CHAPTER

15

Serotonin

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OUTLINE

SEROTONIN, THE NEUROTRANSMITTER

The indolealkylamine 5-hydroxytryptamine (5-HT; serotonin) was initially identified because of its effects on smooth muscle

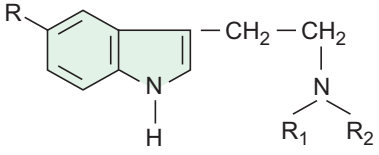
Since the mid-19th century, scientists have known that after blood clots a factor or substance in the resulting serum constricts vascular smooth muscle so as to increase vascular tone. Around the turn of the century, platelets were identified as the source of this substance. It was not until 1948 that Rapport, Green and Page isolated and correctly identified the serum vasoconstrictor factor as the substituted indole 5-hydroxytryptamine (5-HT) (Rapport et al., 1948). Quite appropriately this serum tonic factor, released from platelets during the clotting of blood, was named "serotonin." Independently, Esparmer characterized a substance found in large amounts in the enterochromaffin cells of the gastrointestinal tract, which also constricted smooth muscle. He called this substance "enteramine." In 1952, Esparmer and Areso reported that serotonin and enteramine were the same substance. Twarog and Page in 1953 detected serotonin in extracts of brain using a sensitive bioassay, the mollusk heart. Thus, serotonin was localized to three key systems in the body: platelets, gastrointestinal tract and brain.

The structures of serotonin (5-HT) and related compounds are shown in Figure 15-1. The combination of the hydroxyl group in the 5 position of the indole nucleus and a primary amine nitrogen serving as a proton acceptor at physiological pH makes 5-HT a hydrophilic substance. Therefore, it does not pass the lipophilic blood–brain barrier readily. For this reason, its discovery in brain in 1953 by Twarog and Page was exciting in that it indicated that 5-HT was being synthesized in brain. At about the same time the observation was made that (+)lysergic acid diethylamide (LSD), which was known to have psychedelic or psychomimetic effects, antagonized a response to 5-HT (i.e., the contraction of gastrointestinal smooth muscle produced by serotonin). This further substantiated an important role of 5-HT in brain function. The idea that 5-HT had important behavioral effects stimulated much thought about a central role for 5-HT in mental illness. Subsequently, various theories arose linking abnormalities of 5-HT function in brain to the development of a number of psychiatric disorders, particularly schizophrenia and depression. Psychotherapeutic drugs are now available that are effective in depression, anxiety disorders, and schizophrenia, and have potent, and in some cases selective, effects on serotonergic neurons in brain.

Understanding the neuroanatomical organization of serotonergic neurons provides insight into the functions of this neurotransmitter, as well as its possible roles in mental processes and psychiatric disorders

Serotonin-containing neuronal cell bodies are restricted to discrete clusters or groups of cells located along the midline of the brainstem. Their axons, however, innervate nearly every area of the central nervous system (Figure 15-2). In 1964, Dahlstrom and Fuxe, using the Falck-Hillarp technique of histofluorescence, observed that the majority of serotonergic soma were found in cell body groups that had been previously designated by Taber, Brodal, and Walberg as the raphe nuclei. This earlier description of the raphe nuclei was based on cytoarchitectural criteria, i.e., on cell body structural characteristics and organization. Dahlstrom and Fuxe described nine groups of serotonin-containing cell bodies, which they designated B_1 through B_9, and which correspond for the most part with the raphe nuclei (Tork, 1990) (see Table 15-1). Some serotonergic neuronal cell bodies, however, are found outside the raphe nuclei, and not all of the cell bodies in the raphe nuclei are serotonergic. In most of the raphe nuclei, the majority of neurons are nonserotonergic. For example, the dorsal raphe contains the largest number of serotonergic neurons; however, only 40–50% of the cell bodies in the dorsal raphe are serotonergic (Figure 15-3).

Over the course of the last three decades, a variety of techniques have been used to characterize the circuitry of serotonergic neurons in the central nervous system. The density of serotonergic innervation in the forebrain was initially underestimated because the original histofluorescence method was limited in sensitivity and did not permit the detection of many fine axons and terminals. Subsequent anatomical techniques (e.g., immunohistochemistry of 5-HT or tryptophan hydroxylase, an enzyme unique to the synthesis of 5-HT; retrograde and anterograde axonal transport studies) have allowed a more complete and accurate characterization of the serotonergic innervation of forebrain areas.



Compound	Position		
	R	R_1	R_2
Tryptamine	H	H	H
Serotonin	OH	H	H
Melatonin	OCH_3	$COCH_3$	H
Diethyltryyptamine (DET)*	H	CH_3CH_2	CH_3CH_2
Dimethyltryptamine (DMT)*	H	CH_3	CH_3
Bufotenine*	OH	CH_3	CH_3

*Psychotropic (modifies mental activity)

FIGURE 15-1 **Chemical structure of 5-hydroxytryptamine (5-HT; serotonin) and related indolealkylamines.** The indole ring structure consists of the benzene ring and the attached five-member ring containing nitrogen.

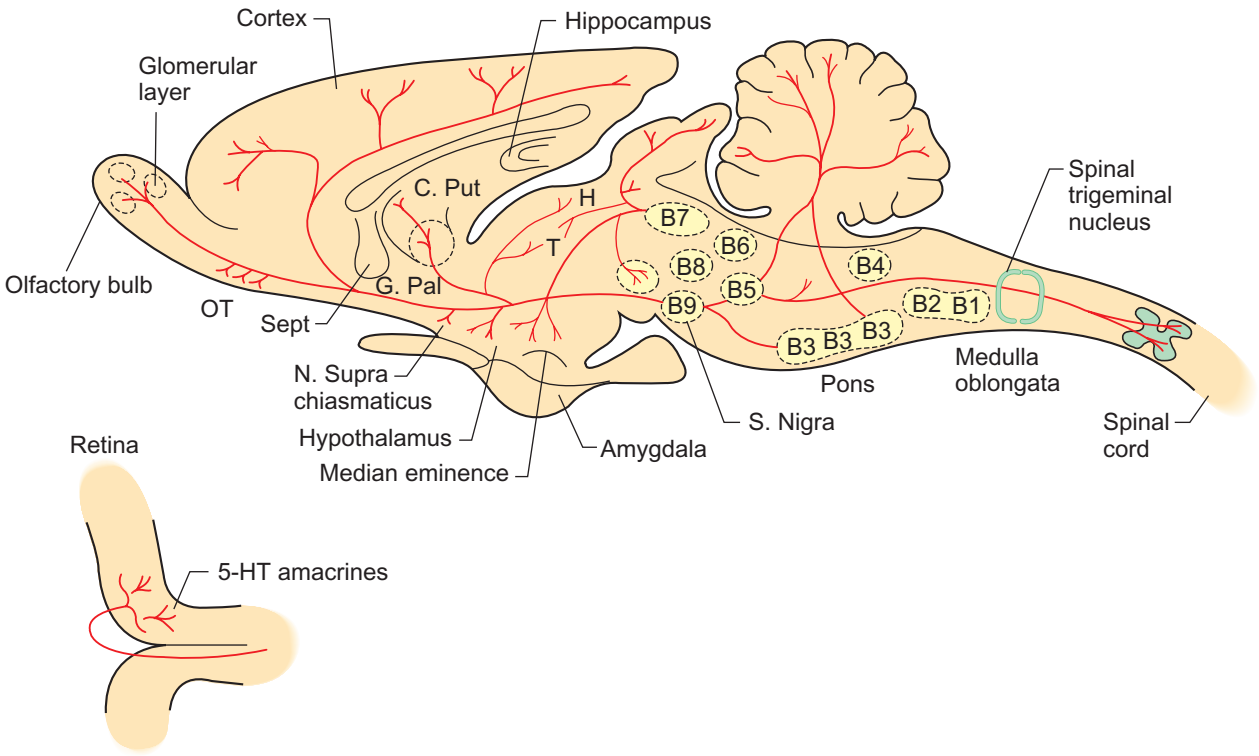


FIGURE 15-2 Schematic drawing depicting the location of the serotonergic cell body groups in a sagittal section of the rat central nervous system and their major projections. OT, olfactory tuberculum; Sept, septum; C. Put, nucleus caudate-putamen; G. Pal, globus pallidus; T, thalamus; H, habenula. (With permission from Consolazione A., et al. CNS Serotonin Pathways. In *The Biology of Serotonergic Transmission.* New York: John Wiley & Sons, 1982, 29–61)

TABLE 15-1 Classification of Serotonergic Cell Body Groups According to Dahlstrom and Fuxe and Corresponding Anatomical Structure

Groups of serotonin-containing cell bodies	Anatomical structure
B_1	Raphe pallidus nucleus
	Caudal ventrolateral medulla
B_2	Raphe obscurus nucleus
B_3	Raphe magnus nucleus
	Rostral ventrolateral medulla
	Lateral paragigantocellular reticular nucleus
B_4	Raphe obscurus nucleus, dorsolateral part
B_5	Median raphe nucleus, caudal part
B_6	Dorsal raphe nucleus, caudal part
B_7	Dorsal raphe nucleus principal, rostral part
B_8	Median raphe nucleus, rostral main part
	Caudal linear nucleus
	Nucleus pontis oralis
B_9	Nucleus pontis oralis
	Supralemniscal region

From Tork, 1990.

The largest group of serotonergic cells is group B_7 of Dahlstrom and Fuxe. B_7 is continuous with a smaller group of serotonergic cells, B_6. Groups B_6 and B_7 are often considered together as the dorsal raphe nucleus, with B_6 being its caudal extension. Another prominent serotonergic cell body group is B_8, which corresponds to the median raphe nucleus, also termed the nucleus central superior. Group B_9, part of the ventrolateral tegmentum of the pons and midbrain, forms a lateral extension of the median raphe and therefore is not considered one of the midline raphe nuclei. Ascending serotonergic projections innervating the cerebral cortex and other regions of the forebrain arise primarily from the dorsal raphe, median raphe, and B_9 cell group. The serotonergic neurons of the median and dorsal raphe nucleus differ in their electrophysiological characteristics and in their inhibition by somatodendritic autoreceptor activation (Beck et al., 2004), as well as in the morphology and topographical organization of their axonal projections to the forebrain (see below). These differences may be extremely important in understanding the role of these two distinct serotonergic systems, arising from the dorsal and median raphe nuclei, in normal brain function and in mental illness.

The two main ascending serotonergic pathways from the midbrain raphe nuclei to the forebrain are the dorsal periventricular path and the ventral tegmental radiations. Both pathways converge in the caudal hypothalamus where they join the medial forebrain bundle. Axons of dopaminergic (A8, A9, A10) and noradrenergic (A6) cell body groups also course anteriorly through the medial forebrain bundle (Molliver, 1987).

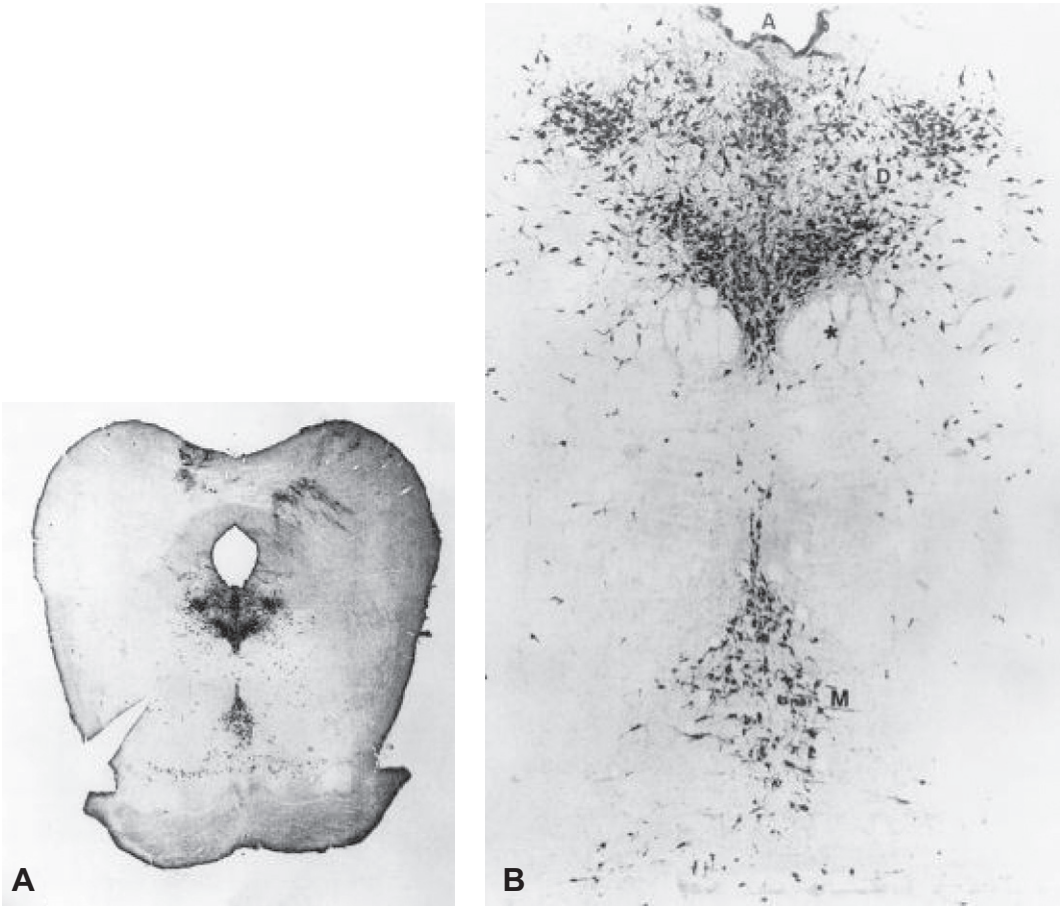


FIGURE 15-3 **Serotonergic cell bodies in the midbrain raphe nuclei demonstrated by 5-HT immunocytochemistry.** (**A**) Low magnification of transverse section through rat midbrain. The serotonergic cell body groups shown give rise to widespread serotonergic projections to cerebral cortex and forebrain structures. (**B**) Higher-magnification micrograph showing serotonergic cell bodies in dorsal and median raphe nuclei. The dorsal raphe nucleus lies in the central gray matter just beneath the cerebral aqueduct. In the transverse plane, the dorsal raphe can be further subdivided into a ventromedial cell cluster between and just above the MLF*, a smaller dorsomedial group just below the aqueduct, and large bilateral cell groups. The median raphe nucleus lies in the central core of the midbrain, below the MLF. (Abbreviations: D = dorsal raphe, M = median raphe, A = aqueduct; * = MLF (medial longitudinal fasciculus) (With permission from Molliver, 1987)

Ascending projections from the raphe nuclei to forebrain structures are organized in a topographical manner. The dorsal and median raphe nuclei give rise to distinct projections to forebrain regions (Figure 15-4), which form dissimilar yet partially overlapping patterns of innervation. The median raphe projects heavily to the dorsal hippocampus, septum and hypothalamus, whereas the dorsal raphe heavily innervates the ventral hippocampus, amygdala and striatum. The dorsal and median raphe nuclei send overlapping neuronal projections to the neocortex, which are also organized in a strict topographical manner and therefore affect different cortical neurons. Within the dorsal and median raphe, cells are organized in particular zones or groups that send axons to specific areas of brain. For example, the frontal cortex receives heavy innervation from the rostral and lateral subregions of the dorsal raphe nucleus. Moreover, raphe neurons send collateral axons to areas of brain that are related in function such as the amygdala and hippocampus, or substantia nigra and caudate putamen. The highly organized innervation of forebrain structures by serotonergic neurons of the raphe is quite interesting in that it implies independent functions of sets of serotonergic neurons dependent on their origin and terminal projections, as opposed to a nonselective or general role for 5-HT in the central nervous system (Molliver, 1987; Hensler, 2006).

Serotonergic axon terminals appear to exhibit morphological differences related to the raphe nucleus of origin (Figure 15-4). Serotonergic axons from the median raphe nucleus (type M) look relatively coarse with large spherical varicosities. By contrast, axons from the dorsal raphe (type D) are very fine and typically have small, pleomorphic varicosities. Dorsal raphe axons appear to be more vulnerable to certain neurotoxic amphetamine derivatives, e.g., 3,4-methylenedioxymethamphetamine (MDMA, or "Ecstasy") and parachloroamphetamine (PCA). Median raphe axons appear to be more resistant to the neurotoxic effects of these drugs. Blockade of the serotonin transporter (SERT) prevents the neurotoxic effects of these amphetamine derivatives, indicating that activity of this transporter is critical for the neurotoxic effects of these drugs.

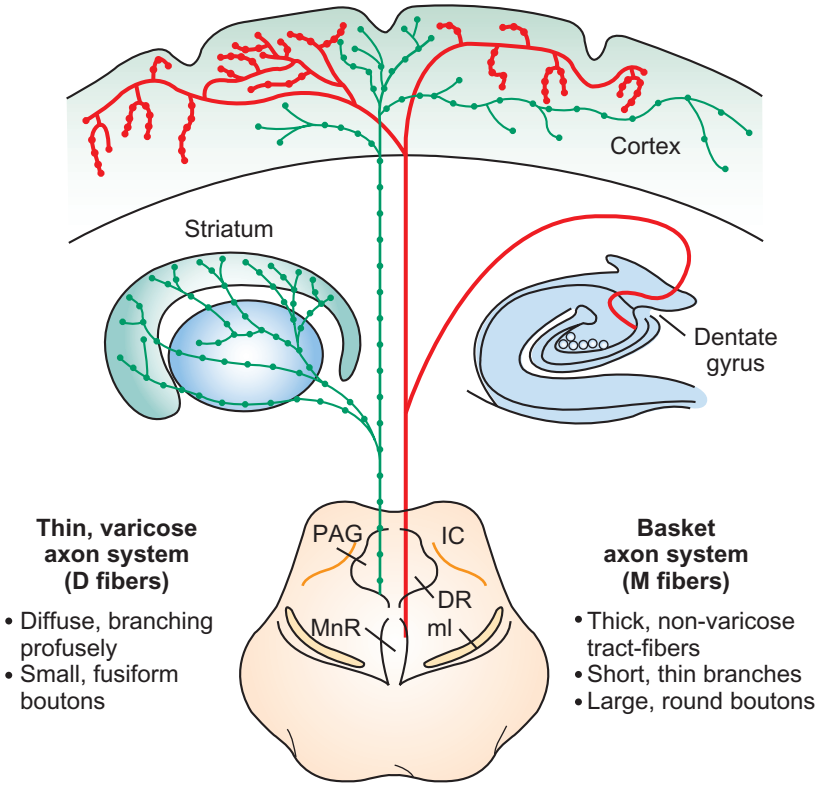


FIGURE 15-4 **Simplified diagram of the main features of the dual serotonergic system innervating the forebrain.** The fine varicose axon system (D fibers) arises from the dorsal raphe (DR) nucleus with fibers that branch profusely in their target areas. It is difficult to demonstrate the synaptic connections of these fibers, and therefore the incidence of synapses on these fibers is still being debated. The basket axon system (M fibers) arises from the median raphe (MnR) nucleus with thick, non-varicose axons, giving rise to branches with characteristic axons that appear beaded, with round or oval varicosities. These large terminals make well-defined synapses with target cells. *PAG*, periaqueductal gray matter; *IC*, inferior colliculus; *ml*, medial lemniscus. (With permission from Tork, 1990)

The other raphe nuclei, B_1 to B_4, are more caudally situated (mid-pons to caudal medulla) and contain a smaller number of serotonergic cells. These cell body groups give rise to serotonergic axons that project within the brainstem and to the spinal cord (Figure 15-2). The spinal cord receives a strong serotonergic innervation. Three principal descending pathways have been described: (1) from the raphe magnus nucleus (B_3) to laminae I and II of the dorsal horn; (2) from the raphe obscurus nucleus (B_2, B_4) to lamina IX of the ventral horn; and (3) from the rostral ventrolateral medulla and the lateral paragigantocellular reticular nucleus (B_3) to the interomediolateral cell column. Projections from the raphe pallidus nucleus (B_1) and raphe obscurus nucleus (B_2, B_4) provide serotonergic input to somatic motor nuclei, such as the motor trigeminal nucleus and the facial nucleus.

Afferent connections to the raphe nuclei include connections between the dorsal and median raphe nuclei, B_9, B_1, and B_3. Connections between the raphe nuclei have been described by retrograde tracing techniques using horseradish peroxidase and wheat germ agglutinin. Such innervation may have considerable physiological and/or pharmacological importance as serotonin released in the vicinity of serotonergic cell bodies regulates the firing of serotonergic neurons through the activation of somatodendritic autoreceptors. The raphe nuclei also receive input from other cell body groups in the brainstem such as the substantia nigra and ventral tegmental area (dopamine), superior vestibular nucleus (acetylcholine), locus coeruleus (norepinephrine), and nucleus prepositus hypoglossi and nucleus of the solitary tract (epinephrine). Other afferents include neurons from the hypothalamus, cortex, and limbic forebrain structures such as the amygdala (Molliver, 1987; Hensler, 2006).

The amino acid L-tryptophan serves as the precursor for the synthesis of 5-HT

The synthesis and primary metabolic pathways of 5-HT are shown in Figure 15-5. The initial step in the synthesis of serotonin is the facilitated transport of the amino acid L-tryptophan from blood into brain. The primary source of tryptophan is dietary protein. Other neutral amino acids, such as phenylalanine, leucine and methionine, are transported by the same carrier into the brain. Therefore, the entry of tryptophan into brain is not only related to its concentration in blood, but is also a function of its concentration in relation to the concentrations of other neutral amino acids. Consequently, lowering the dietary intake of tryptophan while raising the intake of the amino acids

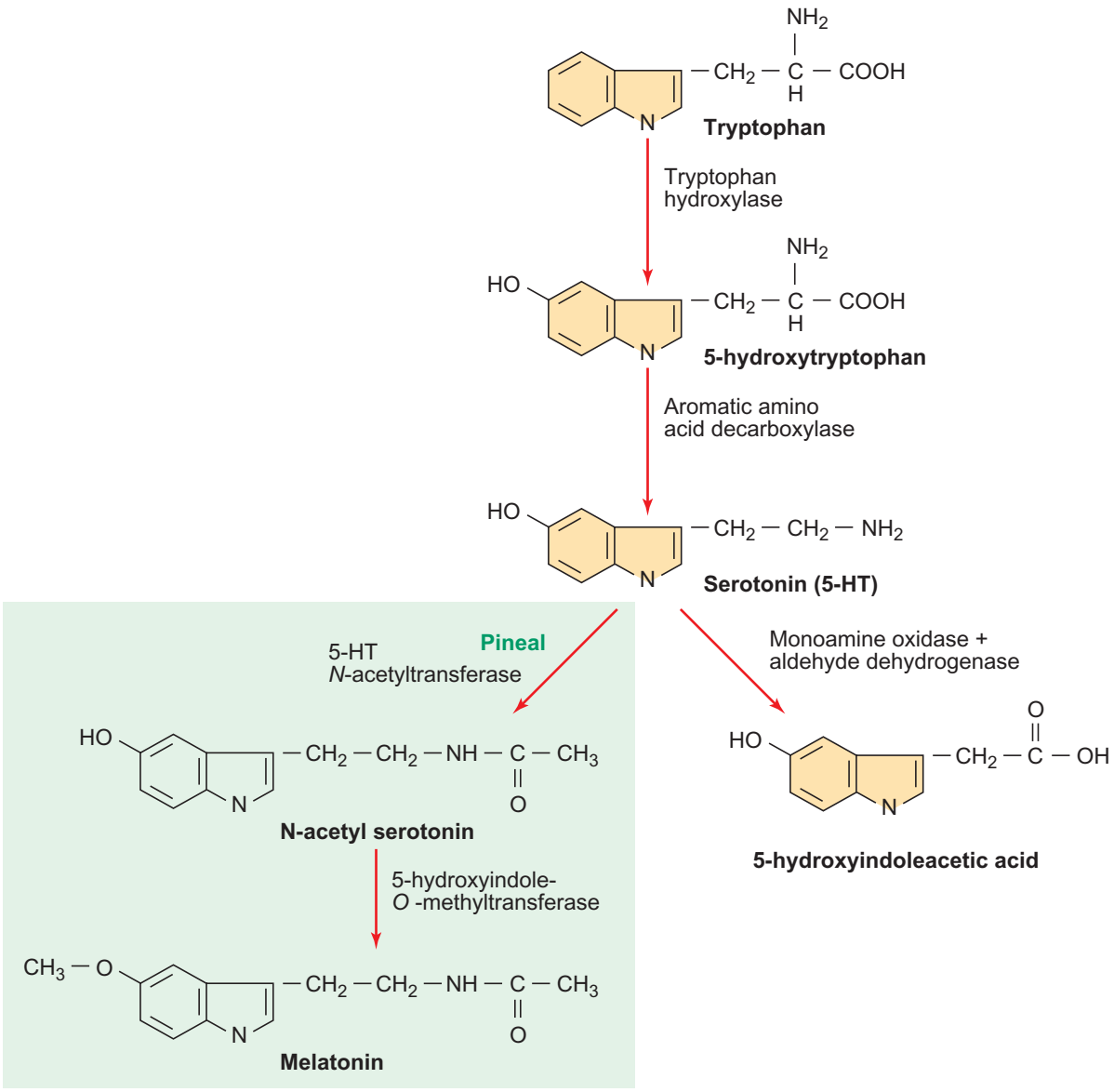


FIGURE 15-5 **The biosynthesis and catabolism of serotonin.** Not only does 5-HT have important physiological effects of its own, but also it is the precursor of the hormone melatonin. Note that in the pineal gland, which lies "outside" the blood–brain barrier, serotonin is converted enzymatically to the hormone melatonin (5-methoxy-N-acetyltryptamine). The synthesis and secretion of melatonin is markedly influenced by the light–dark cycle. A circadian rhythm of melatonin secretion persists in animals housed in continuous darkness. Thus, melatonin synthesis is turned on by an endogenous "clock," located within the SCN of the hypothalamus, with the daily rhythm normally being entrained to the day–night, light–dark cycle (Reiter et al., 2010). Melatonin, which provides circadian and seasonal timing cues through activation of G protein–coupled receptors (Dubocovich et al., 2003), has been implicated not only in the regulation of biological rhythms, but also in sleep and affective disorders.

with which it competes for transport into brain lowers the content of 5-HT in brain and changes certain behaviors associated with 5-HT function. This strategy for lowering the brain content of 5-HT has been used clinically to evaluate the importance of brain 5-HT in the mechanism of action of psychotherapeutic drugs.

Serotonergic neurons contain the enzyme L-tryptophan-5-monooxygenase (EC 1.14.16.4), more commonly termed tryptophan hydroxylase, which converts tryptophan to 5-hydroxytryptophan (5-HTP) (Figure 15-5). Tryptophan hydroxylase contains 444 amino acids, corresponding to a molecular weight of about 51,000 Daltons. In brain, this enzyme is synthesized in serotonergic cell bodies of the raphe nuclei and is found only in cells that synthesize 5-HT. Therefore its distribution in brain is similar to that of 5-HT itself. The Km of tryptophan hydroxylase for tryptophan is approximately 30-60 mM, a concentration comparable to that of tryptophan in brain. If the concentration of tryptophan in serotonergic neurons is assumed to be comparable to that in whole brain, the enzyme would not be saturated with substrate, and the

formation of 5-HT in brain would be expected to rise as the brain concentration of tryptophan increases. This has been found to occur in response to raising the dietary intake of tryptophan specifically.

There are two isoforms of tryptophan hydroxylase, referred to as Tph1, expressed in the periphery, and Tph2, expressed exclusively in brain. Evidence for the existence of isoforms of tryptophan hydroxylase was based on experiments conducted in mice genetically deficient in tryptophan hydroxylase, which surprisingly expressed normal amounts of 5-HT in brain but lacked 5-HT in the periphery. A second tryptophan hydroxylase isoform (Tph2) was cloned from these knockout animals, which was different from the known tryptophan hydroxylase (Tph1) (Walther et al., 2003). The existence of two tryptophan hydroxylase isoforms may offer new opportunities for affecting central or peripheral 5-HT systems by designing drugs that target the expression and activity of Tph1 or Tph2. In addition, the duality of 5-HT synthesis changes our approach to peripheral models of psychiatric disorders. This is particularly important because of efforts to find meaningful correlations between peripheral levels of 5-HT (and its metabolites) and 5-HT function in the brain.

The other enzyme involved in the synthesis of 5-HT, aromatic L-amino acid decarboxylase (AADC) (EC 4.1.1.28), is a soluble pyridoxal-5′-phosphate dependent enzyme that converts 5-HTP to 5-HT (Figure 15-5). It has been demonstrated that administration of pyridoxine increased the rate of synthesis of 5-HT in monkey brain, as revealed using position emission tomography. This presumably reflects a regulatory effect of pyridoxine on AADC activity, and raises the interesting issue of the use of pyridoxine supplementation in situations associated with 5-HT deficiency.

AADC contains 380 amino acids, corresponding to a molecular weight of about 54,000 Daltons, and is present not only in serotonergic neurons but also in catecholaminergic neurons where it converts 3,4-dihydroxyphenylalanine (DOPA) to dopamine (see Chap. 14). However, different pH optima or concentrations of substrate or cofactor are required for optimum activity of the enzyme in brain homogenates when using either 5-HTP or DOPA as the substrate. In cells transfected with the cDNA for AADC, AADC decarboxylates either DOPA or 5-HTP. The mRNA for the enzyme is present in both in serotonergic cells in the dorsal raphe nucleus and in catecholaminergic cells in brain regions containing catecholaminergic soma (Eaton et al., 1993). Taken together, these results and others support the idea that the decarboxylation of both DOPA and 5-HTP are catalyzed by the same enzyme.

Because AADC is not saturated with 5-HTP under physiological conditions, (i.e., the concentration of 5-HTP is much less than the enzyme's Km of 10 μM), it is possible to raise the content of 5-HT in brain not only by increasing the dietary intake of tryptophan but also by raising the intake of 5-HTP. This procedure, though, results in the formation of 5-HT in cells that would not normally contain it (e.g., catecholaminergic neurons) because of the nonselective nature of AADC.

The initial hydroxylation of tryptophan, rather than the decarboxylation of 5-HTP, appears to be the rate-limiting step in serotonin synthesis. Therefore, the inhibition of this reaction results in a marked depletion of the content of 5-HT in brain. The enzyme inhibitor most widely used in experiments is parachlorophenylalanine (PCPA). *In vivo,* PCPA irreversibly inhibits tryptophan hydroxylase, presumably by incorporating itself into the enzyme to produce an inactive protein. This results in a long-lasting reduction of 5-HT levels. Recovery of enzyme activity and 5-HT biosynthesis requires the synthesis of new enzyme. Marked increases in mRNA for tryptophan hydroxylase are found in the raphe nuclei 1–3 days after administration of PCPA (Cortes et al., 1993).

The synthesis of 5-HT can increase markedly under conditions requiring more neurotransmitter

Plasticity is an important concept in neurobiology. In general, this refers to the ability of neuronal systems to conform to either short- or long-term demands placed upon their activity or function. One of the processes contributing to neuronal plasticity is the ability to increase the rate of neurotransmitter synthesis and release in response to increased neuronal activity. Serotonergic neurons have this capability; the synthesis of 5-HT from tryptophan is increased in a frequency-dependent manner in response to electrical stimulation of serotonergic soma (Boadle-Biber, 1993). The increase in synthesis results from the enhanced conversion of tryptophan to 5-HTP and is dependent on extracellular calcium. It is likely that the increased 5-HT synthesis results in part from alterations in the kinetic properties of tryptophan hydroxylase, perhaps due to calcium-dependent phosphorylation of the enzyme by calmodulin-dependent protein kinase II or cyclic AMP dependent protein kinase (PKA).

Short-term requirements for increases in the synthesis of 5-HT can be met by processes that change the kinetic properties of tryptophan hydroxylase, such as phosphorylation, without necessitating the synthesis of more molecules of tryptophan hydroxylase. By contrast, situations requiring long-term increases in the synthesis and release of 5-HT result in the synthesis of tryptophan hydroxylase protein. For example, partial but substantial destruction (>60%) of central serotonergic neurons results in an increase in the synthesis of 5-HT in residual terminals. Although the increase in synthesis initially results from activation of existing tryptophan hydroxylase molecules, the increased synthesis of 5-HT seen weeks after the lesion results from more tryptophan hydroxylase being present in the residual terminals. An increase in tryptophan hydroxylase mRNA has been reported in residual serotonergic neurons of the raphe nuclei after partial lesioning, consistent with the idea of an increase in the synthesis of tryptophan hydroxylase molecules in residual neurons.

As with other biogenic amine transmitters, 5-HT is stored primarily in vesicles and is released by an exocytotic mechanism

Peripheral sources of monoamine-containing cells have been utilized to study the properties of storage vesicles, e.g., chromaffin cells of the adrenal medulla for catecholamines and parafollicular cells of the thyroid gland for 5-HT (Tamir et al., 1996). In some respects, the vesicles that store 5-HT resemble

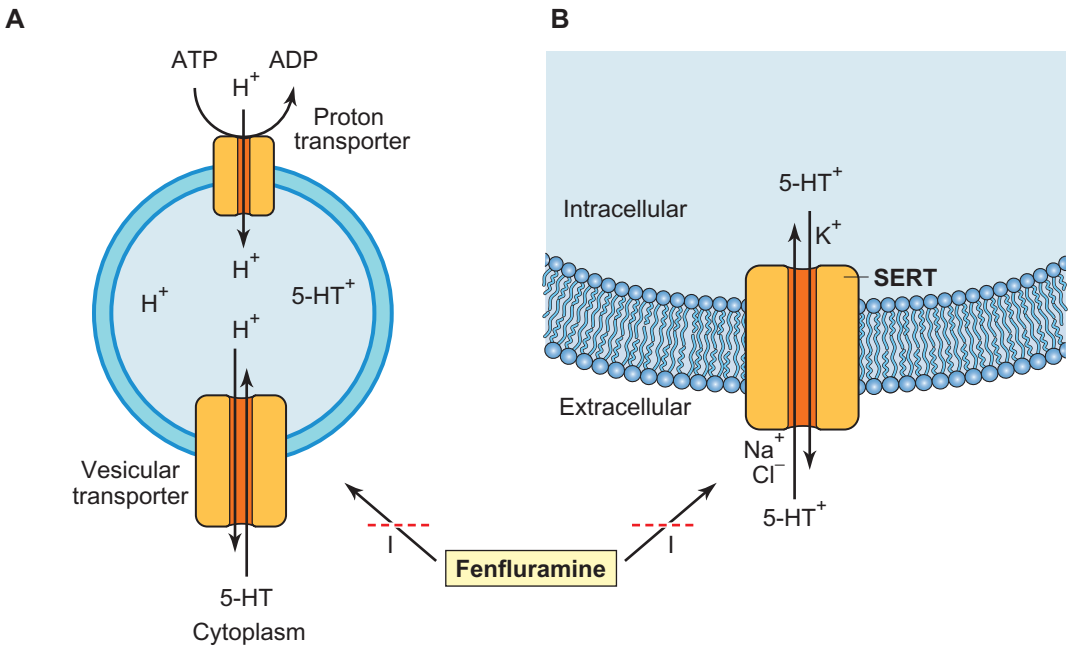


FIGURE 15-6 **The substituted amphetamine fenfluramine inhibits the transport of 5-HT by both (A) the vesicular transporter and (B) the serotonin transporter (SERT).** Substituted amphetamines such as fenfluramine and MDMA stimulate the release of 5-HT from serotonergic terminals. These drugs block the vesicular transporter and disrupt the proton gradient across the vesicle membrane. The increase in intracellular 5-HT favors the release of 5-HT by the reverse action of the SERT. These drugs also act as substrates for the SERT so as to inhibit the transport of 5-HT into cells.

those that store catecholamines. For example, drugs such as reserpine and tetrabenazine, which inhibit the activity of the transporter localized to the vesicular membrane, deplete the brain content of 5-HT as well as catecholamines. Storage of 5-HT in vesicles requires its active transport from the cytoplasm. The vesicular "transporter" uses the electrochemical gradient generated by a vesicular H^+-ATPase to drive transport, such that a cytoplasmic amine is exchanged for a luminal proton, i.e., the uptake of 5-HT is coupled to the efflux of H^+ (Figure 15-6A).

Drugs that inhibit the vesicular transporter do not generally block the serotonin transporter, (SERT) and *vice versa*. However, two drugs that have effects at both the vesicular transporter and the SERT are MDMA (Ecstasy) and the anorexic agent fenfluramine. Fenfluramine and MDMA inhibit the vesicular transporter directly by competing for its substrate-binding site. These agents also dissipate the transmembrane pH gradient to further inhibit 5-HT uptake into the vesicle (Figure 15-6A). The effects of these drugs on the vesicles storing 5-HT raises cytoplasmic 5-HT and facilitates SERT-mediated efflux of 5-HT. Both fenfluramine and MDMA act as substrates for the SERT and not only inhibit the transport of 5-HT into the cell but also facilitate its outward transport by the SERT (Figure 15-6B). The consequence of such pharmacological actions is the stimulation of the release of 5-HT by a calcium-independent (i.e., non-exocytotic) process. In contrast to exocytotic release, this release process is not modulated by terminal auto- or hetero-receptors. The release of 5-HT caused by drugs such as fenfluramine is prevented by drugs that block the SERT.

Vesicles storing 5-HT exhibit some differences from those storing catecholamines. In contrast to catecholamine-containing vesicles, there is virtually no ATP in serotonin vesicles. Also, serotonergic synaptic vesicles, but not chromaffin granules, contain a specific protein that binds 5-HT with high affinity in the presence of Fe^{++}. This serotonin-binding protein (SPB) is packaged in secretory vesicles along with 5-HT, which probably accounts for the observation that newly taken up [^{3}H]-5-HT is rapidly complexed with this protein in brain *in situ*. SPB is secreted along with 5-HT by a calcium-dependent process.

There is considerable evidence that the release of 5-HT occurs by exocytosis, i.e., by the discharge from the cell of the entire contents of individual storage vesicles. First, 5-HT is sufficiently ionized at physiological pH that it does not cross plasma membranes by simple diffusion. Second, most intraneuronal 5-HT is contained in storage vesicles, and other contents of the vesicle including SPB are released together with serotonin. By contrast, cytosolic proteins do not accompany electrical stimulation-elicited release of 5-HT. Third, the depolarization-induced release of 5-HT occurs by a calcium-dependent process; indeed, it appears that the influx of extracellular calcium with or without membrane depolarization can increase the release of 5-HT. Calcium has been reported to stimulate the fusion of vesicular membranes with the plasma membrane.

Serotonin release is regulated in part by the firing rate of serotonergic soma in the raphe nuclei. Numerous studies utilizing a variety of techniques have revealed that an increase in raphe cell firing enhances the release of 5-HT in terminal fields. The opposite effect is observed when raphe cell firing decreases. This means that drugs that change the firing rate of serotonergic soma modify the release of 5-HT as well. Important targets for such drugs are somatodendritic autoreceptors, which, as is discussed later, are 5-HT_{1A} receptors

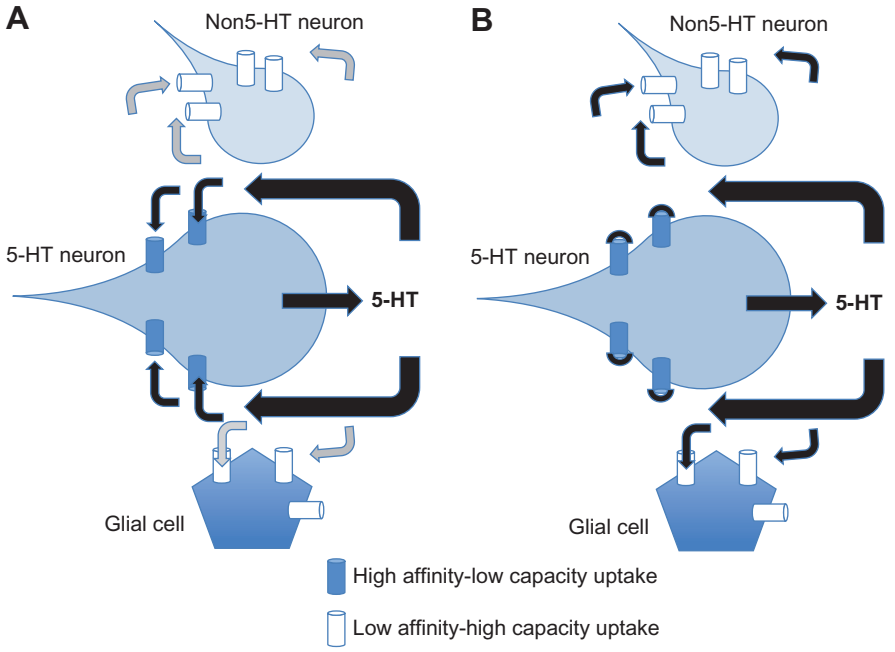


FIGURE 15-7 **The synaptic effects of 5-HT are terminated by transporter proteins.** (**A**) The uptake of 5-HT is mediated primarily by serotonin transporter (SERT) localized to serotonergic neurons. 5-HT reuptake by the SERT is a high-affinity/low-capacity process. The activity of 5-HT in the extracellular space is also terminated by its reuptake by various transporters into other neurons and into glia. 5-HT reuptake by these transporters, i.e., organic cation transporter, plasma membrane monoamine transporter, the norepinephrine transporter and dopamine transporter, is a low-affinity/high-capacity process. **(B)** When SERT function is attenuated, for example as a result of pharmacological blockade or due to a polymorphism in the SERT gene, these low-affinity/high-capacity transporters for 5-HT play an important role in regulating extracellular 5-HT concentrations (Daws, 2009).

(see Figure 15-8). Activation of these receptors by 5-HT or exogenous agonist slows the rate of firing of serotonergic soma. As discussed below, serotonergic autoreceptors on terminals appear to be either the 5-HT_{1B} or 5-HT_{1D} subtype, depending on the species. Activation of these receptors decreases the synthesis and release of 5-HT. However, in contrast to the activation of somatodendritic autoreceptors, such effects are not due to decreases in the firing rate of serotonergic soma.

The activity of 5-HT in the synapse is terminated primarily by its reuptake into serotonergic terminals

The synaptic effects of many neurotransmitters, including 5-HT, are terminated by the binding of these molecules to specific transporter proteins. The uptake of 5-HT is mediated primarily by serotonin transporter (SERT). 5-HT reuptake by the SERT is a high affinity-low capacity process (previous termed "uptake-1"). The SERT is located on serotonergic neurons along the axon rather than within the presynaptic area. The mRNA for the SERT has been localized in brain exclusively to the serotonergic cells in the raphe nuclei. Activity of the SERT regulates the concentration of 5-HT in the extracellular space, thereby influencing synaptic transmission.

The high-affinity uptake system for 5-HT, primarily due to the action of the SERT, is saturable, with a Km value for 5-HT of approximately 0.2-0.5μM. The uptake of 5-HT is an active process that is temperature dependent and has an absolute requirement for external Na^+ and Cl^-; it is inhibited by metabolic inhibitors as well as by inhibitors of Na^+/K^+ ATPase activity. From these and other data, it has been inferred that the energy requirement for 5-HT uptake is not directly used to transport 5-HT but rather is necessary to maintain the gradient of Na^+ across the plasma membrane upon which 5-HT uptake is dependent. The current model of transport has one Na^+, one Cl^- and one protonated 5-HT binding to the transporter extracellularly to form a quaternary complex that subsequently undergoes a conformational change to release the neurotransmitter and the ions into the cytoplasm. The conformational change may involve the "opening" of a pore formed by some portion of the transmembrane domains of the SERT (see below). In the cytoplasm, K^+ associates with the SERT to promote the reorientation of the unloaded carrier for another transport cycle (Figure 15-6B).

The activity of 5-HT in the extracellular space is also terminated by its reuptake by various transporter proteins into glia and other neurons (Figure 15-7). For example, although mRNA for the SERT has not been detected in glia, primary cultures of astrocytes in vitro can take up 5-HT. Such 5-HT uptake into glia and into other non-serotonergic neurons is due to other recently discovered transporters such as organic cation transporter (OCT) and plasma membrane monoamine transporter (PMAT), as well as related transporters such as the norepinephrine transporter (NET) and dopamine transporter (DAT). All of these transporters are capable of removing 5-HT, as well as their native substrates from extracellular fluid in the brain. 5-HT reuptake by these transporters

is a low affinity-high capacity process (previously termed "uptake-2"). When SERT function is compromised, for example genetically or pharmacologically, these low-affinity/high-capacity transporters for 5-HT play an important role in regulating extracellular 5-HT concentrations (Figure 15-7) (Daws, 2009).

The cloning, sequencing and expression of several transporter proteins, including those for 5-HT, has aided considerably in understanding structure/function relationships of transporter proteins (Zahniser & Doolen, 2001). The cDNA for the SERT isolated from rat brain predicts a protein containing 630 amino acids with a molecular weight of about 68,000 Daltons. The putative structure of the SERT has 12 transmembrane domains (TMDs), with both the amino- and carboxy-termini being intracellular, and a large extracellular loop containing glycosylation sites connecting TMDs 3 and 4. The serotonin transporter exhibits about 50% absolute homology with the transporters for norepinephrine (NET) and dopamine (DAT), with the greatest homology being found in TMDs 1 and 2 and in TMDs 4–8. The least-conserved regions are the intracellular amino- and carboxy-terminal tails. Glycosylation seems necessary for optimal stability of the transporter in the membrane, but not for 5-HT transport or ligand binding. There are also numerous potential sites of phosphorylation by protein kinase A (PKA) and protein kinase C (PKC) on the human SERT. The predicted structure of the SERT is similar to the predicted structure of other cloned neurotransmitter transporters and quite distinct, for example, from the seven-transmembrane-domain structure of G protein–coupled receptors. These transporters are considered members of the Na^+ and Cl^- dependent neurotransporter family, distinct from the vesicular transporter family described earlier. All members of this family are fragmented by multiple introns, raising the possibility of multiple transcripts by alternative RNA processing.

Although there is structural homology among the transporters for 5-HT, norepinephrine (NE) and dopamine (DA), some drugs exhibit great selectivity at inhibiting the activity of just one of these proteins. For example, the selective NE reuptake inhibitor reboxetine is 150 fold more potent for inhibiting NE transport than that of 5-HT. By contrast, the selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, sertraline and paroxetine, are 25–100 times more potent in inhibiting the uptake of 5-HT than the uptake of NE. There are also drugs such as cocaine that are nonselective inhibitors of all three transporters.

It has been known for over 25 years that many of the tricyclic antidepressants (TCAs), e.g., imipramine and amitriptyline, are potent inhibitors of both NE and 5-HT reuptake. Some tricyclic antidepressants, e.g., desipramine, inhibit the uptake of NE much more potently than the uptake of 5-HT. Thus, it was unclear for some time whether the inhibition of 5-HT uptake played any role in the antidepressant action of those TCAs that possessed this pharmacological property. Recently, though, effective antidepressants such as fluoxetine, paroxetine and sertraline have been marketed and these SSRIs are much more potent inhibitors of the uptake of 5-HT than of that of NE (Figure 15-8). Thus, selective inhibition of the uptake of either NE or 5-HT can result in an antidepressant effect.

The SSRIs, which were initially developed as antidepressants, are now the most widely used agents in the treatment of many additional neuropsychiatric disorders (e.g., eating disorders, anxiety, obsessive–compulsive disorder) (Figure 15-8). Clomipramine, a TCA that is somewhat selective *in vivo* as an inhibitor of 5-HT uptake, also produces clinically significant amelioration of the symptoms associated with obsessive–compulsive disorder (OCD). Thus, the inhibition of 5-HT uptake not only has an antidepressant effect, but also reduces the symptoms of anxiety disorders, including OCD. This clinical effect is not found with drugs such as desipramine that selectively inhibit the uptake of NE.

Acute and chronic regulation of SERT function provides mechanisms for altering synaptic 5-HT concentrations and neurotransmission

Long-term exposure to drugs that block the SERT have been shown to downregulate the SERT. A number of studies have shown that chronic administration of SSRIs produces a reduction in SERT activity and expression. In postmortem samples of dorsal raphe nucleus from cocaine users, SERT binding sites are reduced, suggesting a downregulation of the SERT with chronic cocaine exposure. In addition to this long-term regulation of SERT function by drugs that block the SERT, there is a more dynamic, short-term regulation of SERT function by substrate exposure, membrane potential changes, and presynaptic receptor activation/inhibition. Depolarization of the plasma membrane is associated with reduced transport velocity, whereas hyperpolarization results in enhance transport. The transient inhibition of uptake during neuronal depolarization may allow 5-HT to diffuse away from the presynaptic terminal to interact with postsynaptic receptors. Substrate occupancy and/or transport activity promotes retention of the SERT on the cell surface, thereby maintaining SERT function (Zahniser & Doolen, 2001). Acute presynaptic receptor–mediated regulation of SERT function has been recently demonstrated in experiments using *in vivo* chronoamperometry; the activation of 5-HT_{1B} terminal auto receptors increases SERT activity (Daws et al., 2000).

The regulation SERT function can occur at the level of transcription or translation, or by posttranslational modifications (e.g., glycosylation or phosphorylation). Research carried out *in vitro* using cells that express the SERT has indicated that second-messenger systems, particularly those activating protein kinases, play a role in the regulation of SERT function. SERT gene expression is influenced by cyclic AMP-dependent pathways, and rapidly regulated by changes in intracellular calcium, treatment with calmodulin inhibitors, or by activation of protein kinase C (PKC) as well as nitric oxide (NO)/cyclic GMP pathways. Most of the changes in enzyme kinetics reflect change in maximal transport capacity (V_{max}) rather than changes in apparent affinity (K_m). Activation of PKC causes a loss of SERT protein on the cell surface. Thus, occupancy of the SERT by substrate (5-HT) may induce a conformational change of the transporter that either cannot be phosphorylated by PKC (thereby inhibiting PKC-induced internalization of the SERT), or enhances access of phosphatases (thereby reducing phosphorylation and internalization) (Zahniser & Doolen, 2001).

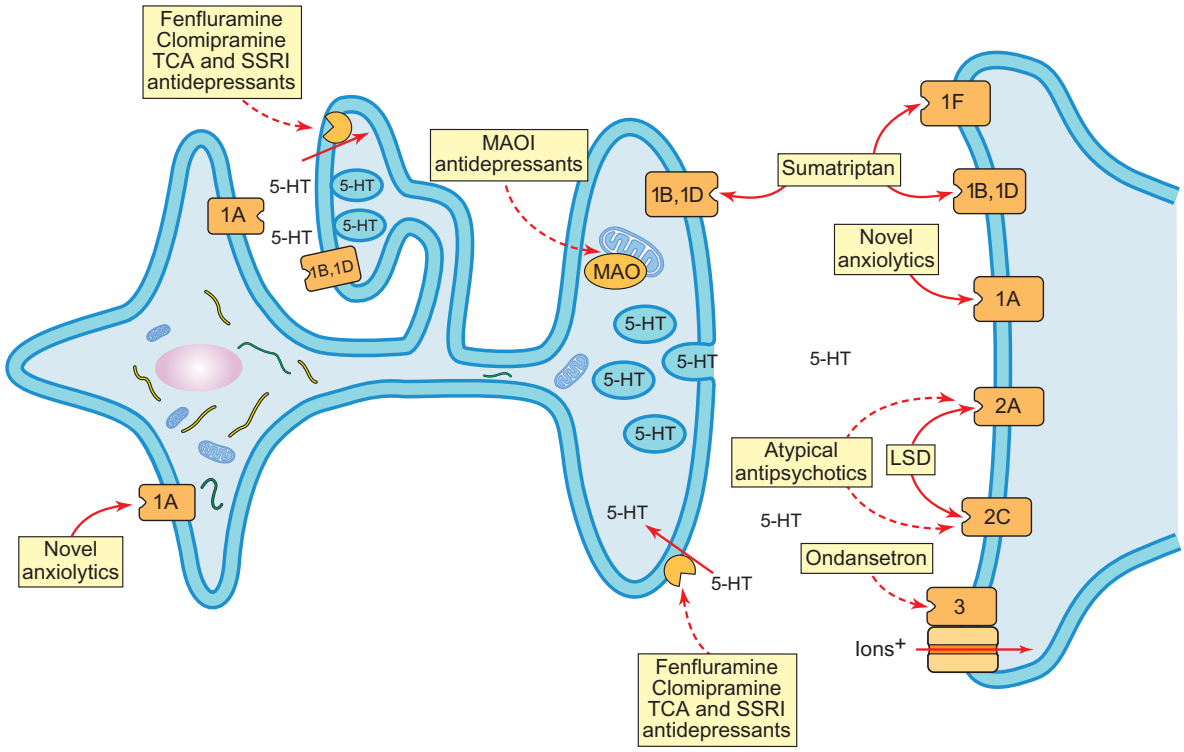


FIGURE 15-8 Serotonin neurons and receptors are targets for a wide variety of therapeutic drugs. Drugs that act as agonists to activate receptors are indicated by solid-line arrows, whereas antagonists or inhibitors are shown with broken-line arrows. The 5-HT_{1A} receptor acts as both the somatodendritic autoreceptor and a postsynaptic receptor; anxiolytic drugs such as buspirone are agonists at this receptor. Postsynaptic 5-HT_{1A} receptors are also potential targets of novel antipsychotic drugs. In terminal fields, the autoreceptor is either the 5-HT_{1B} or 5-HT_{1D} subtype; these receptors also function as postsynaptic receptors. The anti-migraine drug sumatriptan is an agonist at these receptors as well as the 5-HT_{1F} receptor. Selective 5-HT_{1F} receptor agonists are of great interest as potential anti-migraine drugs. Hallucinogenic drugs such as LSD are agonists at 5-HT_{2A} and 5-HT_{2C} receptors whereas atypical antipsychotic drugs such as clozapine and olanzapine are antagonists. The 5-HT_3 receptor, a ligand-gated ion channel, is blocked by drugs effective in the treatment of chemotherapy-induced nausea and emesis, such as ondansetron. Another important target for psychotherapeutic drugs is the serotonin transporter, which is blocked by drugs effective in the treatment of depression or obsessive–compulsive disorder (clomipramine). The enzyme responsible for the catabolism of serotonin, MAO, is inhibited by another class of antidepressants.

The SERT is encoded by a single gene located on the long arm of chromosome 17. The human serotonin transporter gene possesses a functional polymorphism within the promotor region, which affects the transcription and therefore expression of the gene. The long (l/l) variant, compared to the short (s/s) or heterozygous (s/l) forms, is associated with greater SERT expression and function. Alterations in the expression and function of the SERT would be expected to affect 5-HT neurotransmission. The discovery of these polymorphisms has led to much research examining the potentially exciting association of these polymorphisms with different personality traits, a variety of neuropsychiatric disorders, and differing responses to drugs, in particular the SSRIs (Murphy & Lesch, 2008). For example, SSRIs are not effective in many patients carrying SERT polymorphisms that impair the function of this high-affinity transporter of 5-HT. Therefore targeting other transporters, which remove 5-HT with greater capacity, may be a better strategy for the treatment of psychiatric disorders wherein reduced 5-HT neurotransmission is thought to play a major role (Figure 15-7) (Daws, 2009).

The primary catabolic pathway for 5-HT is oxidative deamination by the enzyme monoamine oxidase

Monoamine oxidase (MAO) (E.C.1.4.3.4.) converts serotonin to 5-hydroxy-indoleacetaldehyde, and this product is oxidized by a NAD^+-dependent aldehyde dehydrogenase to form 5-hydroxyindoleacetic acid (5-HIAA) (see Figure 15-5). There are at least two isoenzymes of MAO, referred to as type A and type B. These isoenzymes are integral flavoproteins of outer mitochondrial membranes in neurons, glia, and other cells. Evidence for the existence of isoenzymes of MAO was based initially on differing substrate specificities and sensitivities to inhibitors of MAO. For example, 5-HT and NE are metabolized preferentially by type A MAO. Selective inhibitors of each form of MAO exist, e.g., clorgyline or moclobemide for type A and deprenyl for type B. Definitive proof of the existence of these two forms of MAO comes from the cloning of cDNAs encoding type A and type B MAO from human liver (Shih, 1991). The deduced amino acid sequences of type A and type B MAOs show about 70% homology and have masses of 59,700 Daltons and 58,800 Daltons, respectively.

The activity of the proteins expressed in recombinant cell systems resembles that of the endogenous enzymes from human brain, e.g., the expressed type A MAO prefers 5-HT as a substrate and is preferentially inhibited by clorgyline.

Several different techniques have been used to study the neuroanatomical localization of the two forms of MAO in brain, e.g., immunohistochemical techniques, and *in situ* hybridization histochemistry. It is of some interest that there is more type A enzyme than type B enzyme throughout rat brain, whereas human brain contains more type B MAO than type A. Interestingly, serotonergic cell bodies contain predominantly type B MAO so that serotonergic neurons contain the form of MAO (type B) that does not preferentially metabolize 5-HT. This has led to the hypothesis that type B MAO in serotonergic neurons prevents the cell from accumulating various natural substrates (e.g., dopamine) that could interfere with the storage, release, and uptake of 5-HT. Furthermore, treatment of rats with clorgyline, a selective inhibitor of type A MAO, raises the brain content of 5-HT and reduces the conversion of 5-HT to 5-HIAA in brain. Thus, 5-HT may well be oxidized preferentially by type A MAO *in vivo*, just as it is *in vitro*, even though serotonergic neurons do not contain much of this form of the enzyme.

Recently, techniques have been developed that permit the selective elimination or "knockout" of genes encoding specific proteins in mice. Using such methodology, mice that have either type A- or type B MAO knocked out have been developed (Cases et al., 1995). In the brains of mice deficient in type A MAO, the content of 5-HT is markedly elevated for about 12 days after birth and then slowly declines, reaching values comparable to those in normal mice after about seven months. In the type A MAO–deficient mice, the selective inhibitor of type B MAO, deprenyl, had a greater effect on serotonin metabolism than it did in normal mice. Such observations indicate that in the absence of type A MAO, the type B isoform can metabolize 5-HT *in vivo*. However, mice lacking the MAO B isoenzyme do not have elevated levels of 5-HT in brain. Of interest are the aggressive behaviors exhibited by the mice deficient in type A MAO, consistent with a postulated role of serotonergic neurons in human aggressive behaviors.

Another class of antidepressant drug is the monoamine oxidase inhibitors (MAOIs), e.g., phenelzine and tranylcypromine. These drugs are nonselective in that they irreversibly inhibit the activity of MAO type A and type B (Figure 15-8). Because MAO metabolizes biogenic amines such as 5-HT, DA, and NE, these neurotransmitters have been implicated in the mechanism of action of these drugs. Interestingly, studies have been carried out from which it was inferred that 5-HT is needed for SSRIs or MAOIs to produce a beneficial clinical response in depressed patients. Such data are consistent with the idea that drug-induced enhancement of serotonergic transmission can produce amelioration of depressive symptomatology.

In addition to classical synaptic transmission, 5-HT may relay information by volume transmission or paracrine neurotransmission

As expected, serotonergic terminals make the usual specialized synaptic contacts with target neurons and release 5-HT following nerve stimulation. In some areas of the mammalian CNS, however, there are sites where 5-HT is released and no evidence for synaptic specializations has been found. For example, it is difficult to demonstrate the synaptic contacts of fine varicose fibers, whereas large terminals make well-defined synapses with target cells. The percentage of 5-HT terminals associated with synaptic specializations appears to vary in particular brain regions. This may have important implications for the type of information processing in which 5-HT is involved in these brain areas. The appearance of specialized synaptic contacts suggests relatively stable and strong associations between a presynaptic neuron and its target. Conversely, the lack of synaptic specialization implies a dynamic and perhaps less specific interaction with target neurons. For example, neurotransmitter is released and then diffuses over some distance (as great as several hundred microns).

Consistent with the idea that a significant mode of transmission for 5-HT is through volume or extra-synaptic transmission is the observation that the SERT is localized perisynaptically and along axons and dendrites, and not confined to the terminals (Tao-Cheng & Zhou, 1999). Thus 5-HT may diffuse away from the site of release before its action is terminated by reuptake into serotonergic neurons. 5-HT_{1B} receptors, which function as both auto- and hetero-receptors in serotonergic terminal field areas, are also found predominantly at extrasynaptic and nonsynaptic sites (Riad et al., 2000). The fact that serotonergic neurons contain predominantly type B MAO, the form of MAO that does not preferentially metabolize 5-HT, is also consistent with the volume of extrasynaptic transmission of 5-HT. As discussed above, 5-HT is oxidized preferentially by type A MAO, even though serotonergic neurons do not contain this form of the enzyme. Thus 5-HT may have a paracrine or neuromodulatory role in brain, adjusting or tuning ongoing activity at synapses some distance from the serotonergic terminal.

5-HT may be involved in a wide variety of behaviors by setting the tone of brain activity in relationship to the state of behavioral arousal/activity

Serotonin has been implicated in practically every type of behavior, e.g., appetitive, emotional, motoric, cognitive, and autonomic. However, from a physiologic perspective, it is not clear whether 5-HT affects such behaviors specifically, or does so more generally by coordinating the activity of the nervous system, particularly to set the tone of activity in conjunction with the organism's level of arousal.

The primary body of data that has contributed to the view that 5-HT has a general effect on behavior by modulating the tone of nervous system activity comes from studies of the firing rate of serotonergic soma in raphe nuclei (Jacobs & Fornal, 1993). Under quiet waking conditions, serotonergic neurons display a slow, clock-like activity of about 1–5 spikes/sec, which shows a gradual decline as the animal becomes drowsy and enters slow wave sleep. A decrease in the regularity of firing accompanies this overall slowing of neuronal activity. During REM (rapid eye movement) sleep, the activity of these neurons becomes silent. In response to certain types of arousing stimuli, the firing rate of these serotonergic

neurons increases. Not surprisingly, such data led to the idea that the activity of serotonergic neurons is related to the level of behavioral arousal/activity. Such data have contributed also to the idea that the activity of serotonergic neurons is associated with motoric output, since atonia of the major skeletal muscle groups occurs during REM sleep. Oral–buccal motor activity, such as chewing, biting, licking or grooming, causes a marked increase in the firing rate of a subgroup of serotonergic soma that are also activated by somatosensory stimuli applied to the head, neck, and face. However, exposing a cat to environmental stressors such as a loud noise or a dog, although producing strong sympathetic activation and typical behavioral responses, does not alter the firing rate of serotonergic neurons. Thus, the type of motoric activity that activates serotonergic cell bodies seems to be repetitive, of the type mediated by central pattern generators. Furthermore, activation of serotonergic transmission inhibits information processing in afferent systems. From all such data, it has been suggested that the serotonergic neuronal system functions at the organismic level to integrate functions needed for the animal's behavioral output, i.e., facilitation of motor output with suppression of activity in sensory systems irrelevant to the ongoing behavior.

Perturbation of the 5-HT system can elicit changes in a wide variety of behaviors. Furthermore, drugs that target serotonergic neurons and their receptors are used to treat diseases such as depression, anxiety disorders and schizophrenia. Thus, 5-HT has been implicated in the regulation of many behaviors and physiological processes. The involvement of 5-HT in three areas—neuroendocrine function, circadian rhythms and feeding behavior—will be highlighted for illustrative purposes.

5-HT modulates neuroendocrine function

The hypothalamus secretes several releasing factors and release-inhibiting factors to control the secretion of hormones from the anterior pituitary gland. 5-HT is among the many neurotransmitters that participate in the hypothalamic control of pituitary secretion, particularly in the regulation of the secretion of adrenocorticotrophin (ACTH), prolactin and growth hormone. A direct synaptic connection between serotonergic terminals and corticotrophin-releasing hormone (CRH)-containing neurons in the paraventricular nucleus of the hypothalamus has been described. Precursors of 5-HT or drugs that enhance the effect of 5-HT increase CRH in portal blood and ACTH in plasma. In addition to effects at the hypothalamus, 5-HT may also have direct effects on the anterior pituitary to stimulate the release of ACTH, and at the level of the adrenal cortex to regulate release of corticosterone or cortisol. However, what role, if any, is played by 5-HT in regulating stress-induced elevations of CRH or the circadian periodicity of the HPA axis is unclear.

Measurement of these endocrine responses after administration of drugs that increase brain serotonin function provides one of the few methods currently available for assessing such function in humans. Precursors of 5-HT, releasing agents, reuptake inhibitors, and receptor agonists and antagonists have all been used to probe serotonergic function. For example, intravenous administration of the serotonin precursor L-tryptophan consistently increases plasma concentrations of prolactin and growth hormone, but not ACTH or cortisol. Fenfluramine (Figure 15-6) causes a dose-dependent increase in plasma prolactin. When administered to humans, 5-HT receptor agonists that stimulate 5-HT_{1A} or 5-HT_2 receptors also increase plasma ACTH, cortisol, prolactin, and perhaps growth hormone levels. The neuroendocrine response in humans to such agents has been used clinically to assess the functioning of the central serotonergic system in patients with psychiatric disorders.

5-HT modulates circadian rhythmicity

Serotonin also appears to be involved in the regulation of circadian rhythms. The suprachiasmatic nucleus (SCN) of the hypothalamus generates electrophysiological and metabolic cycles that repeat approximately every 24 hours. When isolated *in vitro*, the SCN continues to produce 24-hour rhythms in metabolism, vasopressin secretion, and spontaneous electrical activity, indicating that circadian time-keeping functions and pacemaker activity are endogenous characteristics of the SCN. Ordinarily, this rhythm is synchronized or entrained to the environmental photoperiod, also about 24 hours. A serotonergic contribution to circadian rhythm regulation has been postulated because the SCN receive very dense serotonergic innervation from the midbrain raphe nuclei. In addition, there is a serotonergic innervation to the intergeniculate leaflet, an area of brain through which photic information indirectly accesses the SCN.

Serotonin appears to function as an inhibitory transmitter that modulates the effects of light on circadian rhythmicity. Direct application of 5-HT or receptor agonists to the SCN block light-induced phase shifts during the subjective night but cause phase advances during the subjective day. Such agents inhibit the excitatory effect of light, measured electrophysiologically, in either the SCN or the lateral geniculate complex. The nonselective 5-HT agonist quipazine has been shown to reset or shift the rhythm of spontaneous electrical activity of single cells recorded extracellularly in SCN isolated in brain slices.

Lesions of serotonergic neurons in laboratory animals have been reported by some, but not all, investigators to disrupt locomotor rhythms or to result in the loss of the daily rhythm of corticosterone. Recent results show that in the hamster the median raphe nucleus projects to the SCN whereas the dorsal raphe nucleus innervates the intergeniculate leaflet (IGL); furthermore, the serotonergic innervation to the SCN and not the IGL is necessary for the photic entrainment of locomotor activity (Meyer-Bernstein & Morin, 1996). It appears then, that the SCN circadian pacemaker or clock is modulated by stimulation of serotonergic receptors in the SCN and that serotonergic projections to the SCN may modulate the phase of the SCN in intact animals.

5-HT modulates feeding behavior and food intake

Pharmacological studies primarily have contributed to the idea that 5-HT has an inhibitory effect on feeding behavior. Drugs that either directly or indirectly activate postsynaptic

5-HT receptors decrease food consumption whereas agents that inhibit serotonergic transmission increase food intake. Precisely how this occurs is controversial, with claims that 5-HT governs the selection of macronutrients in the diet, or influences responses to the taste qualities of food, or modulates gastric activity to reduce feeding. Perhaps the most comprehensive and enduring view is that enhanced serotonergic activity enhances satiety, particularly by increasing the rate of satiation and prolonging the state of satiety (Simansky, 1996).

Fenfluramine, originally the racemate and more recently the d-isomer, has been the prototypical drug for studying serotonergic mechanisms in feeding behavior. As mentioned previously, fenfluramine elicits the release of 5-HT and inhibits its reuptake (see Figure 15-6). Fenfluramine decreases meal size, the rate of eating, and eating between meals. This is probably related to its ability in humans to decrease the sensation of hunger and to increase the feeling of "fullness." Serotonin reuptake inhibitors such as fluoxetine and serotonin precursors mimic these effects of fenfluramine. Fenfluramine's effects on feeding behavior are blocked by the nonselective serotonin receptor antagonist metergoline, the 5-$HT_{2A/2C}$ antagonist ritanserin and the 5-$HT_{1A/1B}$ antagonist cyanopindolol.

Multiple mechanisms in brain appear to be responsible for the effects of serotonergic drugs on satiety, e.g., postsynaptic 5-HT_{1B} receptors are involved in regulating the size of meals but 5-HT_{2C} receptors influence the rate of eating. Much of the weight loss produced by fenfluramine has been attributed to the direct activation of 5-HT_{2C} receptors in the central nervous system via the active desmethyl-metabolite of fenfluramine, norfenfluramine. Mice lacking functional 5-HT_{2C} or 5-HT_{1B} receptors are obese and resistant to d-fenfluramine's effects on feeding. 5-HT_6 receptors may also play an important role in feeding and body weight regulation. Woolley and colleagues (Woolley et al., 2001) found that reducing 5-HT_6 receptor function either by intracerebroventricular administration of an antisense oligonucleotide or systemic administration of the putative 5-HT_6 receptor antagonist Ro 04-6790 enhanced cognitive performance, and also reduced food intake and body weight. When on a high-fat diet, 5-HT_6 knockout mice eat less and gain less weight than wild-type mice (Frassetto et al., 2008).

Because obesity continues to be a serious health problem worldwide, there is great interest in the development of effective and safe anorexic agents. Fenfluramine and the more active enantiomer dexfenfluramine were considered to be among the most effective of weight loss agents before they were removed from the market due to increased incidence of cardiac valve defects and pulmonary hypertension in patients taking these drugs. Because the anorexic effects of fenfluramine are attributed to activation of 5-HT_{2C} receptors in the central nervous system, the 5-HT_{2C} receptor remains an attractive target for drug development. However, it will be necessary for these new drugs to be selective for 5-HT_{2C} receptors to avoid activation of additional serotonin receptors responsible for the serious unwanted side effects of fenfluramine, i.e., 5-HT_{2A} and 5-HT_{2B}, which likely mediated the heart valve hypertrophy, or 5-HT_{1B} receptors responsible for the development of pulmonary hypertension (Miller, 2005).

SEROTONIN RECEPTORS

Pharmacological and physiological studies have contributed to the definition of the many receptor subtypes for serotonin

The initial suggestion that there might be more than one type of receptor for serotonin came from experiments of Gaddum and Picarrelli in 1957. Using the isolated guinea pig ileum, they demonstrated that only a portion of the contractile response to 5-HT was blocked by high concentrations of morphine. The remainder of the response to 5-HT was blocked by low concentrations of dibenzyline (phenoxybenzamine). In the presence of maximally effective concentrations of dibenzyline, the remaining contractile response elicited by 5-HT was blocked by low concentrations of morphine. They speculated that there were two different receptors for 5-HT in the ileum, one blocked by morphine (termed the M receptor) and one blocked by dibenzyline (termed the D receptor). The D receptor was thought to be on the smooth muscle of the ileum whereas the M receptor was considered to be on ganglia or nerves within the muscle.

In the 1970s, the development of radioligand binding assays furthered our understanding of subtypes of receptors for 5-HT. Initially, a number of radioligands, such as [^{3}H]-5-HT, [^{3}H]-LSD and [^{3}H]-spiroperidol, were used to label sites related to 5-HT receptors. In 1972, Farrow and Van Vunakis observed high-affinity, stereospecific binding of [^{3}H]-LSD in cortex that was inhibited more potently by serotonin than by other neurotransmitters. Peroutka and Snyder in 1979 demonstrated the presence of two classes of serotonin receptors in brain. Binding sites that were labeled with high affinity by [^{3}H]-5-HT were designated the 5-HT_1 receptor; binding sites labeled with high affinity by [^{3}H]-spiroperidol were termed the 5-HT_2 receptor (Peroutka & Snyder, 1979).

The binding of [^{3}H]-5-HT to 5-HT_1 receptors was shown to be displaced by spiperone in a biphasic manner, suggesting that what was termed the 5-HT_1 receptor might be a heterogeneous population of receptors. The [^{3}H]-5-HT binding site that showed high affinity for spiperone was termed the 5-HT_{1A} subtype, whereas the component of [^{3}H]-5-HT binding that showed low affinity for spiperone was called the 5-HT_{1B} subtype. The different neuroanatomical localization of these two 5-HT binding sites, as demonstrated by quantitative autoradiography, was further evidence that these were distinct subtypes of receptor for 5-HT. For example, the 5-HT_{1A} subtype was shown to be present in high density in the hippocampus; the 5-HT_{1B} subtype was shown to be present in high density in the globus pallidus and substantia nigra. A high density of binding sites for [^{3}H]-5-HT were also found in the choroid plexus. These [^{3}H]-5-HT binding sites were termed the 5-HT_{1C} subtype as they did not show the pharmacological characteristics used to classify the 5-HT_{1A} or 5-HT_{1B} binding site, or the 5-HT_2 binding site. Subsequently, a fourth binding site for [^{3}H]-5-HT was identified in bovine brain and was called the 5-HT_{1D} receptor. The 5-HT_{1D} receptor was identified by pharmacological criteria only in brains of species devoid of the 5-HT_{1B} receptor such as pig, cow, guinea pig and human.

Bradley and associates in 1986 proposed a classification scheme with three major types of receptors for serotonin, using

pharmacological criteria and functional responses primarily in peripheral tissues. The receptors were called "5-HT_1-like," 5-HT_2 and 5-HT_3. The development of potent and selective antagonists of the 5-HT_2 receptor, such as ketanserin, facilitated the assignment of certain effects mediated by 5-HT to the 5-HT_2 receptor. The D receptor of Gaddam and Picarelli, originally described in guinea pig ileum, and the 5-HT_2 receptor were shown to be pharmacologically indistinguishable. The M receptor of Gaddum and Picarelli, which is pharmacologically distinct from all of the binding sites associated with 5-HT receptors just described, was renamed the 5-HT_3 receptor. The development of potent selective antagonists and an agonist, 2-methyl-5-HT, provided useful tools for the pharmacological characterization of 5-HT_3 receptors.

The application of techniques used in molecular biology to the study of 5-HT receptors led to the rapid discovery of additional 5-HT receptor subtypes and furthered our understanding of the structure and function of 5-HT receptors

The first 5-HT receptor to be cloned and fully sequenced was the 5-HT_{1A} receptor, by Kobilka and co-workers in 1987. Over the course of the next five years, the 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, and 5-HT_3 receptors were also cloned. Techniques used in molecular biology also led to the rapid discovery of additional subtypes of receptor for 5-HT, making it necessary to establish an unambiguous system of nomenclature for serotonin receptors. The current classification scheme takes into account not only operational criteria (drug-related characteristics such as selective agonists, selective antagonists, and ligand-binding affinities), but also information about intracellular signal transduction mechanisms and molecular structure (amino acid sequence of the receptor protein) (Hoyer et al., 1994). For example, the 5-HT_{1C} receptor was reclassified as a 5-HT_2 receptor based on the sequence homology, similar pharmacological characteristics and effector coupling of the 5-HT_2 and 5-HT_{1C} receptors. The 5-HT_2 receptor was renamed the 5-HT_{2A} receptor, and the 5-HT_{1C} was renamed the 5-HT_{2C} receptor. Although the amino acid sequences of many new 5-HT receptors have been reported, the classification of some of these receptors remains tentative due to limited knowledge of their operational and transductional characteristics, which have only been described for these recombinant receptors in transfected cell systems. Because the functions mediated by these 5-HT receptors in intact tissue are unknown, lower-case appellations are presently used (Hannon & Hoyer, 2008) (see Table 15-2).

Three families of serotonin receptor, the 5-HT_1 family, the 5-HT_2 family, and the family that includes the 5-HT_4, 5-HT_6 and 5-HT_7 receptors, represent the three major classes of 5-HT receptors that are G-protein–coupled receptors. The 5-HT_3 receptor is a ligand-gated ion channel and is a separate family. Although each serotonin receptor can be potently activated by 5-HT, differences in signal transduction mechanisms, neuroanatomical distribution and affinities for synthetic chemicals creates opportunities for drug discovery and makes each 5-HT receptor subtype a potential therapeutic target.

The 5-HT_1 receptor family is composed of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-HT_{1F} receptors

The five receptor subtypes in the 5-HT_1 receptor family share 40–63% overall sequence homology. These 5-HT receptors couple preferentially to the inhibition of adenylyl cyclase via the Gi/o family of G proteins (see Chapter 21). The 5-HT_{1C} receptor appellation is vacant because, as described above, this receptor was renamed the 5-HT_{2C} receptor due to structural, operational and transductional similarities with the 5-HT_2 receptor family. A lower case appellation is used for the 5-ht_{1E} receptor because a physiological role for these receptors in intact tissue has not been found (Hannon & Hoyer, 2008).

The 5-HT_{1A} receptor

The 5-HT_{1A} receptor is present in high density in cortical and limbic structures (i.e., hippocampus, entorhinal cortex, septum, amygdala, frontal cortex). The distribution of the 5-HT_{1A} receptor in brain suggests that this 5-HT receptor subtype may have a role in cognitive or integrative functions, as well as in emotional states. 5-HT_{1A} receptors in terminal field areas of serotonergic innervation are located postsynaptically. 5-HT_{1A} receptors also present in high density on serotonergic cell body areas, in particular the dorsal and median raphe nuclei. Here they function as somatodendritic autoreceptors, involved in the negative feedback modulation of serotonergic neuronal activity. Therefore somatodendritic 5-HT_{1A} autoreceptors play a key role in regulating serotonergic neurotransmission. In terminal field areas of serotonergic innervation such as the hippocampus, 5-HT_{1A} receptors are coupled to the inhibition of adenylyl cyclase activity, as well as the opening of potassium channels, which results in neuronal hyperpolarization (Table 15-2). However, in the dorsal raphe nucleus 5-HT_{1A} receptors are coupled to the opening of potassium channels, but do not appear to be coupled to the inhibition of adenylyl cyclase.

Activation of 5-HT_{1A} receptors in the central nervous system results in a variety of physiological and behavioral responses. 5-HT_{1A} receptors modulate feeding, sexual behavior and body temperature. Activation of 5-HT_{1A} receptors stimulates the release of adrenocorticotrophic hormone (ACTH). The 5-HT_{1A} receptor has been implicated in affective disorders such as anxiety and depression. Newer anti-anxiety medications, specifically the substituted azapirones (e.g., buspirone, Buspar), are agonists with high affinity for 5-HT_{1A} receptors (Figure 15-8). Postsynaptic 5-HT_{1A} receptors mediate the effects of antidepressant drugs in behavioral animal models and on cell proliferation and survival in the hippocampus. The desensitization of somatodendritic 5-HT_{1A} autoreceptors following chronic administration of drugs used to treat anxiety and depression is also of interest given the critical role of this receptor in regulating serotonergic neuronal firing and therefore serotonergic neurotransmission. Drugs with agonist activity at 5-HT_{1A} receptors are the subject of intense research in the treatment of schizophrenia, and of behavioral disorders such as impulsivity and aggression (Figure 15-8).

TABLE 15-2 **Serotonin Receptors Present in the Central Nervous System**

Receptor	Distribution in brain	Effector mechanism
5-HT$_1$ receptor family		
5-HT$_{1A}$	hippocampus, amygdala, septum entorhinal cortex, hypothalamus, raphe nuclei	inhibition of adenylyl cyclase, opening of K^+ channels, activation of MAP kinase pathway
5-HT$_{1B}$ (5-HT$_{1D\beta}$)	substantia nigra, basal ganglia frontal cortex, superior colliculus, lateral geniculate, deep nuclei of the cerebellum	inhibition of adenylyl cyclase
5-HT$_{1D}$ (5-HT$_{1D\alpha}$)	globus pallidus, substantia nigra, caudate putamen hippocampus and cortex	inhibition of adenylyl cyclase
5-ht$_{1E}$	?	inhibition of adenylyl cyclase
5-HT$_{1F}$	cerebral cortex, striatum, hippocampus, nucleus accumbens, amygdala	inhibition of adenylyl cyclase
5-HT$_2$ Receptor Family		
5-HT$_{2A}$ 13q14-21	claustrum, cerebral cortex, olfactory tubercle, striatum, nucleus accumbens	stimulation of phospholipase C closing of K^+ channels
5-HT$_{2B}$ 2q36.3-37.1	cerebellum, lateral septum, dorsal hypothalamus, medial amygdala	stimulation of phospholipase C
5-HT$_{2C}$ Xq24	choroid plexus, globus pallidus, cerebral cortex, hypothalamus septum, substantia nigra, spinal cord dorsal and median raphe	stimulation of phospholipase C
5-HT$_3$ receptor family		
5-HT$_3$ (5-HT$_{3A}$) (5-HT$_{3B}$)	hippocampus, entorhinal cortex, amygdala, nucleus, accumbens, solitary tract nerve, trigeminal nerve, motor nucleus of the dorsal vagal nerve, area postrema, spinal cord	ligand-gated cation channel
5-HT$_4$	hippocampus, striatum, olfactory tubercle, substantia nigra	stimulation of adenylyl cyclase
5-ht$_{5A}$	?	inhibition of adenylyl cyclase
5-ht$_{5B}$	?	?
5-HT$_6$	striatum, nucleus accumbens, olfactory tubercle cortex, amygdala, hypothalamus, hippocampus	stimulation of adenylyl cyclase
5-HT$_7$	cerebral cortex, septum, thalamus hypothalamus, amygdala, hippocampus	stimulation of adenylyl cyclase

Lower-case appellations are used in some cases because the functions mediated by these receptors in intact tissue are not known.

5-HT_{1A} RECEPTOR POLYMORPHISMS AND MENTAL ILLNESS

Julie G. Hensler

Strong evidence supports an important role of the serotonin system in the etiology and treatment of mental illness. Genetic polymorphisms that affect the expression or function of key components of neurotransmitter systems or signaling cascades are believed to affect an individual's predisposition to psychiatric disorders. In addition to the SERT, 5-HT_{1A} receptors located on serotonergic cell bodies and dendrites play a critical role in the regulation of serotonergic neurotransmission. These 5-HT_{1A} receptors function as somatodendritic autoreceptors and when activated by 5-HT released from axon collaterals serve to inhibit serotonergic neuronal firing. Decreased serotonergic neurotransmission is associated with a number of mood disorders, e.g., bipolar illness, panic disorder, major depression and suicide. In response to antidepressant treatments, or long-term administration of novel anxiolytics such as Buspar® (Figure 15-8), 5-HT_{1A} somatodendritic autoreceptors become desensitized. Attenuation of 5-HT_{1A} somatodendritic autoreceptor sensitivity and function results in an increase in serotonergic neuronal firing rate and 5-HT neurotransmission, and is believed to contribute to the therapeutic effect of antidepressant drugs (Artigas et al., 2001; Blier et al., 2003). Post-synaptic 5-HT_{1A} receptors are also important components of antidepressant drug action, mediating behavioral and neurochemical responses to antidepressant treatments (De Vry, 1995; Mayorga et al., 2001; Santarelli et al., 2003).

A common C(1019)G polymorphism has been identified in the promotor region of the human 5-HT_{1A} receptor gene. This polymorphism is located in a sequence that is recognized by two transcription factors, Deaf-1 and Hes5, in an allele-dependent manner with the C-allele binding to these proteins but not the G-allele. Deaf-1 is co-localized with pre-and postsynaptic 5-HT_{1A} receptors in brain, consistent with a role in the regulation of both pre- and postsynaptic 5-HT_{1A} receptors, but displays differential activity. In serotonergic cells, Deaf-1 suppresses 5-HT_{1A} receptor expression. In non-serotonergic cells, Deaf-1 exhibits enhancer activity. Both enhancer and repressor activities of Deaf-1 are abolished in the G-allele. The G-allele therefore is expected to result in decreased expression of postsynaptic 5-HT_{1A} receptors in forebrain areas and over-expression of 5-HT_{1A} autoreceptors on serotonergic neurons, which would translate to reduced serotonergic neuronal firing. The G-allele is associated with major depression, anxiety and panic disorder, and increased raphe 5-HT_{1A} binding potential in depressed patients. Increasing evidence supports a functional role for the C(-1019)G site in the dysregulation of 5-HT_{1A} receptor expression and predisposition to mental illness (Le François et al., 2008).

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The 5-HT_{1B} and 5-HT_{1D} receptor subtypes

The 5-HT_{1B} and 5-HT_{1D} receptor subtypes are also linked to inhibition of adenylyl cyclase activity (Table 15-2). Binding sites that have been pharmacologically defined as 5-HT_{1B} receptors were characterized in certain rodents (rat, mouse, hamster), whereas the 5-HT_{1D} receptor was characterized using pharmacological criteria in species such as guinea pig, dog, pig, cow, and human. The 5-HT_{1B} and 5-HT_{1D} receptors were considered to be species variants of the same receptor because the pharmacological profiles of these two receptors are similar, although not identical, and the distribution of these two receptors in brain is very similar.

Although biochemical, pharmacological and functional data suggest that the 5-HT_{1B} receptor found in rats and mice, and the 5-HT_{1D} receptor found in other species, including human, are functionally equivalent species homologues of the same receptor, the story has been somewhat complicated by the discovery of two genes encoding the human 5-HT_{1D} receptor, 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. The human 5-$HT_{1D\beta}$ receptor was shown to have a high degree of homology with the rodent 5-HT_{1B} receptor. Subsequently a gene homologous to the human 5-$HT_{1D\alpha}$ was discovered in the rat. This gene encoded a receptor with the pharmacological profile of the 5-HT_{1D}. Thus, both rat and human express a 5-HT_{1B} and 5-HT_{1D} receptor. As a result, the nomenclature for the 5-$HT_{1B/1D}$ receptors was reassessed (Hartig et al., 1996). This new nomenclature scheme recognizes that the human 5-$HT_{1D\beta}$ receptor is a species equivalent of the rodent 5-HT_{1B} receptor, as discussed above, despite differing pharmacology, and realigned the 5-$HT_{1D\beta}$ to the 5-HT_{1B} receptor classification. Prefixes are often used to denote species-specific 5-HT_{1B} receptors because of the significant differences in the pharmacology of the 5-HT_{1B} receptor across species, e.g., r5-HT_{1B} and h5-HT_{1B} denote the rat and human receptors, respectively. The 5-$HT_{1D\alpha}$ receptor expressed in both rat and human is reclassified as the 5-HT_{1D} receptor (Table 15-2).

Much of what is known about the function and neuroanatomical distribution of the 5-HT_{1B} receptor is attributed to the receptor designated under the older classification scheme as the 5-HT_{1B} receptor in rodents and the 5-HT_{1D} receptor in bovine, guinea pig and human brain. The 5-HT_{1B} (5-$HT_{1D\beta}$, r5-HT_{1B}, h5-HT_{1B}) receptor is located in high density in the basal ganglia, particularly in the globus pallidus and the substantia nigra (Table 15-2). The neuroanatomical localization of 5-HT_{1B} receptor raises the interesting possibility that this receptor may be involved in diseases of the brain that involve the basal ganglia, such as Parkinson's disease. Functional studies indicate that 5-HT_{1B} receptors are located on presynaptic terminals of serotonergic neurons and modulate the release of serotonin. 5-HT_{1B} receptors are also located postsynaptically, where they may modulate the release of other neurotransmitters, such as acetylcholine in the hippocampus and dopamine in the prefrontal cortex. Postsynaptic 5-HT_{1B} receptors are also located on cerebral arteries and other vascular tissues. Agonists at the 5-HT_{1A} and 5-HT_{1B} receptors have been termed "serenics" because of their selective ability to inhibit aggressive behavior without sedation in rats and mice. Drugs that are agonists at 5-$HT_{1B/1D}$ receptors (e.g., sumatriptan, zolmitriptan) (Figure 15-8) are currently used clinically in the treatment of migraine. These drugs constrict cranial blood vessels and inhibit the neurogenic inflammation in the dura mater that gives rise to plasma extravasation.

It has been difficult to determine the distribution of the 5-HT_{1D} (5-$HT_{1D\alpha}$) receptor in brain due to the lack of selective radioligands. Receptor binding sites attributed to the 5-HT_{1D} receptor are present in basal ganglia (globus pallidus, substantia nigra, caudate putamen) and also hippocampus and cortex (Bruinvels et al., 1993). Using the technique of *in situ* hybridization histochemistry, the distribution of cells in the brain expressing the mRNA for 5-HT_{1D} receptors was characterized. 5-HT_{1D} receptor mRNA is expressed at low levels in the basal ganglia, dorsal raphe nucleus and locus coeruleus, indicative of the 5-HT_{1D} receptor being located predominantly on axon terminals of both serotonergic and non-serotonergic neurons. The release of 5-HT from dorsal raphe nucleus appears to be under the control of 5-HT_{1D} receptors, presumably located on serotonergic terminals. It has been proposed that neurogenic inflammation and trigeminal nociception may be 5-HT_{1D} receptor mediated. Thus, there is much interest in the 5-HT_{1D} receptor as a useful therapeutic target for migraine.

The 5-ht_{1E} receptor

The 5-ht_{1E} receptor was originally identified in homogenates of human frontal cortex by radioligand binding studies using [^{3}H]-5-HT in the presence of 5-CT to block 5-HT_{1A} and 5-HT_{1D} receptor sites. Because of the lack of specific radioligands for the 5-ht_{1E} receptor, the overall distribution of this receptor in brain is unknown. 5-ht_{1E} receptor mRNA has been found in the cortex (particularly entorhinal cortex) and caudate putamen. The function of the 5-ht_{1E} receptor in intact tissue is not known due to the lack of selective agonists or antagonists. In transfected cells, the 5-ht_{1E} receptor is coupled to the inhibition of adenylyl cyclase activity (Zgombick et al., 1992). The 5-ht_{1E} receptor displays a higher degree of homology with the 5-HT_{1D} receptor (64%) than any other 5-HT_1 receptors. Although 5-ht_{1E} receptor mRNA and binding sites have been mapped in the rodent and human brain, confirmation of a true physiological role for 5-ht_{1E} receptors is still lacking; hence, they retain their lower-case appellation (Hannon & Hoyer, 2008).

The 5-HT_{1F} receptor

The 5-HT_{1F} receptor was cloned and sequenced in 1993 and shares the greatest sequence homology with the 5-ht_{1E} receptor (61%). 5-HT_{1F} receptor mRNA is found in cortex, hippocampus, dentate gyrus, nucleus of the solitary tract, spinal cord, uterus and mesentery. In transfected cells, the 5-HT_{1F} receptor is coupled to the inhibition of adenylyl cyclase. Because selective agonists or antagonists for the 5-HT_{1F} receptor have not been available until very recently, little was known about the distribution or function of the 5-HT_{1F} receptor in brain. With the development of the selective 5-HT_{1F} radioligand [^{3}H] LY334370, 5-HT_{1F} receptor sites have been found in cortex, nucleus accumbens, striatum, amygdala and hippocampus. The 5-HT_{1F} receptor has been the subject of intense research as a target for anti-migraine drugs. Activation of 5-HT_{1F} receptors *in vivo* is associated with the inhibition of plasma extravasation in the dura, a component of neurogenic inflammation thought to be a possible cause of migraine. Because activation of 5-HT_{1F} receptors does not mediate constriction of human vasculature (unlike 5-$HT_{1B/1D}$ receptors), 5-HT_{1F} receptor agonists would be devoid of unwanted cardiovascular side effects, an advantage in the treatment of migraine.

The 5-HT_2 receptor family is composed of the 5-HT_{2A}, 5-HT_{2B} and 5HT_{2C} receptors

The three receptor subtypes in the 5-HT_2 receptor family share 46–50% overall sequence homology. Members of the 5-HT_2 receptor family are coupled via the $G_{q/11}$ family of G proteins to the phospholipase C signaling cascade, which involves the hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP2) and increases in intracellular calcium (see Chap. 23). The 5-HT_{2A} receptor nomenclature refers to the classical D receptor of Gaddum and Picarelli, and 5-HT_2 receptor of (Peroutka & Snyder, 1979). The 5-HT_{2C} receptor was formerly the 5-HT_{1C} receptor. It is important to note that because selective drugs able to differentiate between members of the 5-HT_2 receptor family have not been available until very recently, many of the functional and clinical correlates of the 5-HT_{2A} receptor may very well involve or be attributed to the 5-HT_{2C} or 5-HT_{2B} receptor.

5-HT_{2A} receptors

5-HT_{2A} receptors are found postsynaptically to serotonergic neurons, and are particularly concentrated in the frontal cortex. 5-HT_{2A} receptors are also found in high density in the claustrum, a region that is connected to the visual cortex, in parts of the limbic system (i.e., amygdala and hippocampus), and in the basal ganglia (Table 15-2). In the cortex, the 5-HT_{2A} receptor is located on local GABAergic interneurons, as well as on pyramidal projection neurons, which are known to be glutamatergic. The high level of 5-HT_{2A} receptor expression throughout the cortex suggests that this 5-HT receptor subtype may play

a role in higher cognitive or integrative functions. Activation of 5-HT_{2A} receptors in the central nervous system results in an increase in body temperature (hyperthermia) and increased secretion of ACTH. In the periphery, 5-HT_{2A} receptors mediate the contractile responses of vascular smooth muscle, and a component of the contractile response of smooth muscle in the gut, to 5-HT. The 5-HT_{2A} receptor has been implicated in the hallucinogenic effects of 5-HT_2 receptor agonists. There is also considerable interest in the role of the 5-HT_{2A} receptor in antipsychotic drug action. Newer antipsychotic medications (e.g., clozapine, olanzapine) (Figure 15-8) are antagonists with high affinity for the 5-HT_{2A} receptor.

The cloning of the 5-HT_{2A} receptor was used to gain insight into a controversy over the nature of agonist binding to the 5-HT_{2A} receptor. The hallucinogenic amphetamine derivative [^{3}H]-DOB, an agonist, binds to receptor sites with properties similar to those of the receptor labeled with the antagonist [^{3}H]-ketanserin. Some investigators interpreted these and other data as evidence for the existence of a new subtype of 5-HT_{2A} receptor, whereas others interpreted these data as indicative of agonist high-affinity and agonist low-affinity states of the 5-HT_{2A} receptor. In experiments in which the cDNA encoding the 5-HT_{2A} receptor was transfected into clonal cells, binding sites for both the 5-HT_{2A} receptor antagonist [^{3}H]-ketanserin and the 5-HT_2 receptor agonist [^{3}H]-DOB were found. Furthermore, agonists had higher affinities for [^{3}H]-DOB binding than for [^{3}H]-ketanserin binding. Thus, a single gene produces a protein with both binding sites, substantiating the view that agonist and antagonist binding are to different states of the 5-HT_{2A} receptor rather than two different subtypes of the 5-HT_{2A} receptor.

The 5-HT_{2B} receptor

Although the 5-HT_{2B} receptor is the most recently cloned of the 5-HT_2 receptor class, it was among the first of the 5-HT receptors to be characterized using pharmacological criteria. The first report of the sensitivity of rat stomach fundus to 5-HT was published by Vane in 1959. This receptor, whose activation results in the contraction of fundus smooth muscle, was originally placed in the 5-HT_1 receptor class because of its sensitivity to serotonin and because responses mediated by this receptor were not blocked by 5-HT_2 or 5-HT_3 receptor antagonists. It has been reclassified as a 5-HT_2 receptor because of its similar pharmacological profile to the 5-HT_{2C} receptor (Table 15-2). The recombinant receptor expressed in clonal cells is coupled to hydrolysis of membrane phosphoinositides (PI). However, in rat stomach fundus, the 5-HT_{2B} receptor appears not to be coupled to PI hydrolysis. 5-HT_{2B} receptor-mediated contraction of rat stomach fundus is dependent upon the influx of calcium through voltage-sensitive channels, intracellular calcium release, and activation of PKC (Cox & Cohen, 1996). The effector system to which this receptor is coupled in the CNS remains to be established.

5-HT_{2B} receptor mRNA has been detected in the stomach fundus, intestine, kidney, heart, and lung, as well as in the brain (cerebellum, cerebral cortex, amygdala, substantia nigra, caudate, thalamus, hypothalamus and retina). The lack of truly selective 5-HT_{2B} receptor agonists and antagonists has limited our knowledge about the functional role of the 5-HT_{2B} receptor in brain. The 5-HT_{2B} receptor is also expressed in a number of blood vessels. Given the vasodilatory role of the 5-HT_{2B} receptor, recently developed 5-HT_{2B} receptor antagonists may be indicated for the treatment of migraine. Activation of the 5-HT_{2B} receptor appears to be responsible for the cardiac valve defects reported in obese patients treated with appetite suppressant preparations containing dexfenfluramine (Hannon & Hoyer, 2008).

The 5-HT_{2C} receptor

The 5-HT_{2C} receptor is present in high density in the choroid plexus. High-resolution autoradiography has shown that 5-HT_{2C} receptors are enriched on the epithelial cells of the choroid plexus. It has been proposed that 5-HT-induced activation of 5-HT_{2C} receptors could regulate the composition and volume of the cerebrospinal fluid. 5-HT_{2C} receptors are also found throughout the brain, in particular areas of the limbic system (hypothalamus, hippocampus, septum, neocortex) and in areas associated with motor behavior (substantia nigra, globus pallidus). 5-HT_{2C} receptors are also localized to GABAergic cells in the dorsal raphe, giving anatomical support to the proposed negative feedback loop involving reciprocal connections between GABAergic interneurons and 5-HT neurons in the dorsal raphe. The excitation of GABAergic interneurons through activation of 5-HT_{2C} receptors would result in the suppression of 5-HT cell firing.

The 5-HT_{2C} receptor is one of the few G protein–coupled receptors whose mRNA undergoes editing. RNA editing occurs when gene transcripts are altered co- or post-transcriptionally. 5-HT_{2C} receptor mRNA undergoes post-transcriptional editing to yield multiple 5-HT_{2C} receptor isoforms with different distributions in brain (Burns et al., 1997; Fitzgerald et al., 1999). Messenger RNA editing alters both the binding affinity and functional potency of agonists for recombinant 5-HT_{2C} receptor isoforms. This is potentially of great significance as the isoforms expressed endogenously may have different functional and regulatory properties, and thus provide a novel mechanism for the regulation of 5-HT synaptic signaling and plasticity.

5-HT_2 receptors are thought to mediate hallucinogenic properties of some 5-HT agonists, such as LSD (Figure 15-8). Evidence for this comes from experiments in which animals are trained to discriminate 5-HT_2 receptor agonists, and therefore are able to recognize or discriminate other 5-HT_2 receptor agonists but not, for example, 5-HT_1 receptor agonists. Thus, activation of 5-HT_2 receptors is said to lead to a discriminative stimulus. The production of drug-discriminative stimulus properties of 5-HT_2 receptor agonists, such as the hallucinogenic amphetamine derivatives, can be blocked by 5-HT_2 receptor antagonists. There is also a close correlation between the human hallucinogenic potency of 5-HT_2 receptor agonists and their affinity for 5-HT_2 (i.e., both 5-HT_{2A} and 5-HT_{2C}) receptor–binding sites.

Unlike the other subtypes of receptor for 5-HT, the 5-HT_3 receptor is a ligand-gated ion channel

The 5-HT_3 receptor

The 5-HT_3 receptor corresponds to the classical M receptor of Gaddum and Picarelli. Based on its overall electrophysiological

features and sequence, it is a member of the ligand-gated ion channel superfamily of receptors. The 5-HT_3 receptor is a pentamer, i.e., five subunits come together to form a cation channel that is gated by 5-HT. Like other members of this receptor superfamily, it possess additional pharmacologically distinct recognition sites where the function of the receptor can be modulated by alcohols and anesthetic agents (Parker et al., 1996).

The 5-HT_3 receptor is made up of five subunits that surround a central ion channel. The 5-HT_{3A} receptor subunit was the first to be cloned, in 1991. The cloned receptor subunit exhibits sequence similarity to the alpha subunit of the nicotinic acetylcholine receptor. A second subunit, 5-HT_{3B}, was subsequently cloned. Although single subunits of members of the ligand-gated ion channel receptor family can form functional homomeric receptors (receptors composed of subunits of a single type), they generally lack some of the properties of the native receptor. This appears to be the case for the 5-HT_3 receptor. Expression of 5-HT_{3B} receptor subunits in mammalian cells or Xenopus oocytes does not result in the formation of functional receptors or specific 5-HT_3 binding sites. The heteromeric combination of 5-HT_{3A} and 5-HT_{3B} subunits is necessary to provide the functional characteristics of native 5-HT_3 receptors. Three additional human 5-HT_3 receptor subunit genes are been identified and cloned. The 5-HT_{3C}, 5-HT_{3D} and 5-HT_{3E} subunits do not traffic to the cell surface when singularly expressed in mammalian cells. However, when co-expressed with 5-HT_{3A} receptor subunits, these receptors are functional but exhibit characteristics or functional properties that are different when compared to homomeric 5-HT_{3A} receptors. The exact subunit composition of native 5-HT_3 receptors is not known. It is entirely possible that the subunit composition of native 5-HT_3 receptors may vary with their location in the body, or area of the brain, raising the exciting possibility of differences in the pharmacology and regulation of native 5-HT_3 receptors depending on the tissue or brain region in which they are expressed (Hannon & Hoyer, 2008; Barnes et al., 2009).

5-HT_3 receptors are found postsynaptically to serotonergic neurons in the central and peripheral nervous systems. 5-HT_3 receptors initially appeared to be confined to peripheral neurons, where they mediate depolarizing actions of 5-HT and modulate neurotransmitter release. Serotonin regulates both motility and intestinal secretion throughout the gastrointestinal tract through activation of 5-HT_3 receptors. 5-HT_3 receptors are found in high density in peripheral ganglia and nerves (superior cervical ganglion and vagus nerve) as well as in the substantia gelatinosa of the spinal cord. Their localization in spinal cord and medulla suggest that 5-HT could modulate nociceptive mechanisms via 5-HT_3 receptors. 5-HT_3 receptors facilitate the release of substance P in the spinal cord. The localization of 5-HT_3 receptor–binding sites in cortical and limbic areas of the brain is consistent with behavioral studies in animals, which suggest that 5-HT_3 receptor antagonists (Figure 15-8) may have potential anxiolytic, antidepressant and cognitive effects. The observation that 5-HT_3 receptors modulate the activity of dopaminergic neurons in the ventral tegmental area has led to the hypothesis that 5-HT_3 receptor antagonists may have potential as antipsychotic drugs, and may posses the ability to reduce the rewarding effects of alcohol and certain drugs of abuse. The highest density of 5-HT_3 receptor sites in the brain is in the area postrema, the site of the chemoreceptor trigger zone (Table 15-2).

Antagonists at 5-HT_3 receptors, such as ondansetron and granisetron (Figure 15-8), are an important class of drugs for the treatment of nausea and emesis (vomiting) in cancer patients receiving chemotherapy. Chemotherapeutic drugs such as cisplatin and decarbazine induce the release of 5-HT from enterochromaffin cells of the gastrointestinal tract. The 5-HT released activates 5-HT_3 receptors located on the enteric nerves innervating the smooth muscle of the gut, causing depolarization of visceral afferent nerves and increasing their rate of firing. The enhanced afferent input leads to stimulation of the chemoreceptor trigger zone, which produces nausea and vomiting. Antagonism of 5-HT_3 receptors prevents or reduces this chain of events. The site of action of these drugs appears to be 5-HT_3 receptors in the gastrointestinal tract even though the central area regulating emesis, i.e., the chemoreceptor trigger zone, possesses a high density of 5-HT_3 receptors. Unfortunately, there is a more prolonged, often milder, form of emesis caused by chemotherapeutic drugs that is not dependent on the release of 5-HT and is resistant to improvement with 5-HT_3 receptor antagonists.

The 5-HT_4, 5-HT_6 and 5-HT_7 receptors are coupled to the stimulation of adenylyl cyclase

The 5-HT_4, 5-HT_6 and 5-HT_7 preferentially couple to the stimulation of adenylyl cyclase, increasing cAMP formation, via the Gs family of G proteins. These receptors, however, share only >35% overall sequence homology. For this reason, they are classified as distinct receptor groups or classes, and not subtypes of a family (Hannon & Hoyer, 2008).

The 5-HT_4 receptor

The 5-HT_4 receptor was originally described in cultured mouse collicular neurons, and subsequently in mouse and guinea pig brain, by Bockaert and co-workers in the late 1980s. This 5-HT receptor coupled to the stimulation of adenylyl cyclase activity, and possessed pharmacological characteristics distinct from that of the 5-HT_1, 5-HT_2 or 5-HT_3 receptors. It had been known for number of years prior to the pharmacological definition of the 5-HT_4 receptor that 5-HT could stimulate adenylyl cyclase in brain. Studies of the 5-HT_4 receptor were hampered by the absence of a high-affinity radioligand. The synthesis and development of specific radioligands provided the necessary tools for the study and characterization of the 5-HT_4 receptor. 5-HT_4 receptor–binding sites are localized with high density in the striatum, substantia nigra, and olfactory tubercle and have been reported in the hippocampus as well (Table 15-2). The 5-HT_4 receptor is located postsynaptically to serotonergic neurons and modulates the release of several neurotransmitters (i.e., acetylcholine, dopamine and GABA), as well as indirectly modulating 5-HT release. 5-HT_4 receptor activation enhances cognitive performance in rats and monkeys. In humans, a role for the 5-HT_4 receptor in memory enhancement remains to be demonstrated in clinical studies (Bockaert et al., 2008).

In the periphery, the 5-HT_4 receptor mRNA is found in vascular smooth muscle. Newly developed drugs that activate 5-HT_4 receptors are of interest for their potential in treating cardiac arrhythmia. The 5-HT_4 receptor is also located on

neurons of the alimentary tract, for example the myenteric plexus of the ileum, and on smooth muscle cells and secretory cells of the gastrointestinal tract, where they evoke secretions and the peristaltic reflex. 5-HT_4 receptor agonists (e.g., cisapride, prucalopride) are used therapeutically in the treatment of constipation-predominant irritable bowel syndrome, and in functional motility disorders of the upper gastrointestinal tract.

Multiple isoforms of the 5-HT_4 receptor have been shown to exist, the result of alternative splicing of 5-HT_4 receptor mRNA. These splice variants of the 5-HT_4 receptor differ in the length and composition of their carboxyl termini. Studies indicate specificity in the pattern of expression of these splice variants in different tissues. For example, the human 5-HT_{4a}, 5-HT_{4b} and 5-HT_{4c} receptor isoforms are expressed in the brain, atrium and gut, whereas expression of the 5-HT_{4d} receptor isoform appears to be restricted to the gut. The physiological significance of the differential distribution of many splice variants of the 5-HT_4 receptor in the body remains to be understood. The existence of different isoforms of the 5-HT_4 receptor raises interesting possibilities as to the distinct pharmacological characteristics and regulation of these different receptor isoforms.

The 5-HT_6 receptor

The 5-HT_6 receptor, when expressed in transfected cells, stimulates adenylyl cyclase and shows high affinity for [^{125}I]-LSD and [^{3}H]-5-HT. The pharmacology of this receptor is unique. Interestingly, this receptor has high affinity for various antipsychotic and antidepressant drugs such as clozapine, amitriptyline, clomipramine, mianserin and ritanserin. In striatum, 5-HT_6 receptors mediate an increase in cAMP accumulation.

5-HT_6 receptor mRNA has been located in the striatum, nucleus accumbens, amygdala, hippocampus and cerebral cortex. 5-HT_6 receptors have been found in these brain regions with immunohistochemical and radioligand binding approaches, with a primarily postsynaptic localization. Like the 5-HT_{2C} receptor, 5-HT_6 receptor mRNA has not been found in the peripheral tissue, suggesting that compounds acting at this receptor may have limited peripheral side effects (Hannon & Hoyer, 2008). Given the distribution of 5-HT_6 receptors in brain, particularly in areas associated with learning and memory, much research has focused on the role of this receptor in cognitive function. 5-HT_6 receptor antagonists increase cholinergic neurotransmission and have positive effects on learning and memory. 5-HT_6 receptor activation modulates glutamate, GABA, dopamine and norepinephrine release. 5-HT_6 receptor–selective ligands are of great interest as potential therapeutic agents in Alzheimer's disease and schizophrenia as well as depression and obesity.

The 5-HT_7 receptor

The 5-HT_7 receptor is the most recently identified member of the family of G protein–coupled 5-HT receptors, although it was known for some time in the cardiovascular or GI systems, and was originally termed "5-HT_1-like." The distinct pharmacological profile of 5-HT_7 receptor sites has been used to delineate the function and distribution of this receptor *in vivo*. 5-HT_7 receptor–binding sites in the rat brain were originally described using nonselective radioligands in the presence of drugs to mask 5-HT_{1A} and 5-HT_{1B} receptors. The distribution of 5-HT_7 receptors has been confirmed in autoradiographic studies using nonselective ligands in the brains of mice lacking 5-$HT_{1A/1B}$ receptors (Bonaventure et al., 2002). More recently, analysis of the binding of selective radioligands revealed the presence of high densities of 5-HT_7 receptors in the anterior thalamus and dentate gyrus region of hippocampus, with intermediate levels in septum and hypothalamus, CA1 and CA2 regions of hippocampus and cortex (Hannon & Hoyer, 2008) (Table 15-2).

The 5-HT_7 receptor has been implicated in the regulation of sleep, circadian rhythms and mood. As discussed above, 5-HT has been known for some time to induce phase shifts in behavioral circadian rhythms and modulate neuronal activity in the suprachiasmatic nucleus, the likely site of the mammalian circadian clock. The pharmacological characteristics of the effect of 5-HT on circadian rhythms are consistent with the 5-HT_7 receptor. Moreover, mRNA for the 5-HT_7 receptor is found in the suprachiasmatic nucleus. There is also increasing evidence that the 5-HT_7 receptor may play a role in psychiatric disorders. The regional distribution of 5-HT_7 receptors in brain includes limbic areas and cortex. Atypical antipsychotics, such as clozapine and risperidone, and some antidepressants display high affinity for this receptor. In the periphery, 5-HT_7 receptors have been shown to mediate relaxation of vascular smooth muscle.

Alternative mRNA splicing has been reported to generate four 5-HT_7 receptor isoforms. Although these isoforms differ in their C-termini, they have not been shown to differ in their respective pharmacology, signal transduction or tissue distribution.

The 5-ht_5 receptor and the 5-ht_{1P} receptor are orphan receptors

Two subtypes of the 5-ht_5 receptor have been cloned (5-ht_{5A} and 5-ht_{5B} receptors). These receptor proteins are 77% identical to each other, whereas the homology with other serotonin receptors is low. 5-ht_{5A} receptor mRNA transcripts have been detected by *in situ* hybridization in the cerebral cortex, hippocampus, granule cells of the cerebellum, medial habenula, amygdala, septum, several thalamic nuclei and olfactory bulb of the rat and mouse. 5-ht_{5B} mRNA has been detected by *in situ* hybridization in the hippocampus, habenula and the dorsal raphe nucleus of rat. In human, the 5-ht_{5B} receptor gene fails to encode a functional protein. Immunohistochemical studies using antibodies to the 5-ht_{5A} receptor have shown this receptor to be expressed predominantly by astrocytes, although some neurons in cortex were labeled as well. When expressed in recombinant cell systems, the 5-ht_{5A} receptor is coupled to the inhibition of adenylyl cyclase. At the present time, the functional correlate and transductional properties are unknown for the 5-ht_{5B} receptor. Because no evidence has been obtained to confirm that the recombinant 5-ht_5 receptor is expressed and functions in an endogenous tissue, this receptor retains its lower-case appellation (Hoyer et al., 1994; Hannon & Hoyer, 2008).

The existence of an atypical 5-HT receptor on the enteric neurons of the gut has been shown in a series of investigations

by Gershon and associates. This receptor has high affinity for [^{3}H]-5-HT and mediates a slow depolarization of particular myenteric neurons that is not blocked by selective 5-HT_3 antagonists. This receptor has been termed the 5-ht_{1P} receptor as it has a high affinity for 5-HT and is found in the periphery, in high density in the gut. The pharmacology of this receptor is somewhat similar to that of the 5-HT_4 receptor. The available functional and radioligand binding data confirm the orphan status of the 5-ht_{1P} receptor and emphasize the need to establish a rigorous basis for its positive identification (Hoyer et al., 1994; Hannon & Hoyer, 2008).

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