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THE PHARMACOLOGY OF PARKINSON'S DISEASE THERAPY: AN UPDATE [1]

Irwin J. Kopin
National Institutes of Health, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland 20892

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INTRODUCTION

Parkinson's disease is characterized by the slow onset, usually beyond the fourth decade of life, of tremor and difficulties with movement and posture. Although many areas of brain become involved, the major and most consistent pathology in Parkinson's disease is a striking loss of neurons in the pars compacta of the substantia nigra (SNc). Until the discovery of dopamine in brain (1) and the demonstration of a striking decrease in brain dopamine levels in the brains of patients dying with Parkinson's disease (2), treatment of this disease was empirical, based on chance observations that drugs given for other purposes alleviated, to some degree, parkinsonian symptoms. Belladona alkaloids were found to have moderate antiparkinsonian effects when they were administered to stem the drooling of saliva and remained the only available antiparkinsonian drugs for nearly 100 years until the development in late 1940s of synthetic anticholinergic agents that appeared to have more specific antiparkinsonian effects (3, 4). The antiparkinsonian effects of amantidine (5), an antiviral drug, benadryl (6), an antihistamine, amphetamine (7), and apomorphine (8) were all discovered serendipitously. These drugs had relatively marginal efficiency limited by side effects. The age of empirical treatment of Parkinson's disease ended with the discovery of the marked depletion of dopamine in the striatum (caudate and putamen) and other brain regions innervated by the neurons in the SNc.

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Dopamine does not readily penetrate the blood-brain barrier, but its amino acid precursor, 3,4-dihydroxyphenylalanine (levodopa), was known to reverse the tranquilizing effects of reserpine. This behavioral effect and biochemical studies indicated that levodopa readily enters the brain. Early efforts at dopamine substitution with dopa failed, however, because of the severe side effects, particularly nausea and vomiting. In 1967, Cotzias et al (9, 10), showed that by gradually increasing the doses of levodopa, adverse side effects could be avoided or reduced and that, at doses of up to 16 grams a day, remarkable symptomatic improvement could be achieved. Attention was then focused on the pharmacokinetics and metabolism of levodopa. The objectives were to reduce the dose by enhancing bioavailability of levodopa to the brain, prolonging the actions of dopamine formed and released in brain, and reducing acute adverse side effects.

The enzyme that converts levodopa to dopamine (Figure 1), aromatic amino acid decarboxylase (AADC), is present in the liver, kidney, and many other tissues, including the endothelium of brain capillaries as well as in the brain. Blocking AADC with an inhibitor that could not enter the brain would be expected to slow metabolism of levodopa only in the peripheral tissues. In the early 1970s, carbidopa and benserazide (Figure 2), two highly effective hydrazine derivatives which effectively blocked AADC but were excluded from brain, were introduced to clinical practice (11–13). Three mechanisms contribute to the potentiation of levodopa by inhibition of peripheral AADC.

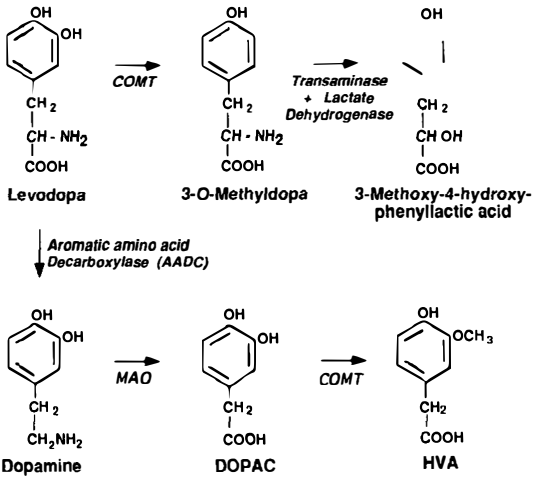


Figure 1 Major pathways of levodopa metabolism. COMT (catechol-0-methyltransferase) is responsible for 0-methylation of levodopa whereas aromatic amino acid decarboxylase converts levodopa to dopamine.

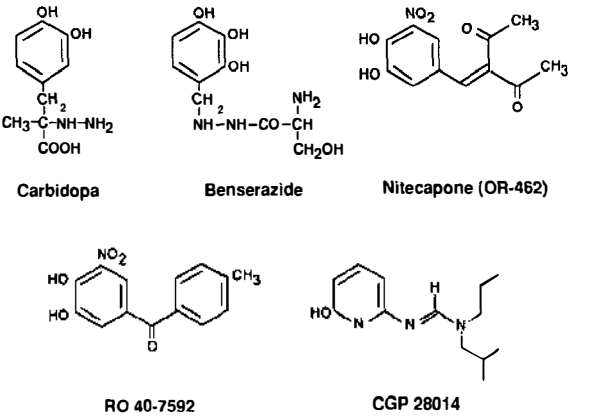


Figure 2 Inhibitors of levodopa metabolism. Carbidopa and benserazide inhibit aromatic amino acid decarboxylase, but are excluded from brain and act only in peripheral tissues. Nitecapone, RO 40-7592 and CGP 20814 inhibit catechols-0-methyltransferase. Nitecapone is excluded from brain, whereas RO 40-7592 and CGP 20814 inhibit brain, as well as peripheral tissue COMT.

First, inhibition of decarboxylation of orally administered levodopa in the intestine and liver enhances entry of the amino acid from the intestine into the systemic circulation. Second, by preventing levodopa decarboxylation in the peripheral tissues, such as kidney, higher blood levels are sustained. Finally, by blocking levodopa decarboxylation in brain capillary endothelial cells, the enzymatic barrier to entry of the amino acid into brain has been made ineffective.

Another means of increasing dopamine levels in brain is by preventing its destruction. Since dopamine is a substrate for monoamine oxidase (MAO), several attempts were made to use MAO inhibitors, alone or in combinations with levodopa, to treat patients with Parkinson's disease. Because of relative inefficacy alone and severe hypertension when administered with levodopa, it was not until a "safe" MAO inhibitor, deprenyl, was introduced that this approach was considered. This drug is discussed in more detail below because it has been claimed to retard the progression of Parkinson's disease as well as to potentiate the effects of levodopa.

The response to levodopa is generally stable and reasonably predictable for the first several years, but with chronic levodopa treatment dyskinesias, fluctuations in efficacy ("on-off responses" and "wearing off"), freezing, mental changes, and loss of efficacy (requiring higher levodopa doses and inviting side effects) emerge. The complicated pharmacokinetics of levodopa, progression of the disease with diminished ability to convert levodopa to dopamine in brain or to regulate its extracellular concentration by uptake into

dopaminergic terminals, and alterations in dopamine-receptor sensitivity and responsivity have been cited as mechanisms contributing to these adverse effects. The strategies explored during the 70s and 80s to prevent or alleviate these symptoms included adjustments of dosage and time of administration, slow-release formulations, continuous intravenous infusions, and drug "holidays" to allow for normalization of dopamine receptors and potentiation and prolongation of the effects of dopamine using a "safe" monoamine oxidase inhibitor (see below).

During the past ten years, additional strategies to enhance levodopa therapy have been investigated, particularly by inhibiting its 0-methylation, as well as other means of stimulating dopamine receptors with specific agonists or blocking adverse affects of dopamine replacement therapies with specific antagonists. Cloning of subtypes of dopamine receptors has given impetus to development of new drugs to target more specifically dopamine receptors that regulate movement or block those responsible for adverse effects. The discovery that in primates, MPTP causes degeneration of nigrostriatal neurons and produces a motor deficit indistinguishable from Parkinson's disease has stimulated searches for mechanisms of the degenerative process and methods to prevent the development or retard the degenerative process of Parkinson's disease. It is the purpose of this update to describe the current status of this rapidly advancing frontier.

CATECHOL-0-METHYLTRANSFERASE INHIBITORS POTENTIATE LEVODOPA

In addition to decarboxylation, levodopa is metabolized by 0-methylation and transamination (Figure 1). When decarboxylation is prevented, 0-methylation predominates and plasma levels of 3-0-methyl-DOPA (3-OMD) are elevated. Both levodopa and 3-OMD are transported by a saturable carrier system for which many large neutral amino acids (LNAA) are substrates. Since levodopa enters the body from the gut and is transported from the blood into the brain by the LNAA transport system, 3-OMD and dietary amino acids influence the efficacy of levodopa treatment. Furthermore, 3-0-methylation contributes to the short half-life of levodopa. On the basis of these considerations attention has been directed at development and testing effects of inhibition of catechol-0-methyltransferase (COMT); recently effects in humans of three potent relatively nontoxic COMT inhibitors (Figure 2) have been reported.

NITECAPONE Nitecapone, a 3-nitrocatechol (Figure 2), 3-(3,4-dihydroxy-5-nitrobenzylidene)-2,4-pentadione (OR 462), was encountered during a search for a potent inhibitor of COMT (14). In addition to inhibiting COMT in vitro, it reduced formation of 3-OMD and enhanced elevations of striatal levodopa

and dopamine when administered to rats along with levodopa/carbidopa (15, 16). Except in high doses, nitecapone did not blunt the increment in brain HVA, indicating that brain COMT was not inhibited, presumably because this drug does not readily cross the blood-brain barrier. When measured ex vivo, brain COMT activity was not reduced significantly by nitecapone in doses up to 30 mg/kg, whereas a related COMT inhibitor, OR-486, did appear to enter the brain at such doses (17, 18). In cynamologus monkeys, nitecapone reduced the rate of formation of 3-0-methyldopa and prolonged the disappearance of plasma levodopa (19, 20). In human volunteers, nitecapone (150 mg) inhibited, by about 50%, COMT activity in red blood cells and in gastroduodenal biopsies (21) and increased the relative bioavailability of levodopa (administered with carbidopa) while decreasing excretion of 0-methylated dopamine metabolites (22).

RO 40-7592 Another 3-nitrocatechol (Figure 2), RO 40-7592 (3, 4-dihydroxy-4′-methyl-5-nitrophenone) was found to be a competitive inhibitor of COMT in both the brain and peripheral tissues (23). When combined with benserazide and levodopa, this drug blocked formation of 3-OMD and elevated levodopa levels in plasma and brain. Brain dopamine was found to reach higher levels than with levodopa/benserazide alone. In experimental animals, the effects of levodopa/benserazide on locomotor activity, reserpine-induced hypothermia, and neuroleptic-induced catalepsy are potentiated by the COMT inhibitor (24). Microdialysis studies have shown that RO-40-7592 enhances and prolongs increases in striatal extracellular concentrations of levodopa, dopamine, and dihydroxyphenylacetic acid produced by administration of levodopa/carbidopa-treated rats, whereas the increases in homovanillic acid are blunted markedly (25, 26). In rhesus monkeys, pretreatment with the COMT inhibitor RO-40-7592 appeared to interfere with the specificity of carbidopa as a peripheral decarboxylase inhibitor (27). Uptake of ^{11}C-levodopa was increased when RO-40-7592 was combined with benserazide, but not when the COMT inhibitor was combined with carbidopa. These results suggest that COMT is effective in blocking inactivation of carbidopa as an inhibitor of the decarboxylating enzyme, whereas it does not appear to have this effect on benserazide, perhaps because of the structural differences between the two drugs (Figure 2).

CGP 28014: A NONCATECHOL COMT INHIBITOR Recently, CGP 28014, N-(2-pyridone-6-yl)-N-N-di-n-propyl-formamidine, a noncatechol (Figure 2), which is a weak inhibitor of COMT in vitro, was found to reduce HVA and elevate DOPAC levels in rat striatum (28, 29). Also, this compound inhibited the rise in 3-methoxytyramine usually found after the administration of the monoamine oxidase inhibitor, clorgyline, and diminished the increase in

3-OMD after administration of levodopa. In five humans pretreated with 200–600 mg CGP 28014, increments in 3-OMD plasma levels following administration of levodopa were decreased by about two thirds, whereas DOPAC levels were elevated (30). If the availability of dopamine can be stabilized by such treatment, fluctuations in responses might be attenuated.

The usefulness of long-term administration of COMT inhibitors to potentiate the effects of levodopa in the treatment of Parkinson's disease is still to be established, but nitecapone has proven useful in enhancing resolution during brain imaging with ^{18}F-6-fluorodopa by diminishing the nonspecific background radioactivity due to formation of ^{18}F-6-fluoro-0-methyldopa (31, 32).

DOPAMINE RECEPTORS AS TARGETS FOR DRUGS TO TREAT PARKINSON'S DISEASE

The common failures encountered with long-term levodopa treatment and the development of dopamine receptor agonists encouraged the hope that these agents, which act directly on the dopamine receptors and are independent of the degenerated dopaminergic neurons, might successfully control parkinsonian symptoms in patients who could no longer respond to the indirectly acting levodopa.

The first dopamine receptor agonists to be discovered were the aporphines, apomorphine and N-propylnorapomorphine, and the ergot derivatives, bromocriptine, lisuride, quinpirole (LY 171555), and pergolide (Figure 3). The effects of various dopamine agonists are not identical and do not precisely

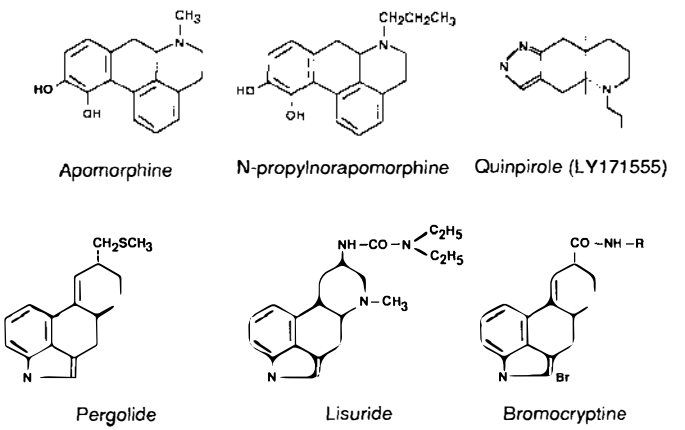


Figure 3 Chemical structures of some dopamine agonists used in treatment of Parkinson's disease.

mimic dopamine, presumably because they each interact differently, from dopamine and from each other, at dopamine receptors. To appreciate the variety of mechanisms by which dopamine or drugs acting at dopamine receptors influence brain motor control, it is necessary to recognize the various dopamine receptor subtypes, to consider their distribution at sites that may affect neuronal function and to have delineated the neuronal pathways that control motor cortical function.

Dopamine Receptor Subtypes

The first indication of the existence of dopamine receptor subtypes was the report in 1979 by Kebabian & Calne (33) that there are two categories of dopamine receptors, which they designated as D_1 and D_2 receptors. The distinction between these two receptors initially was biochemical. D_1 receptors activate adenylcyclase and thereby stimulate cyclic AMP production, whereas D_2 receptors fail to affect adenylcyclase or may even diminish cyclic AMP formation. Subsequent demonstration of two types of binding sites for radioactively labeled dopamine agonists and antagonists further supported the division of dopamine receptors into at least two categories (34).

Butyrophenones, such as haloperidol and spiperone, which have been used in the treatment of schizophrenia and cause parkinsonian symptoms, are potent D_2 dopamine receptor antagonists. Benzamides (sulpiride, raclopride) are also potent D_2 antagonists and have neuroleptic properties. The distinction between the two subtypes of dopamine receptors also made possible characterization of dopamine agonists with regard to their actions at these receptor subtypes. Furthermore, binding studies with labeled spiperone have revealed that D_2 receptors have interconvertable high- and low-dopamine affinity states; the affinity for dopamine differs by more than two orders of magnitude between the two states, whereas the affinity for spiperone is similar for both states.

The ergoline derivatives, which are relatively specific in stimulating D_2 receptors, cause marked behavioral changes in animals, producing increased locomotion, rotation of unilateral dopamine depleted (with 6-hydroxydopamine intracerebral injections) rats, emesis, and psychotomimetic behavior. Aporphines are nonselective, stimulating both D_1 and D_2 receptors; they also enhance locomotion, cause emesis, and elevate prolactin levels by preventing dopamine inhibition of prolactin secretion.

Whereas many D_2 receptor agonists and antagonists have been discovered, there are relatively few selective D_1 agonists or antagonists. SKF 38393 and SKF 82526 (fenoldopam) are selective D_1 agonists, whereas the N-methyl, 7 halogenated derivative of SK 38393, SCH 33390, is a potent D_1 antagonist. Many biochemical, pharmacological, neurophysiological, and behavioral studies have been carried out using these D_1 agonists and antagonists and the effects compared with the drugs acting at D_2 receptors (see below).

Distribution of Dopamine Receptors

The distribution and density of D_1 and D_2 receptors have been examined using quantitative autoradiography to identify binding sites of radiolabeled ligands to receptors. With ligands that are highly specific for a particular receptor, there is a good relationship between binding and receptor density. To enhance specificity of less specific ligands, competitive displacement of binding at other receptors can be attempted (34). In such studies, the regions with the highest density of dopamine receptors are found in the caudate, putamen, nucleus accumbens, and olfactory tubercle (34). In the striatum, the distribution of D_1 and D_2 receptors is not homogeneous, but there does not appear to be a good correspondence with striatal compartments (see below). In most species, including humans, D_1 receptor density is greater than that of D_2 receptors. In the substantia nigra, D_2 receptors predominate, presumably as autoregulatory dopamine receptors, but the density is lower than in the striatum.

During the past three years, not only have D_1 and D_2 receptors been cloned and their molecular structures been characterized, but structural variants of these receptors have been identified and three additional types of dopamine receptors have been discovered (see below). In view of the pharmacological similarities among D_2, D_3, and D_4 receptors and their subtypes, and among D_1 and D_5 receptor subtypes, anatomical distribution studies based on ligand binding may require some modifications. Also, pharmacological characteristics of the many dopamine receptor agonists and antagonists, which have been characterized almost entirely with reference to the first two dopamine receptor subtypes, may in the future become defined more specifically.

The Basal Ganglia, Dopamine, and Motor Control

The basal ganglia are components of major parallel cortical-subcortical-cortical circuits (Figure 4), which are important in the control of movement (see for example Ref. 35, 36). Within the basal ganglia are many neurotransmitters/neuromodulators and a complex mosaic organization of neuronal structures with distinct biochemical and neuroanatomic characteristics (see reviews 37–39). Glutaminergic excitatory neurons in layer 5 and the supragranular layer of the cerebral cortex send axons to the striatum. Here they arborize and form synaptic contacts with the ends of the dendritic spines of the medium spiny neurons, which constitute over 90% of the neurons in the striatum. About 40–45% of these GABAergic inhibitory neurons innervate the neurons in the inner layer of the globus palliclus (GPi) in primates (or the endopeduncular nucleus in rodents) and the neurons of the substantia nigra reticulate. The target neurons of this "striatonigral" projection are also GABAergic and send

inhibitory axons to the thalamus where they innervate excitatory glutaminergic neurons which project to the cortex. These four neurons constitute a direct cortical-subcortical-cortical loop (Figure 4), which contains two inhibitory synapses and accounts for a disinhibition mechanism for activation of descending motor activation. In addition to this direct pathway, another 45–50% of the medium spiny neurons project to the external layer of the globus pallidus (GPe) where synapses are formed with GABAergic neurons that innervate glutaminergic excitatory neurons in the subthalamic nucleus. The subthalamic neurons innervate GABAergic neurons in the GPI and thus provide an alternative, indirect, five-neuron cortical-subcortical-cortical circuit (Figure 4) with three inhibitory synapses, inhibiting disinhibition. Coordination and balance of the output of these opposing pathways presumably is the basis for smooth and efficient motor control. This coordination and balance is attained through the influence of modulatory mechanisms that are only beginning to be understood. The bases for these mechanisms are the organization and special characteristics of subpopulations of the neurons that comprise the direct and indirect pathways and the neurons that provide means for regulating the output of these pathways.

Compartments and Subpopulations of Striatal Neurons

There appear to be at least two histologically distinct compartments within

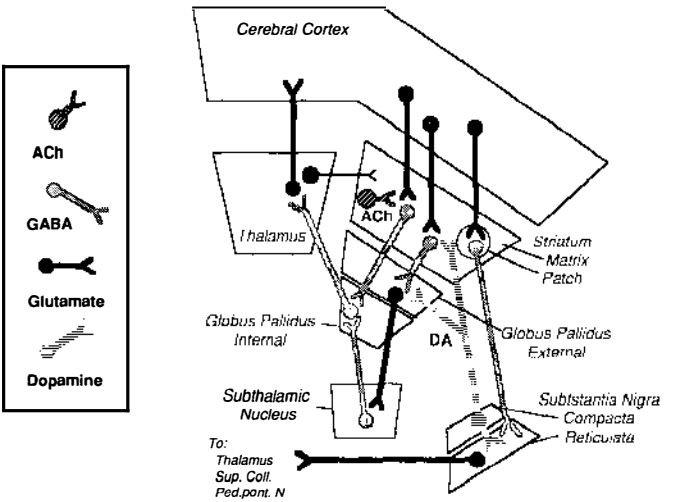


Figure 4 Schematic representation of major neuronal circuits involving the basal ganglia and their modulation by dopaminergic nigrostriatal projections and striatal cholinergic interneurons. See text for details.

Table 1 Differential patch and matrix densities of neurochemical markers*

Neurotransmitter/modulator	Patch vs matrix
Dopaminergic	
D_1 receptors	P > M
D_2 receptors, DA uptake, TH-IR	M > P
Cholinergic	
m_1 Receptors	P > M
Choline uptake, ChAT, AChE	M > P
Peptides	
SP, Dyn, NT	P > M
Enk, SOM, Calbindin	M > P

*from Graybiel (37)

the striatum; a network of "striasomes" or "patches", which makes up 10–20% of the striatal volume, characterized by their low density of acetylcholine esterase (AChE) and high density of μ-opiate receptors (μ-R) is set within a matrix of AChE-rich, μ-R-poor bulk of the striatum (37). The distributions of a number of neurochemical markers appear to distinguish patches and matrix (Table 1). In addition to the patch-matrix compartments, there are distinct subpopulations of neurons in both regions. The GABAergic medium spiny neurons of the direct and indirect circuits described above are both found in the matrix; however, they differ in several important respects. The striatonigral neurons express D_1 receptors and contain dynorphin (Dyn) and Substance P (SP), whereas most (about 70%) of the striatopallidal neurons express D_2 receptors and contain enkephalin (Enk). Some of the striatal neurons express both D_1 and D_2 receptor mRNAs. About 30% of the striatopallidal neurons contain neurokinin B and express neither D_1 nor D_2 receptors (39).

Medium spiny neurons in the patch regions of the striatum project to the dopaminergic neurons of the SNc and to islands of dopaminergic neurons in the SNr. They are innervated by projections from glutaminergic neurons in the deep portion of layer 5 of the cerebral cortex and in the thalamus and by dopaminergic neurons in the SN, which they in turn also innervate. A small number of large aspiny striatal neurons contain choline acetyltransferase and are presumably cholinergic. The dendrites of these neurons reach a relatively large surrounding volume and the axonal arborizations extend even further. These cholinergic neurons show irregular tonic activity, which presumably is modulated by their muscarinic (M_2) autoreceptors and the D_2 and SP receptors, which are expressed by these neurons.

Dopaminergic Modulation of the Cortical-Subcortical-Cortical Circuits

Because of the multiple sites of dopamine release and the arrays dopamine receptors present on the axons, dendrites, and somata of neurons that constitute these circuits, there is ample opportunity for dopaminergic modulation of nerve activity through these pathways. Furthermore, each of these sites is a potential target for the action of drugs. The importance of dopamine in modulating these circuits is apparent from the striking impairment of motor activity comprising the parkinsonian syndromes that develop as a consequence of toxin-induced or naturally occurring destruction of nigrostriatal dopaminergic neurons or blockade of dopamine receptors. The dopamine deficiency states are largely reversed by treatment with levodopa and markedly benefited by drugs that activate dopamine receptors. As is evident from consideration of the distribution of dopamine receptors, modulation of motor activity by dopamine may be the result of direct actions at several sites: at glutaminergic afferent neurons and their axon terminals, on medium spiny neuron dendrites, on striatal interneurons, and at the axonal synapses and target neurons of the basal ganglia efferents as well as the striatal neurons that express either D_1 and D_2 receptors or both.

The first potential site of dopaminergic modulation of these neuronal circuits is at the level of the terminals of the corticostriatal projections. In microdialysis studies of the striatum, the D_2 receptor agonist, LY171555, was found to inhibit K^+-induced depolarization-induced release of glutamate and aspartate from the terminals of corticostriatal neurons, whereas the D_1 agonist, SKF38393, inhibited only the release of aspartate (40). D_1 receptors on the Dyn-SP-containing medium spiny neurons and D_2 receptors on the Enk-containing medium spiny neurons (32), are sites at which dopamine may influence activity of the striatal efferent GABAergic neurons.

The D_2 receptors on striatal cholinergic interneurons are yet another site of dopamine modulation of striatal neuronal activity. Ibotenic acid lesions in the striatum produce loss of D_1 receptor binding and of D_1 receptor mRNA in the caudate-putamen; the axon projections to the substantia nigra degenerate and there is loss of D_1 receptor binding sites in the pars reticulata (41), consistent with earlier studies that demonstrated localization of D_1 receptors on striatonigral projections (42). The similarity in distribution of [^{3}H]-SCH 23390 binding sites and Substance P or dynorphin B immunoreactivity in the substantia nigra of human brain suggests that, in humans also, D_1 binding sites are located mainly on the striatonigral projections (43). The D_1 receptors present on GABA-releasing striatal afferents may modify transmitter output at synapses at the target neurons. Also, the D_2 autoreceptors of nigrostriatal neurons serve to modulate activity of dopaminergic input to the striatum. By

direct injection of D_1 and D_2 agonists into small regions of brain, LaHoste & Marshall (44) showed that nigral D_1 and striatal D_2 receptors mediate behavioral effects of dopamine agonists. Thus, actions on separate neurons having different dopamine receptors could contribute to effects of dopamine agonists.

There is also considerable evidence that there are important functional interactions between D_1 and D_2 receptors. In normal rats, the effects of apomorphine on reticular neuronal activity are reversed by treatment with other D_1 or D_2 antagonists (45), and combined administration of D_1 and D_2 agonists appears to have a synergistic effect (46). Behavioral studies in dopamine-depleted experimental animals have also supported such interactions. For example, the prevention or reversal of the decline in bromocriptine efficacy in MPTP-induced parkinsonism by a selective D_1 agonist (46), is evidence for such an interaction. In such animals, while the D_2 agonist, LY171555, suppresses resting tremor when administered alone, the effects of small doses of the D_2 agonist are potentiated by the D_1 agonist CY 208-243, although the D_1 agonist alone, at the doses used, has no significant effects (48). The complex array of dopamine receptors on different neurons, the extensive medium spiny neuron axon collateral cross innervation and the indirect connections via interneurons, is an important basis for interactions among D_1 and D_2 receptor-mediated effects.

In addition to the neuronal pathways that could account for D_1–D_2 receptor interactions, striatal neurons that express both receptor subtypes are sites at which such interactions may be mediated. Although there is some controversy over the proportion of striatal neurones that express both D_1 and D_2 receptors (38, 49), there is general agreement that these receptors are coexpressed by a subpopulation of medium spiny neurons. Inhibition by D_2 agonists of D_1 receptor-mediated adenylcyclase stimulation in the striatum may be the result of opposing actions of these receptors in the same neuron (50). In isolated rat striatal neurons, inhibition of $(Na^+–K^+)$-ATPase occurs through a synergistic effect on D_1 and D_2 receptors, demonstrating unequivocally that interactions between D_1 and D_2 receptors can occur on the same neuron (51). Furthermore, in homogenized tissue, pretreatment with the D_1 antagonist SCH 23390 prevented D_2 agonist-induced decrease in ^{3}H-raclopride binding to D_2 receptors in the striatum, but not in the pituitary, which lacks D_1 receptors (52). The D_1 antagonist reduced the number of high-affinity agonist labeled D_2 receptors; a similar effect on ^{3}H-raclopride binding was obtained with a nonhydrolysable guanine nucleotide analogue. ^{3}H-SCH 23390 binding to D_1 receptors was prevented by the D_2 antagonist eticlopride. These results suggest that D_1-D_2 receptor interactions in individual neurons could be mediated by guanine-nucleotide binding proteins.

The complexity of interconnections via neuronal networks, feedback

circuits and D_1-D_2 in the same cell defies simple analysis, but it is evident that the net effects of dopamine deficiency produce parkinsonian symptoms. Failure to disinhibit motor tone may account for rigidity; predominance of episodic disinhibition may be the basis of tremor; inability to excite appropriately disinhibition in balance with inhibition of striatal outflow to the cortex could account for postural instability and difficulty in initiating and executing volitional movement. Nonuniform degeneration and partial compensatory effects of dopamine denervation might be the basis for the abnormal movements produced by levodopa in advanced Parkinson's disease. Such considerations have prompted trials of dopamine receptor-specific agonists as novel therapeutic agents. More complete knowledge of changes in the receptors and compensatory mechanisms as well as understanding of the roles of the peptide cotransmitters, interneurons, and feedback pathways will be required before rational therapeutic approaches to optimal dopamine agonist therapies can be developed, but at present some dopamine agonists are clearly useful in the management of Parkinson's disease.

Dopamine Agonists Used in Treating Parkinsonism

Three predominantly D_2 dopamine receptor agonists that are ergoline derivatives (Figure 3) have been used to treat early symptoms of Parkinson's disease or as adjuncts to levodopa/carbidopa (or levodopa/benserazide) in later stages of the disease. Bromocryptine has been used both as an initial treatment for Parkinson's disease, and in later stages of the disease, in combination with levodopa, when responses to the primary therapy has diminished or fluctuations in efficacy of levodopa developed (53). In experimental animals, the dopaminomimetic effects of bromocriptine are diminished by inhibition of tyrosine hydroxylase with α-methyl-*p*-tyrosine, which suggests that dopamine from intact dopaminergic neurons is required for some of its effects (54). Since bromocriptine is a mixed agonist-antagonist at D_2 receptors (55), presumably because it is unable to induce the high-affinity state (56), it might enhance release of endogenous dopamine by an inhibitory action at dopamine autoreceptors. This is consistent with the need for higher doses of bromocriptine in patients with advanced parkinsonism. As cited earlier, the decline in efficacy of bromocriptine reversal of MPTP-induced parkinsonism in monkeys is prevented or reversed by treatment with SKF 38393, a selective D_1 agonist (47), possibly because bromcryptine also may act as a D_1 antagonist, but the role of this action in its effects is not clearly defined.

Lisuride is about an order of magnitude more potent as a D_2 agonist than bromocriptine and about 1000 times more potent than dopamine. Like bromocriptine it has some D_1 antagonist activity. Although it binds also to serotonin and adrenergic receptors, most of its pharmacological effects appear to be the result of dopamine D_2 receptor stimulation. Lisuride is effective in

reversing all the major motor deficits of parkinsonism and has been used alone in the early stages of Parkinson's disease as well as an adjunct to levodopa when its efficacy wanes (57). The severity of fluctuations, abnormal involuntary movements, and dystonic features that attend chronic levodopa treatment may be lessened by lisuride.

Pergulide, unlike bromcryptine and lisuride, has significant agonist activity at D_1 receptors. Although its affinity for D_1 receptors is lower than for D_2 receptors, there is evidence that some of the effects of pergolide in experimental animals may be attributed to an action at D_1 receptors. There is, however, no clear evidence that its action at D_1 receptors is significant for its therapeutic benefit in Parkinson's disease (58).

In 1985, (+)-4-propyl-9-hydroxynaphthoxazine (PHNO) was shown to be a potent antiparkinsonian agent (59), but this compound has not come into clinical use. Although it has the advantage of being absorbed through the skin (60), tolerance to the levodopa-sparing effects develop (61), presumably as a result of down-regulation of dopamine receptors, as has been found in PHNO-treated MPTP parkinsonian monkeys (62).

Levodopa is generally more effective in relieving parkinsonian symptoms than are direct-acting dopamine agonists, presumably because optimal antiparkinsonian treatment may depend upon attaining an appropriate balance of dopamine receptor activation. As indicated above, cloning and characterization of the molecular structures of the D_1 and D_2 receptors not only validated their pharmacological distinction but led to the discovery of other types of dopamine receptors. The D_2 receptor was the first of the dopamine receptors to be cloned and characterized (63). The cloning strategy was based on presumptive homology of G-protein-coupled receptors; the hamster β_2-adrenergic receptor gene was used as a hybridization probe and the rat gene cDNA for the D_2 receptor was among several new genes belonging to this family that have been cloned. Shortly thereafter, cloning of the D_1 receptor was reported simultaneously from four different laboratories (64–67). To date, three additional dopamine receptors have been cloned and characterized. D_2 and D_3 receptors have similar affinities for ^{125}I sulpiride but their distribution, based on in situ hybridization for the mRNAs encoding these receptors, differs (68, 69). D_3 mRNA is most abundant in dopaminergic projections areas associated with cognitive and emotional functions, e.g. N accumbens and other A10 projection areas. There exist alternative transcripts of the D_3 receptor but their functional significance, if any, has not been determined (70, 71). The D_4 receptor has been reported to be highly homologous with the D_2 and D_3 receptor genes. Although pharmacologically similar to these receptors, its affinity for clozapine, an "atypical" neuroleptic that does not produce parkinsonian side effects, is an order of magnitude higher than for D_2 or D_3 receptors (72). In rats, it has been claimed that central nervous system

abundance of mRNA encoding D_4 receptors is relatively low compared to the levels in the cardiovascular system (73), but the significance of this observation has not yet been determined.

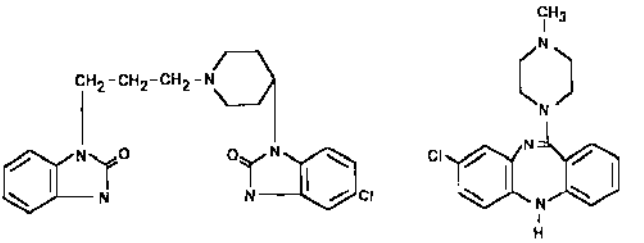
Recently, five genetically determined variants of the D_4 receptor have been distinguished in humans, differing in the number (2, 3, 4, 5, or 7) of repeats of a 48-base pair sequence encoding the amino acids in the third cytoplasmic loop of the receptor (74). Such structural differences may be a basis for individual differences in responses to endogenous transmitters or to therapeutic agents. Finally, a D_5 receptor mRNA was deduced to encode a 477-amino acid protein with 49% homology with the D_1 amino acid sequence (75). This receptor, which appears to be neuron-specific, was found to display a tenfold higher affinity for dopamine than D_1 receptors. Like D_1 receptors, D_5 receptors stimulate adenylcyclase activity. Two pseudogenes, 95% identical with each other, have been reported (76). Neither of the pseudogenes can encode a functional receptor, but one of the mRNAs that encodes 154-amino acid proteins is transcribed in several brain areas (77). The variety and distribution of these multiple dopamine receptor subtypes holds promise that more specific therapeutic agents may be developed to treat a number of psychiatric and neurological disorders, among which is Parkinson's disease. Unless more highly specific dopamine receptor agonists are developed, dopamine antagonists may be useful in limiting undesirable side effects of dopamine and dopamine receptor agonists.

Dopamine Antagonists as Adjuncts to Treatment of Parkinsonism

The therapeutic objective of providing dopaminergic stimulation in brain to reverse parkinsonian symptoms has been achieved by administration of levodopa or dopamine agonists, as discussed above. Use of peripheral decarboxylase inhibitors has generally proven effective in limiting the side effects of peripheral dopamine, as well as sparing dopa from useless decarboxylation in tissues outside of the brain and diminishing the enzymatic barrier in brain endothelium to entry of levodopa into the brain, but inhibition of decarboxylation confers no protection against peripheral dopaminergic effects of dopamine agonists. Thus, drugs that selectively block peripheral dopamine receptors may be useful adjuncts to dopamine-replacement therapy of Parkinson's disease. Domperidone (Figure 5) is a benzimidazolinic dopamine receptor antagonist that does not penetrate readily into the brain and, therefore, at doses that inhibit peripheral dopamine receptors, is devoid of central effects. After systemic administration, the drug does not reach effective concentrations in the brain (78). It is effective in blocking dopamine-mediated behavioral effects only when administered intracerebrally (79), and is unable to antagonize central effects of apomorphine unless given in

very high doses (80), but is effective in blocking peripheral effects, including emesis, induced by dopamine agonists (see 81, 82). Dopamine and dopamine agonists produce emesis when applied to the area postrema, a region of brain devoid of a blood-brain barrier. Since this site is accessible to domperidone, it is effective in preventing dopamine agonist-induced vomiting (83). Thus, disturbing gastrointestinal symptoms, whether due to dopaminergic stimulation in the gastrointestinal tract or in the area postrema, are largely prevented by the doses of domperidone (10–60 mg/day in three divided doses), which do not antagonize the antiparkinsonian effects of levodopa or the directly acting dopamine agonists. Since domperidone has no significant effect on orthostatic hypotension in parkinsonian patients being treated with dopa/decarboxylase inhibitors (84, 85), or with bromocriptine (81, 86), this side effect of levodopa/carbidopa or dopamine agonist antiparkinsonian treatment appears to be mediated centrally or by a dopamine receptor subtype that is not blocked by domperidone.

Clozapine (Figure 5) is prototypic of antipsychotics that are considered to be atypical because they have little tendency to cause parkinsonian movement disorders or produce tardive dyskinesia (87). The drug has multiple actions at histamine, serotonin, and acetylcholine, as well as dopamine, receptors and there has been considerable discussion regarding the basis of its antipsychotic effects (88). As indicated above, clozapine has a tenfold greater affinity for D_4 than D_2 or D_3 receptors; its special characteristics of being devoid of parkinsonian side effects is presumably the result of this specificity. Clozapine has proven useful in treating levodopa-induced psychosis in Parkinson's disease (89), which suggests that its therapeutic benefit is related to its efficacy in blocking dopamine receptors that are not essential for motor regulation but are associated with the psychotic effects of levodopa. Furthermore, it has been claimed that clozapine ameliorates tremors in nonpsychotic as well as psychotic parkinsonian patients (90–92).



Domperidone Clozapine

Figure 5 Chemical structure of dopamine receptor antagonists which have found use in Parkinson's disease treatments.

RETARDING THE PROGRESSION OF PARKINSON'S DISEASE

Tips From a Toxin

The discovery that inadvertent self-administration of 1-methyl-4-phenyi-1,2,3,4-tetrahydropyridine (MPTP), formed from a side reaction during the illicit synthesis of a narcotic substitute, was responsible for the rapid development of severe parkinsonism has spawned a host of studies directed towards understanding the etiology and pathogenesis of Parkinson's disease. In man and nonhuman primates, MPTP selectively destroys the dopaminergic neurons of the nigrostriatal pathway and produces a motor disorder that closely resembles idiopathic parkinsonism. Soon after the discovery of MPTP toxicity, it became evident that the toxic effect was not due to MPTP itself, but was the result of its metabolism to 1-methyl-4-phenyi-pyridinum (MPP^+). It is MPP^+ that is found in brain; however, when MPP^+ is administered systemically it is not neurotoxic, presumably because of its inability to penetrate the blood-brain barrier.

The current view of the mechanisms involved in MPTP-induced dopaminergic neurotoxicity (93, 94) is summarized in Figure 6. MPTP enters the brain where it is captured by acidic organelles in astrocytes; these cells contain monoamine oxidase type B (MAO-B), which oxidizes MPTP to MPP^+ that escapes into the extracellular space. MPP^+ is taken up via catecholamine transporters into dopaminergic and noradrenergic cells and is further concentrated in mitochondria where it inhibits mitochondrial respiration. It is primarily the oxidation of NADH-linked substrates that are inhibited, indicating that the site of electron-transport blockade is at complex I. Consequences of the blockade of mitochondrial respiration include ATP depletion, failure

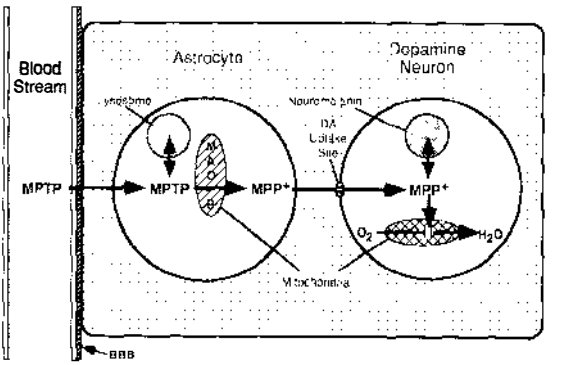


Figure 6 Schematic representation of mechanisms involved in MPTP toxicity targeting of dopaminergic neurons (see text).

of energy dependent ion transport, elevation of cytosolic Ca^{++} to toxic levels, increased release of superoxide from the mitochondria, and disruption of vital cell functions. A combination of these factors leads to cell death.

The realization that a toxin could cause selective destruction of the dopaminergic neurons and cause a parkinsonian syndrome clinically indistinguishable from the spontaneously occurring movement disorder, rekindled interest in previously proposed mechanisms of nigrostriatal degeneration and stimulated new hypotheses regarding the etiology and pathogenesis of Parkinson's disease. Three major hypotheses have emerged. The first, and most obvious, was that a neurotoxin or protoxin, similar to MPTP, either in the environment or endogenously produced, is responsible for the degeneration of catecholaminergic neurons, particularly of the dopaminergic neurons of the substantia nigra. The second, and not necessarily exclusive of the neurotoxic hypothesis, is that a mitochondrial abnormality in electron transport, possibly genetically determined, increases the vulnerability of some individuals to a destructive process involving the nigrostriatal neurons. The third, also not necessarily exclusive of the other hypotheses, is that oxidative stress, generated as a result of special characteristics of the dopaminergic nigrostriatal neurons, is the basis for the degenerative process causing death of these neurons.

SEEKING THE CAUSE OF PARKINSON'S DISEASE

Extensive searches for environmental or endogenous toxins have yielded provocative epidemiologic evidence in support of a neurotoxin in the environment (95), but no such toxin has been found and only traces of endogenously produced potential neurotoxins have been demonstrated in vivo.

ABNORMAL MITOCHONDRIA Deficiencies in mitochondrial electron transport at complex I similar to that caused by MPP^+ have been reported in platelets (96, 97), in muscle (98, 99), and in substantia nigra (100), of Parkinson's disease patients. It has been claimed that the mitochondrial defect is confined to the substantia nigra (101). This putative mitochondrial abnormality has prompted reconsideration of the genetic contribution to vulnerability to Parkinson's disease (102). The demonstrated mitochondrial abnormality, however, is not necessarily a primary etiological factor; it might be secondary to another metabolic deficit. For example, the inhibitory effect of MPP^+ on mitochondrial respiration is reversible (103), but free radicals formed during inhibition of complex I (104), appear to inhibit irreversibly electron transport at complex I (105). Excess free radicals produced from any source might be responsible for the deficiency in complex I activity reported in Parkinson's disease.

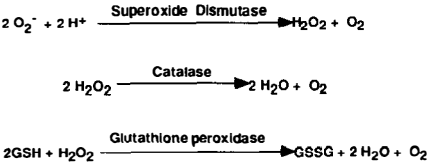


Figure 7 Inactivation of reactive oxygen species.

OXIDATIVE STRESS There is considerable evidence that oxidative stress and free radical formation might play an important role in the pathogenesis of Parkinson's disease (106). Deficiencies in the mechanisms protective against oxidative stress (Figure 7) might contribute to vulnerability to the degenerative process leading to Parkinson's disease. Elevated levels of superoxide dismutase demonstrated post mortem in the substantia nigra of parkinsonian patients (107) might signal enhanced superoxide formation. This enzyme converts superoxide to hydrogen peroxide (Figure 7). Levels of glutathione and glutathione peroxidase, the enzyme catalyzing the removal of hydrogen peroxide by oxidation of glutathione (Figure 7), have been reported to be abnormally low in the substantia nigra of Parkinson's disease patients (108). In view of the relative paucity of brain catalase (109), the enzyme that converts hydrogen peroxide to water and oxygen (Figure 7), deficiencies in glutathione, or glutathione peroxidase might increase vulnerability of the brain to oxidative stress.

In addition to its formation from superoxide, hydrogen peroxide is produced by oxidases, among which is MAO. Oxidative deamination of dopamine, by either intraneuronal MAO-A or astrocytic MAO-B, yields hydrogen peroxide (Figure 8). Thus, rapid release of dopamine in regions of high dopamine concentration might confer particular vulnerability to these regions. In support of this view, procedures that increase dopamine release have been shown to increase levels of oxidized glutathione (110, 111). Furthermore, the increase in oxidized glutathione is prevented by pretreatment with a MAO-B inhibitor (112). Increased lipid peroxidation in the substantia nigra of parkinsonian patients (113) supports the notion that free radicals have damaged the dopaminergic neurons in the nigrostriatal pathway.

Transition elements such as manganese and iron catalyze the formation of free radicals and enhance their toxicity (114). Such metal ion-catalyzed autoxidation of dopamine (and levodopa) to toxic quinones (Figure 8), attended by formation of superoxide ions, has been implicated in the pathogenesis of parkinsonism (115). At physiological pH, spontaneous oxidation of catechols to quinones is slow but results in formation of hydrogen peroxide. The conversion of superoxide and hydrogen peroxide to the highly

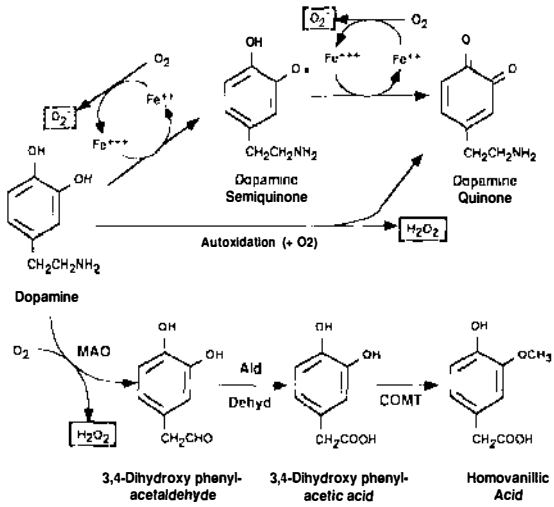


Figure 8 Dopamine oxidations. Autoxidation of dopamine to its quinone results in formation of hydrogen peroxide. This reaction is accelerated by redox cycling of iron with formation of superoxide. The major route of dopamine metabolism is by MAO-catalyzed oxidative deamination, which also produces hydrogen peroxide.

reactive hydroxyl ion (Haber-Weiss reaction) is normally slow, but in the presence of iron, which catalyzes conversion of hydrogen peroxide to hydroxide and a free radical, the reaction is rapid (Figure 9). Thus, hydroxyl ion formation, from superoxide, as well as from hydrogen peroxide is catalyzed by iron.

Several studies (116–119) have reported that post mortem levels of iron, particularly ferric ion, are increased in the substantia nigra of parkinsonian patients. The iron is deposited in astrocytes, macrophages, reactive glia,

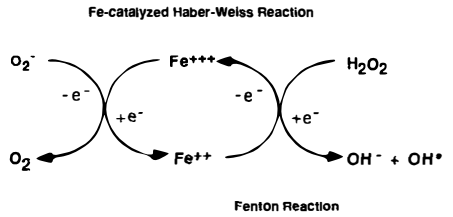


Figure 9 The highly reactive hydroxyl radical is formed from superoxide and hydrogen peroxide. This reaction is slow, (Haber-Weiss), but in the presence of iron is markedly accelerated, presumably because of the redox cycling of iron in the Fenton reaction that converts hydrogen peroxide to hydroxide ions and hydroxy radicals.

nonpigmented neurons (117), and in damaged areas devoid of neuromelanin (117, 118), but iron appears to be diminished on neuromelanin aggregates (117). In one study (112), elevated zinc and decreased copper levels were reported, whereas others (116) report normal zinc but elevated aluminum levels. Nigral ferritin levels have been reported to be diminished when measured by radio-immunoassay (117) but elevated when assayed in paraffin sections (118). Thus, although there appears to be increased iron deposited in the substantia nigra of parkinsonian patients, it is unclear if this is a result of the neuronal degeneration or due to an abnormality in iron metabolism. Neuromelanin is a complex redox polymer that contains free radicals and it has been proposed that neuromelanin-iron interactions might account for the vulnerability of pigmented neurons to oxidative stress (120).

DEPRENYL: SYMPTOMATIC EFFECTS VERSUS NEUROPROTECTION

Although a host of hydrazide and nonhydrazide MAO inhibitors were studied after the discovery of iproniazid, a distinction between subtypes of the enzyme did not become apparent until nearly 20 years later. In 1968, Johnston (121) noted that clorgyline inhibited deamination of serotonin, but not of benzylamine and on this basis distinguished a clorgyline-sensitive form of MAO, which he designated MAO-A, from a clorgyline-insensitive MAO-B. Knoll & Magyar (122) showed that deprenyl was a potent, selective inhibitor of MAO-B. Although inhibition of MAO, with or without concurrent administration of levodopa, had been attempted as a treatment for Parkinson's disease, MAO inhibitors alone were marginally effective and although potentiating levodopa, the combination of drugs could produce severe hypertension and levodopa was contraindicated in patients receiving a MAO inhibitor (123). When it became apparent that selective inhibition of MAO-B with deprenyl did not potentiate sympathomimetic amines and that levodopa could be safely administered with deprenyl (124, 125), although dopamine was proven to be a substrate for MAO-B in humans (126), many studies were initiated to determine optimal use for the levodopa potentiating effects of deprenyl. Deprenyl appeared to be of use in management of fluctuating responses to levodopa/carbidopa, in diminishing the "on-off" effects, and in prolonging the efficacy of levodopa treatment. In addition to these clinical observations, however, Birkmayer et al (127) reported that patients who had received deprenyl along with levodopa therapy appeared to have a longer life expectancy that those treated with levodopa alone. These observations came at about the time that MPTP toxicity had been shown to be prevented by inhibition of MAO-B with deprenyl. Fueled by these observations and the newly emerging hypotheses regarding the role of oxidative stress, prospective,

randomized, double-blind studies were initiated to examine the possibility that deprenyl alone or in combination with an antoxidant, tocophenol, might retard the progression of the degenerative process of Parkinson's disease. In two studies (128, 129), deprenyl (selegiline) and tocopherol were administered to previously untreated parkinsonian patients and deprenyl was found to delay highly significantly the onset of disability sufficient to warrant initiation of levodopa therapy. Although the findings were dramatic, whether they were the result of symptomatic improvement due to potentiation of endogenous dopamine (by any of several potential mechanisms) or a really neuroprotective effect is the subject of considerable controversy (see Ref. 130), particularly since treatment with deprenyl did not produce any significant biochemical changes to substantiate a free radical scavenging effect (131).

GLUTAMATE RECEPTOR ANTAGONISTS IN PARKINSON'S DISEASE

As is evident from Figure 4, increased glutaminergic outflow as a result of selective activation of the indirect net inhibitory pathway could contribute to parkinsonian motor abnormalities. Consistent with this hypothesis, lesions of the subthalamic nucleus in MPTP parkinsonian monkeys reduced all of the major motor disturbances in the contralateral limbs, including akinesia, rigidity, and tremor (132). Antagonists of the NMDA (N-methyl-D-aspartate) subtype of glutamate receptor have been claimed to potentiate the therapeutic effect of dopaminergic agonists (133). Also, administration of either an AMPA (alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid)-antagonist, NBQX, or a competitive NMDA-antagonist, CPP, while not effective (in marmosets) or only somewhat effective (in aged monkeys) as an antiparkinsonian agent when given alone, enhance the efficacy of levodopa in ameliorating parkinsonian symptomatology and stimulating unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra (134, 135).

Furthermore, it has been suggested that glutamic acid and its analogs are excitotoxins that might contribute to the pathogenesis of Parkinson's disease. Although NMDA-type glutaminergic receptors are sparse in the pars compacta, AMPA and NNKQ (nonkainate, nonquisqualate) binding sites appear to be located on dopamine neurons in parkinsonian brains (136).

FUTURE DIRECTIONS

Symptomatic treatment of advanced Parkinson's disease requires more detailed understanding of the mechanisms responsible for the resistance to levodopa and for the abnormal movements that develop after long-term levodopa therapy. Enhancing and stabilizing dopamine formation from

administered levodopa might diminish adverse effects of levodopa treatment. Selective stimulation or judicious blocking of receptor subtypes may allow optimization of symptomatic treatment. The recent attention given to determining the cause of the degenerative process could yield a means for identifying and protecting vulnerable individuals against the development of the disease. Taken together, the mitochondrial deficiency in electron transport, enhanced lipid peroxidation, elevated superoxide dismutase, diminished capacity to remove excess hydrogen peroxide (suggested by low glutathione and glutathione peroxidase levels) and the presence of increased iron, which catalyzes formation of highly reactive hydroxyl ions from hydrogen peroxide, make attractive the suggestion that oxidative stress could play an important role in the degenerative process leading to Parkinson's disease. These considerations provide a rationale for therapeutic strategies to diminish oxidative stress in dopaminergic regions of brain. If oxidative stress is a major factor, the agents that selectively and safely chelate iron may be of value. The high concentrations of dopamine that can be deaminated extraneuronally by MAO-B with attendant hydrogen peroxide formation, and the importance of MAO-B in bioactivation of MPTP to form a neurotoxin that selectively destroys dopaminergic neurons suggest that use of an MAO-B inhibitor, particularly with coadministration of an antoxidant, might diminish the oxidative stress and retard the progression of Parkinson's disease. Recently, increasing attention is being attracted to the potential use of selective glutamate antagonists as means to retard disease progression as well as to treat symptoms of Parkinson's disease.

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