

14

Catecholamines

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OVERVIEW OF CATECHOLAMINES

Catecholamines belong to the group of transmitters called monoamines

Catecholamines contain a nucleus catechol group, that is, a benzene group with two adjacent hydroxyl groups, as well as an ethylamine side chain with a single amine group that may have additional substitutions. The predominant catecholamines in the brain are dopamine, norepinephrine and epinephrine. Catecholamine neurotransmitters were first found in the autonomic nervous system. In 1954 Marthe Vogt demonstrated that norepinephrine was not uniformly

distributed in the brain, and that its distribution did not necessarily coincide with blood vessel density. This suggested that norepinephrine could function as a central neurotransmitter. Soon after, it was discovered that dopamine had a separate distribution in brain, indicating that it could function independently as a neurotransmitter and not simply be a metabolic intermediate of norepinephrine synthesis. The catecholamines exist in low (micromolar) concentrations in the brain with respect to amino acid neurotransmitters such as glutamate and γ -aminobutyric acid. However, they have considerable importance in the regulation of multiple aspects of central nervous system function and are vital therapeutic targets.

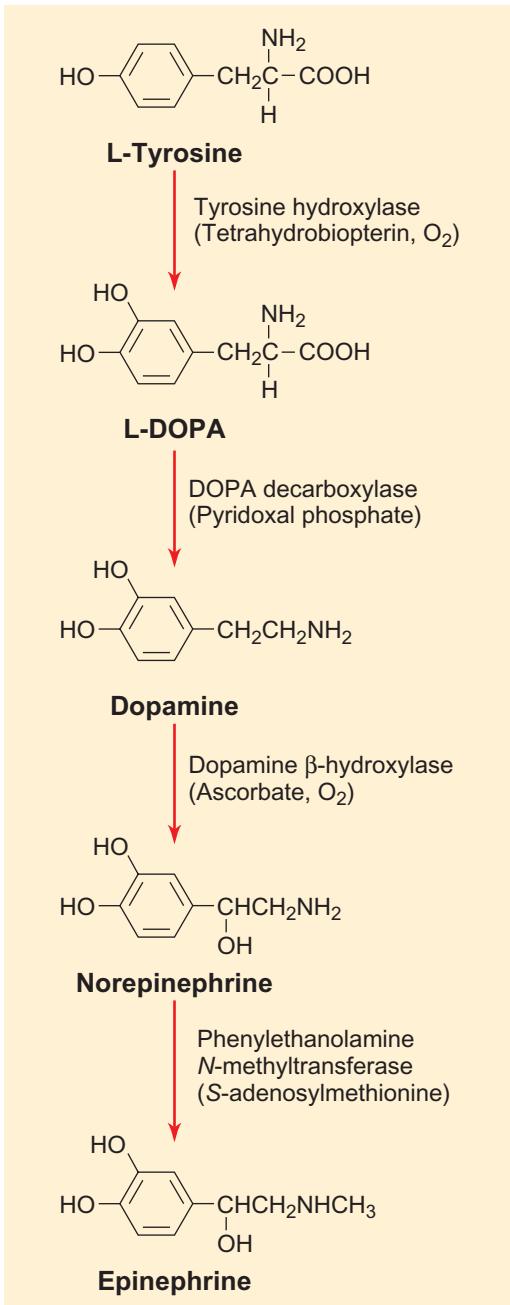


FIGURE 14-1 Biosynthetic pathway for catecholamines.

Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine biosynthesis

The catecholamines are synthesized in the nerves at the site of their release, both at the terminals and in the cell bodies. Tyrosine hydroxylase (TH, tyrosine 3-monooxygenase) catalyzes the conversion of its substrates tyrosine and molecular oxygen to 3,4-dihydroxy-l-phenylalanine (L-DOPA). It is the most important enzyme in catecholamine synthesis, being the initial and rate-limiting step in the synthesis of dopamine,

norepinephrine and epinephrine (Fig. 14-1). Its importance is also underscored by the fact that disruption of the TH gene is lethal to the embryo (Table 14-1). The monooxygenase requires a pteridine, (6R)-L-erythro-tetrahydrobiopterin (BH₄), and ferrous iron as cofactors. Fe²⁺ is bound directly to TH, and is oxidized to Fe³⁺ by O₂. Other iron-containing, pteridine-binding enzymes related to tyrosine hydroxylase are tryptophan hydroxylase and phenylalanine hydroxylase. TH is a homomeric enzyme, consisting of four identical 60 kDa subunits, each of which binds Fe²⁺ and BH₄ (Nakashima et al., 2009). The N-terminal of each subunit has a regulatory domain containing numerous phosphorylation sites while the C-terminal contains a catalytic domain. The enzyme is both soluble and membrane bound.

As the rate limiting and arguably the most important enzyme in catecholamine biosynthesis, TH is subject to complex regulation. TH is modulated at both transcriptional and post-translational levels by nearly every physiological mechanism. On a rapid time scale, tyrosine hydroxylase is regulated by two types of end-product inhibition, both of which are modulated by the phosphorylation status of N-terminal serines (Kumer et al., 1996). One type of feedback inhibition is a kinetically mediated, readily reversible inhibition that acts as a sensor of the local concentration of the catecholamines. Catechols bind to the same enzyme intermediate as does the cofactor, BH₄, competitively preventing the binding of BH₄ and thus decreasing enzyme activity. Levels of BH₄ are limiting, so the affinity for this cofactor regulates enzyme activity. The levels of tyrosine in the brain are greater than the micromolar affinity of TH for tyrosine, so levels of tyrosine are unlikely to significantly regulate enzyme activity. The second type of end product inhibition results from a long-term, nearly irreversible binding of catechols to the Fe³⁺ ion at the catalytic site. This binding sequesters the enzyme in a more stable but less active form. Phosphorylation relieves both types of inhibition.

Phosphorylation of TH at N-terminal serines alters the conformation of TH such that the catechol binding becomes more dissociable and BH₄ binding is increased. This is observed kinetically as an increase in V_{max} for the enzyme and a decrease in K_m for BH₄. Four serine residues in the N-terminus of TH have been identified as selective substrates for a number of protein kinases (See Fig. 14-2). Despite numerous elegant studies delineating these serines and the enzymes responsible for their phosphorylation, there are still many questions concerning the kinases and phosphatases that modulate select serines *in situ* and the ability of these phosphorylations to activate the enzyme. Direct phosphorylation of Ser40 and Ser31 increases TH activity and catecholamine synthesis. See Dunkley et al. (2004) for a more complete discussion of these issues. Phosphorylation-related changes in enzyme activity may also mediate the inhibition of TH by presynaptic dopamine auto-receptors and the increase in TH activity mediated by impulse flow in the striatum.

In an alternate pathway, TH can hydroxylate phenylalanine to form tyrosine, which is converted to L-DOPA. This route is of significance in patients diagnosed with phenylketonuria, a condition in which phenylalanine hydroxylase activity is depressed. Experimentally, the activity of TH is inhibited by the compound α-methyl-p-tyrosine. This

TABLE 14-1 Studies With Knockout Mice

Protein	Phenotype	Reference
Tyrosine hydroxylase	Not viable	(L. Chen & Zhuang, 2003)
Dopamine deficient	Hypoactive and aphagic; die weeks after birth	(L. Chen & Zhuang, 2003)
Dopamine-β-hydroxylase	Hypotension, sleep dysregulation, hypersensitivity to psychostimulants	(Swoap et al., 2004; Weinshenker et al., 2002)
MAO-A	Aggressive behaviors	(L. Chen & Zhuang, 2003)
COMT	Sexually dimorphic effects, aggression in heterozygotes only	(Gogos et al., 1998)
VMAT2	Homozygote: not viable Heterozygote: supersensitivity to psychostimulants	(L. Chen & Zhuang, 2003)
DAT	Growth defects, hyperlocomotion, impaired working memory, sleep dysregulation	(L. Chen & Zhuang, 2003)
NET	Enhanced sensitivity to psychostimulants, enhanced morphine-induced analgesia	(Gainetdinov & Caron, 2003)

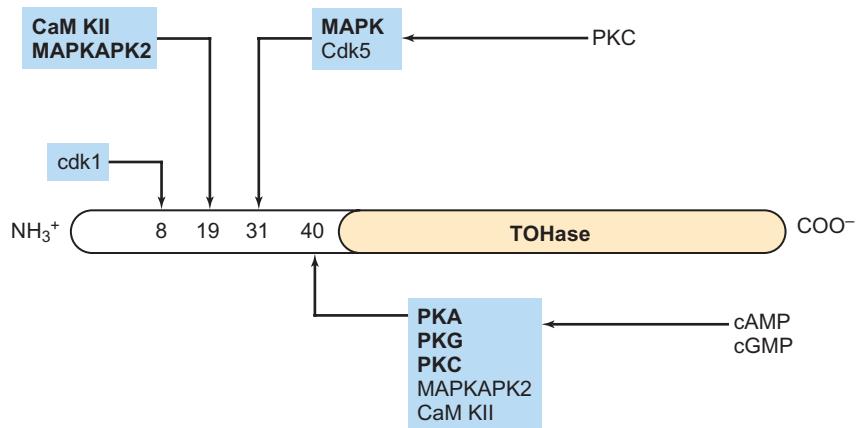


FIGURE 14-2 Schematic diagram of the phosphorylation sites on each of the four 60 kDa subunits of tyrosine hydroxylase (TOHase). Serine residues at the N-terminus of each of the four subunits of TOHase can be phosphorylated by a number of protein kinases. For the kinases underlined, there is reasonable evidence that they phosphorylate the enzyme *in situ* (Dunkley et al., 2004). Serine-40 can be phosphorylated by protein kinase A (PKA) and protein kinase G (PKG), MAPK-activated protein kinase 2 (MAPKAP2), calcium/calmodulin-dependent protein kinase II (CaM KII), and protein kinase C (PKC). Phosphorylation of serine-40 by PKA results in enzyme activation. Serine-31 can be phosphorylated by MAPK and cyclin-dependent kinase 5 (Cdk5). Phosphorylation of serine-31 leads to an increase in enzyme activity. Serine-19 is phosphorylated by CaM kinase II and MAPKAP2. Phosphorylation by CaM kinase II will activate the protein but only upon addition of the 14-3-3 protein. There is no evidence that phosphorylation of the serine-8 residue leads to tyrosine hydroxylase activation.

compound is used to examine the role of catecholamines in various physiological or pathological states. A very small number of humans have tyrosine hydroxylase deficiency, an autosomal recessive disorder caused by a mutation in the promoter region of the tyrosine hydroxylase gene (Willemsen, et al., 2010). These patients express either a hypokinetic-rigid syndrome with dystonia that responds well to L-DOPA, or a complex encephalopathy.

Aromatic amino acid decarboxylase (AAAD), also called DOPA decarboxylase, catalyzes the conversion of L-DOPA to dopamine

AAAD is a preferred name because the enzyme also catalyzes the decarboxylation of 5-hydroxytryptophan to

5-hydroxytryptamine, or serotonin. AAAD is a pyridoxine-dependent dimer of 53 kDa that is widely distributed in the body. AAAD has been identified in monoaminergic neurons and neurons that do not contain other monoamine synthetic enzymes, as well as in glia, kidney and blood vessels. The K_m for L-DOPA is 165 μM and the V_{max} is 1.5 nmol/min/mg protein (Siow & Dakshinamurti, 1990). Despite its high activity and seemingly constitutive nature, the enzyme is subject to regulation and has important clinical and physiological roles. AAAD is regulated at pre- and posttranslational levels by a number of receptors, pharmacological agents and protein kinases (Berry et al., 1996). The enzyme is solely responsible for the synthesis of ‘trace amines’ such as 2-phenylethylamine, tryptamine and tyramine. Trace amines may act as endogenous modulators of central transmission. They activate G-protein-coupled trace amine receptors

(Bunzow et al., 2001), and many are substrates for monoamine transporters. AAAD is of clinical importance in Parkinson's disease, in which it becomes the rate-limiting step for the conversion of therapeutically administered L-DOPA to dopamine. The pathology of Parkinson's disease includes a degeneration of dopaminergic cells in the substantia nigra, resulting in a progressive loss of dopamine in the basal ganglia (see Chap. 49). In Parkinson's disease AAAD activity in cells other than dopaminergic neurons becomes therapeutically important. Inhibition of peripheral AAAD is critical to increase the CNS bioavailability of L-DOPA. L-DOPA is commonly combined with CNS-impermeable inhibitors of AAAD, such as carbidopa or benserazide. AAAD can be inhibited experimentally by methyldopa, hydralazine and aminoxyacetic acid.

In noradrenergic and adrenergic neurons, dopamine is further converted to norepinephrine by Dopamine- β -hydroxylase (DBH)

DBH is located in adrenal medulla, sympathetic neurons, and noradrenergic and adrenergic neurons of the brain. Unlike

TH and AAAD, DBH exists in the synaptic vesicle and chromaffin cells in both membrane-bound and soluble forms. DBH characterized from bovine chromaffin cells is a glycoprotein consisting of four 75 kDa subunits linked by disulfide bonds. Two copper ions are bound per subunit and are required for enzymatic activity. DBH is also referred to as dopamine β -monoxygenase, as molecular oxygen is the source of the hydroxyl oxygen and there is a stoichiometric oxidation of the electron donor, ascorbic acid. During the reaction, ascorbic acid is oxidized to semihydroascorbate, which is then reduced back to ascorbate by the action of cytochrome *b*-561 (Diliberto et al., 1991). The K_m for dopamine is ~ 5 mM but the concentration of catecholamine in the synaptic vesicle or chromaffin cell is high. In addition to dopamine, phenylethylamines can serve as substrates. Enzyme that is not bound to the vesicle membrane is released upon stimulation of nerve terminals or chromaffin cells, but most is bound to the membrane. Because DBH is a Cu²⁺-containing enzyme, it can be inhibited by copper chelating agents, including diethyldithiocarbamate, disulfiram, D-cysteine, and L-cysteine. More selective inhibitors for DBH include fusaric acid and 3-phenylpropargylamine.

NEURONAL-SPECIFIC MISSPLICING IN FAMILIAL DYSAUTONOMIA

George J. Siegel

Familial dysautonomia (FD, also known as Riley-Day syndrome) is a disease consisting of autonomic and sensory neuropathy inherited as an autosomal recessive trait, present almost exclusively in Ashkenazi Jews with an incidence of 1/3600 live births. The carrier frequency in this population is 1/30. The disease is manifested at birth with poor sucking, failure to thrive, unexplained bouts of fever, and episodes of lung infections. In the growing child, there are manifestations of autonomic nervous system abnormalities such as lack of tears, defective temperature control, labile blood pressure, postural hypotension, excessive sweating, GI symptoms, absence of tongue fungiform papillae and autonomic crises with sweating, hypertension and vomiting. There is marked impairment of pain and temperature sensation. There is poor development and progressive degeneration of the sensory and autonomic nervous system. Nerve biopsies reveal decreases in the numbers of small myelinated and unmyelinated fibers, which explains the impairment of pain and temperature sensory function. Autopsy studies show diminished numbers of neurons in sympathetic and parasympathetic ganglia, in intermediolateral gray column, and, to a lesser extent, diminished neurons in sensory dorsal root ganglia (Pearson & Pytel, 1978).

Serum levels of dopamine beta hydroxylase, which hydroxylates dopamine to form norepinephrine (NE) in presynaptic NE-containing vesicles, are significantly reduced. This reduction is diagnostic. Patients excrete in their urine increased amounts of homovanillic acid (HVA), which is a dopamine metabolic product, and decreased amounts of vanillylmandelic acid (VMA) and methoxyhydroxyphenylglycol (MHPG), which are norepinephrine metabolic products [see text in this chapter]. Also, reduced

levels of monoamine oxidase A have been found in cells and tissues of FD patients (Rubin & Anderson, 2008). These biochemical findings are consistent with decreased NE-containing terminals and NE synthesis. Abrupt uncompensated changes in levels of either dopamine or NE could be involved in hypertensive crises.

The gene mutated in FD (*IKBKAP*) encodes the IKB kinase-complex associated protein (IKAP/hELP1) which is the human homologue of the yeast ELP 1 protein (Blumenfeld et al., 1993). The latter protein is part of the RNA polymerase II-associated elongator complex, but it is not known whether hELP1 serves the identical function. Results from various experimental models implicate its function in both transcription and translation (Svejstrup, 2007).

The mutation found in 99.5% of FD patients is a T-to-C change in base pair 6 of the 5'-splice donor site on intron 20, resulting in skipping of exon 20 and causing a frameshift (with exon 19 spliced to exon 21), therefore producing a truncated protein. However, this deletion of exon 20 is tissue specific. Lymphoblast RNA from FD patients showed normal length RNA containing exon 20. RNA extracted from postmortem brain stem and temporal lobe samples showed complete absence of exon 20 from the *IKBKAP* mRNA. Fibroblast lines from FD patients homozygous for the mutation yielded variable results: some lines displayed about equal amounts of wild-type and mutant-type mRNAs, whereas others displayed primarily wild type. The absence of exon 20 in the brain samples of RNA together with the preponderance of wild-type RNA or variable proportions of mutation type to wild type in lymphoblast and fibroblast cell lines, all from homozygous FD patients, has led to the

NEURONAL-SPECIFIC MISSPLICING IN FAMILIAL DYSAUTONOMIA (cont'd)

hypothesis that the efficiency of splicing between exon 20 and 21 in the presence of the T to C mutation at base pair 6 at the splice donor site is reduced in a tissue specific manner, and that the autonomic and sensory neuronal systems have a particularly greater sensitivity to this inefficiency. The mutation does not completely abolish the splicing of exon 20 to 21 but weakens it. The degree of weakening may, of course, depend on other tissue-specific factors (Blumenfeld et al., 1993; Slaugenhaupt & Gusella, 2002).

The creation of a transgenic mouse line harboring the human FD-*IKBKAP* gene shows that the presence of the FD mutation causes missplicing of human *IKBKAP* in mice and that the efficiency of exon 20 inclusion varies in a tissue-specific manner that closely models that seen in FD patients. Additionally, this study showed in tissue culture experiments that missplicing of human *IKBKAP* in mouse cells can be corrected by kinetin treatment, demonstrating conservation of cellular factors required for kinetin activity (Hims et al., 2007).

It is thought that the IKAP/hELP1 functions in general gene-activation mechanisms. The FD disease may be caused by aberrant expression of genes crucial to the development of the sensory and autonomic nervous systems, secondary to the loss of a completely functional IKAP/hELP1 protein in specific tissues. However, the pathogenic mechanism for the FD phenotype is not known (Slaugenhaupt & Gusella, 2002).

A microarray expression study using RNA extracted from postmortem cerebrum of an 11-year-old male and a 47-year-old female patient with FD revealed no genes upregulated but a twofold decrease in expression of 25 genes in comparison to results from normal age- and gender-matched control samples. Of these, 13 are known to be involved in oligodendrocyte differentiation and/or myelin formation. Their downregulation was confirmed by PCR and protein analyses. These data support a view that IKAP/hELP1 controls a complex process responsible for axon development and myelination in the CNS and PNS. However, many questions remain for investigation (Cheishvili et al., 2007). A study of genes affected by IKAP/hELP1 in HeLa cells transfected with RNAi oligomers that target the IKAP/hELP1 transcript disclosed about 100 genes that were significantly downregulated, while about 15 genes were upregulated in a microarray analysis of total extracted mRNA. It was reported that 15% of the 100 downregulated genes encode proteins involved in regulating the actin cytoskeleton, cell motility and migration. Moreover, it was shown that decreases in levels of IKAP/hELP1, in neuronally derived and other cell types, did in fact result in migration defects. It was proposed that reduced motility of neuronal-derived cells may be relevant to the neurodevelopmental disorder in FD (Close et al., 2006). Another study utilizing RNAi with primary neuronal cells also revealed that cells expressing very low amounts of IKAP displayed significant defects in migration and adhesion and that these defects were associated with the inability of filamin A to localize at leading

edges of migrating cells and with disorganized actin cytoskeleton. These defects could be rescued by co-expression of wild-type IKAP but not co-expression of the truncated FD-IKAP (Naumanen et al., 2008).

The fact of tissue-specific missplicing of the *IKBKAP* transcript is a platform from which to initiate investigation of potential therapeutic or preventive strategies. Potential strategies for therapy, such as kinetin, may be tested in the transgenic mouse model mentioned above (Hims et al., 2007). Kinetin was administered orally for eight days to 29 healthy carriers of the mutated gene, which resulted in elevations of *IKBKAP* mRNA in leukocytes, thus indicating (1) a potential therapeutic or preventive effect in FD and (2) that splicing disorders might be modulated by pharmacologic means (Gold-von Simson et al., 2009).

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In select neurons and adrenal medulla, norepinephrine is metabolized to epinephrine by phenylethanolamine-n-methyltransferase (PNMT)

Chromaffin cells and a small group of neurons in the brain stem utilize epinephrine as a transmitter. Using S-adenosylmethionine as a methyl donor, PNMT catalyzes the N-methylation of norepinephrine. A number of phenylethanolamines can serve as substrates for the enzyme. PNMT is a soluble, 30kDa, monomeric enzyme.

Catecholamines are stored in small, clear synaptic vesicles or large, dense-core granules

After synthesis, catecholamines are stored in synaptic vesicles. This storage is crucial to maintaining a supply of catecholamines that can be released, and also to mediate the process of release through exocytosis. The majority of monoamine-labeled synaptic terminals are characterized by the presence of small, clear vesicles (40–60 nm) and large, granular or dense-core vesicles (70–120 nm) within the terminal (Maley et al., 1990). In the adrenal medulla, catecholamines are stored in large dense-core chromaffin granules (~300 nm). In both the large, granular vesicles and the chromaffin granules, catecholamines are stored with proteins, notably chromogranin, peptides, and DBH at sites where norepinephrine is synthesized. Synaptic vesicles and granules both contain adenosine triphosphate in a molar ratio of catecholamine to ATP of about 4:1.

Catecholamines are transported into synaptic vesicles and chromaffin granules through vesicular monoamine transporters (VMATs) (see Table 14-2). As stated above, the concentration of catecholamines inside vesicles and granules is very high, so the catecholamines must be pumped in against their gradient. The energy for the transport is provided by the coupling of VMAT to an H⁺-adenosine ATPase in the vesicle membrane. The ATPase is similar in composition to the ATPase found in the lysosomes, endosomes, Golgi membranes and clathrin-coated vesicles. The proton pump is electrogenic such that the inside of both catecholamine synaptic vesicles and chromaffin granules are acidic, with an internal pH of ~5.6. Two forms of the vesicular monoamine transporter have been identified and cloned. VMAT1 is expressed in adrenal chromaffin granules and intestinal enterochromaffin cells. VMAT2 is expressed in CNS synaptic vesicles, in postganglionic sympathetic cells and in chromaffin granules. VMAT2 has substrate specificity for catecholamines, serotonin and histamine. Hydropathy plots derived from the protein sequence predict that VMAT2 contains 12 putative transmembrane domains (TMDs) with both N- and C- termini in the cytoplasm and a large, hydrophobic, N-glycosylated loop between TMDs 1 and 2 facing the vesicle lumen. VMAT has sequence similarity to a family of bacterial antibiotic-resistant transporters that exclude antibiotics, suggesting that it may also have functioned as a detoxification system. VMAT2 protects neurons by sequestering the dopamine neurotoxin, 1-methyl-4-phenyl-pyridinium (MPP⁺). Two drugs, reserpine and tetrabenazine, which have been instrumental in defining catecholamine function in the brain, deplete catecholamines via their inhibition of VMAT. Reserpine is an alkaloid

derived from the roots of the *Rauwolfia serpentine* (snake root) plant. Tetrabenazine is a synthetic compound. Reserpine will inhibit both VMAT1 and VMAT2 while tetrabenazine is active only against VMAT2.

Catecholamines are stored relatively stably in chromaffin granules, turning over with a half-life of greater than one day. On the contrary, neuronal catecholamine storage is much more dynamic. Dopamine escapes within minutes from purified brain synaptic vesicles, by both a VMAT-dependent route and a VMAT-independent route that is probably diffusion (Floor, et al., 1995). Half-times for escape of endogenous norepinephrine from synaptic vesicles in peripheral nerves range from 3 to 43 minutes. Therefore, catecholamine storage in neurons is very dynamic as compared to that in chromaffin granules. The demands of exocytosis require the constant filling of the synaptic vesicle, further emphasizing the high activity of VMAT2 in central nerve terminals.

Catecholamines are released from synaptic vesicles and the vesicles recycle

Stimulus-induced release of catecholamines in the central nervous system and chromaffin granules occurs by exocytosis. Following an influx of Ca²⁺ into the cell through voltage-sensitive Ca²⁺ channels, the synaptic vesicle fuses to the membrane and discharges its contents: catecholamines, ATP, and DBH, if a chromaffin granule. Vesicles are targeted to a specialized site on the nerve terminal overlapping the synapse called the active zone, where they dock and fuse to the membrane. Following exocytosis of the vesicular contents, the vesicle undergoes endocytosis and recycling. This process is described in detail in Chapter 7. Catecholamines are stored and released from both small clear vesicles and larger granular vesicles. Although there are similarities in the mechanisms of release from small synaptic vesicles and large dense-core vesicles, there are noted differences (Edwards, 1998). Release from large dense-core vesicles is not restricted to an active zone, requires a stronger stimulation and takes place over a longer time scale than release from small synaptic vesicles. Catecholamine release from these two different compartments may thus serve distinct roles in signaling. Following exocytosis, synaptic vesicles are quickly subject to endocytosis through a clathrin- and dynamin-dependent mechanism. It is possible that Ca²⁺ stimulates both exocytosis and endocytosis. Due to the demands of exocytosis on the neuron, synaptic vesicles recycle locally and do not need to be replenished from the soma. In neurons, synaptic vesicles primarily regenerate directly from the plasmalemmal membrane or can undergo a more complex recycling pathway involving endosomal intermediates. For a more complete discussion of neurotransmitter storage and release see Fon & Edwards (2001).

The physiological actions of catecholamines are terminated by reuptake into the neuron, catabolism and diffusion

The primary mechanism of inactivation of catecholamine action is reuptake into the terminal via plasmalemmal transporters. In 1970 Julius Axelrod won the Nobel Prize

for discovering that norepinephrine was taken back up into nerve terminals from which it was released. Despite the significance of this finding, the transporter proteins involved were considered to be low-affinity, constitutively active sites that conducted a mundane albeit important 'housekeeping' activity. Cloning of these transporters and development of reagents and techniques to better study them led to the discovery that they are highly mobile proteins which traffic in and out of the plasmalemmal membrane, and are subject to regulation by substrates, inhibitors and psychoactive drugs. Moreover, the transporters play a critical role in maintaining normal synaptic function and presynaptic catecholamine homeostasis.

The dopamine and norepinephrine transporters (DAT and NET, respectively) belong to the SLC6 family of Na^+/Cl^- -dependent transporters (Table 14-2). The co-transport of Na^+ and Cl^- provides the energy for the transporters to pump catecholamines into the terminals against their concentration gradient. The Na^+ gradient in the membrane is maintained by the sodium-potassium pump Na^+/K^+ -ATPase. The uptake is saturable and obeys Michaelis-Menton kinetics. Following exocytotic release, the catecholamine diffuses to the transporter, which exists outside the active zone. Upon binding to the transporter, the catecholamine is taken up into the

terminal and subsequently pumped into the synaptic vesicle to protect it from catabolism. Concentrations of catecholamines in the synapse can reach low micromolar levels following exocytosis, and the affinities of the transporters for the catecholamines reflect those levels. Although transporters are generally specific for their substrate, there is overlap between DAT and NET. K_m values for dopamine uptake at cloned DAT and NET are $5.2\text{ }\mu\text{M}$ and $0.24\text{ }\mu\text{M}$, while the K_m for norepinephrine at DAT and NET are $17\text{ }\mu\text{M}$ and $0.58\text{ }\mu\text{M}$, respectively (Gu et al., 1994). Therefore, NET has higher affinity for the endogenous catecholamines than does DAT, but DAT displays greater substrate selectivity for dopamine versus norepinephrine than does NET. This becomes especially important in an area such as the prefrontal cortex where there are both DAT and NET and there is competition for dopamine uptake. In the rat prefrontal cortex, the DAT content is low and dopamine uptake is primarily through NET. In the basal ganglia, however, an area important for locomotion, cognition and reward, there is very little NET and DAT is a key determinant of the duration and intensity of dopaminergic transmission (Jones et al., 1998).

There is a 66% homology in the amino acid sequence between human DAT and NET, which is not surprising considering the similarities of their pharmacological profiles. Molecular cloning has demonstrated that these proteins are highly conserved among species. Hydropathy analysis of the amino acid sequence predicted structures with 12-transmembrane rings with a glycosylated large second extracellular loop and intracellular amino- and carboxy-termini (Fig. 14-3). Functionally, the transporter is a pump, or a gated channel, whereby gates can alternate access to substrate at either side of the membrane. A greater understanding of the Na^+/Cl^- -dependent transporter structure and function was attained upon the solution of the crystal structure of a homologous bacterial leucine transporter (Yamashita et al., 2005). An important feature of the structure was the binding of substrate and Na^+ ions close together in a hydrophobic binding pocket contained within partially unwound membrane-spanning domains. DAT and NET undergo clathrin-dependent endocytosis and recycling to the surface; substrates, inhibitors and phosphorylation regulate the trafficking. DAT and NET exist

TABLE 14-2 Properties of Amine Transporters

	DAT	NET	VMAT-2
Mechanism	Na^+/Cl^- dependent	Na^+/Cl^- dependent	H^+ dependent
Transmembrane segments	12	12	12
Amino acids	617	620	742
Chromosome	16	5	10
Blockers	GBR12909, benztropine, cocaine, methylphenidate	Nisoxetine, desipramine, atomoxetine	Reserpine, tetrabenazine

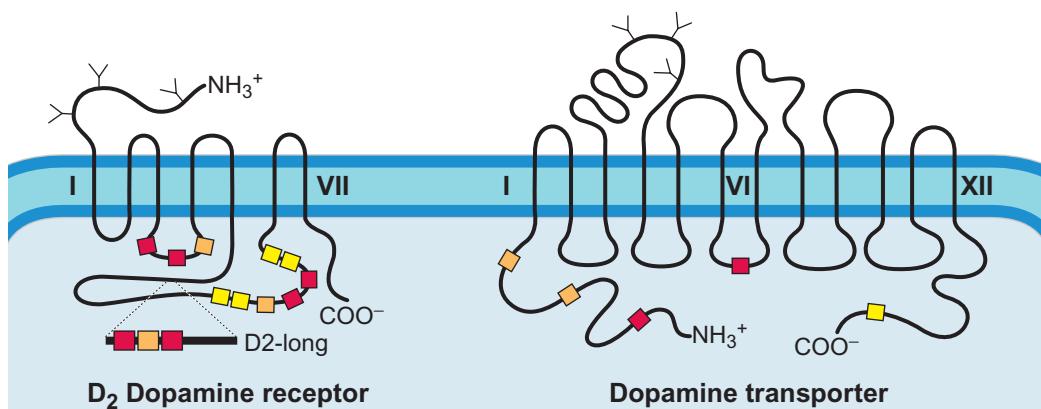


FIGURE 14-3 Schematic of the D2 receptor and dopamine transporter. There is evidence that the D2S autoreceptor and the dopamine transporter bind to each other through the i3 of the D2S receptor and the amino terminal of the dopamine transporter (Lee et al., 2007).

as functional oligomers, although individual homomers are still functional as transporters. Evidence suggests that substrates may act to dissociate oligomers prior to internalization (Chen & Reith, 2008).

A number of psychoactive and therapeutically useful drugs have their main sites of action at DAT and NET. Amphetamines, such as *d*-amphetamine and methamphetamine, are substrates at both DAT and NET. As substrates, they are transported along with Na^+ and Cl^- ions into the cytoplasm. Upon dissociation of the amphetamines, the buildup of Na^+ at the inner face of the membrane promotes the binding of available dopamine, which is then transported out of the terminal into the synapse. Amphetamines also bind to VMAT2, blocking storage of catecholamines in vesicles. Although they have somewhat greater affinity at NET, the ability of the amphetamines to promote locomotion and reward are due primarily to their action at DAT. Cocaine, on the other hand, is a competitive reuptake blocker with greater affinity for DAT than NET. Both amphetamines and cocaine act to increase synaptic dopamine. Methylphenidate (Ritalin) is an amphetamine by structure, but is a DAT inhibitor like cocaine. Both amphetamine and methylphenidate are therapeutically useful in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy. Modifications have been made to the formulations to reduce abuse liability. Neurotoxins, such as MPP⁺ and 6-hydroxydopamine (6-OHDA), which cause lesions in dopaminergic cells, are transported into the cells through DAT. Benztrapine (Cogentin), a compound used in the treatment of Parkinson's disease, is selective for blockade of DAT over NET. Compounds such as GBR12909, a diphenylpiperazine derivative, are used experimentally as selective inhibitors of DAT.

Blockade of norepinephrine and/or serotonin reuptake has proven to be a successful therapeutic strategy for the treatment of depression (Chapter 60). Secondary amine tricyclic antidepressants, such as nortriptyline, have greater selectivity for norepinephrine than serotonin reuptake, and are used to treat depression and neuropathic pain. Tricyclic anti-depressant drugs, especially the tertiary amines, also bind many monoamines, eliciting a number of untoward effects. Recently drugs have been developed that either are specific for both norepinephrine and serotonin transporters (venlafaxine (Effexor) and duloxetine (Cymbalta)), or are specific only for the norepinephrine transporter (atomoxetine (Strattera)). In the United States, atomoxetine is used primarily to treat ADHD.

Diffusion also plays an important role in the inactivation of catecholamines

Neither DAT nor NET is located directly in the synaptic cleft, so that dopamine and norepinephrine must diffuse to the reuptake sites. Brain regions differ in their density of reuptake sites; catecholamines need to diffuse farther to reuptake sites in the cerebral cortex than in the striatum (Lewis, et al., 2001). The presence of catecholamine receptors at sites removed from the synapses signals that transmitters must diffuse to these sites; this is called volume transmission. Volume transmission has been described for both dopamine (Smiley et al., 1994) and norepinephrine (Aoki et al., 1998).

Catecholamines are primarily metabolized by monoamine oxidase and catechol-o-methyltransferase

Metabolism of catecholamines takes place locally in the neuron and largely independently of exocytosis (Eisenhofer et al., 2004). The preponderance of catecholamine metabolism occurs in the terminal following leakage of the catecholamine from the vesicle. Because turnover is a reflection of synthesis and degradation, this means that catecholamine turnover is also largely dependent on vesicle leakage.

Monoamine oxidase (MAO)

Within the terminal, catecholamines are metabolized by monoamine oxidase (MAO) (Fig. 14-4). MAO is a flavin-containing enzyme that catalyzes the oxidative deamination of a number of monoamines, including the catecholamines, serotonin and trace amines. There are two isozymes of MAO, termed MAO-A and MAO-B, and they are located on the outer membrane of mitochondria. The subtypes were originally defined based on differences in substrate and inhibitor sensitivity. MAO-A has a higher affinity for serotonin and norepinephrine, while MAO-B prefers β -phenethylamine. Pharmacological criteria, however, best identify the two isozymes: MAO-A is sensitive to low concentrations of clorgyline, while MAO-B is best inhibited by low concentrations of deprenyl (selegiline). The two isozymes have 70% homology in their amino acid sequence; the shared pentapeptide sequence (Ser-Gly-Gly-Cys-Tyr) binds the cofactor FAD. In the brain, MAO-A is predominantly found in catecholaminergic neurons, while MAO-B is the form most abundant in serotonergic and histaminergic neurons and especially in glial cells (Shih et al., 1999). MAO-A is concentrated in noradrenergic cell bodies in the locus caeruleus, while MAO-B is the predominant form in serotonergic cell bodies in the raphe nucleus. MAO degrades catecholamines into their corresponding aldehydes, which are then oxidized into acids by aldehyde dehydrogenases or converted into alcohols or glycals by aldehyde reductase. The byproducts of these reactions include potentially neurotoxic species, such as hydrogen peroxide.

MAO has a profound effect on the concentrations of central and peripheral monoamines, and thus MAO inhibitors have been useful in clinical states, such as depression, where it is advantageous to increase monoamine levels. Many of the older MAO inhibitors used therapeutically are irreversible inhibitors; that is, after binding, they form a 'dead-end' complex with the enzyme. Restoration of enzyme activity involves the synthesis of new enzyme. Examples of irreversible inhibitors are phenelzine and iproniazid, which are nonselective for the isoforms. Slightly newer, yet irreversible, inhibitors are clorgyline and selegiline, which are selective for MAO-A and MAO-B, respectively. Newer, reversible inhibitors are now available, such as moclobemide, which is selective for MAO-A. MAO inhibitors were widely, and successfully, used as antidepressant drugs before the development of newer, more targeted approaches. Their use is limited because of potentially dangerous side effects such as liver toxicity and interactions with other drugs that elevate catecholamines, causing hypertensive crisis. Selective MAO-B inhibitors, however, notably

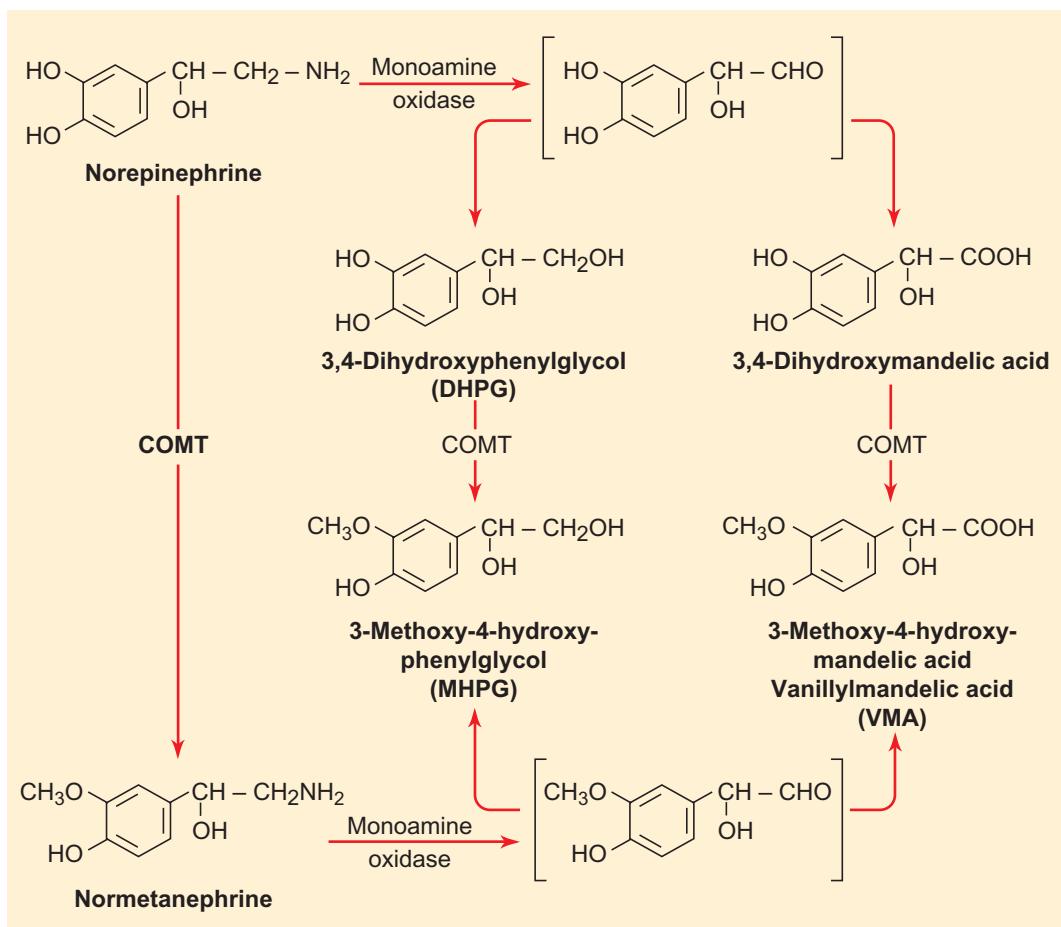


FIGURE 14.4 Pathways of norepinephrine degradation. Unstable glycol aldehydes are shown in brackets. COMT, catechol-O-methyltransferase.

selegiline and rasagiline, are used therapeutically as adjunct therapy in Parkinson's disease to elevate dopamine levels. MAO-B is not located in the dopamine nerve endings in the basal ganglia, but instead is located in the glia.

Catechol-O-methyltransferase (COMT)

COMT is synthesized in two forms from a single gene. The allozymes are referred to as COMT-S, for soluble, and MB-COMT for membrane bound; the latter form contains 50 hydrophobic amino acids not present in S-COMT. The molecular weights of the allozymes are 29.6 kDa for S-COMT and 30 kDa for MB-COMT. MB-COMT is the predominant transcript in brain, and will be referred to simply as COMT. There is controversy surrounding both the cellular and subcellular location of COMT in the brain (Tunbridge et al., 2006). Cellularly, COMT appears to be on postsynaptic neurons, but low levels may also exist on glia. The subcellular localization of MB-COMT has been assigned to the plasmalemmal membrane and the outer membrane of mitochondria, but more recent evidence demonstrates its presence on the rough endoplasmic reticulum. Because the preponderance of studies of dopamine metabolism have focused on the basal ganglia, COMT was ascribed a relatively minor role in

dopamine metabolism and termination of dopamine activity as compared to MAO and dopamine reuptake. However, the examination of areas sparsely populated with the dopamine transporter, such as the prefrontal cortex, has revealed an important role for COMT. Interestingly, COMT appears to be important for regulating dopamine but not norepinephrine levels in the prefrontal cortex.

COMT catalyzes the transfer of the methyl group of S-adenosylmethionine to the 3-position hydroxyl group of a number of compounds including the catecholamines, their hydroxylated metabolites, L-DOPA, catecholestrogens and ascorbic acid. Other dietary and medicinal products are also substrates for COMT, such as α -methyldopa, benserazide and carbidopa. The general purpose of COMT is to eliminate biologically active and toxic catechols and other hydroxylated metabolites. The enzyme is dependent on Mg^{2+} and inhibited by Ca^{2+} . The K_m s for MB-COMT for DA (3 μ M) and norepinephrine (24 μ M) are significantly less than those of S-COMT (280 μ M for dopamine and 369 μ M for norepinephrine). The V_{max} values for MB-COMT are also less than those for S-COMT. The higher affinity of MB-COMT, however, gives greater relevance to COMT-MB in normal metabolism of synaptic catecholamines.

Early COMT inhibitors had dissociation constants in the micromolar range and thus were of limited use therapeutically. However, a second generation of inhibitors, the nitrocatechols, was developed and these exhibit reversible, tight binding to the enzyme. They also demonstrate good selectivity for COMT as compared to other enzymes using catechols as substrates. Tolcapone and entacapone are two orally administrable inhibitors that have been used clinically as adjunct therapy in Parkinson's disease. They have a significant effect in increasing the absorption and availability of L-DOPA by blocking its metabolism in the intestine. L-DOPA is readily metabolized to 3-O-methyldopa by COMT in the intestine. Tolcapone readily crosses the blood brain barrier but entacapone does not. Tolcapone was withdrawn from the market because it can trigger severe hepatotoxicity.

A functional polymorphism in the coding sequence leads to a tripartite distribution of COMT in humans. A G→ A substitution results in a valine to methionine substitution at position 158 in MB-COMT. The met¹⁵⁸ form has lower thermostability and thus lower activity at physiological temperatures. COMT genotype appears to affect prefrontal cortex physiology (Akil et al., 2003).

Dopamine metabolites

A principal metabolite of dopamine resulting from MAO and aldehyde dehydrogenase activity is 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC is then metabolized by COMT to homovanillic acid (HVA), which is the primary metabolite in humans (Kopin, 1985). 3-Methoxytyramine is formed by direct metabolism of dopamine by COMT, such that it has been considered a reflection of neuronal dopamine release. The levels in the CSF are low, indicating that it is rapidly metabolized. Both DOPAC and HVA enter the cerebral spinal fluid from which they are actively transported out of the brain and into the bloodstream. Accumulation of HVA in the CSF has been used as an index of functional activity of dopaminergic neurons in the brain. Generally, increased impulse flow in dopaminergic neurons leads to release of dopamine and an increase in dopamine metabolites. The brain, however, contributes at most 12% of the circulating plasma levels of HVA (Eisenhofer et al., 2004); most of circulating levels of HVA are due to intestinal metabolism of dopamine.

Norepinephrine metabolism

Whereas dopamine is primarily converted to the acidic metabolite, DOPAC, by MAO, norepinephrine is principally converted to the alcohol metabolite, 3,4-dihydroxyphenylglycol (DHPG). The presence of the β-hydroxyl group on norepinephrine and epinephrine favors metabolism via aldehyde reductase. DHPG is then further metabolized by COMT to 3-methoxy-4-hydroxyphenylglycol (MHPG). Patterns of norepinephrine metabolites differ among animal species. In human CSF, the predominant metabolite is MHPG followed by DHPG and low levels of vanillylmandelic acid (VMA). Considerable MHPG is formed in peripheral nerves, which is then metabolized to VMA in the liver (Eisenhofer et al. 2004). VMA is the major urinary metabolite of norepinephrine and epinephrine metabolism in humans.

NEUROANATOMY

Catecholamine systems have cell bodies in restricted regions of the brain stem and hypothalamus with neurons that project diffusely throughout the brain. For instance, there are about 15,000 to 20,000 dopamine neurons per side in the rat brain, but each projecting axon can branch to give rise to thousands of boutons in forebrain terminal areas (Anden et al., 1966). Catecholamine pathways in the brain were delineated in the 1960s. Using the technique of formaldehyde histofluorescence, Falck and Hillarp identified norepinephrine and dopamine in discrete neuronal areas in the brain. Dahlstrom and Fuxe (1964) subsequently utilized this technique, in conjunction with chemical and pharmacological tools, to map monoamine pathways in the rat brain. The nomenclature these investigators assigned to the monoamine nuclei is still used today and is convenient when comparing different species.

Dopaminergic nuclei exist primarily in the midbrain (groups A8 to A10) or in the diencephalon, olfactory bulb and retina (groups A11 to A17). Groups A8 to A10 comprise long projection systems, linking the substantia nigra (A9) and the ventral tegmental area (A8, A10) with the neostriatum (caudate putamen), limbic cortex (medial prefrontal, cingulated and entorhinal areas) and other limbic structures, such as the olfactory tubercle, nucleus accumbens, amygdaloid complex and piriform cortex (see Fig. 14-5). The more rostrally located A9 nucleus projects primarily to the dorsal caudate-putamen and comprises the nigrostriatal pathway. This pathway is part of the basal ganglia, which includes globus pallidus and subthalamic nucleus. The projections of the A8 and A10 nuclei to limbic and frontal cortical areas comprise the mesolimbic and mesocortical pathways, respectively. These distinctions are not absolute, as there is overlap among the midbrain cell groups. However, dopamine cells that are near each other are more likely to innervate a common region than cells that are more removed from each other.

Intermediate-length projection systems emanate from hypothalamic nuclei. One in particular, the tuberoinfundibular pathway, projects to the median eminence of the hypothalamus. Axons of this pathway release dopamine into the hypothalamic-hypophyseal portal system, where it is transported to the anterior pituitary gland. There, the dopamine acts on lactotrophs to inhibit the release of prolactin. Very short DA projection systems are located in the olfactory bulb and the retina.

A number of functions of dopamine can be inferred from the knowledge of the neuroanatomy of the dopamine system. Dopamine has a role in endocrine regulation (hypothalamic neurons), movement (nigrostriatal neurons), motivational processes, learning, affective behavior and cognition (nigrostriatal, mesolimbic and mesocortical neurons). The dopamine system is targeted by antipsychotic drugs (Chapter 58), anti-Parkinson's drugs (Chapter 49), stimulants, and drugs of abuse (Chapter 61).

There are three main groupings of norepinephrine cell bodies: the locus coeruleus (A6 and A4), the lateral tegmental system (A5 and A7) and the dorsal medullary group (A2). Axons from the most important nucleus, the locus coeruleus, project rostrally as the dorsal noradrenergic bundle, dorsally

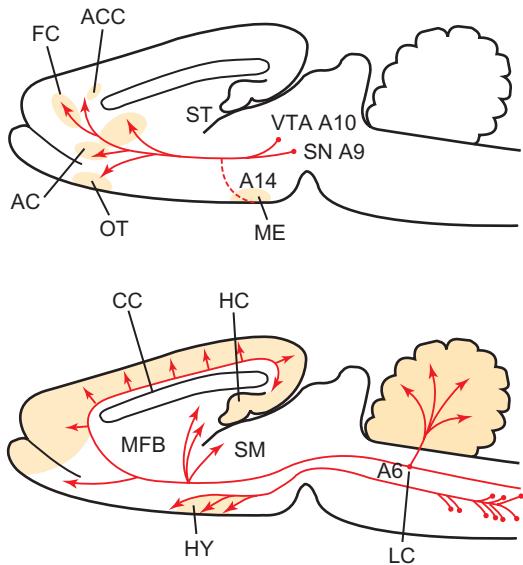


FIGURE 14-5 Some catecholaminergic neuronal pathways in the rat brain. **Upper.** Some dopaminergic neuronal pathways. A9, substantia nigra cell group; A10, ventral tegmental cell group. **Lower:** noradrenergic neuronal pathways. A6, locus coeruleus; AC, nucleus accumbens; ACC, anterior cingulated cortex; CC, corpus callosum; FC, frontal cortex; HC, hippocampus; HY, hypothalamus; LC, locus coeruleus; ME, median eminence; MFB, median forebrain bundle; OT, olfactory tubercle; SM, striated medullaris; SN, substantia nigra; ST, striatum. (Courtesy of J.T. Coyle and S.H. Snyder).

into the cerebellum and caudally into the spinal cord. The LC has a comparatively small number of neurons (~1500/side in the rat, 12,000 in human) but they widely innervate the cerebrum. The LC–norepinephrine system plays a major role in attention, arousal and vigilance. As discussed in Chapter 60, the NE system is targeted by antidepressant drugs. Some epinephrine-containing cells are intermingled with norepinephrine cells in the lateral tegmentum and dorsal medulla.

Catecholamines elicit their effects by binding to cell-surface receptors

The two primary catecholamine neurotransmitters in the brain, dopamine and norepinephrine, elicit an astounding number of diverse functional responses. Diversity in the action of the catecholamines initially arises from the location of the receptors and the multiple subtypes of the receptors that exist for each catecholamine neurotransmitter. In addition to occupying postsynaptic sites within the terminal fields, catecholamine receptors are also strategically located on their respective neurons and axonal terminals. On these sites the receptors function as autoreceptors, where upon activation by locally released transmitter, they regulate the release and, in some cases, synthesis of the neurotransmitter. Multiple subtypes of catecholamine receptors lead to further divergence in activity. The effects of dopamine are mediated through activation of five different receptors, referred to as D1-like (D1, D5)

and D2-like (D2, D3, D4). Norepinephrine and epinephrine carry out their physiological effects by acting at nine different receptors that are grouped into three families (α_1 , α_2 and β), each of which contains three subtypes encoded by different genes.

All catecholamine receptors are G-protein-coupled receptors (GPCRs) and have the typical GPCR structural characteristics: seven hydrophobic, transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. All of the receptors are metabotropic, that is, they function by activating G-proteins, which then affect enzymatic or ion channel activities. Because none of the catecholamine receptors are ion channels, they modulate but do not elicit rapid synaptic transmission. The receptors were initially characterized by the type of G-protein which they could activate, either G_s (D1-like, β -adrenergic) or G_i/G_o (D2-like, α_1 , α_2). The multiple numbers of subtypes for each receptor likely accounts for the fact that knockout of one receptor subtype does not have a strong phenotype. As shown in Fig. 14-6, a number of biochemical pathways are stimulated in response to activation of a catecholamine receptor, further delineating the multiplicity of responses to one catecholamine. Evidence now suggests that a specific conformation of a receptor is responsible for stimulating a specific activity, so that a catecholamine might serve as an activator (agonist) of one pathway and an inhibitor (antagonist) of another pathway requiring an alternate conformation. This is referred to as ligand-induced substrate selectivity. Different ligands selectively recruit various intracellular signaling proteins to produce diverse phenotypic effects in cells. The crystal structure for the β -adrenergic receptor has recently been solved and will aid in understanding the different conformations and ligand interactions. This concept is important not only for physiological responses to the catecholamines but also for drug development, because the catecholamines and GPCRs are important drug targets.

Dopamine receptors primarily exist in the brain but are also found in the kidney and peripheral vascular beds. The first dopamine receptor identified was the D1 receptor, which was found to couple to G_s (and G_{olf} in the striatum) and stimulate cyclic AMP formation. Subsequently the D2-like receptor was identified by antagonist binding studies, but was found to have a different pharmacology and location within the synapse from the D1 receptor. D2 receptors are coupled to G_i/G_o GTP-binding proteins and inhibit cAMP formation (Chapter 22). Subsequently subtypes within the D1 and D2 groups were identified by molecular cloning. Characteristics of each of the dopamine receptors are shown in Table 14-3. Although selective drugs are being developed, pharmacological overlap among the subtypes within each major group still makes it difficult to precisely functionally differentiate the individual subtype receptors. Receptor deletion mutants have helped in this respect. Although D1-like and D2-like dopamine receptors are both located in many dopaminergic terminal areas, there is some selectivity in localization, as shown in Table 14-3.

Both D1-like and D2-like dopamine receptors have a ‘typical’ GPCR structure, but differ in that the D1 receptor has a smaller third intracellular loop (i_3) but a longer intracellular C-terminal tail than does the D2 receptor. Dopamine receptors are subject to posttranslational modifications including glycosylation, palmitoylation and phosphorylation. Consensus

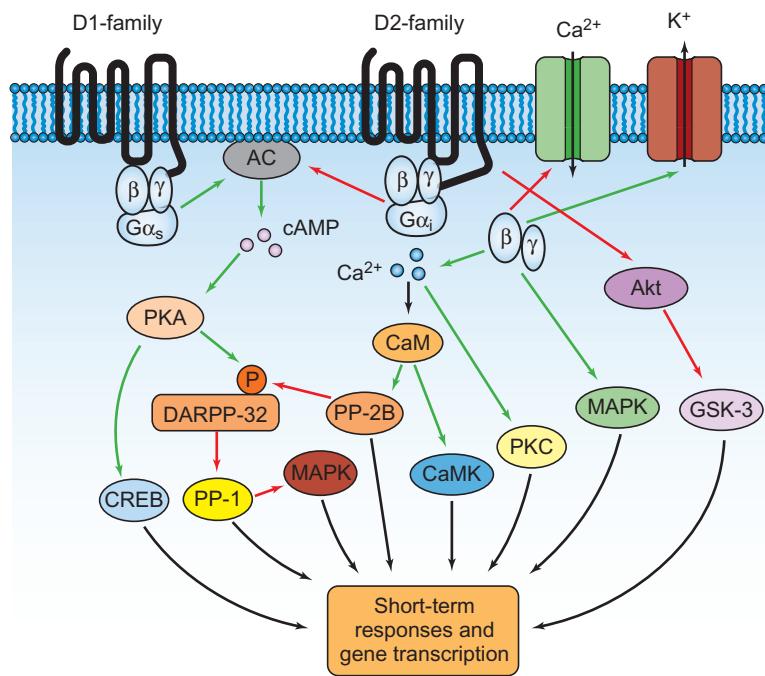


FIGURE 14-6 Effect of dopamine on intracellular signaling pathways. Stimulation of receptors by agonists can change enzyme activities as well as gene expression. The D₁ family of receptors (D₁ and D₅) are coupled to adenylyl cyclase (AC) via a stimulatory GTP-binding protein (G_s) which consists of G_{αs}, a β and a γ subunit. The D₂ family of receptors (D₂, D₃ and D₄) inhibit adenylyl cyclase activity via coupling to an inhibitory GTP-binding protein (G_i). Activation of adenylyl cyclase leads to formation of cyclic AMP (cAMP) and activation of protein kinase A (PKA). The activated PKA phosphorylates, among other substrates, DARPP-32, which, when phosphorylated, will inhibit protein phosphatase-1. Activation of D₁-family receptors will result in activation of mitogen-activated protein kinase (MAPK). A prominent substrate of PKA that alters gene transcription is CREB (cAMP-response element binding protein). In addition to inhibition of AC by G_{iα}, activation of the D₂ family of dopamine receptors results in dissociation of the βγ subunit, which affects numerous activities. When dissociated from the G_{iα} subunit, the βγ subunit inhibits voltage-sensitive Ca²⁺ channels and activates voltage-sensitive K⁺ channels. The βγ subunit will also activate a phospholipase C isozyme, leading to an increase in intracellular Ca²⁺. The Ca²⁺ leads to activation of kinases and phosphatases including MAPK, protein kinase C (PKC) and calmodulin (CaM)-stimulated enzymes such as Ca²⁺/calmodulin-stimulated protein kinases (CaMK), as well as protein phosphatase-2B (PP-2B, calcineurin). One substrate of PP-2B is DARPP-32. Through a mechanism involving β-arrestin but independent of cAMP, activation of the D₂ family of receptors inhibits Akt activity, leading to an activation of glycogen synthase-kinase 3 (GSK-3) activity, which has direct effects on gene transcription (Beaulieu et al., 2007).

TABLE 14-3 Properties of Human Dopamine Receptor Subtypes

	<i>D₁-like</i>		<i>D₂-like</i>		
	D ₁	D ₅	D ₂	D ₃	D ₄
Amino acids	446	477	415/444	400	387
Chromosome	5	4	11	3	11
Effector pathways	↑cAMP	↑cAMP	↓cAMP ↑K ⁺ channel ↓Ca ²⁺ channel	↓cAMP ↑K ⁺ channel ↓Ca ²⁺ channel	↓cAMP ↑K ⁺ channel ↓Ca ²⁺ channel
Distribution	Caudate/putamen, nucleus accumbens, olfactory tubercle, cerebral cortex	Hippocampus, hypothalamus, cerebral cortex	Caudate/putamen, nucleus accumbens, midbrain	Olfactory tubercle, hypothalamus	Frontal cortex, medulla, midbrain, nucleus accumbens

sequences for phosphorylation have been identified within the i₃ and C-terminal tail. The D₂ dopamine receptor has two alternatively spliced mRNA variants, resulting in a short form (D_{2S}) and a long form (D_{2L}) of the receptor. The development

of a D_{2L} knockout mouse revealed that the D_{2S} form serves primarily as a presynaptic autoreceptor, while the D_{2L} form has a predominantly postsynaptic function (Usiello et al., 2000; Wang et al., 2000).

In the adult human brain D1 receptors are expressed at a higher level than any other dopamine receptor. D1-like receptors have a high affinity for benzazepines, such as SCH23390, a receptor antagonist, and a low affinity for benzamides, such as sulpiride. D1 and D5 receptors share similar pharmacology, with the exception that D5 receptors have a higher affinity for DA than D1 receptors. Through activation of either G_s or G_{olf} in the striatum, the primary action of D1-like receptors is to activate PKA. Prominent substrates of D1-activated PKA are DARPP-32, a protein phosphatase inhibitor enriched in dopaminergic cells (Chapter 25), voltage- and ligand-gated ion channels and the cyclic AMP response element-binding protein, CREB. PKA-independent activities of D1-like receptors have been noted, such as activation of mitogen-activated protein kinase (MAPK) (Neve et al., 2004).

D2-like dopamine receptors are found in a variety of brain regions and were originally identified by their high affinity for radiolabeled antipsychotic drugs, such as [3 H]haloperidol. The importance of these receptors in schizophrenia (Chapter 58) was reinforced by the finding that there was a direct relationship between the potency of binding of many therapeutically effective antipsychotic drugs to the D2-like receptor and their clinical potency (Creese et al., 1976). Although both D1-like and D2-like dopamine receptors are important in basal ganglia and frontal cortical dopamine function, the D2-like receptors are the ones targeted therapeutically by antipsychotic and anti-Parkinson's drugs.

D2, D3 and D4 have overlap in their pharmacology, but some differences are emerging with the development of more specific ligands. D4 receptors have a particularly high affinity for the antipsychotic drug clozapine. All three subtypes have postsynaptic locations in dopamine terminal areas. Both D2 and D3 receptors are also located on dopamine neurons and thus would have autoreceptor activity. Evidence in knockout mice demonstrates that the D2 receptor is the functional dopamine autoreceptor in mice (L'Hirondel et al., 1998). In the basal ganglia, dopamine autoreceptors reduce both synthesis and release of dopamine, while synthesis-regulating autoreceptors are not evident in the prefrontal cortex.

D2-like receptor signaling is mediated through the activation of heterotrimeric $G\alpha_{i/o}$ proteins. In the brain, D2S and D2L receptors predominantly couple to $G\alpha_o$, while D3 and D4 receptors demonstrate more varied coupling. Through coupling to the $G\alpha_{i/o}$ proteins, the D2-like receptors inhibit adenylyl cyclase activity. The behavioral importance of this pathway to the action of D2-like receptors is demonstrated by the lack of inhibition of locomotor activity by antipsychotic drugs in mice null for adenylyl cyclase 5, the predominant adenylyl cyclase in postsynaptic neurons in the basal ganglia. Inhibition of adenylyl cyclase may also serve as a mechanism by which dopamine synthesis-inhibiting D2 autoreceptors act, but other mechanisms involving phosphatases may also have a role. D2-like receptors modulate many signaling pathways independent of adenylyl cyclase, including phospholipases, ion channels, MAP kinases, and the Na^+/H^+ exchanger. Many of these pathways, including those regulating ion channel activity, are mediated by $G\beta\gamma$. Evidence strongly suggests that release inhibiting dopamine autoreceptors act through $G\beta\gamma$ -mediated activation of G-protein regulated inwardly rectifying K^+ channels-2 (GIRK₂) channels.

ADRENERGIC RECEPTORS

Receptors for norepinephrine and epinephrine are referred to as adrenergic receptors. Because these catecholamines function as important peripheral neurotransmitters, adrenergic receptors are widely located throughout the body. Studies on peripheral tissues by Ahlquist (Ahlquist, 1948) in the 1940s led to the discovery of two major types of adrenergic receptor, differentiated based on agonist selectivity. α -Adrenergic receptors were potently activated by the natural catecholamines norepinephrine (NE) and epinephrine (Epi) but not the synthetic compound isoproterenol (ISO), giving the order $\text{NE} \approx \text{Epi} > \text{ISO}$. β -Adrenergic receptors, however, were more sensitive to isoproterenol than the natural catecholamines, displaying , ranking $\text{ISO} > \text{Epi} \approx \text{NE}$. Subtypes of the β -adrenergic receptors were identified in the 1960s followed by identification of subtypes of the α -adrenergic receptors in the 1970s (Bylund et al., 1994). Molecular cloning has identified further subtypes giving nine separate adrenergic receptors: three β -adrenergic (β_1 , β_2 , β_3); three α_1 -adrenergic (α_{1A} , α_{1B} , α_{1D}); and three α_2 -adrenergic (α_{2A} , α_{2B} , α_{2C}) receptors.

All adrenergic receptors are GPCRs

The three primary groups are distinguished by their pharmacology and by their separate signal transduction pathways. α_1 -Adrenergic receptors are coupled to G_q/G_{11} , which activates phospholipase C, leading to increased Ca^{2+} release and protein kinase C activation in the cell (Chapter 23). α_1 -Adrenergic receptors also activate phospholipase D and mitogen-activated protein (MAP) kinases (Table 14-4). α_2 -Adrenergic receptors are coupled to G_i/G_o proteins and primarily inhibit adenylyl cyclase and stimulate phospholipase A₂ activities (Table 14-5). Similar to D₂ dopamine receptors, activation of α_2 -adrenergic receptors leads to release of $G\beta\gamma$ resulting in activation of K^+ channels and inhibition of Ca^{2+} channels. All three subtypes of the β -adrenergic receptor are coupled to G_s and activate adenylyl cyclase activity (Table 14-6). G-protein-independent activation of MAPK by β -adrenergic receptor activation has been reported. The functional role of this effect is not yet known and it only occurs at high agonist concentrations. Recently the crystal

TABLE 14-4 Properties of Human α_1 -Adrenergic Receptor Subtypes

	α_{1A}	α_{1B}	α_{1D}
Amino acids	430–476	515	560
Chromosome	8	5	20
Effector pathways	$\uparrow\text{Ca}^{2+}$, protein kinase C	$\uparrow\text{Ca}^{2+}$, protein kinase C	$\uparrow\text{Ca}^{2+}$, protein kinase C
Distribution	Heart, liver, cerebellum, cerebral cortex	Spleen, kidney, fetal brain, blood vessels	Aorta, cerebral cortex

Modified with permission from Hieble et al. 1995; Bylund, 1992.

TABLE 14-5 Properties of Human α_2 -Adrenergic Receptor Subtypes

	α_{2A}	α_{2B}	α_{2C}
Amino acids	450	450	461
Chromosome	10	2	4
Effector pathways	\downarrow cAMP	\downarrow cAMP	\downarrow cAMP
Distribution	Pancreas, small intestine, locus ceruleus, hippocampus	Liver, thalamus	Heart, lung, aorta, hippocampus, olfactory bulb

Modified from Dylund, 1992 with permission.

TABLE 14-6 Properties of Human β -Adrenergic Receptor Subtypes

	β_1	β_2	β_3
Amino acids	477	413	402/408
Chromosome	10	5	8
Effector pathways	\uparrow cAMP	\uparrow cAMP	\uparrow cAMP
Distribution	Heart, kidney, cerebral cortex, hypothalamus	Lung, liver, cerebellum, hippocampus, cerebral cortex, smooth muscle, olfactory bulb	Fat, brain (?)

structures for the β_1 - and β_2 -adrenergic receptors have been solved (Cherezov et al., 2007; Rasmussen et al., 2007; Warne et al., 2008). Knowledge of the crystal structures will no doubt resolve issues of ligand and G-protein functional selectivity. Although all subtypes within a grouping utilize the same signal transduction pathways, they are not redundant in their activities, as demonstrated by the specific knockout mice (Table 14-7).

All subtypes of adrenergic receptors have postsynaptic locations at the synapse, but the α_2 -adrenergic receptor is also located presynaptically, serving as an autoreceptor to inhibit the release of norepinephrine and a presynaptic regulator of the release of other neurotransmitters. Evidence suggests that the α_2 -adrenergic receptor serves an autoreceptor function by releasing $G_{\beta\gamma}$ from $G_{i/o}$, which then binds to and inhibits N- and P/Q-type Ca^{2+} channels. α_{2A} and to some extent α_{2C} -adrenergic receptors serve as autoreceptors. Many β receptors are found on glia, where they have multiple actions such as reducing the uptake of glutamate, regulating glucose availability, and regulating inflammatory cascades.

Adrenergic receptors, like dopamine receptors, are subject to multiple posttranslational modifications. They are glycosylated, palmitoylated and phosphorylated on various residues. Mechanisms by which phosphorylation regulates GPCR activity were first elucidated using the β -adrenergic receptor and rhodopsin (Lefkowitz, 2004). Both PKA and G-protein receptor kinase (GRK) can phosphorylate and downregulate β -adrenergic receptors. PKA mediates heterologous or agonist nonspecific desensitization. Binding of agonist to the receptor, however, unmasks sites that can be phosphorylated by receptor G-protein-coupled receptor kinase 2 (GRK-2). Subsequent binding of a protein named arrestin to the phosphorylated

TABLE 14-7 Characteristics of Catecholamine Receptor Knockout Mice

Receptor Knockout	Phenotype	Reference
D_1 dopamine	Hyperlocomotion, aphagia, background dependence	(L. Chen & Zhuang, 2003)
D_2 dopamine	Parkinsonian-like motor impairment	(L. Chen & Zhuang, 2003)
D_3 dopamine	Mild hyperactivity	(L. Chen & Zhuang, 2003)
D_4 dopamine	Reduced spontaneous activity and reduced response to novelty	(L. Chen & Zhuang, 2003)
D_5 dopamine	Elevated locomotor and rearing activities, high blood pressure	(L. Chen & Zhuang, 2003)
α_{1A} -adrenergic	Decreased blood pressure	(Rokosh & Simpson, 2002)
α_{1B} -adrenergic	Decreased blood pressure, decreased response to CNS stimulants	(Cavalli et al., 1997)
α_{1D} -adrenergic	Decreased blood pressure	(Tanoue et al., 2002)
α_{2A} -adrenergic	Increased sympathetic activity, disrupted presynaptic inhibition of NE release at high frequencies	(Hein et al., 1999)
α_{2B} -adrenergic	Inability to develop salt-induced hypertension	(Makaritsis et al., 1999)
α_{2C} -adrenergic	Disrupted presynaptic inhibition of NE release at low frequencies	(Hein et al., 1999)
β_1 -adrenergic	Most die prenatally, normal resting heart rate	(Rohrer & Kobilka, 1998)
β_2 -adrenergic	Altered vascular tone and energy metabolism	(Chruscinski et al., 1999)
β_3 -adrenergic	Increased fat storage	(Rohrer & Kobilka, 1998)

receptor precludes coupling to G_s, resulting in desensitization of the receptor.

As stated above, norepinephrine in the CNS plays an important role in attention, arousal and vigilance. Activation of α₁-adrenergic receptors in the medial preoptic area leads to wakefulness, while blockade of these receptors elicits sedation. However, α₁-, α₂- and β-adrenergic receptors in the hypothalamus also are important components of the ascending arousal pathway (Mitchell & Weinshenker, 2010). Norepinephrine enhances long-term memory consolidation in the amygdala and hippocampus through actions at α₁- and β-adrenergic receptors. Further, α₂-adrenergic receptor agonists enhance prefrontal cortex cognitive function, including working memory. In contrast, release of high concentrations of norepinephrine acting on α₁-adrenergic receptors in the prefrontal cortex impairs prefrontal cortex function. Many antipsychotic drugs block α₁-adrenergic receptors and may ameliorate some prefrontal deficits by this mechanism. Norepinephrine, injected into the paraventricular nucleus of the hypothalamus, activates postsynaptic α₂-adrenergic receptors to elicit a robust eating response. Many therapeutically active antidepressant drugs increase norepinephrine levels, which would act by binding to adrenergic receptors.

AGONIST-INDUCED DOWNREGULATION

Chronic exposure to agonists diminishes receptor responsiveness. Through a number of molecular mechanisms, many involving phosphorylation, continued exposure of receptors to their agonists generally decreases the activity of the receptors. Agonists can reduce receptor responsiveness by dissociating the coupling of the receptor to the requisite G-protein (desensitization) or by promoting the removal of the receptor from the cell surface (internalization). As described above for the β-adrenergic receptor, agonist binding to its receptor elicits a conformational change promoting phosphorylation of the receptor by GRKs, leading to the recruitment of β-arrestin. Binding of β-arrestin to the receptor uncouples the receptor from G-proteins, resulting in desensitization, but can also target the receptor into clathrin-coated pits, leading to an internalization of the receptor. Interestingly, internalized β-arrestin itself elicits signal transduction activities, such as MAPK activation, and thus transduces signals from the GPCRs. With variations in subtypes of GRKs and β-arrestins, these mechanisms apply to all of the catecholamine receptors. The catecholamine receptors are also subject to heterologous desensitization. For instance, phosphorylation of the D2 dopamine receptor by protein kinase C, which is activated by G_q-coupled GPCRs, desensitizes the receptor.

Agonist-induced downregulation of receptor responsiveness has behavioral and clinical consequences. Blockade of catecholamine reuptake significantly increases the levels of catecholamines in the synapse. Repeated treatment of laboratory animals with tricyclic antidepressant drugs, which block the reuptake of norepinephrine and serotonin, downregulates presynaptic α₂-adrenergic receptors and postsynaptic β-adrenergic receptors. Similarly, amphetamine and cocaine, which increase synaptic dopamine, regulate the responsiveness of postsynaptic

dopamine receptors. Amphetamine is especially effective at increasing synaptic dopamine because, as a substrate, it competitively blocks dopamine reuptake and reverses the dopamine transporter to release more dopamine into the synapse. An acute dose of amphetamine leads to downregulation of both D₁-like and D₂-like dopamine receptors. Mice in which a select form of GRK has been deleted display enhanced locomotor behavior in response to amphetamine (Gainetdinov et al., 2004), due, presumably, to a blockade in dopamine-induced receptor downregulation. Amphetamine-stimulated D₂ receptor downregulation has diagnostic significance. In positron emission tomography (PET) studies in humans, D₂ receptor imaging is combined with intravenous injection of amphetamine to examine the interaction of endogenously released dopamine with the binding of radioligands to the D₂ receptor. It was puzzling to investigators that the binding capacity of the labeled D₂ receptor would be decreased long past the half-life of amphetamine. Recently, PET studies in β-arrestin knockout mice demonstrated that the prolonged decrease in D₂ receptor binding after amphetamine is due to internalization of D₂ receptors, not continued displacement by released dopamine (Skinbjerg et al., 2010).

REPEATED ANTAGONIST TREATMENT

In the converse of chronic agonist activity, repeated treatment with a receptor antagonist often results in an enhanced response to agonist. This is referred to as 'reverse tolerance' or supersensitivity. Changes in physiological responses to sympathetic amines after denervation were first described and characterized in the peripheral nervous system (Fleming et al., 1973; Trendelenburg, 1966). Denervation supersensitivity has two components: (1) a presynaptic component due to loss of the nerve terminal and its uptake system, resulting in enhanced sensitivity to agonist; (2) a more slowly developing component resulting from changes at the postsynaptic membrane, such as an increase in numbers of receptors or efficiency of receptor signaling.

Investigation of denervation supersensitivity to catecholamines was made possible by the use of the neurotoxin 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1971). 6-hydroxydopamine is a congener of dopamine and is taken up into catecholamine nerve terminals through the plasmalemmal monoamine transporter systems. Injection of 6-OHDA along with a selective norepinephrine reuptake inhibitor into the basal ganglia produces a degeneration of dopamine nerve endings in the terminal fields. Unilateral injection of the toxin creates a postsynaptic supersensitivity to dopamine agonists that is behaviorally demonstrated by circling behavior of the animal contralateral to the side of injection. The apparent 'change' in sides is due to the crossover of extrapyramidal neurons in the spinal cord. Conversely, if a dopamine mimetic drug such as amphetamine, which depends on the 'missing' presynaptic terminal for its activity, is given, ipsilateral turning is evident. This is an important behavioral model that is still used today to analyze changes in pre- and postsynaptic sensitivity of dopaminergic neurons.

Repeated treatment with dopamine receptor antagonists increases the concentration of postsynaptic D₁-like and D₂-like

dopamine receptors in the brain (Strange, 2001). This has clinical consequences because all antipsychotic drugs used clinically today, with one exception, are D₂-like dopamine receptor blockers. The one exception is aripiprazole, a weak dopamine agonist that when present in a synapse containing excessive dopamine will function as an antagonist. It has been postulated that postsynaptic supersensitivity due to repeated antipsychotic drug treatment could contribute to tardive or emergent dyskinesias, which result from chronic, potent D₂-like blockade (see Chapter 58).

Interestingly, repeated treatment with dopamine mimetic drugs, such as amphetamine, cocaine and even L-DOPA, also results in the behavioral supersensitivity often referred to in this literature as sensitization. The important factor in this treatment appears to be the intermittent nature of the drug administration. Continuous administration of L-DOPA or amphetamine through a pump will give the expected downregulation in dopamine receptor activity. Giving these drugs once a day, or even once a week, results in an exaggerated behavioral response to the same dose of the drug. Clinically, intermittent L-DOPA results in the development of dyskinesias, but it is not clear that continuous L-DOPA administration does not produce dyskinesias. Nevertheless, the use of drugs that extend half-life occupation of the D₂-like receptor is advised in the treatment of Parkinson's disease.

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