Release of Glutamate

VESICULAR GLUTAMATE TRANSPORTER

Glutamate is stored in synaptic vesicles from which the transmitter is released in a calcium-dependent manner on depolarization of the nerve terminal. Although the vesicular storage of glutamate was convincingly demonstrated long ago and was well characterized biochemically, the cloning of the (multiple) vesicular glutamate transporters lagged behind. This is in part due to the fact that the vesicular glutamate transporter protein is not related to other mammalian transmitter transporters. The first vesicular glutamate transporter was initially identified as a protein mediating the sodium-dependent transport of inorganic phosphate across the membrane. There are now three known vesicular glutamate transporters, all of which are densely expressed in axon terminals of glutamate neurons (El Mestikawy et al., 2011). The mRNA encoding VGLUT1 is expressed predominantly by cortical pyramidal cells, while that encoding VGLUT2 is mainly expressed by subcortical glutamate neurons. The third vesicular glutamate transporter is much less abundant. There are now examples of colocalization of all three types of vesicular glutamate transporters, although the functional significance of this arrangement is not clear.

REGULATION OF GLUTAMATE RELEASE

The originally identified glutamate receptors were multimeric *ionotropic* receptors that when activated form an ion channel that allows cations to enter the cell. Subsequently, another group of glutamate receptors was identified that were *metabotropic* receptors that are coupled to the second messenger cAMP (see Chapter 10 for a discussion of metabotropic receptors). The release of glutamate from nerve terminals is regulated by a metabotropic autoreceptor. There are eight different metabotropic glutamate receptors (mGluRs) that comprise three distinct classes based on their coupling to cAMP. The release-modulating autoreceptor are class II mGluRs, which are negatively coupled to adenyl cyclase. These two mGluRs, the $mGluR_2$ and $mGluR_3$ receptors, are localized to presynaptic glutamatergic axon terminals; a large body of work indicates that these class II mGluRs function as release-modulating glutamate receptors. In addition, the mGluR3 receptor is expressed by astrocytes, a type of glial cell. Electrophysiological studies have suggested the presence of an impulse-modulating glutamate autoreceptor, which is thought to be either an $mGluR_I$ or $mGluR_5$ site. The $mGluR_4$ receptor has been localized to presynaptic nerve terminals but is present on

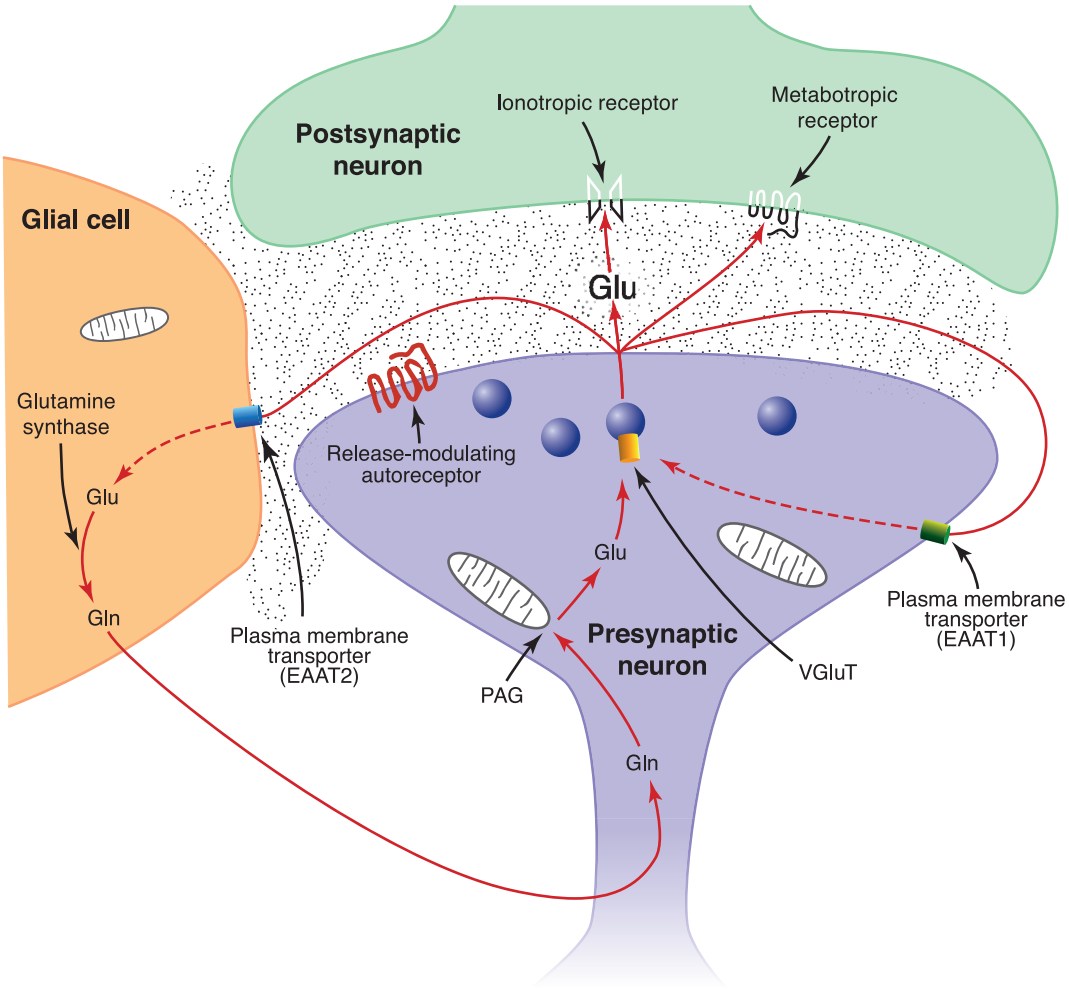

FIGURE 7.7 **Depiction of an excitatory amino acid (glutamate) synapse.** Glutamate, synthesized via metabolic pathways, is accumulated by a vesicular transporter into vesicles. After release from the presynaptic terminal, glutamate can interact with postsynaptic and/or release-modulating receptors. Glutamate is then cleared from the synaptic region by the high-affinity plasma membrane transporters or by recycling through adjacent glia.

nonexcitatory nerve terminals and thus is likely to be a heteroceptor rather than an autoreceptor. With eight different primary metabotropic glutamatergic receptors, it is reasonable to ask the question: Why have so many? The answer may be that these receptors subserve an extremely broad array of functions and appear to be critically involved in regulating not only glutamatergic function but also the activity (including release) of a dizzying number of transmitters, ranging from classic transmitters such as dopamine to peptide transmitters such as substance P (Cartmell and Schoepp, 2000; Niswender and Conn, 2010). There is currently considerable attention devoted to developing the use of drugs that target metabotropic glutamate receptors in neuropsychiatric disorders (Krystal et al., 2010; Moghaddam and Javitt, 2012).

Inactivation of Glutamate

Glutamate inactivation occurs predominantly by reuptake of the amino acid by dicarboxylic acid plasma membrane transporters. In contrast to GABA and other classic transmitters, there does not appear to be a significant role for enzymatic inactivation of glutamate. The extent to which diffusion regulates synaptic and extracellular levels of glutamate is not clear.

Five glutamate transporters have been cloned, with some localized to glia and others to neurons (Danbolt, 2001). For example, the glutamate transporter EAAT2 (known as GLT-1 in rodents) is almost exclusively expressed in astrocytes, whereas EAAT3 (EAAC1) is restricted to neurons. The glutamate transporters accumulate D- and L-aspartate as well as L-glutamate; although the affinities of the transporters are similar

for glutamate they differ for other amino acids. The transporters have distinct brain distributions, and even the glial transporters exhibit regional and intracellular differences in expression (Oberheim et al., 2012), underscoring the heterogeneity of glia as well as neurons.

The presence of certain glutamate transporters on glial cells is consistent with the intricate interplay of glial and neuronal elements in the synthesis of glutamate. Recent data have found that glutamate is not only taken up by glia cells, but also released from glia as a signaling molecule (Haydon and Carmignoto, 2006). Thus, because glutamate released from neurons is accumulated by glia and then metabolized to glutamine, there is ultimately a complex multicellular recycling of glutamate. The fate of glutamate accumulated by the neuronal glutamate transporter is unclear. It has not been established if glutamate released from a given neuron is taken up by a glutamate transporter on that particular neuron or, alternatively, by glutamate transporters on other neurons or glia.

Acetylcholine

Much of our basic understanding of chemical synaptic transmission is founded on studies of acetylcholine (ACh), the first transmitter identified. First noted as the vagal stuff of Loewi (1921) and subsequently demonstrated to be responsible for transmission at the neuromuscular junction by Loewi and Navratil, it has been a century since ACh was first proposed as a transmitter.

A key reason ACh has assumed such a prominent role in guiding studies of neurotransmitters has been the relative ease with which ACh can be studied. Acetylcholine is the transmitter at the neuromuscular junction, and thus both the nerve terminal and its target can be readily accessed for experimental manipulations. Subsequent investigations also focused on another peripheral site, the superior cervical ganglion, which was also easy to isolate and study. Lessons learned from experiments conducted on these peripheral tissues have shaped our current approaches to defining the characteristics of neurotransmitters in the brain.

The ability to expose and maintain isolated preparations of the neuromuscular junction permitted electrophysiological and biochemical studies of synaptic transmission. Electrophysiological studies revealed fast excitatory responses of muscle fibers to stimulation of the nerve innervating the muscle. The presence of miniature end-plate potentials (MEPPs) in the muscle fiber was noted, and Fatt and Katz (1952) demonstrated that these MEPPs resulted from the slow "leakage" of ACh, with each MEPP representing the release of transmitter in one vesicle (termed a *quantum*) (see Chapter 15 for additional details on MEPPs). When neurons are depolarized there is a sharp increase in the number of quanta released over a given period. In addition, studies of the neuromuscular junction allowed detailed analyses of the enzymatic inactivation of ACh, setting the reference for subsequent studies.

Over the past half-century many of the rules that govern ACh neurotransmission have been shown to be general principles that apply to other transmitters. For example, the concept of the quantal nature of neurotransmission is central to current ideas of transmitter release. Although the discovery of different neurotransmitters has expanded our knowledge, studies of ACh continue to provide a foundation for modern concepts of chemical neurotransmission (see Fisher and Wonnacott, 2012).

Acetylcholine Synthesis

The synthesis of ACh has only a single step: the acetyl group from acetyl-coenzyme A is transferred to choline by the enzyme choline acetyltransferase (ChAT). The requirements for ACh synthesis are also correspondingly few: the substrate choline, the donor acetyl-coenzyme A, and the enzyme ChAT (see Fig. 7.8).

The acetyl-CoA that serves as the donor is derived from pyruvate generated by glucose metabolism. This obligatory dependence on a metabolic intermediary is similar to the situation present in GABA synthesis, where the immediate precursor glutamate is formed from α-ketoglutarate. Acetyl-CoA is localized to mitochondria. Because the synthetic enzyme ChAT is cytoplasmic, acetyl-CoA must exit the mitochondria to gain access to ChAT; the specifics of this process are poorly understood.

CHOLINE ACETYLTRANSFERASE

Choline acetyltransferase is considered a definitive marker of cholinergic neurons (Wu and Hersch, 1994). Multiple mRNAs encode ChAT, resulting from differential use of three promoters and alternative splicing of the 5′ noncoding region of the enzyme (Trifonov et al., 2009). In the rat the different transcripts encode the same protein but in humans give rise to multiple forms of the enzyme, including both active and inactive (truncated) forms. The functional significance of the different transcripts under normal conditions is a topic of considerable interest. Myasthenia gravis, a disease marked by decreased muscle activity, is linked to a variety of deficits in neuromuscular cholinergic function; in congenital forms of myasthenia mutations in both nicotinic cholinergic receptors and the enzyme acetylcholinesterase (AChE) have been found. Recently, a particular ChAT mutation has been linked to a form of myasthenia that

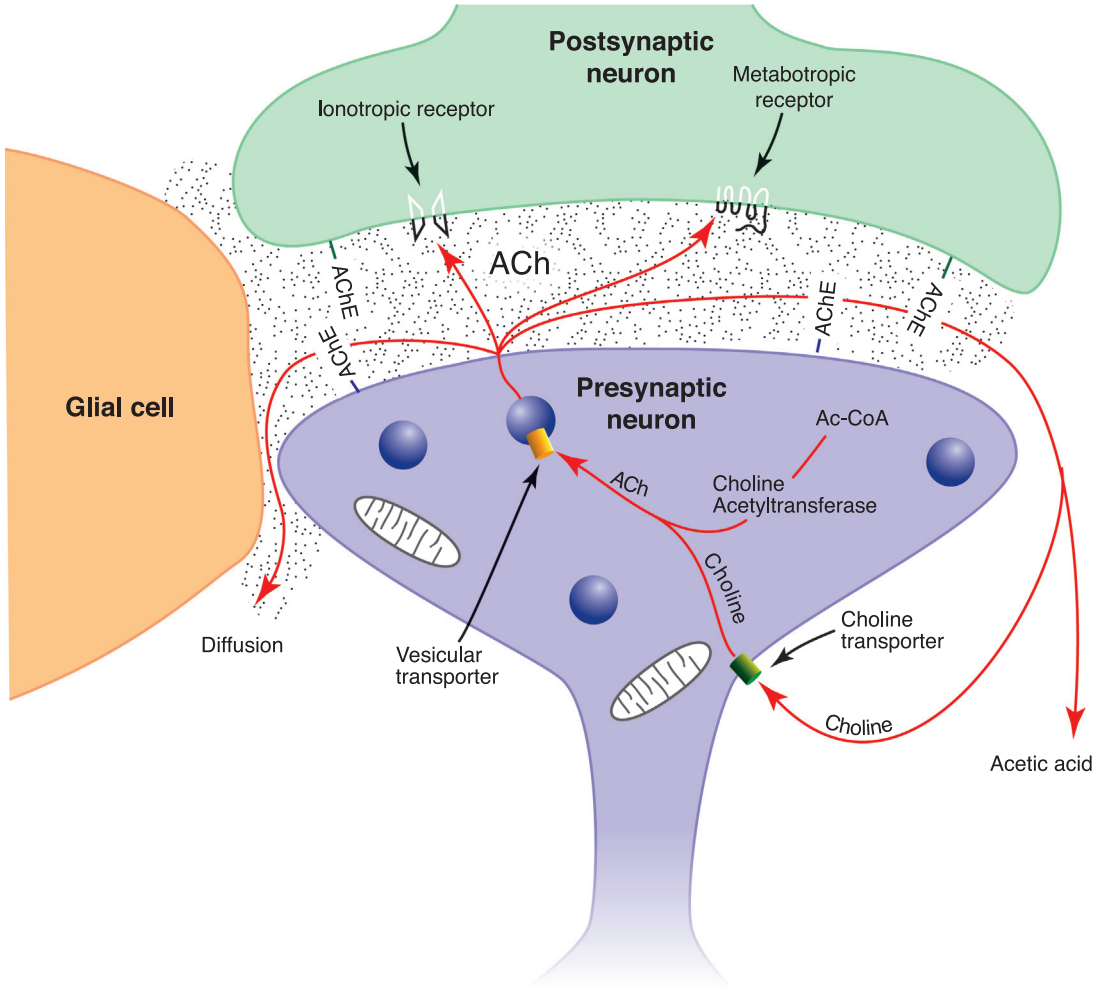

FIGURE 7.8 **Acetylcholine (ACh) synthesis, release, and termination of action.** A choline transporter accumulates choline. The enzyme choline acetyltransferase (ChAT) acetylates the choline using acetyl-CoA (Ac-CoA) to form the transmitter ACh, which is accumulated into vesicles by the vesicular transporter. The released ACh may interact with postsynaptic muscarinic or nicotinic cholinergic receptors, or can be taken up into the neuron by a choline transporter. Acetylcholine can be degraded after release by the enzyme acetylcholinesterase (AChE).

is characterized by often fatal episodes of apnea (Ohno et al., 2001).

Although ChAT is the sole enzyme in ACh synthesis, ChAT is not the rate-limiting step in ACh synthesis. When ChAT activity is measured *in vitro* it is much greater than would be expected on the basis of ACh synthesis *in vivo*. The reason for this discrepancy was thought to be related to the need to transport acetyl-CoA from the mitochondria to the cytoplasm, which may be rate limiting in ACh synthesis. Alternatively, intracellular choline concentrations may ultimately determine the rate of ACh synthesis. This latter speculation has led to the use of choline precursors in attempts to enhance ACh synthesis in Alzheimer's disease, in which there is a marked decrease in ACh in the cerebral cortex. Attempts have been made to treat Alzheimer's disease with lecithin, a choline precursor. Unfortunately, lecithin does not appear to diminish dementia, although it does cause bad breath!

Acetylcholine Storage and Release

VESICULAR CHOLINERGIC TRANSPORTER

ACh is synthesized by ChAT and transported into vesicles by the vesicular cholinergic transporter (VAChT). The VAChT was cloned on the basis of homology to a *C. elegans* gene (unc-17) that encodes a protein that is homologous with VMAT (Roghani et al., 1994). VAChT is expressed in cholinergic neurons throughout the brain.

When the mammalian VAChT was initially cloned it was noted that the human VAChT is present in chromosome 10, near the gene for ChAT. It was subsequently demonstrated that the VAChT is unique in

that its entire coding region is contained in the first intron of the ChAT gene (Usdin et al., 1995). This suggests that both genes are coordinately regulated, a suspicion that has been confirmed (Bernard et al., 1995).

CHOLINERGIC AUTORECEPTOR FUNCTION

Cholinergic release-modulating autoreceptors have been identified both in peripheral tissues and in the brain. This receptor is a muscarinic cholinergic receptor, rather than the nicotinic cholinergic receptor found at the neuromuscular junction. There are five types of muscarinic receptors, designated M_1–M_5. The release-modulating ACh autoreceptor in the cortex and other brain regions is an M_2 muscarinic receptor. The M_2 receptor is also found on noncholinergic (cholinoceptive) neurons, where it serves as a conventional heteroceptor. There are few direct data that point to the presence of a synthesis-modulating cholinergic autoreceptor.

Inactivation of Acetylcholine

ACETYLCHOLINESTERASE

The primary mode of inactivation of ACh appears to be enzymatic. Several enzymes that hydrolyze choline esters can degrade ACh, but the major esterase in the central nervous system is acetylcholinesterase (AChE; see Box 7.3).

The enzymatic inactivation of ACh is simply the hydrolysis of ACh to choline. Two groups of cholinesterases have been defined on the basis of substrate specificity, acetylcholinesterases and butyrylcholinesterases (Taylor and Brown, 2006). The first are relatively specific for ACh and present in high concentration in the brain, where there are multiple AChE species (Fernandez et al., 1996). Butyrylcholinesterases also efficiently hydrolyze choline esters but are found primarily in the liver, with lower levels being present in the adult brain.

AChE is present in high concentration in cholinergic neurons; however, AChE is also present in moderately high concentration in some noncholinergic neurons that receive cholinergic inputs (i.e., that are cholinoceptive). This observation is consistent with the fact that AChE is a secreted enzyme that is associated with the cell membrane. Thus, ACh hydrolysis takes place extracellularly, and the choline generated is conserved by the high-affinity reuptake process.

In addition to its role in inactivating released acetylcholine, AChE has been proposed to function as a chemical messenger in the CNS (Greenfield, 1991). Acetylcholinesterase appears to be released in a calcium-dependent manner from neurons in the substantia nigra and cerebellum; the cerebellar release is enhanced by electrical stimulation of cerebellar afferents. Electrophysiological studies have revealed that AChE elicits changes in the threshold for Ca^{2+} spikes, and local application of AChE enhances the responses of cerebellar neurons to glutamate and aspartate, the transmitters present in the climbing and mossy fiber innervations of the cerebellum (Appleyard and Jahnsen, 1992).

There are several AChE species, all of which are encoded by a single gene that is alternatively spliced (Schumacher et al., 1988), with tissue-specific expression of different transcripts (Seidman et al., 1995). Among the multiple mRNAs encoding AChE is one

BOX 7.3

ACETYLCHOLINESTERASE INHIBITORS, NERVE GASES, AND PHARMACOTHERAPY

The enzymatic inactivation of acetylcholine (ACh) has been fertile ground for the development of a large number of pharmaceutical agents. Anticholinesterases such as sarin are potent neurotoxins and have been used as nerve gases since World War I. Other anticholinesterases include organophosphates (such as parathion), which are widely used insecticides. Anticholinesterases, whether the target is a human or a tomato hornworm, function in the same way: instead of the released ACh leading to discrete single depolarizations of muscle fibers, the accumulation of acetylcholine at the neuromuscular junction leads to muscle fibrillation and ultimately depolarization inactivation of the muscle; i.e., the muscle is so excited it stops!

Anticholinesterases have some less aggressive uses as well. Competitive neuromuscular blocking agents such as succinylcholine are used as an adjunct to anesthetics during surgery to increase muscle relaxation; conversely, anticholinergics can be used to reverse the muscle paralysis caused by succinylcholine. Anticholinesterases are the mainstay of treatment of myasthenia gravis, a disorder of the neuromuscular junction that is usually marked by the presence of anti-nicotinic receptor antibodies. Attempts have also been made to treat Alzheimer's disease, in which there is a sharp decrease in cortical ACh, by administering an anticholinesterase to inhibit breakdown of ACh. Unfortunately, this approach has not proven very effective.

that represents the primary form of the enzyme expressed in brain and muscle.

HIGH-AFFINITY CHOLINE TRANSPORTER

Choline is found in the plasma in high concentration. A low-affinity reuptake process for choline is widely distributed in the body; however, both low-affinity and high-affinity choline uptake processes are present in brain. Cholinergic neurons in the brain express a sodium-dependent transporter that is saturated at plasma levels of choline, consistent with the high-affinity component of choline uptake.

The choline transporter, in contrast to other plasma membrane transmitter transporters, is not directly involved in termination of the action of release transmitter. Since ACh is hydrolyzed by AChE, enzymatic inactivation is the major means of terminating cholinergic transmission. In the absence of direct evidence that choline binds with high affinity to cholinergic receptors, it appears that the function of the choline transporter is conservation of choline for subsequent ACh synthesis. The high-affinity choline transporter appears to play important roles in a variety of homeostatic processes relating to cholinergic function, including cholinergic receptors (Bazalakova and Blakely, 2006; Bazalakova et al., 2007).

Paralleling the situation surrounding the identification of the vesicular glutamate transporter gene, which was cloned on the basis of a related worm gene, a cDNA encoding the rat high-affinity choline transporter was recently cloned based on similarities to *cho−1* in *C. elegans*. The vesicular cholinergic transporter is not homologous to other neurotransmitter transporters, being related instead to the sodium-dependent glucose transporter family.

Why do Neurons have so many Transmitters?

We have discussed in varying amounts of detail a moderate number, but not all, classic transmitters. About a dozen classic transmitters and literally dozens of neuropeptides function as transmitters, and still more molecules serve as "unconventional" transmitters, including growth factors and gases such as nitric oxide. If the role of neurotransmitters is to serve as a chemical bridge that conveys information between two spatially distinct cells, why have so many chemical messengers?

Afferent Convergence on a Common Neuron

Perhaps the simplest explanation for multiple transmitters is that tens of thousands of axons synapse with a single neuron. A neuron must be able to distinguish between the multiple inputs that bring information to the neuron. To some degree, this can be accomplished by the site on a neuron at which an afferent (input) terminates: at the cell body, axon, or dendritic shaft or spine. However, because many afferents terminate in close proximity, another means of distinguishing the inputs and their information is necessary. Chemically coding different signals onto neurons allows one to distinguish the multiple inputs, with the information conveyed by distinct transmitters distinguished by the different receptors on the targeted neuron.

Neurotransmitter Colocalization

A major conceptual change in the neurosciences over the past generation has been the realization that a cell can use more than one neurotransmitter (Deutch and Bean, 1995; Hnasko and Edwards, 2012). The idea that a neuron is limited to one transmitter can be traced to Henry Dale, or, more properly, to an informal restatement of what is termed *Dale's principle*. About 60 years ago, Dale posited that a metabolic process that takes place in the cell body can reach or influence events in all the processes of the neuron. John Eccles restated Dale's view to suggest that a neuron releases the same transmitter at all its processes. Illustrating the dangers of the scientific equivalent of sound bites, this principle was soon misinterpreted to indicate that only a single transmitter can be present in a given neuron. This is clearly not the case. Neurons can colocalize two or more transmitters. For example, a neuron can use both a classic transmitter such as dopamine and a peptide transmitter such as neurotensin. Indeed, it now appears that few if any neurons contain only one transmitter, and there are many cases in which three or even four transmitters are found in a single neuron.

The presence of multiple transmitters in a single neuron may indicate that different transmitters are used by a neuron to signal different functional states to its target cell. For example, the firing rates of neurons differ considerably, and thus it may be useful for a neuron to encode fast firing by one transmitter and slower firing by another transmitter. In addition, transmitters may be released by different patterns of neuronal firing. For example, a neuron may have an average firing rate of five impulses/second. This frequency could result from a neuron discharging every 200 ms or alternatively by discharging five times in an initial 200 ms period followed by 800 ms of silence ("burst" firing). Recent data indicate that in cases in which there is a colocalization of a peptide and a classic transmitter, peptide transmitters are often released at higher firing rates and particularly under burst-firing patterns.

In many ways, the different biosynthetic strategies used by peptides and classic transmitters may lead to differential release. Classic transmitters can be rapidly replaced because their synthesis occurs in nerve terminals. In contrast, peptide transmitters are synthesized

in the cell body and transported to the terminal. It is therefore useful to conserve peptide transmitters for situations of high demand, because they would otherwise be rapidly depleted.

Transmitter Release from Different Processes

The restatement of Dale's principle by Eccles held that a transmitter or other protein is present in all processes of a neuron. However, it now appears that a transmitter can be specifically localized to different parts of a neuron (see Deutch and Bean, 1995). For example, in the marine mollusk *Aplysia*, different transmitters are targeted to different processes of a single neuron (Sossin et al., 1990). If a transmitter is restricted to a particular part of a neuron, it follows that the neuron would need multiple transmitters to account for different release sites. Considerable evidence supports distinct spatial localizations of receptors (e.g., for ionotropic glutamate receptors) on a neuron, and even indicates that there is movement and clustering of receptors to maximize information transfer from pre- to postsynaptic neurons.

Synaptic Specializations versus Nonjunctional Appositions between Neurons

In addition to the diversity in transmitters that may result from transmitters being targeted to different intraneuronal sites, the anatomical relationships between one cell and its follower may contribute to the need for different transmitters. We usually think of synaptic specializations (see Chapters 1 and 2) as the morphological substrate of communication between two neurons. However, there may also be nonsynaptic forms of communication between two neurons. These could occur across distances that are smaller (e.g., gap junctions) or much larger than the separation of pre- and postsynaptic neurons by the synaptic cleft. The requirements for transmitter action would differ from those discussed previously if the distance traversed by a transmitter is larger than that typically present at a synaptic apposition. Thus, transmitters that lack an efficient reuptake system, such as peptide transmitters, might be favored at nonsynaptic sites. Because a single neuron can form both synaptic and nonsynaptic specializations, a single neuron may require more than one neurotransmitter.

Fast versus Slow Responses of Target Neurons to Neurotransmitters

We have seen that different firing rates or patterns may be accompanied by changes in the amount of transmitter being released from a neuron. As described in detail in Chapter 16, the postsynaptic response to a transmitter occurs over different timescales. For example, transmitter activation of ionotropic receptors (i.e., those that form ion channels) leads to very rapid changes, because the ionic gradients across the cell are almost instantaneously changed. In contrast, metabotropic receptors that respond to catecholamines and peptide transmitters are coupled to intracellular events via various transduction molecules, such as G proteins, and respond to neurotransmitter stimulation on a slower timescale than is seen when ionotropic receptors are activated. This difference in temporal response characteristics is useful, because it allows the receptive neuron to respond differently to a stimulus, depending on the antecedent activity in the cell. A transmitter can change the response characteristics of a particular cell to subsequent stimuli on the order of seconds or even minutes, and thus short-term changes can occur independent of changes in gene expression.

Stability of Neurotransmitter Identity of Neurons

We have discussed neurotransmitters as constant elements in neurons. However, it has been known for over a quarter of century that neurons can change their transmitter phenotype during maturation, with the cell no longer expressing its original transmitter but assuming a new transmitter identity (see Birren and Marder, 2013). But even mature neurons can undergo a transmitter switch that is associated with different behavior. Dulcis et al. (2013) found that certain neurons in the adult rat hypothalamus that are involved in circadian rhythms change the transmitter they express from the peptide transmitter somatostatin to dopamine depending on whether the animals are exposed to short (5 hours) or long (19 hours) periods of daylight. Moreover, these changes in transmitter identity were associated with depressed behaviors in the animals. The underlying changes that allow such a switch, involving dopamine changes in a number of enzymes and their cofactors, are unknown, but suggest that the ability of neurons to change in response to their environment is even greater than previously realized.

Non-Transmitter Roles of Neurotransmitters

Over the past few years several of the key proteins involved in regulating chemical neurotransmission have been identified based on homologies to proteins found in invertebrate species, such as the worm *Caenorhabditis elegans* and the fly *Drosophila melanogaster*.

It now appears that some of the molecular players in neurotransmission are found even in plants! Plant homologs of glutamate receptors have been identified and shown to be important in regulating diverse functions, ranging from calcium utilization to morphogenesis (Brenner et al., 2000; Kim et al., 2001), and geneological analysis has suggested that these glutamate receptors

may predate the divergence of plants and animals (Chiu et al., 1999). As nervous systems have become elaborate through evolution, many neurotransmitter-related proteins have roles that are not related to transmitter function or alternatively are involved in less discrete and more spatially elaborate signaling.

An example is acetylcholine. As discussed previously, ChAT is a cytosolic protein that drives the synthesis of ACh from choline and acetyl-CoA. However, one form of human ChAT is localized to the nucleus, where it seems unlikely to play a transmitter role (Resendes et al., 1999). ChAT mRNA is found in the testes, where it is translated and the protein appears in spermatozoa (Ibanez et al., 1991). Moreover, ChAT mRNAs have been reported to be present in lymphocytes, as have certain muscarinic cholinergic receptors (Kawashima et al., 1998).

In addition, AChE mRNAs appear to be present in lymphocytes, where both acetylcholinesterase and butyrylcholinesterase enzyme activities have been reported to be decreased in Alzheimer disease (Bartha et al., 1987; Inestrosa et al., 1994). AChE is present in high abundance in bone marrow cells and peripheral blood cells in certain types of leukemias (Lapidot-Lifson et al., 1989). Recent data indicate that inhibition of AChE gene expression in bone marrow cultures suppresses apoptosis (programmed cell death) and leads to progenitor cell expansion (Soreq et al., 1994), suggesting a role for AChE in the development of leukemias.

The presence of neurotransmitter-related proteins in peripheral tissues is not restricted to molecules related to ACh function. Three dopamine receptor mRNAs, encoding the D3, D4, and D5 receptors, are present in lymphocytes, and expression of the D3 receptor transcript has been suggested to be increased in schizophrenic subjects (Ilani et al., 2001; Kwak et al., 2001).

It is relatively easy to envision how transmitter receptors that are expressed on peripheral nonneural tissues can respond to transmitters present in the periphery, essentially functioning as hormonal signals. Thus, dopamine or other catecholamines that are circulating at low levels in the periphery may bind to DA receptors present on lymphocytes. However, another means of signaling is via axonal noradrenergic innervation of nonneural immune tissues in the periphery. Thus, sympathetic noradrenergic fibers innervate not only the vasculature but also primary (bone marrow) and secondary (spleen, lymph nodes) immune lymphoid structures (Felton et al., 1985). This noradrenergic innervation may be the central regulator of peripheral immune and stress responses, since chemical lesions of the sympathetic nervous system markedly alter T- and B-cell proliferation and activity (Madden et al., 2000). Not only are the transmitters of neurons communicating between neural and immune system cells the same, but there are similarities between the structural substrates of communication between different nervous system cells (neurons) and immune system cells, through junctional specializations called *synapses* (see Trautmann and Vivier, 2001).

SUMMARY

Classic neurotransmitters are small molecules that are derived from amino acids or intermediary metabolism and share several characteristics. The sequential actions of key enzymes result in the biosynthesis of these transmitters, usually in the general vicinity of where they will be released. The synthesized transmitter is stored in vesicles where it is poised for release and protected from degradation; the vesicular transporters also sequester xenobiotics and thus protect the neuron from certain toxins. Neurotransmitter release is elicited by depolarization and is calcium dependent. The action of the released neurotransmitter is terminated by a reuptake mechanism involving plasma membrane transporters and by enzymatic means.

The criteria for designation as a classic transmitter have been based on experiments conducted in sites that were easily accessible (such as the neuromuscular junction). Although many of the key principles of chemical synaptic transmission have been found to be the same in other areas that are less accessible to experimental manipulation (neurons in the brain), our ideas of the defining characteristics of transmitters have evolved to account for new knowledge and the emergence of many exceptions to the rules enunciated previously. The relatively high concentrations of classic transmitters permitted the easy measurement of these compounds, and thus the measurement of transmitter release became a key criterion for defining a neurotransmitter. Unfortunately, transmitter release has proven to be a difficult criterion to meet for many putative transmitters discovered over the past 30 years. Nevertheless, the increasing sensitivity of analytical techniques coupled with the ingenuity of neuroscientists led to the uncovering of a large number of peptides, growth factors, and even gases that function as transmitters. We explore in the next chapter the similarities and differences of the classic transmitters with these new kids on the block. These differences have often illuminated unknown fundamental processes of neurons and expanded our concept of information flow between neurons.

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