Pathophysiology of Motor Fluctuations in Parkinson’s Disease

Katherine Widnell, MD, PhD*

Abstract: Loss of dopamine neurons in Parkinson’s disease (PD) initiates a complex series of changes in neuronal function that results in clinical signs of tremor, bradykinesia, and rigidity. Although levodopa remains the most-effective drug for the symptomatic treatment of PD, its chronic use is complicated by the development of motor fluctuations and dyskinesias. A full review of the mechanisms underlying the development of motor complications is beyond the scope of this article, which will instead focus on some observed changes in dopamine pathway signal transduction proteins.

Alterations in neuronal activity associated with prolonged use of L-dopa occur gradually over time and may underlie the emergence of dyskinesias and/or motor fluctuations. The risk of developing motor fluctuations has been linked to disease severity, dosage of L-dopa, and the age of the patient. A growing number of preclinical and clinical studies suggest that pulsatile stimulation of striatal dopamine receptors may increase the frequency, and possibly even the development, of motor fluctuations. However, the hypothesis that treatments that offer more continuous dopaminergic stimulation may decrease the development of motor fluctuations and dyskinesias has not yet been tested directly in patients with PD.

BASAL GANGLIA ANATOMY

The basal ganglia consists of input and output stations (see Fig. 1). The input stations include the striatum and the subthalamic nucleus (STN), which receive excitatory glutamatergic input from various regions of the cortex, modulatory dopaminergic input from the substantia nigra pars compacta (SNc), and serotonergic input from the dorsal raphe nucleus. The output stations include the globus pallidus pars interna (GPI) and substantia nigra pars reticulata (SNr). The cortical region of origin defines the function of the basal ganglia circuit as either “motor,” “oculomotor,” “associative,” or “limbic”.

Voluntary movement involves activation of a motor circuit that originates in the pre- and postcentral sensory-motor regions. As shown in Figure 1, neurons project directly from the cortex to the putamen or indirectly by means of the centromedian nucleus (CM) of the thalamus. The majority of neurons in the striatum are medium γ-aminobutyric acid (GABA)ergic projection cells. Cortical glutamatergic projections terminate at the spine heads of distal dendrites of the striatal medium spiny neurons and excite these neurons by means of N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-
isoxazolepropionic acid (AMPA), and kainate glutamate receptors. In contrast, dopamine plays a modulatory role in the basal ganglia. Nigral dopaminergic projections terminate on dendritic shafts of the striatal medium spiny neuron.6

Two distinct pathways connect the striatum to the basal ganglia output stations. The striatum projects directly to the globus pallidus pars externa (GPe) and SNr by means of inhibitory GABA pathways and indirectly to the GPi by means of (1) GABA-ergic projections to the GPe and SNr by means of inhibitory γ-aminobutyric acid (GABA, black) pathways and (2) GABA-ergic projections from the GPe to the STN. Subthalamic projections use glutamate as a neurotransmitter and result in excitation of neurons in the GPi/SNr.

FIG. 1. Basal ganglia anatomy: The striatum and subthalamic nucleus (STN) receive excitatory glutamatergic (gray) input from various regions of the cortex, modulatory dopaminergic input from the substantia nigra pars compacta (SNc), and serotonergic input from the dorsal raphe nucleus (serotonergic input not shown). The output stations include the globus pallidus pars interna (GPi) and substantia nigra pars reticulata (SNr). The striatum projects directly to the GPe and SNr by means of inhibitory γ-aminobutyric acid (GABA, black) pathways and indirectly to the GPi by means of (1) GABA-ergic projections to the globus pallidus pars externa (GPe) and then (2) GABA-ergic projections from the GPe to the STN. Subthalamic projections use glutamate as a neurotransmitter and result in excitation of neurons in the GPi/SNr. CM, centromedian nucleus; VA/VL, pallidal-receiving area of the thalamus; PPN, pedunculopontine nucleus.

isoxazolepropionic acid (AMPA), and kainate glutamate receptors. In contrast, dopamine plays a modulatory role in the basal ganglia. Nigral dopaminergic projections terminate on dendritic shafts of the striatal medium spiny neuron.6

Two distinct pathways connect the striatum to the basal ganglia output stations. The striatum projects directly to the globus pallidus pars externa (GPe) and SNr by means of inhibitory GABA pathways and indirectly to the GPi by means of (1) GABA-ergic projections to the GPe and then (2) GABA-ergic projections from the GPe to the subthalamic nucleus (STN).7,8 Subthalamic projections use glutamate as a neurotransmitter, and result in excitation of neurons in the GPi/SNr.

GPi/SNr projections to the thalamus are GABA-ergic and therefore inhibit the excitatory (glutamatergic) pathway from the thalamus to the cortex. Striatal neurons that form the direct pathway express high levels of the type 1 dopamine receptor (D1 receptors) and contain the neuropeptides dynorphin and substance P. In contrast, striatal neurons that form the indirect pathway express high levels of the type 2 dopamine receptor (D2 receptors) and contain the neuropeptide enkephalin.9

**BASAL GANGLIA PHYSIOLOGY**

As shown in Figure 2, there are two distinct populations of dopamine receptors, which differentially regulate second messenger pathways in the striatum. D1 receptor activation leads to stimulation of adenyl cyclase, the enzyme responsible for the synthesis of cyclic adenosine monophosphate (cAMP). D2 receptor activation inhibits adenyl cyclase, which results in decreased synthesis of cAMP. Levels of cAMP regulate the activity of cAMP-dependent protein kinase (PKA), which plays an important role in cell phosphorylation events such as ion channel modulation and regulation of gene expression.

Dopamine differentially regulates the direct and indirect pathways. The direct and indirect pathways work together to balance the motor control wielded by the basal ganglia. While the direct pathway facilitates movement by means of decreases in tonic inhibition of basal ganglia output and disinhibition of thalamocortical and brainstem pathways, the indirect pathway suppresses movement by increasing the inhibitory basal ganglia output to the thalamus. One of the roles of the direct and indirect pathways is to scale movement by first inhibiting GPi/SNr neurons by means of the direct pathway and then disinhibiting the same GPi/SNr neurons to terminate the movement.10–12

Decreased dopaminergic input to the striatum from the SNc in Parkinson’s disease results in a complex alteration in activity between input and output stations of the basal ganglia. Decreased levels of dopamine result in increased activity along the indirect pathway and decreased activity along the direct pathway, which together result in increased excitation of GPi and SNr neurons, then to increased inhibition of thalamic neurons, and finally to decreased excitation of the cortex. Patterns of neuronal discharge within the basal ganglia are disturbed in PD. In particular, there is a tendency for neuronal elements to synchronize at around 20 Hz in the absence of dopaminergic treatment, whereas this activity can be replaced by spontaneous synchronization at much higher frequencies (>70 Hz) after dopaminergic treatment.13–15

Again, this simplistic approach to explain the clinical features of PD does not take into account observed changes in spontaneous discharge patterns observed in the parkinsonian state.16–18

The mechanisms by which chronic L-dopa results in further perturbations of firing patterns of basal ganglia
neurons is not well understood. It is also not known whether L-dopa–induced motor fluctuations result from chronic effects of dopamine denervation, chronic effects of L-dopa administration or both. Attempts have been made in animal models to correlate the behavioral changes of motor complications to the neurobiological and physiological adaptations observed at a single cell level. These adaptations include changes in neurotransmitters and receptor expression, resulting in functional alterations in signal transduction proteins that will be detailed below.

**CELLULAR ADAPTATIONS IN PARKINSON’S DISEASE**

Long-lasting, activity-dependent changes in neuron function are thought to require alterations in gene expression. As shown in Figure 2, neurotransmitter receptor activation initiates intracellular signaling events that culminate in (1) activation of a preexisting transcription factor or (2) de novo synthesis of a transcription factor. Transcription factors are proteins that bind to specific DNA sequences in the regulatory regions of genes. The binding of transcription factors to these regulatory sequences increases or decreases the rate at which those genes are transcribed. Studies of the regulation of gene expression in the striatum have focused on transcription factors activated by both mechanisms. Basal levels of cAMP response element binding protein (CREB) are present in the striatum and can be activated by various stimuli. CREB belongs to a family of leucine zipper transcription factors that share certain structural motifs and are activated after phosphorylation by PKA and other protein kinases. Phosphorylated CREB regulates expression of downstream target genes (such as fos) by binding to DNA sequences (CRE sites) in their promoter regions. In contrast, levels of c-Fos, c-Jun, and products of other related immediate early genes (IEGs) are low and must be expressed and translated in striatal cells.19 The newly synthesized fos messenger RNA (mRNA) is translated into cFos protein, which associates with a member of the Jun family, forming a complex that binds to specific DNA sequences known as activator protein-1 (AP-1) sites in the promoter regions of target genes and regulates their expression.19

Alterations in levels of IEGs, neuropeptides, and neurotransmitter receptors have been observed in animal models of PD. It has become clear that dopamine depletion results in significant changes in cellular signaling pathways. More recently, studies have suggested that different patterns of treatment with dopaminergic agents (L-dopa or dopamine agonists) results not only in differential behavior but also significant differences in patterns of gene expression. It is not clear whether the observed changes in gene expression result in a behavioral phenotype of motor fluctuations, but correlations have been made between abnormal patterns of gene expression and the development of motor complications.

**FIG. 2.** Two classes of dopamine (DA) receptors exist (D1R and D2R) and differentially activate signal transduction pathways. The D1 receptor increases the activity of adenyl cyclase (AC), which increases levels of cyclic adenosine monophosphate (cAMP), resulting in phosphorylation and activation of protein kinase A (PKA). Activated protein kinase phosphorylates and activates cAMP-response element binding protein (CREB), which leads to changes in gene expression. The D2 receptor decreases the activity of AC, which decreases levels of cAMP resulting in less phosphorylation and decreased activity of PKA. Arrows represent either a positive (full line) or negative (dashed line) effect on the activity of the protein represented in the figure. IEG, immediate early gene.
Immediate Early Genes

The induction of IEGs, such as c-fos, zif268, and other transcription factors, is followed temporally by the expression of “late response genes”, which include many neuropeptides. Alterations in patterns of IEG expression may explain the development of functional responses of striatal neurons to dopamine.2 For example, in animal models, when cocaine (an indirectly acting dopamine agonist) was administered to normal rats, a homogenous induction of fos and related IEGs was observed in both striosome and matrix compartments of the caudate and putamen.2 This effect of dopamine agonist treatment was even more profound in the setting of dopamine depletion. In dopamine-depleted mice, D1 agonist treatment resulted in an abundance of IEG induction, suggesting that dopamine depletion results in alterations of signal transduction mechanisms that regulate gene expression. Near complete depletion of dopamine in the striatum must occur for such a profound IEG response to be seen. In normal rats, treatment with combinations of dopamine D1 and D2 receptor agonists produces alterations in normal behavior, including stereotyped behaviors such as gnawing and biting.20 These changes are not seen after treatment with either D1 or D2 agonists given alone. Similarly, systemic administration of D1-selective agonists produces a strong and widely distributed pattern of c-Fos expression in the striatum that was not seen after D2-selective agonist treatment.20 Just as the induction of behavioral changes was altered by coadministration of D1 and D2 agonists, patterns of c-Fos expression in the striatum changed when both D1 and D2 agonists were given together, suggesting some sort of synergistic effect. However, such interactions between dopamine receptors appear to be significantly altered after dopamine denervation, when stereotyped behaviors occur with activation of either class of receptor alone.20 In addition, patterns of gene induction by D1 or D2 agonists in dopamine-lesioned animals were significantly altered, suggesting circuit-level modifications in the output pathways of the basal ganglia.

Further evidence that alterations in striatal expression of IEGs may underlie clinical changes seen in Parkinson’s disease has been provided by studies that demonstrate that more than 40 IEGs are induced after D1-agonist treatment in animal models of Parkinson’s disease.2 These IEG changes are not observed in a dopamine-intact striatum. It has been postulated that the development of dyskinesias results from aberrant activation of IEGs and resulting changes in expression of downstream targets of the IEGs by D1-receptor activation in direct striatal pathway neurons.2 Further evidence that aberrant D1-receptor effects in the dopamine-depleted striatum has been provided by studies of the Fos-related IEG δ FosB (a truncated form of FosB) expression. In monkeys rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment, there was a modest increase in striatal levels of delta-FosB–like proteins, primarily in striatopallidal neurons that express the D2 receptor.21 The development of dyskinesias produced by twice daily (b.i.d.) injections of the D1-agonist SKF-82958 was associated with large increases in delta-FosB proteins. In contrast, injections (every 48 hours) of the D2-agonist cabergoline did not result in dyskinesias and reduced levels of delta-FosB proteins in MPTP-lesioned animals to normal.21 Whereas it is unlikely that changes in IEGs per se result in the development of dyskinesias, it is tempting to suggest that IEG-induced changes in signaling proteins, neuropeptides, or neurotransmitters result in alterations in cell signaling, firing, and responsivity to dopamine.

Neuropeptides

After dopamine depletion in animal models of PD, increases in striatal levels of preproenkephalin (PPE) mRNA, along with decreases in levels of preproenkaycin (PPT) mRNA have been observed.22,23 Levels of PPE mRNA in the striatum have been correlated with the presence of more severe dyskinesias.22,24 Increased levels of PPE may reflect abnormal activity in the indirect striatopallidal pathways, resulting in an imbalance in the direct and indirect pathways that could explain the emergence of dyskinesias.24

When MPTP-lesioned animals were treated with pulse treatment (injections of agonist twice a day) or continuous treatment (pump infusion) of the D2-dopamine receptor agonist U91536A, different behavioral responses were observed: animals treated in a pulsatile mode developed dyskinesia, whereas after continuous infusion, behavioral tolerance was observed but no dyskinesias developed. In the putamen and lateral caudate nucleus, elevated PPE mRNA expression generally was not corrected by chronic L-dopa treatment. In general, pulsatile administration of U91356A partially corrected the lesion-induced elevation of PPE mRNA levels in the putamen and lateral caudate, whereas the correction was most pronounced and widespread when MPTP monkeys received continuous administration of this drug.22

Additional studies have supported the concept that more continuous stimulation of D2 receptors results in a reversal of MPTP-induced alterations in levels of PPE mRNA expression. Elevated PPE and decreased PPT
mRNA levels observed in monkeys after MPTP-lesioning were corrected after treatment with cabergoline (0.25 mg/kg every other day), a dose that had antiparkinsonian effects but did not produce sustained dyskinesia. Not only did pulsatile administration of the D1 agonist SKF-82958 (3 times/day) not reverse the increase of PPE mRNA levels, it actually significantly increased levels of PPE mRNA and resulted in a shorter duration of motor benefit (wearing-off) and dyskinesias in the animals. Continuous treatment with SKF-82958 produced no clear antiparkinsonian benefit in the animals, nor did it alter the MPTP-induced increases in PPE or decreases in PPT mRNA levels. The mechanism underlying changes in PPE mRNA levels is unknown; it has been postulated that increases in PPE mRNA due to dopamine depletion may result from unchecked glutamatergic excitation after denervation.

**NEUROTRANSMITTER RECEPTORS**

In general, in monkey models of untreated Parkinson’s disease, levels of D2 receptors have been shown to be either normal or upregulated in the striatum, whereas levels of D1 receptors are not clearly altered. In contrast, studies have suggested that, after treatment with L-dopa or other D2 agonists, D2 receptors are downregulated to normal levels, whereas D1 receptors are decreased but not affected greatly in monkey models as well as patients with Parkinson’s disease.

The location of alterations in dopamine receptor expression may also be important. In situ histochemistry revealed that D1 receptor mRNA levels in rostral striatum decreased, whereas D2 receptor mRNA levels in caudal striatum increased in MPTP monkeys compared to control animals. As previously noted, pulse treatment of the D2 dopamine receptor agonist U915356A resulted in dyskinesias in MPTP-lesioned monkeys, an effect not seen after continuous infusion of U915356A. Of interest, continuous administration of U91356A reversed the MPTP-induced increase of D2 receptor mRNA, whereas the pulsatile administration did not significantly correct these mRNA changes. Both continuous or pulse treatment with U91356A partially corrected the D1 receptor messenger RNA lesion-induced decrease in the striatum. Other studies have shown that continuous occupancy of the D2 dopamine receptor is required to maintain normal levels of D2 receptor mRNA but that the decreases in D1 receptor mRNA are not corrected by continuous treatment with D1 receptor agonists and instead require single and repeated treatment.

**CONTINUOUS DOPAMINERGIC STIMULATION**

The theory of continuous dopaminergic stimulation suggests that the development of motor fluctuations is related to pulsatile stimulation of dopamine receptors and that treatments that offer more continuous dopaminergic stimulation may decrease the risk that motor fluctuations and dyskinesias will develop.

Clinical evidence has suggested that chronic (continuous) infusion of L-dopa or dopamine agonists (lisuride or apomorphine) dramatically ameliorates motor fluctuations. An increasing body of preclinical data supports these findings. Administration of pulsatile doses of L-dopa b.i.d. in MPTP-treated common marmosets resulted in marked dyskinesias. Further evidence supports the view that the combination of L-dopa with agents that provide more continuous dopaminergic stimulation can decrease the development of dyskinesias. This finding has been accomplished by treating MPTP-treated common marmosets with frequent L-dopa doses (4 times/day) in addition to entacapone or ropinirole, without losing improvements in disability score or improvements in on time. Similarly, MPTP-treated monkeys with a long-standing and stable parkinsonian syndrome (that had never received dopaminergic agents) were treated for a month with L-dopa administered orally or with L-dopa in addition to threshold doses of cabergoline. L-Dopa–induced dyskinesias were observed in the L-dopa group but not in the group receiving L-dopa and cabergoline.

**CONCLUSIONS**

In summary, changes in the pattern of gene expression and behavior occur after dopamine depletion and chronic dopaminergic treatment. Although symptoms of PD decrease with chronic use of L-dopa, the development of dyskinesias represents a serious side effect of this treatment strategy. The development of dyskinesia cannot be attributed solely to the D1 or D2 receptors, and cooperation between the two receptors appears to be necessary for this manifestation. However, the mechanism of dopaminergic administration may play a crucial role in the development of motor fluctuations. A significant body of preclinical data suggests that avoiding pulsatile dopaminergic stimulation may prevent the onset of dyskinesias and motor fluctuations. Continuous dopaminergic stimulation may be achieved with combinations of L-dopa with dopamine agonists or with agents that prolong the half-life of L-dopa.
REFERENCES


