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Dopamine Receptor Diversity: Anatomy, Function, and Relevance to Parkinson's Disease

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INTRODUCTION

The importance of dopamine in the motor functions of the striatum is evident in Parkinson's disease (PD). The striatum controls motor activity by processing the flow of information arising from the cerebral cortex and projecting via direct and indirect pathways to the output nuclei of the basal ganglia. The degenerative loss of dopamine is a hallmark of this disease and leads to severe motor impairments that are relieved by dopamine agonists. However, dopamine plays a role not only in the execution of complex movement, but also in higher-order cognitive processes, including motor planning and sequencing, motor learning, and motivational drive and affect. Of the biogenic amine neurotransmitters, dopamine has been the best studied in the central nervous system (CNS). The actions of dopamine are segregated in different neural circuits. For example, dopamine in the nigrostriatal pathway is involved in the generation and execution of voluntary movement. In this function, dopamine is a prime modulator of

various other basal ganglia neurotransmitters, including gamma-aminobutyric acid (GABA), acetylcholine, glutamate, enkephalin, and substance P. Dopamine in the mesolimbic pathway plays a role in the control of various cognitive functions, including drive, reinforcement, attention, and in the addiction to psychostimulants.

Five different receptor subtypes that are members of the large G-protein–coupled receptor superfamily mediate the central effects of dopamine. Dopamine receptors are divided into two major subclasses, D1-like and D2-like receptors, which differ in their second messenger transduction systems and anatomical locations. The cloning of these receptors and their genes in the last decade has led to the identification of multiple dopamine receptor subtypes termed D1, D2, D3, D4, and D5. The D1 and D5 subtypes of dopamine receptors exhibit overlapping functional and pharmacological properties that are related to the D1 receptor (D1-like), whereas the remaining members of this receptor family share pharmacological characteristics that are similar to the D2 receptor subtype (D2-like). The two receptor families have overlapping but distinct neuroanatomical distributions as determined by radioligand binding autoradiography and immunocytochemical localization. Thus, the various functions of dopaminergic neurotransmission appear to be mediated by the regional expression of these different receptor subtypes.

The molecular cloning of dopamine receptor subtype genes and the identification of their different locations in the brain and distinct pharmacology has advanced medication development for the treatment of PD and serious mental illnesses. The focus on dopaminergic neurotransmission as a target for medication development is due largely to the recognition that alterations in dopamine function are involved in neurodegenerative and psychiatric brain disorders. Degeneration of the nigral dopamine-containing neurons contributes to the pathogenesis of PD (1). The antiparkinson effects of the indirect dopamine agonist levodopa and other direct-acting agonists are mediated by dopamine receptors localized to striatal neurons (for review, see Ref. 2). The chorea of Huntington's disease is due to a deterioration of the dopaminoceptive cells localized to the striatum. Schizophrenia and other psychotic disorders are thought to be due to an imbalance in corticolimbic dopamine signaling. Dopamine receptor antagonists are used for the clinical management of these disorders (3–5). Chronic dopamine receptor blockade leads to a dysregulation of central dopaminergic tone and the development of extrapyramidal syndromes, while involuntary movements and psychosis are observed with chronic administration of the indirect-acting agonist levodopa in PD (2). Antipsychotic medications act through the D2-like family of receptors. Although none of the dopamine receptor subtypes have been linked to the

etiology of schizophrenia, the distinct regional locations of D3 and D4 receptors in cerebral cortical and associated subcortical limbic brain areas suggest that subtype-selective neuroleptics that lack extrapyramidal side effects may be developed. The advent of new subtype-selective dopamine receptor agonists may provide neuroprotective effects in PD and modify symptom progression (for review, see Ref. 6).

MOLECULAR SUBTYPES OF DOPAMINE RECEPTORS

The molecular cloning and characterization of dopamine receptor heterogeneity was advanced by the early recognition that G-protein–coupled receptors are evolutionarily related (for review, see Ref. 7). The existence of a G-protein–coupled receptor supergene was proposed based on the reported sequences for rhodopsin and beta$_2$-adrenergic receptors (7). Both of these receptors have a membrane typology of seven highly conserved transmembrane domains of amino acid residues. Several structural features are common to all biogenic amine receptors. These include the specific aspartate and serine residues that interact with the neurotransmitter, sites for N-linked glycosylation located on putative extracellular regions, and consensus sites for phosphorylation by protein kinase A or C found on putative intracellular domains. These similarities suggested that all G-protein–coupled receptors had similar structural characteristics, a hypothesis that was immediately strengthened by the cloning and sequencing of the m2 muscarinic receptor (8). The identification of primary shared sequence homologies among G-protein–coupled receptors advanced the development of technical approaches for, first, the cloning of the D2 receptor (9) and, then, the D1 receptor (10,11) subtypes.

The complementary deoxyribonucleic acid (cDNA) for the D2 receptor was first isolated in 1988 (9), and subsequently alternative splice variants were identified (12,13). The cDNA encodes a protein of 415 amino acids, with three glycosylation sites in the N-terminus, a large third intracellular loop between transmembrane regions 5 and 6, and a short C-terminus. The D3 receptor was isolated by screening rat libraries with the known D2 sequence followed by polymerase chain reaction (PCR) extension (14). The topography of the D3 receptor includes a glycoprotein of 400 amino acids with a glycosylation site in the N-terminus and a short C-terminus. The D4 receptor was cloned by screening a library from the human neuroblastoma cell line SK-N-MC (15). The D4 glycoprotein is 387 amino acid residues in length with the characteristic seven transmembrane spanning domains, a large third intracellular loop, and a short C-terminus. The dopamine D1 (or D1$_a$) receptor was independently cloned by four separate groups of investigators (10,11,16,17). The isolation of cDNAs or

genes from rat or human DNA libraries was done by homology screening with a D2 receptor probe and by polymerase chain reaction with degenerate primers. Both the rat and human D1 receptor genes encode a protein that is 91% homologous for amino acid sequence. The second member of the D1-like receptor family, D5 was isolated using the sequence of the D1 receptor (18). The coding region for the carboxy terminal of the protein is about seven times longer for D1-like than for the D2-like receptors (19,20). The cloned D1 and D5 receptors are 446 residues in length and exhibit 91% amino acid sequence homology within the highly conserved seven transmembrane spanning regions.

The gene structure of D2 receptors demonstrated that the coding region contains six introns, the D3 receptor contains five introns, and the D4 has three introns (19,20). The presence of introns within the coding region of the D2 receptor family allows generation of splice variants of the receptor. For example, alternative splicing of the D2 receptor at the exon between introns 4 and 5 results in functional D2S (short) and D2L (long) isoforms (13). Putative nonfunctional proteins encoded by alternative splice variants of the D3 receptor also have been demonstrated (22–24). The human D4 receptor gene, located on the short arm of chromosome 11, has eight different polymorphic variants. The existence of polymorphic variations within the coding sequence of the D4 receptor demonstrated a 48-base-pair sequence in the third cytoplasmic loop that exists with multiple repeated sequences (25). The number of repeated sequences is related to ethnicity, with most humans (70%) having four repeats. Nonfunctional, truncated isoforms of the D5 receptor have been reported on human chromosomes 1 and 2 (20,25,26).

NEUROANATOMICAL LOCALIZATION OF DOPAMINE RECEPTOR PROTEIN AND MESSAGE

The dopaminergic systems in the brain comprise three distinct pathways, including the nigrostriatal, mesocortical, and mesolimbic projections (27). The nigrostriatal pathway originates in the ventral tier of neurons of the substantia nigra pars compacta and terminates in the striatum. The mesolimbic pathway originates in the ventral tegmental area (VTA) and paranigral area and projects to the limbic sectors of the striatum, amygdala, and olfactory tubercle. The mesocortical pathway originates in the VTA and terminates within particular sectors of the cerebral cortical mantle, including the prefrontal, orbitofrontal cingulate, and entorhinal cortices.

D1-like and D2-like receptors and message are abundant in the CNS, having a widespread distribution across the three dopaminergic projection systems. The anatomical localization of D1 receptors correlates with dopamine-stimulated adenylyl cyclase and radioligand-binding activities.

High densities of radioligand-binding sites are found within the caudate, putamen, and nucleus accumbens with lower levels in the thalamus and cerebral cortex (Fig. 1). D1 receptor messenger ribonucleic acid (mRNA) is localized to medium-sized neurons of the striatonigral projection that also express substance P (28). D5 mRNA is distributed in a more restricted pattern than D1 mRNA with the highest expression seen in limbic and cerebral cortical brain areas (29). Very low levels of D5 mRNA are found within the rat and human striatum.

Radioligand binding and mRNA studies have demonstrated a good correlation for the D2-like receptors. D2 receptors and message are found in the striatum and substantia nigra of the rat and human brain (Fig. 1). D2 receptors are expressed by medium spiny neurons containing enkephalin that project to the external segment of the globus pallidus (28). The globus pallidus is a major efferent projection system of the striatum that has high densities of D2 receptors (29). However, neurons expressing D2 receptor mRNA are lower in the globus pallidus than in the caudate and putamen, suggesting that most of the D2 protein is located on projections extrinsic to this structure. D2 receptor mRNA is co-localized with enkephalin expression cells in many brain areas, including the periaquaductal grey, suggesting a role for these sites in the modulation of analgesia.

The D3 dopamine receptor is highly expressed in limbic brain and has low expression in motor divisions of the striatum (6,30). In vitro receptor autoradiography demonstrates that D3 receptors in the human brain have a distinct localization pattern that is less dense than either D1 or D2 binding sites (Fig. 1). The highest densities of D3 receptors are seen over subcortical limbic brain regions. Low levels of D3 binding sites are seen over the ventromedial (limbic) sectors of the striatum. The highest levels of D3 message expression are found within the telencephalic areas receiving mesocortical dopaminergic inputs, including the islands of Calleja, bed nucleus of the stria terminalis, hippocampus, and hypothalamus. In the cerebellum, Purkinje cells lobules IX and X express abundant D3 mRNA, whereas binding sites are only found in the molecular layer (30,31). Since no known dopaminergic projections are known to exist in this area, it has been suggested that the D3 receptor may mediate the nonsynaptic (paracrine) actions of dopamine (31). D4 receptor message is localized to dopamine cell body fields of the substantia nigra and VTA. This pattern suggests that the D4 receptor protein may function as a presynaptic autoreceptor in dendrites and/or presynaptic terminals (32). The highest areas of D4 expression are found in the frontal cortex, amygdala, and brainstem areas. The very low levels of D4 receptor message in the terminal fields of the striatum are in keeping with the lack of extrapyramidal side effects observed following treatment with putative D4 selective atypical neuroleptics.

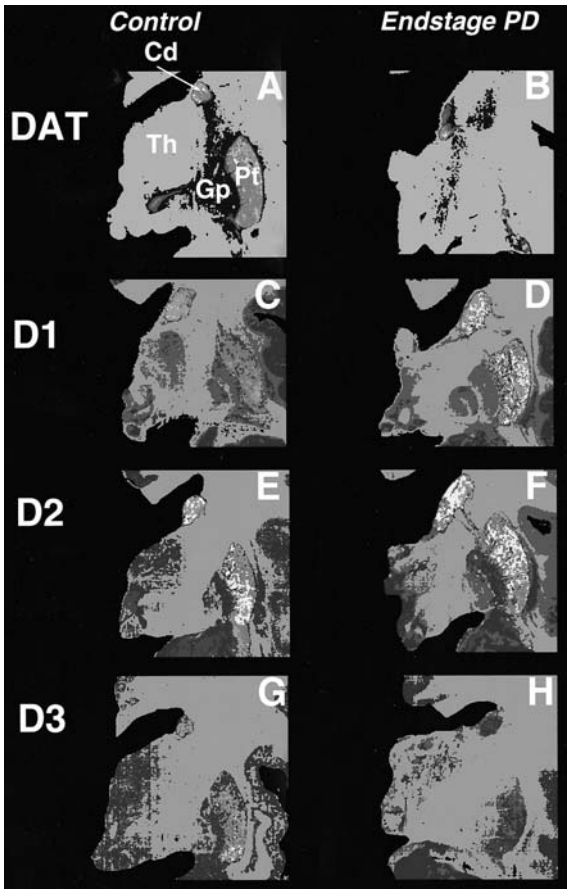

FIGURE 1 Autoradiographic localization of the distribution of D1, D2, and D3 receptors in representative coronal half-hemisphere sections of the human brain. Brain autoradiograms are shown in pseudocolor codes corresponding to a rainbow scale (red = high densities; green = intermediate densities; purple = low densities) for a control subject (male, age 72 yrs) and a patient with Parkinson's disease (male, age 67 yrs). The dopamine transporter was labeled with [3H]WIN 35, 428 (panels A and E) and shows the severity of the loss of dopamine terminals in end-stage Parkinson's disease. Panels B and F illustrate the distribution of D1 receptors with 1 nM [^{3}H]SCH 23390 in the presence of 10 nM mianserin to occlude labeling of the 5-HT$_2$ receptor. Panels C and G show the distribution of D2 receptors labeled with 2 nM [^{3}H] raclopride. Panels D and H illustrate the distribution of D3 receptors labeled with [^{3}H]7OH DPAT. Panels C and F show the distribution of D3 receptors labeled with [3H]7OH-DPAT (for method see Ref. 68). Cd, caudate; Gp, globus pallidus; Pt, putamen; Th, thalamus. (See color insert.)

Previous studies have suggested that D1-like and D2-like receptors may be colocalized in a subpopulation of the same neostriatal cells (33). This hypothesis has been questioned by recent data from Gerfen and coworkers (34), which demonstrated that the interactions may occur at an intercellular level as opposed to an intracellular second messenger integration. This latter hypothesis suggests that the D1-like and D2-like receptor proteins are on distinct populations of neurons with extensive axon collateral systems subserving the integration across neural subfields. However, there is considerable evidence from anatomical and electrophysiological studies that direct cointegration may occur at the single cell level (32,33). This anatomical arrangement would afford D1-mediated cooperative/synergistic control of D2-mediated motor activity and other psychomotor behaviors. Most studies have demonstrated opposing roles of D1 and D2 receptor–mediated actions in the striatum resulting from the stimulation and inhibition of adenylyl cyclase, respectively (35). While more studies are needed to clarify the precise nature and extent of these functional interactions on cyclic adenosine monophosphate (cAMP) second messenger systems, species-specific differences may limit the extrapolation of rodent studies to monkeys and humans (36).

Isolated activation of D1 and D2 dopamine receptors produces short-term effects on striatal neurons, whereas the combined stimulation of dopamine and glutamate receptors produces long-lasting modification in synaptic excitability (37). Dopamine terminals arising from the substantia nigra constitute, along with corticostriatal afferents containing glutamate, the majority of axon terminals in the striatum. Morphological studies have demonstrated close proximity of glutamatergic and dopaminergic synaptic boutons contacting dendritic spines of striatal spiny neurons (for review, see Ref. 38). Repetitive stimulation of both glutamate and dopamine receptors produces either long-term depression (LTD) or long-term potentiation (LTP) of excitatory synaptic transmission (37). Corticostriatal synaptic plasticity is severely impaired following dopaminergic denervation. The physiological and pharmacological features of corticostriatal transmission as an excitatory drive to striatal cells is important for understanding development of dyskinesias and treatment-related fluctuations in PD. D1 receptor occupation by dopamine stimulates adenylyl cyclase activity and augments the direct striatal output pathway, while D2 receptors inhibit adenylyl cyclase and inhibit neurons projecting from the external segment of the globus pallidus forming the first neuron in the indirect pathway. Pathological inhibition of striatal output neurons may be due to repetitive D1 receptor stimulation and functional uncoupling of D1 and D2 receptor subtypes from their respective second messenger pathways (39).

SECOND MESSENGER PATHWAYS

Dopamine receptors transduce the effects of agonists by coupling to specific heterotrimeric guanosine triphosphate (GTP) binding proteins (i.e., G-proteins) consisting of alpha, beta, and gamma subunits (for review, see Ref. 40). Within the dopamine receptor family, the adenylyl cyclase stimulatory receptors include the D1 and D5 subtypes. Although the D1 and D5 share sequence homology that is greater than 80%, the receptors display 50% overall homology at the amino acid level (41). D5 receptors have been suggested to have higher affinity toward dopamine and lower affinity for the antagonist (+) butaclamol. However, when the D1 and D5 subtypes are expressed in transfected cell lines derived from the rat pituitary, both D1 and D5 receptors stimulate adenylyl cyclase and have identical affinities for agonists and antagonists (for review, see Ref. 42). Studies done in transfected cell lines are complicated by the fact that transection systems may not express the relevant complement of G-proteins as in the native tissue environment. In the primate brain, there is an overlap in the regional brain expression of D1 and D5 receptors. Thus, because of the identical affinities of D1 and D5 receptors for agonists and antagonists and the lack of subtype selective drugs that fully discriminate between these receptor subtypes, it is not yet possible to assign with certainty specific functions to D1 vs. D5 receptor activation.

Although G-protein–coupled receptors were initially believed to selectively activate a single effector, they are now known to have an intrinsic ability to generate multiple signals through an interaction with different α subunits (43). D1 and D5 receptors have been shown by a variety of methodologies to couple to the Gsα subunit of G-proteins. The Gsα subunit has been linked to the regulation of Na^+, Ca^+, and K^+ channels, suggesting that D1 receptor activation affects the functional activity of these ion channels. To complicate this picture, D1 receptors inactivate a slow K^+ current in the resting state of medium spiny neurons in the striatum (44) through an activation of Goα in the absence of D1 receptor Gsα coupling (42,45). These studies provide evidence for the involvement of this G-protein subunit in the D1-mediated regulation of diverse ion channels.

The ability of the D5 receptor to stimulate adenylyl cyclase predicts that this subtype couples to Gsα. D5 receptors inhibit catecholamine secretion in bovine chromaffin cells (46). The negligible dopamine stimulation of adenylyl cyclase demonstrated in these cells suggests the possibility that this activity of the D5 receptor is mediated by a different G-protein. Recent studies have demonstrated that the D5 receptor can couple to a novel G-protein termed Gzα (47), which is abundantly expressed in neurons. Thus, despite similar pharmacological properties, differential

coupling of D1 and D5 receptors to distinct G-proteins can transduce varied signaling responses by dopamine stimulation. However, since the precise function of Gzα has not been established, the molecular implications of D5/Gzα coupling is not yet known. For example, Gzα has been shown to inhibit adenylyl cyclase activity in certain cell types (48). Even though it is unclear which signaling pathways are linked to D_5/Gzα coupling, the co-localization of D5, Gzα, and specific cyclase subtypes may provide a clue to the physiological relevance. For example, Gzα inhibits adenylyl cyclase type I and V (48). Both type V cyclase and D1 receptors are expressed in very high amounts in striatum, which has rich dopaminergic input (49). D1 receptor activation in the striatum is known to stimulate the activity of adenylyl cyclase type V (50). In contrast, the hippocampus is rich in D5 but not in D1 receptors, and type I cyclase is abundantly expressed in this brain region (51). Taken together, these studies suggest the functional relevance of co-localization of specific cyclases with a particular member of the D1-like receptor family.

D2, D3, and D4 receptors have introns in their coding region and exist in various forms by alternate splicing in the region of the third cytoplasmic loop. These receptors produce rapid physiological actions by two major mechanisms, involving either the activation of inward K^+ channels or the inhibition of voltage-dependent Ca^+ channels, or involving activation of Gi/Go proteins to inhibit adenylyl cyclase activity (20). D2 and D4 receptors inhibit adenylyl cyclase by coupling to inhibitory G-proteins of the Gi/Go family (20,21), whereas D3 receptors demonstrate weak inhibition of adenylyl cyclase activity (52). This weak effect on inhibiting cAMP production led to the conclusion that the D3 receptor does not couple to G-proteins (21,52). Both isoforms of the D2 receptor inhibit adenylyl cyclase activity, although the short isoform requires lower concentrations of agonist to cause half maximal inhibition than the long isoform expressed in transfected cell lines (53,54). The short D2 receptor isoform couples to K^+ currents via a pertussis-toxin–insensitive mechanism (55), whereas the long isoform couples to the same current via a pertussis-toxin–sensitive mechanism (56). Thus, D2 receptors, if expressed by the same cells, can influence transmembrane currents in similar ways, but through independent transduction pathways. D2-like receptors that couple to G-proteins modulate a variety of other second messenger pathways, including ion channels, Ca^+ levels, K^+ currents, arachidonic acid release, phosphoinositide hydrolysis, and cell growth and differentiation (for review, see Ref. 57).

PHARMACOLOGICAL SELECTIVITY

Central dopamine systems have properties that make them unique in comparison to other neurotransmitter systems. For example, dopaminergic projections are mainly associated with diffuse neural pathways. This anatomical arrangement argues for dopamine to act as a neuromodulatory molecule in addition to its role as a neurotransmitter in brain. Dopamine neurons are highly branched with elongated axons capable of releasing neurotransmitters from many points along their terminal networks en route to the striatum (58,59). This mode of volumetric transmission of action potentials suggests that dopamine release mediates paracrine (i.e., neurohumoral) signals across the network. This view is supported by the observation that dopamine is released by axon terminals and dendrites, providing a double polarity for regulating basal ganglia function, simultaneously gating signaling at nigral, striatal, and pallidal levels. These properties have important implications in the clinical expression of human disorders involving dopamine neuron dysfunction.

The members of the D1 receptor subfamily have several characteristics that distinguish them from the D2 subfamily. All members of the D1 subfamily bind benzazepines with high affinity and bind butyrophenones and benzamides with low affinity (12). Subtypes in the D1 family have approximately 50% homology overall and 80% homology in the highly conserved transmembrane region. All of the receptors in this family have short third intracellular loops and a long carboxy terminus. These regions are important for the generation of second messenger signals as explained above. D5 and the rat D1b are species homologs because they map to the same chromosomal locus (26). D5 and D1b have a 10-fold higher affinity for dopamine, suggesting that D5 receptors are activated at neurotransmitter concentrations that are subthreshold for the D1 receptor (21). The D2-like receptors bind butyrophenones and benzamides with high affinity and bind benzazepines with low affinity (10,15,16).

The pharmacological distinction of dopamine receptor subtypes holds tremendous potential for treatment of nervous system dysfunction. Dopamine receptors are the primary targets for the pharmacological treatment of PD, schizophrenia, and several other nervous system disorders. Presently used drugs have significant limitations that are in part due to their nonselective binding to many receptor subtypes. For example, drug-related side effects, including dyskinesias and delirium, are frequent and important problems in parkinsonian patients receiving levodopa or dopamine agonist therapy. These adverse effects result from stimulation of dopamine receptors in motor and cognitive circuits, respectively (21). Conversely, treatment of schizophrenia with dopaminergic antagonists, although intended to

selectively block receptors in cortical and limbic circuits, may induce parkinsonian symptoms or even tardive dyskinesias by interaction with dopamine receptor subtypes in motor pathways. Clearly, drugs aimed at molecular subtypes of dopamine receptors offer the potential for specific therapeutic interventions for motor and psychiatric disorders of the nervous system.

Although there are agonists and antagonists that are highly selective and that can discriminate between D1-like and D2-like receptor subfamilies, there are few agents that are highly selective for the individual receptor subtypes (Table 1). Some progress has been made in the development of antagonists for the D2 receptor family. For the D1/D5 receptor subtypes, there are currently no compounds that exhibit high selectivity. Thus, the high overall sequence homology between dopamine receptors of the same subfamily have made it difficult to develop specific ligands that do not interact with related receptors. The high affinity of the "atypical" neuroleptic, clozapine, for D4 receptors and the low level of D4 receptor expression in the striatum and high levels in the cerebral cortex and certain limbic brain areas led to the suggestion that the antipsychotic properties of the neuroleptics may be mediated through blockade of D4 receptors, whereas the side effects may be mediated through blockade of D2 receptors (15,60). This hypothesis was strengthened by the low incidence of extrapyramidal side effects for clozapine. However, clozapine at therapeutic doses also blocks many other types of receptors in addition to D4 receptors making it difficult to draw definitive conclusions. For example, clozapine binds to muscarinic acetylcholine receptors and is 20- to 50-fold more potent at these sites than at D2 receptors (for review, see Ref. 61)

Recently, it has been suggested that clozapine and other related antipsychotic drugs that elicit little or no parkinsonism bind more loosely than dopamine to brain D2 receptors, yet have high occupancy of these receptors (61). By determining fractional occupancies of receptors bound by therapeutic drug levels, it has been demonstrated that the dominant factor for deciding if a particular antipsychotic drug will elicit parkinsonism is whether it binds more tightly or more loosely than dopamine at the D2 receptor subtype. Thus, for those antipsychotic drugs that elicit little or no parkinsonism, it appears that the high endogenous dopamine in the human striatum must outcompete the more loosely bound neuroleptic at the striatal D2 receptor subtype. Dopamine less readily displaces the more hydrophobic radioligands of the haloperidol type, providing an additional correlate between the magnitude of in vivo competition with endogenous agonists and parkinsonism. The separation of antipsychotic drugs into "loose" and "tight" binding to D2 receptors is consistent with the observation that catalepsy induced by olanzapine and loxapine (more loosely bound than

TABLE 1 Properties of Dopamine Receptor Subtypes

	D1-like		D2-like		
Receptor subtype	D1	D5	D2	D3	D4
Amino acids	446	477	443	400	387
Chromosome	5q35.1	4p15.1-16.1	11q22-23	3p13.3	11p15.5
Second messenger	cAMP	cAMP	cAMP K^+ channel Ca^+	K^+ channel Ca^+ channel	cAMP K^+ channel
mRNA	Striatum	Hippocampus Kidney	Striatum	Nucleus Accumbens	Cerebral cortex
Selective agonists	SKF38393	SKF38393	Bromocriptine Butaclamol Pergolide Ropinirole	7-OH-DPAT Pramipexole Pergolide Ropinirole PD128,907	—
Selective antagonists	SCH23390	SCH23390	Spiperone Raclopride Sulpiride	Spiperone Raclopride Sulpiride Nafodotride	Spiperone Clozapine

Source: Data from Refs. 15,16,19,68.

dopamine) but not haloperidol (more tightly bound than dopamine to D2 receptors) was fully reversible (61). Taken together, these observations suggest that D2 blockade may be necessary for achieving antipsychotic action. This suggestion is in keeping with the observation that many patients will suddenly relapse when stopping clozapine, perhaps due to a sudden pulse of endogenous dopamine arising from emotional or physical activity which displaces the loosely bound neuroleptic from the receptor. Clinical dosing schedules can be adjusted to obtain sufficient but low occupancies of D2 receptors in order to minimize the development of parkinsonism. The psychosis caused by levodopa or bromocriptine can be readily treated by low doses of either clozapine or remoxipride (62), since there is very little endogenous dopamine to compete with the antagonist. Further studies are needed to determine whether newer antipsychotic drugs with low affinity for D2 receptors and with low risk for parkinsonism will cause less tardive dyskinesia.

The success of treating parkinsonian symptoms with dopamine precursor amino acid levodopa is due to its ability to reverse the dopamine deficiency. Unfortunately, treatment complications emerge shortly after beginning levodopa therapy. In the DATATOP study (63), almost half of the patients developed wearing off (loss of efficacy towards the end of a dosing interval), about one third showed dyskinesias, and about one fourth were showing early signs of freezing (sudden loss of capacity to move) with a mean duration of treatment of only 18 months. Modern pharmacological treatment of PD has been advanced by the increased understanding of the complexity of dopamine receptor pharmacology and the ability to screen drug candidates in vitro against cloned and expressed human dopamine receptor subtypes (2,21).

Symptoms of parkinsonism in primate models are treated with agonists that activate the D2-like receptor subfamily. D2 agonists with long half-lives can relieve parkinsonism in these animals with little risk of motor side effects, while repetitive levodopa doses will induce motor fluctuations and dyskinesias (64). In dyskinetic animals that had received levodopa doses, D2 agonists that had few side effects on their own, now elicit dyskinesias. These observations suggest that repetitive co-activation of denervated striatal dopamine receptor subtypes initiates the development of these disabling side effects by nonselective activation of postsynaptic D1 and D2/D3 receptors. Pramipexole is a novel dopamine agonist with preferential affinity for D3 receptors (Table 1). It has little affinity for the D1-like receptors, and within the D2 receptor subfamily it exhibits its highest affinity at the D3 receptor subtype, distinguishing it from all other dopamine agonists currently used for the treatment of PD (2,65). As PD

progresses, there is marked reduction of D3 receptors in the caudal sectors of the putamen (Fig. 1H).

Dopamine normally inhibits striatal GABAergic cells of the indirect pathway by stimulating D2 receptors and stimulates GABAergic cells of the direct pathway by activating D1 and D3 receptors. These effects result in the inhibition of the globus pallidus (GPi). In PD, when dopamine innervation has been lost, the GPi fires at very high rates to inhibit thalamic relay neurons resulting in bradykinesia (for review, see Ref. 66). Pramipexole stimulates D3 receptors that directly inhibit GPi neurons, removing its inhibitory gate on thalamocortical motor pathways, and stimulates D2 receptors to indirectly inhibit GPi neurons (66). Thus, pramipexole has two synergistic mechanisms to mimic dopamine and restore function in PD. While D3 receptors have a lower density in the striatum as compared to D2 receptors (Fig. 1E and G), chronic administration of indirect-acting agonists may cause an upregulation in the number of D3 binding sites (67). In keeping with this suggestion, chronic cocaine abusers have elevated densities of D3 receptor sites in limbic sectors of the striatum and nucleus accumbens (68). It is not known if this regulatory change occurs in the denervated striatum, early in the course of agonist replacement for PD. However, pramipexole has shown efficacy for the treatment of depression in PD, in keeping with its postsynaptic effects on limbic targets (69). Thus, pramipexole has clinically meaningful antidepressant activity in moderate depression, a property that is possibly tied to its preferential binding to the D3 receptor subtype.

Joyce (6) has suggested that the D3 receptor may provide neuroprotective effects in PD and modify clinical symptoms that D2 receptor–preferring drugs cannot provide. Although D3 receptors are confined to the limbic sectors of the striatum, they may play a role in PD because the limbic striatum is involved in aspects of movement, including the execution of goal-directed behaviors requiring locomotor activity. Experimental models of PD suggest that D3-preferring agonists do act through D3 receptors to provide relief of akinesia (6). The nucleus accumbens, a region rich in D3 receptors that remains relatively spared in advanced PD (Fig. 1), is involved in behavioral sensitization to psycho-stimulants and changes in affective state. Thus, D3 agonists could modulate the effects of dopamine afferents originating from the medial substantia nigra.

The primary dopamine receptors mediating the antiparkinson effects of levodopa and other direct-acting dopamine agonists are D1 and D2 receptors. D3 receptors afford a novel target for medication development in PD. Whether or not other novel subtypes of dopamine receptors exist in the brain is unknown. However, rapid advances in molecular cloning may reveal additional heterogeneity in the expression of synaptic proteins

involved in dopaminergic neurotransmission. At this time, five cloned and expressed dopaminergic receptor proteins provide a complex molecular basis for a variety of neural signals mediated by a single neurotransmitter. At least three of these receptor subtypes are relevant for understanding the pathophysiology and treatment of PD.

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