Part 7

Neurochemistry, Neuropharmacology

Transmitters and Peptides: Basic Principles 47

Lakshmi A. Devi and Lloyd D. Fricker

Brief History

The peptide secretin was the first chemical transmitter to be discovered, in 1902, although it was many decades until the sequence of secretin was determined. The concept of a molecule secreted from one tissue acting on a distant tissue was extended to other systems, and the molecules were named "hormones" in 1905. Neurotransmission in the nervous system was found to rely on chemicals, some identical to hormones used for communication between various organs as well as other molecules unique to neurons. Acetylcholine was identified in 1914, based on its release from the vagus nerve. Dopamine was first synthesized in 1910 but was only recognized as a neurotransmitter in the 1950s. Many additional small molecule neurotransmitters, as well as neuropeptides, have been identified in the past 50 years, and many more are likely to be discovered in the upcoming decades. An emerging concept in chemical neurotransmitters is that of nonclassical neurotransmitters such as small molecules (nitric oxide and endocannabinoids) and potentially also neuropeptides. Nonclassical transmitters are produced in a regulated manner, in contrast to classical neurotransmitters that are produced in advance and stored within secretory vesicles which are released upon stimulation of the cell. These different types of chemical transmitters provide a large variety of mechanisms for cell-cell signaling and add to the great complexity of the nervous system.

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Introduction: Overview of Cell-Cell Signaling Molecules

Signaling between cells is an essential activity within the brain and nervous system, as well as all other tissues. The vast majority of cell-cell signaling involves chemical mediators such as neurotransmitters and neuropeptides, which are collectively referred to as chemical transmitters. Many drugs that affect the brain and nervous system work by either mimicking or blocking cell-cell signaling, and an understanding of these signaling molecules is therefore an essential aspect of neuropharmacology. Although there are differences between neurotransmitters and neuropeptides, both share a number of key properties. First, the chemical transmitters are synthesized and secreted from the signaling cell. Second, either the synthesis or secretion is regulated so that extracellular levels vary over time. Third, the molecules produce a physiological response in another cell; this is usually mediated by the neurotransmitter or neuropeptide binding to a receptor on the cell surface, although other mechanisms of signaling can occur. Fourth, the signaling molecule is removed from the extracellular space to terminate the signal, either by chemical transformation of the neurotransmitter or neuropeptide into an inactive form or by uptake of the neurotransmitter into a cell. These general properties are shared by classical neurotransmitters (such as acetylcholine), nonclassical neurotransmitters (such as nitric oxide), and neuropeptides, although there are fundamental differences between these various classes of signaling molecules as well. These differences are described below.

Classical Neurotransmitters

The first cell-cell signaling molecules to be discovered were simply called neurotransmitters, and the term "classical" was added more recently, after the discovery of signaling molecules that fit most, but not all, of the criteria that were established for neurotransmitters. The original criteria for neurotransmitters were that the molecules be (1) synthesized in neurons and stored in vesicles, (2) released upon stimulation, (3) able to bind to receptors on nearby cells, and (4) either taken back into the cell that secreted them or degraded by extracellular enzymes. Neurotransmitters are relatively small molecules and in many cases are produced from amino acids (e.g., dopamine) or are amino acids themselves (e.g., glutamate). The enzymes involved in the biosynthesis of the classical neurotransmitters are present in the presynaptic terminal of the neuron, either in the cytosol or within the secretory vesicles. The classical neurotransmitters are primarily stored in small synaptic vesicles but can also be found in other vesicle types such as large dense-core vesicles (which store neuropeptides; discussed below). Classical neurotransmitters are secreted from the presynaptic cell at the synapse. After secretion, the molecules can either be taken up into the cell that secreted it, taken up into a nearby cell (such as a glial cell), degraded by an enzyme in the synaptic space, diffuse out of the synapse, or bind to a receptor on a nearby cell. This latter role is the reason

that the molecule is secreted, but due to competing mechanisms for uptake, metabolism, and diffusion that reduce the number of molecules, only a fraction of the secreted molecules bind to the receptor and produce biological effects. Over a dozen classical neurotransmitters are known, and the following examples are chosen as representative of the different types of molecules within this group.

Acetylcholine

Acetylcholine was the first neurotransmitter to be identified (in 1914) and was originally named vagusstoff, based on its release from the vagus nerve. Acetylcholine is an important neurotransmitter in the autonomic nervous system and a major neurotransmitter in motor neurons of the spinal cord and in the parasympathetic nervous system. In the central nervous system, acetylcholine is primarily produced by motor neurons, neurons within the brain stem and in neurons in the basal forebrain that project to many areas of the brain. For example, acetylcholine is produced in neurons in the nucleus basalis, which project widely throughout the neocortex.

Unlike most of the other classical neurotransmitters, acetylcholine is not produced from an amino acid but is synthesized from acetyl CoA and choline by choline acetyltransferase, a cytosolic enzyme present in the axonal terminals. After biosynthesis, the molecule is transported into secretory vesicles where it is stored. Stimulation of the neuron causes release of the entire vesicle's content of acetylcholine.

As with many of the other chemical transmitters, acetylcholine binds to a number of different receptors. These receptors are either ionotropic (ligand-gated ion channels such as the nicotinic receptors) or metabotropic (G protein-coupled receptors such as the muscarinic receptors); these receptors are described in more detail in another chapter. As the names imply, these acetylcholine receptors also bind plant compounds that are of clinical/therapeutic value (muscarine) or self-administered and highly addictive (nicotine).

Following secretion, acetylcholine is rapidly broken down into acetate and choline by the enzyme acetylcholinesterase at the synapse. The importance of the breakdown in neurotransmission is clear from the effect of inhibitors of acetylcholinesterase, which are among the most potent, toxic, and rapidly acting compounds found in nerve gas and pesticides. Less toxic inhibitors of acetylcholinesterase are important drugs used in treating myasthenia gravis, glaucoma, and Alzheimer's disease.

Dopamine

Dopamine was first synthesized over 100 years ago (in 1910), but it was recognized to be a neurotransmitter many decades later, in the 1950s. In the brain, dopamine is produced in hypothalamic neurons as well as neurons of the ventral tegmental area and substantia nigra. These dopaminergic neurons project to a number of brain regions, particularly in the nucleus accumbens, striatum, and prefrontal cortex. In addition, the tuberoinfundibular pathway that connects the hypothalamus to the pituitary also uses dopamine.

Dopamine is produced from the amino acid tyrosine by a two-step process (Fig. 47.1). First, tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by

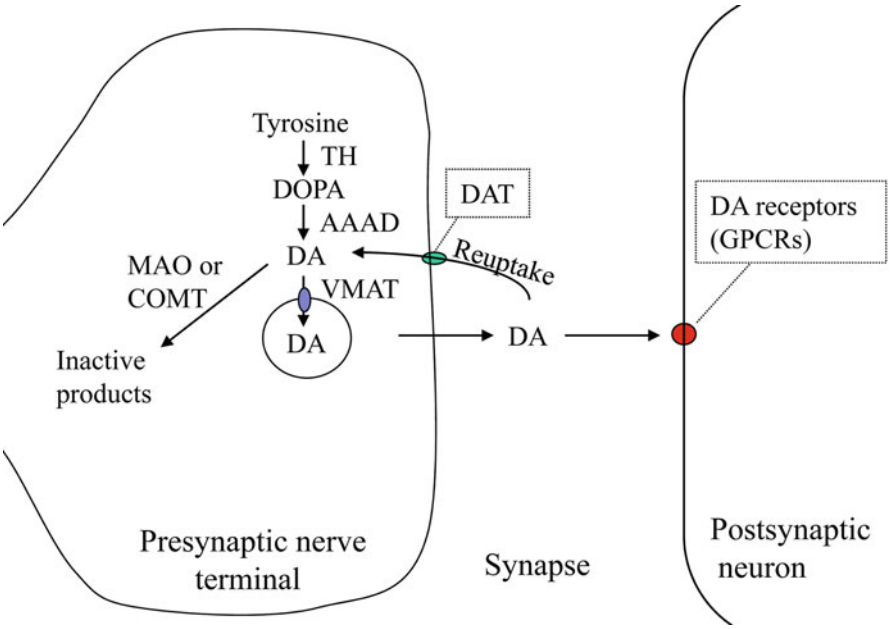

Fig. 47.1 *The dopaminergic synapse*. Abbreviations: *AAAD* aromatic L-amino acid decarboxylase, *COMT* catecholamine *O*-methyl transferase, *DA* dopamine, *DAT* dopamine transporter, *DOPA* dihydroxyphenylalanine, *GPCR* G protein-coupled receptor, *MAO* monoamine oxidase, *TH* tyrosine hydroxylase, *VMAT* vesicular monoamine transporter (Copyright 2011 Lakshmi Devi and Lloyd Fricker)

the enzyme commonly known as tyrosine hydroxylase (tyrosine 3-monooxygenase). DOPA is then converted into dopamine by the enzyme DOPA decarboxylase (aromatic L-amino acid decarboxylase). After biosynthesis in the cytosol of the axon terminal, dopamine is transported into secretory vesicles by vesicular monoamine transporter (VMAT). In some neurons, dopamine is further converted into the neurotransmitter norepinephrine by the enzyme dopamine beta-hydroxylase, which is located within the secretory vesicle. In the adrenal medulla, norepinephrine is further converted into epinephrine by the enzyme phenylethanolamine *N*-methyltransferase, a cytosolic enzyme. Of these three chemical transmitters, dopamine is the most important in the CNS, norepinephrine is second, and epinephrine is not thought to play a major role in CNS function.

There are five distinct dopamine receptors (and additional sets of distinct receptors for the dopamine products, norepinephrine, and epinephrine). All five dopamine receptors are G protein-coupled receptors. Some of these receptors are the target of drugs such as the typical antipsychotic drugs chlorpromazine (Thorazine) and haloperidol (Haldol), which function primarily as D2 dopamine receptor antagonists. DOPA is used as a drug to elevate levels of dopamine in the CNS and is used for treatment of Parkinson's disease (DOPA but not dopamine can cross the blood-brain barrier and get into the CNS).

After secretion of dopamine into the synapse, the intact molecule is reabsorbed into neurons by a specific transporter, the dopamine transporter (DAT). In some brain regions, the primary mechanism of uptake is through a related transporter, the norepinephrine transporter. Dopamine and other biogenic amines are metabolized within cells by monoamine oxidase (MAO) or catecholamine *O*-methyl transferase (COMT); both enzymes convert dopamine into inactive products.

Compounds that inhibit DAT (such as cocaine) cause mood elevation and addiction. Likewise, compounds that inhibit MAO are effective antidepressants. MAO also metabolizes other monoamines, and so it is not clear if the primary effect seen upon MAO inhibition is due to altered dopamine levels alone. Furthermore, although drugs that inhibit MAO or block DAT have relatively rapid effects on synaptic dopamine levels, they are most effective as antidepressants only after weeks of administration. Thus, the antidepressant action of these drugs is not due to short-term alterations in the synaptic levels of dopamine or related biogenic amines.

Serotonin (5-Hydroxytryptamine)

The cell bodies of neurons producing serotonin are primarily located in the raphe nuclei within the brain stem in an area known as the reticular formation. These neurons project broadly throughout the brain and are involved in diverse functions such as modulating mood and sleep/wake cycles.

The precursor of serotonin (also known as 5-hydroxytryptamine, or 5-HT) is the amino acid tryptophan. First, the enzyme tryptophan hydroxylase converts tryptophan into 5-hydroxytryptophan (Fig. 47.2). Then, aromatic L-amino acid decarboxylase converts this intermediate into serotonin (this is the same enzyme that converts DOPA into dopamine and is also called DOPA decarboxylase and 5-hydroxytryptophan decarboxylase). Both the hydrolase and the decarboxylase are located in the cytosol of the axonal terminals. The newly formed serotonin is then transported into the secretory vesicles by VMAT where it is stored until stimulation of the neuron. Prior to transport into the secretory vesicles, serotonin can be metabolized by MAO, the same enzyme that metabolizes dopamine (and other biogenic amines present in brain).

Several serotonin receptors have been identified; most are metabotropic and only one is ionotropic. Drugs that bind to serotonin receptors have diverse functions, depending on the receptors that are affected. Some psychedelics, such as lysergic acid diethylamide (LSD) and psilocybin, are partial agonists at some receptor subtypes (5-HT1A and 5-HT2A), while sumatriptan and related antimigraine drugs are full agonists at other subtypes (5-HT1D and 5-HT1B).

After secretion into the synapse, the primary route of serotonin removal is reuptake into the neurons via a specific transporter, which is appropriately named the serotonin transporter (SERT).

Drugs that inhibit the breakdown of serotonin are antidepressants; an example is the MAO inhibitors (which also affect levels of dopamine because the same enzyme is involved in the metabolism of both dopamine and serotonin). Selective serotonin reuptake inhibitors (SSRIs) are popular antidepressants; the prototype is fluoxetine (Prozac), one of the best-selling antidepressants.

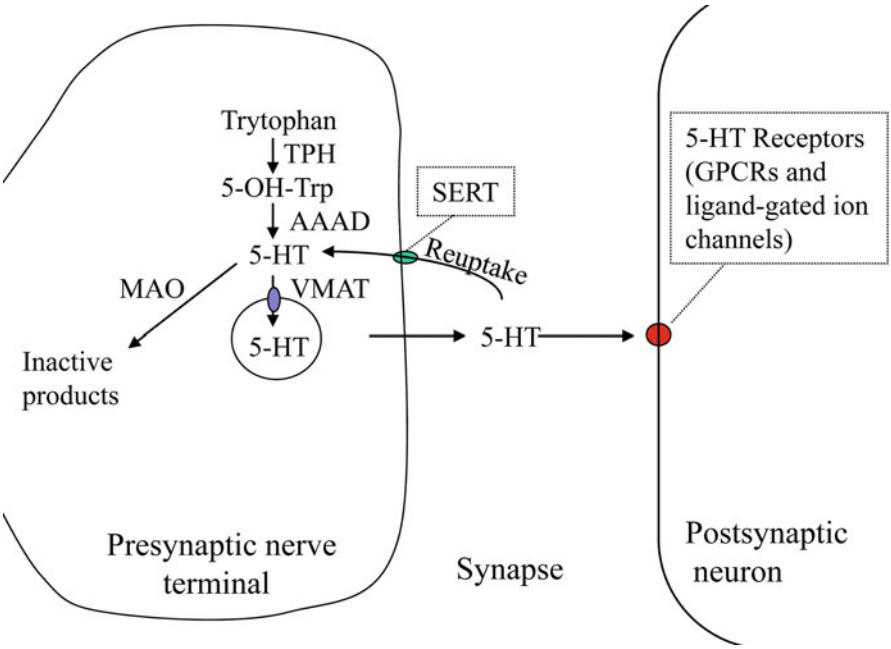

Fig. 47.2 *The serotoninergic synapse*. Abbreviations: *5-HT* 5-hydroxytryptamine (serotonin), *5-OH-Trp* 5-hydroxytryptophan, *AAAD* aromatic L-amino acid decarboxylase, *GPCR* G protein-coupled receptor, *MAO* monoamine oxidase, *SERT* serotonin transporter, *TPH* tryptophan hydroxylase, *VMAT* vesicular monoamine transporter (Copyright 2011 Lakshmi Devi and Lloyd Fricker)

Gamma-Aminobutyric Acid (GABA)

GABA is the major inhibitory neurotransmitter in the mammalian CNS. Binding of GABA to $GABA_A$ receptor produces hyperpolarization, which reduces the likelihood of neuronal firing, therefore serving an inhibitory role to dampen activity in the brain. Many cells use GABA as a primary neurotransmitter, including Purkinje cells of the cerebellum, granular cells of the olfactory bulb, amacrine cells of the retina, inhibitory interneurons of the spinal cord, and many other cell types.

GABA is an amino acid because it contains both an amine group and a carboxylic acid moiety, but it is not one of the 20 amino acids commonly incorporated into proteins. It is produced by many cells as part of the metabolic cycles but is only used as an inhibitory neurotransmitter by a subset of these cells. GABA is produced from glutamate by the enzyme glutamic acid decarboxylase (Fig. 47.3). After synthesis, GABA is transported to secretory vesicles and stored until needed, at which time it is released into the synapse.

Once in the synapse, GABA can bind to one of two receptors, $GABA_A$ or $GABA_B$, which represent distinct receptor families: $GABA_A$ is ionotropic while $GABA_B$ is metabotropic. These receptors are described in more detail in another chapter.

GABA is removed from the synapse by uptake into the presynaptic terminal or into nearby neurons or glia, via GABA-specific transporters (GAT). When

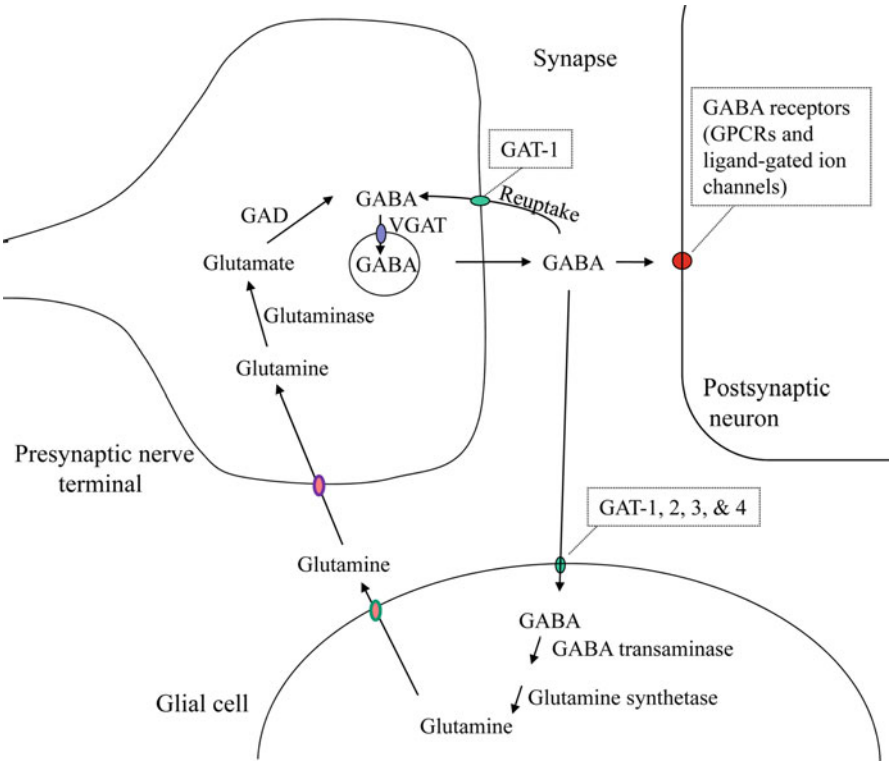

Fig. 47.3 *The GABAergic synapse*. Abbreviations: *GABA* gamma-aminobutyric acid, *GAD* glutamic acid decarboxylase, *GAT* GABA transporter, *GPCR* G protein-coupled receptor, *VGAT* vesicular GABA transporter (Copyright 2011 Lakshmi Devi and Lloyd Fricker)

transported into presynaptic terminals, the GABA can be returned directly into secretory vesicles without further modification. However, when taken into glial cells, GABA is converted into glutamine by a series of enzymes. The glutamine is subsequently released, taken up by the GABAergic neuron, and converted back into glutamate and then GABA. The rationale for the glutamine intermediate (rather than glutamate or GABA itself) is that both GABA and glutamate are major neurotransmitters that have powerful CNS effects whereas glutamine does not. Therefore, nearby glial cells convert GABA into an inactive compound (glutamine) and then return this to the GABAergic neuron where it can be recycled into GABA.

A large number of CNS-active drugs interact with various components of the GABA system, and all are generally used to decrease neuronal activity. Compounds that bind to the $GABA_A$ receptors include sedatives (barbiturates such as phenobarbital), anxyolytic drugs (diazepams), general anesthetics (propofol), and ethyl alcohol (ethanol). All of these compounds function as positive allosteric modulators; rather than directly activating the receptor, these compounds make the receptor more responsive to the action of GABA. Because epilepsy is

associated with excessive neuronal firing, drugs that potentiate the activity of the GABA system are especially important for treatment of epilepsy. Other antiepileptic drugs are inhibitors of the GABA transporters or GABA transaminase; the inhibition of these steps leads to enhanced GABA levels in the synapse and this ultimately reduces neuronal activity.

Glutamate

In addition to being an amino acid used for protein synthesis, glutamate is the most abundant excitatory neurotransmitter in the mammalian brain. It is a nonessential amino acid that is produced in the brain and other tissues. Neurons that use glutamate as a neurotransmitter store the amino acid within secretory vesicles which release glutamate upon stimulation. Glutamate can also be secreted directly from the cell soma. Excessive glutamate in the synapse can lead to cell death due to a process termed "excitotoxicity."

Many different glutamate receptors are known, including ionotropic receptors (such as AMPA, kainate, and NMDA) and metabotropic receptors (mGluR); each of these is comprised of several members encoded by distinct genes.

Glutamate is removed from the synapse by glutamate transporters located on neurons and glial cells. To safely return the glutamate from glial cells back into neurons without causing excitotoxicity, the glutamate is converted into glutamine (as with GABA) which is then released from the glial cells, taken up into the neurons, and converted back into glutamate.

Glutamate has been implicated in a number of disease processes including ischemia/stroke, seizures, Parkinson's disease, Huntington's disease, depression, diabetes, multiple sclerosis, and schizophrenia. Drugs that modulate glutamate receptor activity show some promise for treating these disorders.

Nonclassical Neurotransmitters

The neurotransmitters described above are all synthesized before they are needed, sequestered into secretory vesicles, and ultimately secreted from neurons when the cells are stimulated; this is the definition of the classical pathway of neurotransmitters. In contrast, the nonclassical neurotransmitters are not made in advance and stored in vesicles but are produced only when needed. Because nonclassical neurotransmitters do not depend on secretory vesicles for their secretion, they can transmit information in a retrograde fashion, from the cell bodies back to the presynaptic terminals, and also to surrounding cells. These fundamental differences between classical and nonclassical neurotransmitters increase the complexity of signaling between cells.

Nitric Oxide (NO)

Early studies on "endothelium-derived relaxing factor" identified a compound with unique properties distinct from the classical neurotransmitters. This factor was ultimately identified as the gas nitric oxide, or NO. In addition to its important role

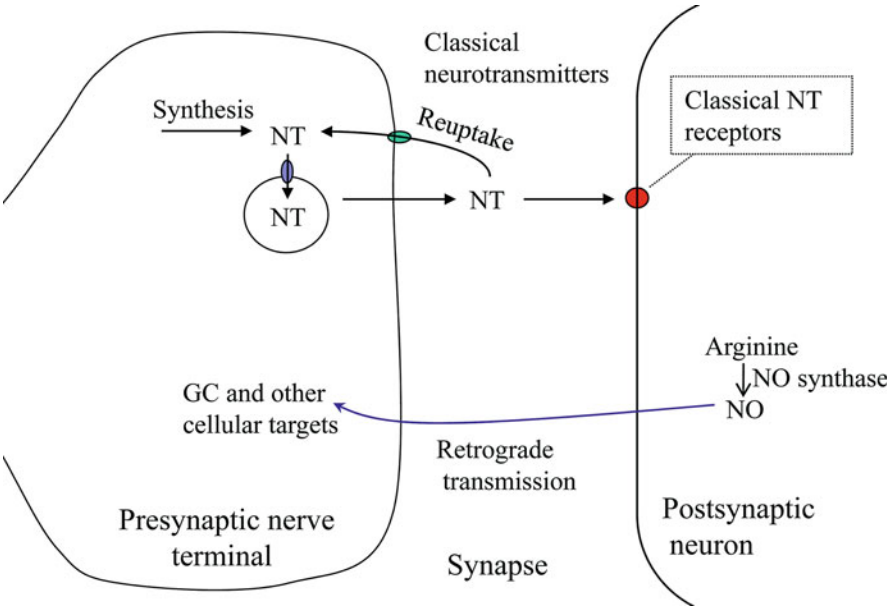

Fig. 47.4 *The nitric oxide synapse*. Abbreviations: *GC* guanylate cyclase, *NO* nitric oxide; NT, classical neurotransmitter (such as dopamine, 5-HT, or GABA) (Copyright 2011 Lakshmi Devi and Lloyd Fricker)

in the periphery, NO is a signaling molecule in the CNS. NO is produced from arginine, an endogenous amino acid, by the enzyme nitric oxide synthase (Fig. 47.4). Several forms of nitric oxide synthase are present in CNS, including constitutive and inducible forms. In the CNS, NO functions as a retrograde signaling molecule, conveying information from postsynaptic neurons to presynaptic neurons. NO is also involved in neuronal protection (at low levels) and neuronal death (at high levels).

Unlike classical neurotransmitters, NO does not interact with conventional receptors. Instead, it interacts with cellular proteins to produce its effects. A major target is the enzyme guanylate cyclase, which catalyzes the production of cyclic GMP. NO also interacts with a large number of other proteins by *S*-nitrosylation of thiol groups (free cysteine residues in proteins). The resulting *S*-nitrosothiol groups can affect the properties of the protein, and so this modification has a regulatory role. In addition, NO can oxidize iron-containing enzymes such as ribonucleotide reductase, thereby affecting cellular processes that depend on the activity of these enzymes.

Lipid-Derived Endocannabinoids

In the mid-1990s, receptors for the major psychoactive ingredient in marijuana, delta-9-tetrahydrocannabinol (D^9-THC), were discovered and named cannabinoid receptors. Searches for the endogenous brain compounds that stimulated these receptors led to the identification of two lipid-derived molecules, named

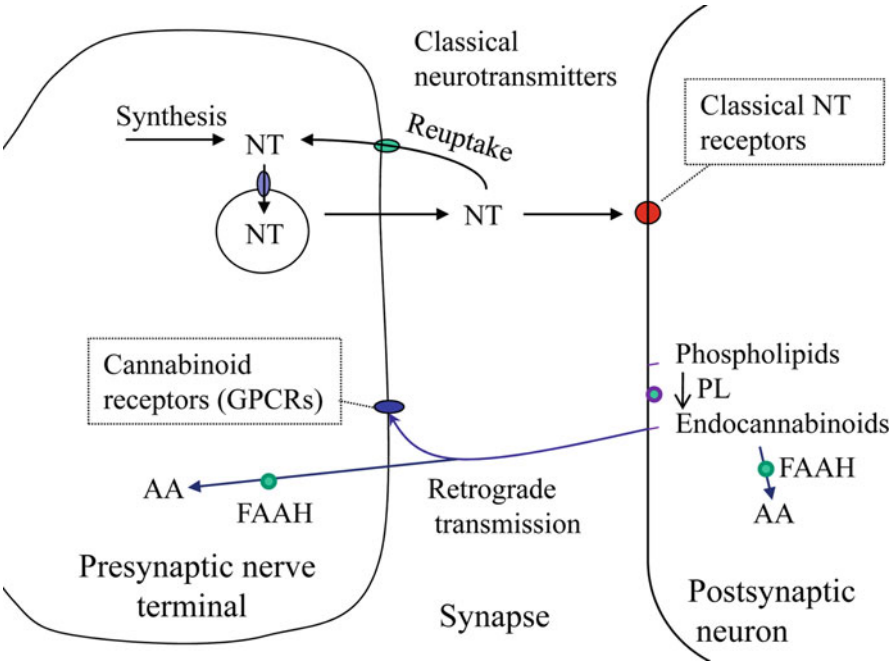

Fig. 47.5 *The endocannabinoid synapse*. Abbreviations: *AA* arachidonic acid, *FAAH* fatty acid amide hydrolase, *GPCR* G protein-coupled receptor; NT, classical neurotransmitter (such as dopamine, 5-HT, or GABA); *PL* phospholipase (Copyright 2011 Lakshmi Devi and Lloyd Fricker)

anandamide and 2-arachidonoylglycerol (2-AG). These lipid endocannabinoids differ in their ability to stimulate the cannabinoid receptors. While anandamide has captured most of the attention due to its catchy name (derived from the Sanskrit word for "bliss"), 2-AG may be the major lipid endocannabinoid in the brain, based on its higher levels and greater efficacy than anandamide. Both anandamide and 2-AG are produced from arachidonic acid, an omega-6 fatty acid, although their biosynthetic pathways differ. Anandamide is produced from N-arachidonoyl phosphatidylethanolamine (the conjugate of arachidonic acid and ethanolamine) by phospholipases A2, C, or D (Fig. 47.5). In contrast, 2-AG is produced by phospholipase C and diacylglycerol lipase. Similar to other nonclassical neurotransmitters, neither anandamide nor 2-AG is stored in secretory vesicles. Instead, their synthesis is upregulated upon stimulation of the neuron, and the molecules diffuse out of the cell to signal nearby cells, often in a retrograde fashion. The lipid endocannabinoids are thought to play a major role in presynaptic inhibition, a phenomenon whereby the postsynaptic neuron signals to the presynaptic neuron to reduce the amount of neurotransmitter released in a negative feedback process.

The lipid endocannabinoids activate members of the G protein-coupled receptor family. Both the endocannabinoids and their receptors are widely distributed throughout the brain, with high levels in the basal ganglia, hippocampus, and

cerebellum. The endocannabinoids are degraded by lipases such as fatty acid amide hydrolase and monoacylglycerol lipase, among others. This inactivation step occurs inside cells, not in the synapse.

Compounds that activate cannabinoid receptors (such as delta-9-THC) modulate a variety of physiological processes including mood, appetite, pain sensation, and memory. Furthermore, drugs that inhibit lipid endocannabinoid degradation are beginning to show promise as potential drugs for these various indications.

In addition to the lipid endocannabinoids, recent studies have identified brain peptides that stimulate cannabinoid receptors. Although further studies are needed to prove that these peptides are part of the endogenous cannabinoid system, the emerging concept is that there is tremendous complexity among the signaling pathways in the brain. These putative peptide endocannabinoids are described below in the section on nonclassical neuropeptides.

Other Classical and Nonclassical Neurotransmitters

In addition to the major neurotransmitter systems described above, there are many additional neurotransmitter systems. Well-established neurotransmitters with a clear biological role include norepinephrine, histamine, glycine, aspartate, and adenosine. Others, such as D-serine and carbon monoxide (CO), have been recently identified as signaling molecules, and many additional compounds have been proposed to function in cell-cell signaling but are not fully established.

Classical Neuropeptides

Neuropeptides represent the largest class of chemical transmitters. Over one thousand peptides have been identified in mammalian brain, but only a subset of these have been shown to be chemical transmitters, thus qualifying as neuropeptides; the function of the other brain peptides is not yet known, and it is possible that some of these are also neuropeptides. Most of the well-studied neuropeptides share many properties in common with classical neurotransmitters, while some are more similar to nonclassical neurotransmitters (these have been termed "nonclassical neuropeptides" and are described in the next section). Classical neuropeptides are involved in many diverse physiological processes including reproduction, feeding and body weight regulation, anxiety, depression, pain, analgesia, memory, alertness, and sleep/wake cycles.

The common properties of classical neurotransmitters and classical neuropeptides include synthesis of the molecule in the presynaptic neuron, storage in secretory vesicles, release into the extracellular space upon stimulation, binding to a cell surface receptor to elicit a physiological effect, and inactivation of the signaling molecule by degradation. Unlike many neurotransmitters, neuropeptides are not recycled into the original cell but are broken down in the extracellular environment by peptidases. Thus, for every neuropeptide molecule secreted, a new

molecule needs to be synthesized. Because of this fundamental difference, neuropeptides are thought to play a modulatory role that affects the signaling of classical neurotransmitters, rather than acting as the primary conveyors of information across a synapse. Another major difference between classical neurotransmitters and classical neuropeptides is that the neuropeptides can be released from the cell body as well as axon terminals, whereas classical neurotransmitters are primarily secreted from nerve terminals at the synapse. Although both types of molecules are stored in regulated secretory vesicles, classical neuropeptides are only found in large dense-core vesicles and not small synaptic vesicles, whereas classical neurotransmitters can be found in both types of vesicles. In some vesicles, the classical neurotransmitters are co-stored with the neuropeptides. The small synaptic vesicles are released more readily from the cells than the large dense-core vesicles, which fits with the concept that classical neurotransmitters play a larger role in the rapid chemical transmission between cells while neuropeptides play a modulatory role.

Classical neuropeptides are produced from specific precursors, termed prohormones, by selective proteolysis at specific sites. Some neuropeptides require additional posttranslational modifications before they are biologically active. Initially, the prohormone is transported into the lumen of the endoplasmic reticulum during protein synthesis. After folding, the protein is transported into the Golgi and then into secretory vesicles where proteolytic processing occurs. Many of the enzymes that process the prohormone into the mature bioactive forms of the peptides are broadly expressed in the CNS and neuroendocrine system where they function to produce many diverse neuropeptides and peptide hormones (such as insulin). These broad peptide-processing enzymes include prohormone convertases 1 and 2, which cleave the precursor to the C-terminal side of basic amino acids, and carboxypeptidase E that removes the C-terminal basic residue(s). Many peptide hormones, and some brain neuropeptides, have C-terminal amide groups; these are produced by the enzyme peptidyl-glycine-alpha-amidating monooxygenase in a two-step process in which a C-terminal glycine is first oxidized and then partially removed, leaving behind the amine of the glycine as the amide group. Other posttranslational modifications are known to occur but are generally restricted to a small number of neuropeptides; examples include phosphorylation of serine and threonine residues, sulfation of tyrosine, acetylation of the N-terminal amine and/or serine side chain, and additional proteolytic cleavages to generate shorter forms of the peptides.

Once secreted, the neuropeptide is cleaved by extracellular peptidases. In some cases, the cleavage products are inactive. But in other cases, the cleavage products are still active at the same receptors as the secreted neuropeptide, although often with differing affinities. This extracellular processing is therefore distinct from degradation. This is a fundamental difference between neuropeptides and neurotransmitters; the latter are metabolized into inactive products. The ability of extracellular peptidases to modify the activity of the neuropeptides adds to the complexity of neuropeptide signaling. A relatively small number of extracellular peptidases are able to cleave most neuropeptides. Although some of these enzymes have been given names that imply specificity for a particular peptide (such as

enkephalinase and angiotensin-converting enzyme), none of these enzymes are peptide specific; all are able to cleave a variety of neuropeptides.

Because most of the extracellular peptidases cleave a large number of distinct peptides, it is possible for a secreted peptide to have biological effects by competing for these peptidases, thereby altering the processing of other peptides; this has been proposed to be the mechanism of action of some biologically active peptides that do not directly bind to receptors. Nonetheless, the majority of well-established neuropeptides are able to bind directly to receptors. Several of these neuropeptides are described in detail in other chapters in this book. In this chapter, two neuropeptide systems are briefly described to illustrate the key points about classical neuropeptides. One of these examples is a precursor that gives rise to a single neuropeptide, and the other example is a more complicated set of three different precursors giving rise to a range of neuropeptides with overlapping biological activities.

Neuropeptide Y (NPY)

NPY is a 36-amino-acid peptide that plays an important role in the regulation of feeding and body weight as well as other functions. This neuropeptide is present in high levels in cells in the arcuate nucleus of the hypothalamus that project to other regions of the brain involved in body weight regulation.

NPY is generated from it's precursor, proNPY by cleavage at a single processing site that separates the N-terminal NPY sequence from the C-terminal peptide of unknown function. The processing site (Gly-Lys-Arg) is cleaved first by one of the prohormone convertases, and then the C-terminal basic residues are removed by carboxypeptidase E. Finally, the amidating enzyme removes the carbon atoms from the Gly to leave behind the nitrogen as an amide group. The mature NPY (and the C-terminal peptide derived from proNPY) is stored within secretory vesicles and secreted upon stimulation. There are several different receptors for NPY, all of which are G protein-coupled receptors.

The Enkephalins

The enkephalins are more complicated than NPY with respect to the number of biologically active forms, number of genes, and the role of processing in affecting biological activity (Fig. 47.6). There are two forms of enkephalin: a five-residue peptide of the sequence Tyr-Gly-Gly-Phe-Met (named Met-enkephalin) and Tyr-Gly-Gly-Phe-Leu (named Leu-enkephalin); these two peptides have similar biological activities. Both of these peptides bind to opioid receptors, named for their ability to bind opiate compounds such as morphine. Enkephalin and enkephalin-containing peptides are broadly distributed in the brain and are also present in other neuroendocrine tissues such as the adrenal medulla.

There are three distinct prohormone precursors that give rise to enkephalin-containing peptides; these are named proenkephalin, prodynorphin, and proopiomelanocortin. Of these precursors, it appears that only proenkephalin is extensively processed into the five-residue forms of enkephalins; the others are mainly processed into larger enkephalin-containing peptides. These longer enkephalin-containing peptides, such as the endorphins and dynorphins, bind to the three

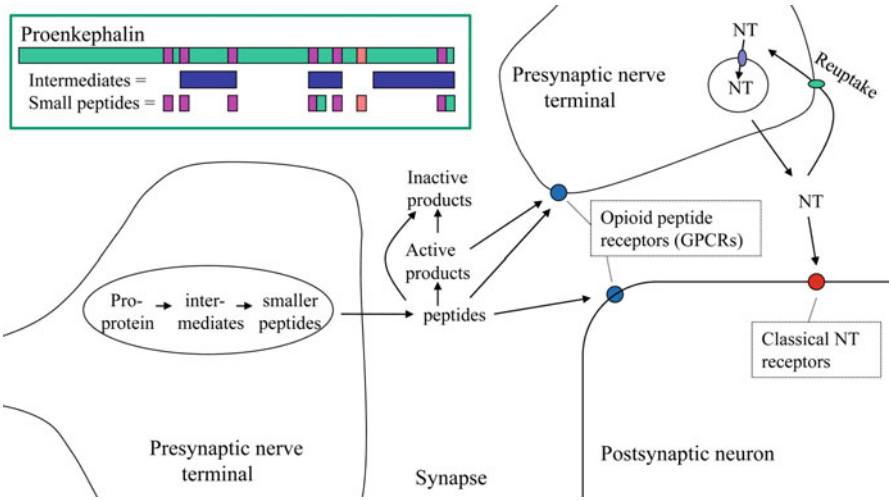

Fig. 47.6 *The opioid peptide synapse*. Abbreviations: *GPCR* G protein-coupled receptor; NT, classical neurotransmitter (such as dopamine, 5-HT, or GABA). Note the absence of a reuptake system for the peptides, in contrast to that of the classical neurotransmitter pathway. Also, synaptic processing of neuropeptides may produce smaller peptides that are still bioactive, in some cases with altered receptor affinities relative to the secreted form of the neuropeptide. Inset: diagram of proenkephalin, with the six copies of Met-enkephalin indicated in purple and the one copy of Leu-enkephalin in orange. Only a small number of the processing intermediates are shown; many more pieces have been identified. The smallest bioactive peptides produced from proenkephalin are either 5 amino acids (Met- or Leu-enkephalin), 7 amino acids (named "heptapeptide" and representing the C-terminal peptide), or 8 amino acids (named "octapeptide" and representing an internal fragment) (Copyright 2011 Lakshmi Devi and Lloyd Fricker)

subtypes of opioid receptors with different affinities than the shorter enkephalin peptides. Thus, the extent of the processing of each precursor, both within the cell and after secretion, has a major influence on the bioactivity of the peptides.

Each of the three precursors contains multiple copies of bioactive peptides. Proenkephalin contains one copy of Leu-enkephalin and six copies of Met-enkephalin, although not all of these are processed into Met-enkephalin. Prodynorphin contains three Leu-enkephalin sequences, which are processed into dynorphin A, dynorphin B, and neo-endorphin. Proopiomelanocortin contains one Met-enkephalin sequence which is the N-terminus of a longer peptide named beta-endorphin. In addition, proopiomelanocortin encodes several other bioactive peptides that bind to distinct receptors.

The three precursors are expressed in different brain regions. Proenkephalin is broadly expressed throughout the brain, with highest levels in the striatum and olfactory bulb. Prodynorphin is also broadly expressed in the brain and spinal cord, whereas proopiomelanocortin is restricted to a small number of cells in the hypothalamus (although these cells project to many brain regions). Proopiomelanocortin is also expressed in the anterior and intermediate lobes of the pituitary, where it is processed into distinct bioactive peptides depending on the cell type. Some of the

proopiomelanocortin-derived peptides bind to opioid receptors, while other peptides bind to melanocortin receptors. Thus, a range of physiological effects can be triggered by stimulation of cells expressing this precursor, with the precise effect depending on the extent of prohormone processing.

In many neurons, the opioid receptors are located on the presynaptic nerve terminal (Fig. 47.6). Stimulation by the enkephalins or other enkephalin-containing peptides (or by opiate drugs) often leads to reduced synaptic transmission by inhibiting the release of excitatory and/or inhibitory neurotransmitters. In other neurons, the opioid receptors are postsynaptic, like many of the classical neurotransmitter receptors.

Other Classical Neuropeptides

Currently, there are several dozen neuropeptide precursors that are known to produce over one hundred bioactive peptides, many with overlapping activities as described above for the enkephalins. Some of the precursors are processed into a single active peptide, like NPY; examples include oxytocin and vasopressin. Other precursors contain multiple forms of bioactive peptides, like the enkephalins; an example is the tachykinins (which includes substance P and neurokinins). The differential processing of neuropeptide precursors is a common mechanism to add further diversity to the range of products formed.

Nonclassical Neuropeptides

An emerging concept in chemical neurotransmitters is that of nonclassical neuropeptides. Some of the evidence for this concept was found decades ago when scientists identified fragments of cytosolic proteins that were active toward extracellular receptors. Recently, a peptide derived from alpha-hemoglobin, named hemopressin, was found to bind to CB1 cannabinoid receptors and function as an antagonist or inverse agonist (i.e., a compound that reduces receptor signaling below endogenous levels). Longer forms of the peptide with 2–3 additional N-terminal residues also bind to the receptor but function as agonists. These N-terminally extended hemopressins appear to represent the major forms of this peptide in the brain. Traditionally, alpha- or beta-hemoglobin are not generally considered to be produced in brain, and therefore, peptides derived from them would not qualify for the criterion of an endogenous neuropeptide. However, recent studies from several laboratories have shown that both alpha- and beta-hemoglobin are produced in the brain in neurons as well as some nonneuronal cells. The production of the N-terminally extended hemopressins is regulated under specific conditions. This has led to the concept of nonclassical neuropeptides, by analogy with nonclassical neurotransmitters like anandamide, 2-AG, and NO. The idea is that the nonclassical neuropeptides are not produced constitutively and secreted upon demand, as are classical neuropeptides, but are produced on demand and

secreted from the cytosol by an undetermined mechanism. Further studies are needed to identify the mechanism of synthesis and secretion of these peptides. It is important to stress that although this concept of nonclassical neuropeptides combines older data in the field with newer results and parallels the nonclassical neurotransmitter system, it remains to be proven that nonclassical neuropeptides are functional within the CNS.

Outlook

Future Perspectives

Based on the recent progress in the field, it is likely that many more chemical signaling molecules will be discovered, both classical (stored in vesicles and released upon demand) as well as nonclassical (not stored in vesicles; made on demand). It is also likely that new roles will be discovered for existing molecules, including those molecules already known to signal as well as precursors and/or metabolites of known compounds. An example of the latter is the trace amine receptors, which appear to bind catecholamine metabolites (tyramine and phenethylamine) and metabolites of the thyroid hormones (decarboxylated and deiodinated thyronamines). This is a very exciting direction of the field, which indicates that we still have much to learn regarding the complexity of cell-cell signaling in the central nervous system. Many undiscovered neuropeptides are also likely to exist, based on the continued discovery of new peptides over the past several decades and the large number of orphan G protein-coupled receptors awaiting identification of their endogenous ligands. Another exciting new concept is the emerging idea of nonclassical peptides, which integrates older studies of bioactive peptides derived from intracellular proteins with newer concepts from the field of nonclassical neurotransmitters like NO and anandamide. Chemical transmission within the CNS is both a well-studied area as well as a new area for further research, with the current trend toward greater complexity in cell-cell signaling within brain.

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