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Glutamate and Parkinson's Disease

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Abstract

Altered glutamatergic neurotransmission and neuronal metabolic dysfunction appear to be central to the pathophysiology of Parkinson's disease (PD). The substantia nigra pars compacta—the area where the primary pathological lesion is located—is particularly exposed to oxidative stress and toxic and metabolic insults. A reduced capacity to cope with metabolic demands, possibly related to impaired mitochondrial function, may render nigral neurons highly vulnerable to the effects of glutamate, which acts as a neurotoxin in the presence of impaired cellular energy metabolism. In this way, glutamate may participate in the pathogenesis of PD. Degeneration of dopamine nigral neurons is followed by striatal dopaminergic denervation, which causes a cascade of functional modifications in the activity of basal ganglia nuclei. As an excitatory neurotransmitter, glutamate plays a pivotal role in normal basal ganglia circuitry. With nigrostriatal dopaminergic depletion, the glutamatergic projections from subthalamic nucleus to the basal ganglia output nuclei become overactive and there are regulatory changes in glutamate receptors in these regions. There is also evidence of increased glutamatergic activity in the striatum. In animal models, blockade of glutamate receptors ameliorates the motor manifestations of PD. Therefore, it appears that abnormal patterns of glutamatergic neurotransmission are important in the symptoms of PD. The involvement of the glutamatergic system in the pathogenesis and symptomatology of PD provides potential new targets for therapeutic intervention in this neurodegenerative disorder.

Index Entries: Basal ganglia; excitotoxicity; excitatory amino acids; bioenergetics; *N*-methyl-D-aspartate.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the clinical triad of tremor, bradykinesia, and rigidity. Affecting about 1% of the population over age 50, PD is a highly debilitating disease that affects profoundly quality of life and shortens life expectancy. It has long been known that the pathophysiology of PD involves dopaminergic

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denervation of the striatum and eventual degeneration of pigmented dopaminergic neurons in the pars compacta of substantia nigra. This has provided the rationale for therapy with L-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine, which relieves symptoms by replacing the deficient neurotransmitter. However, treatment with L-DOPA is only symptomatic, and its efficacy fades as the degeneration of nigral dopaminergic neurons progresses. Also, long-term therapy with L-DOPA is frequently associated with side effects, such as motor fluctuations, dyskinesias, on-off phenomena and, often, psychiatric disturbances, which are extremely problematic for patients.

Development of more effective therapeutic strategies is dependent on a deeper understanding of PD pathophysiology. In this regard, investigations of various aspects of glutamatergic neurotransmission and of neuronal bioenergetic mechanisms, along with a better definition of the functional anatomy of basal ganglia, have recently provided new perspectives.

Glutamate as an Excitatory Neurotransmitter

The amino acid glutamate is the most abundant excitatory neurotransmitter in the central nervous system (CNS). It has been estimated that approx 40% of all brain synapses are glutamatergic *(1–3)*. Glutamate is stored in synaptic vesicles within nerve terminals from which, on depolarization, it is released into the synaptic cleft in a Ca^{2+}-dependent manner. Glutamate's action is terminated by removal from the synaptic cleft via a Na^{+}-dependent, high-affinity uptake system, which is located on neurons and glial cells *(4)*.

Glutamate exerts its physiological actions via the activation of several classes of receptors, which are primarily located on postsynaptic neurons, and which are present in virtually all areas of the CNS. Glutamate receptors were originally classified by pharmacological means into *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. Non-NMDA receptors were further divided into those preferring α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) or kainic acid (KA) as agonists *(5)*. The activation of NMDA, AMPA, or KA receptors leads to opening of associated ion channels ("ionotropic receptors"). An additional class of glutamate receptors, termed "metabotropic," include receptors linked to G proteins. Their activation produces changes in cyclic nucleotides or phosphoinositol metabolism *(6)*.

These pharmacological classifications have largely been borne out by recent investigations of the gene families encoding these receptors. This has led to an improved understanding of NMDA and AMPA receptors. The contribution of other glutamate receptor subtypes to synaptic signaling in the brain is less clear and will be not considered for the purpose of this review.

NMDA Receptors

Because of early pharmacological advances, the NMDA receptor has been extensively studied and is the best characterized of the glutamate receptors. Its activation causes a massive influx of Ca^{2+} and Na^{+} to the intracellular compartment *(7)*. It has been demonstrated that, in addition to glutamate and certain analogs (NMDA, ibotenic acid, quinolinic acid), different substances affect receptor function by binding to other specific sites. Glycine has a binding site on the NMDA receptor that differs from the glycine receptor in that it is strychnine-insensitive. Both the glycine site and the glutamate site must be occupied by ligands for receptor activation to occur *(8,9)*. For this reason, glutamate and glycine are termed "co-agonists." NMDA receptor activation is also modulated by the binding of polyamines, such as spermine and spermidine *(10)*, which increase the ability of glutamate and glycine to open the NMDA receptor ion channel. NMDA receptor function is also influenced by extracellular Zn^{2+} and pH levels. Zn^{2+} acts as an antagonist at a site and with a mechanism that are still unknown *(11,12)*. Reduction in extracellular pH decreases activation of the receptor *(13,14)*.

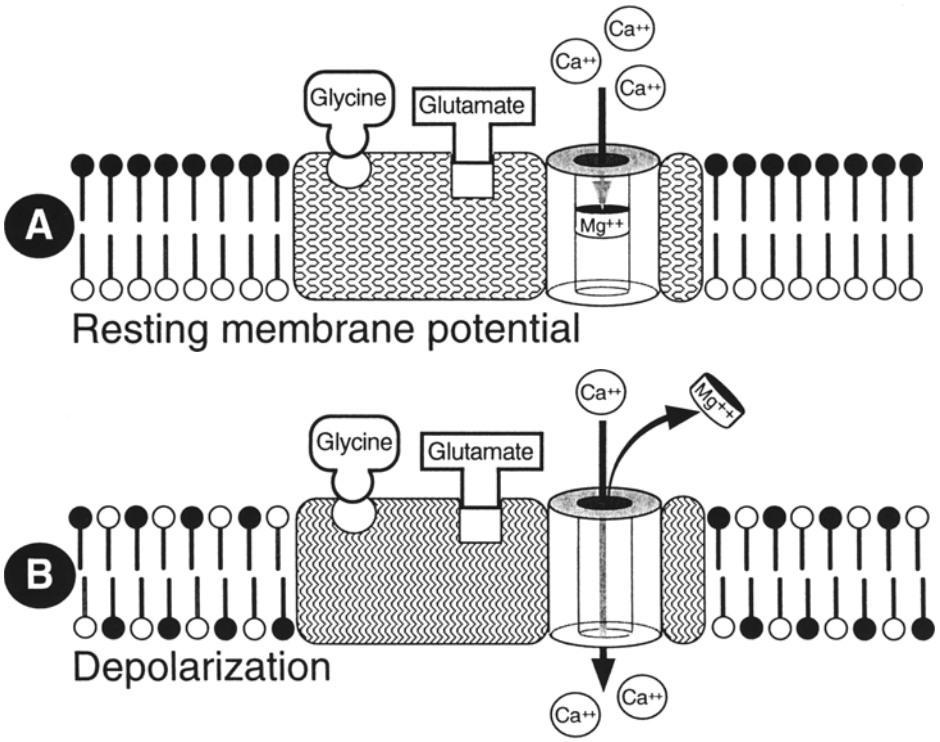

Fig. 1. The NMDA receptor is a calcium-permeable, ligand-gated ion channel that opens in response to the simultaneous binding of glutamate and glycine. **(A)** At resting membrane potential, ambient extracellular magnesium blocks the channel and there is no calcium flux. **(B)** Because the magnesium blockade of the channel is voltage-dependent, depolarization causes extrusion of the magnesium and allows calcium to flow inward. The ability to maintain membrane polarity depends on functional ion pumps, in particular, the Na^+/K^+ ATPase. The Na^+/K^+ ATPase, in turn, depends on an adequate supply of ATP, more than 90% of which is derived from mitochondrial oxidative metabolism. Impaired mitochondrial function depletes ATP, disrupts Na^+/K^+ ATPase activity, and causes depolarization.

An essential feature of the NMDA receptor is that, at normal resting membrane potential, the associated ion channel is blocked by physiological concentrations of extracellular Mg^{2+} *(15)*. Because this Mg^{2+} blockade is voltage-dependent, occupation of the glutamate and glycine sites cannot activate a Ca^{2+} current, if a neuron is kept in a highly polarized or hyperpolarized state. On the other hand, if a neuron is depolarized, Mg^{2+} is extruded from the channel, and glutamate and glycine cause Ca^{2+} (and Na^+) influx (Fig. 1).

It has been recently recognized that the NMDA receptors are multimeric heteromers, composed of different subunits. Thus far, two families of subunits, NMDAR1 and NMDAR2, have been identified *(6)*. The mRNA for NMDAR1 is widely and rather uniformly distributed in the CNS *(6)*. All the regulatory sites found on native NMDA receptors seem to be present on this subunit, but agonist-induced current flow is much greater in native receptors. There are multiple splice variants of the NMDAR1 subunit *(16)*. The second family consists of four members, NMDAR2A through NMDAR2D, which share only a 18–20% amino-acid sequence homology to NMDAR1. NMDAR2 subunits, which show discrete anatomical distributions, can combine with NMDAR1 subunits to form functional NMDA receptors with distinct pharmacological and physiogical properties *(7,17–20)*.

Activation of the NMDA receptor can be antagonized specifically by compounds acting at several distinct sites on the receptor-channel complex. Competitive antagonists block the glutamate binding site, and glycine site antagonists, such as 7-chlorokynurenate, prevent glycine from binding. Drugs like MK-801, phencyclidine, and remacemide prevent current flow by binding to the activated (open) state of the ion channel *(21–23)*.

AMPA Receptors

The AMPA receptor mediates most fast excitatory synaptic transmission in the CNS. Like the NMDA receptor, it is a ligand-gated ion channel. A major functional difference between AMPA and NMDA receptors is the permeability to Ca^{2+}. Binding of glutamate, or analogs like AMPA and quisqualic acid, to the AMPA receptor is associated primarily with an influx of Na^{+} into the neuron. However, some native AMPA receptors have been reported to be highly permeable to Ca^{2+} *(24,25)*. Four subunits, GluR1–GluR4 (also known as GluR A–GluR D) have been cloned *(26,27)*. They can assemble in various combinations that determine the functional characteristics of the receptor. For example, although GluR 1 and GluR 3 can form homomeric or heteromeric ion channels permeable to Ca^{2+}, the inclusion of a GluR 2 subunit prevents Ca^{2+} permeability *(28)*. Further complexity derives from the fact that slightly different mRNA splice variants (called "flip" and "flop") exist for each subunit *(29)*. In terms of physiological and pharmacological profiles, as well as anatomical distribution, this gives rise to a wide spectrum of AMPA receptors. AMPA receptors can be antagonized by drugs, such as 6-nitro-7-sulfamoylbenzo(f)-quinoxaline-2,3-dione (NBQX) *(30)*, that compete for the glutamate binding site, or by compounds, such as GYKI 52466, that enhance receptor desensitization *(31)*.

Glutamate as a Neurotoxin

Excessive stimulation of glutamate receptors by agonists can, under some circumstances, lead to neuronal damage and death. This phenomenon was first described by Olney, who coined the term "excitotoxicity" after the correlated the neurotoxic and the excitatory properties of various glutamate analogs *(32)*. Neurotoxicity mediated by the NMDA receptor is apparently caused by a massive influx of extracellular Ca^{2+} *(33)*. The increase in cytoplasmic Ca^{2+} activates a number of Ca^{2+}-dependent enzymes, including protein kinase C, phospholipase A_2, phospholipase C, Ca^{2+}/calmodulin dependent protein kinase II, nitric oxide synthase, and various proteases and nucleases. Ca^{2+}-induced activation of enzymes involved in the catabolism of proteins, phospholipids, and nucleic acid may lead to cell death through different pathways. The relative contribution of these pathways is still unclear. For example, activation of phospholipase A_2 might result in extensive membrane breakdown *(34)*, whereas Ca^{2+}-mediated activation of proteases seems to determine changes in the microtubular organization of the cytoskeleton that lead to characteristic cytoskeletal alterations (blebbing) *(35,36)*. On the other hand, activation of phospholipase A_2 and subsequent production of arachidonic acid lead to the generation of cytotoxic oxygen radicals *(37,38)*. Also, in certain neurons, Ca^{2+}-mediated activation of nitric oxide synthase causes the release of nitric oxide, which is lethal to surrounding neurons *(39)*. Such an effect may be caused by the generation of peroxynitrite anion from the reaction of nitric oxide with superoxide anion ($\bullet O_2^-$) and the subsequent decomposition to hydroxyl radical ($\bullet$OH) *(40)*.

One of the earliest signs of excitotoxic injury is mitochondrial swelling and dysfunction *(41)*. Recent studies suggest that mitochondria are also the site of NMDA receptor-induced free radical formation *(42)*. Mitochondria serve as a potential "sink" for Ca^{2+} when cytoplasmic levels rise abnormally *(43)*. Laser scanning confocal microscopy reveals that activation of NMDA receptors causes a rapid, reversible accumulation of Ca^{2+} into mitochondria *(44)*. With prolonged receptor activation, the reversibility of mitochondrial Ca^{2+} sequestration

appears to be impaired, which may contribute to the mitochondrial dysfunction associated with excitotoxicity. Moreover, as discussed in the next section, mitochondrial damage may sensitize neurons to further excitotoxic challenges.

Acute neuronal death related to glutamate receptor-mediated overload of Ca^{2+} is of the necrotic type, with swelling of organelles and subsequent cytolysis. However, there is evidence that Ca^{2+} influx might also trigger apoptotic cell death, which is characterized by membrane blebbing, compacting of organelles, and chromatin condensation. Several studies have shown that Ca^{2+} overload can cause DNA fragmentation, probably through the activation of endonuclease *(34)*. It has also been reported that glutamate can trigger cleavage of neuronal internucleosomal DNA, in both in vitro and in vivo experiments *(45)*.

There is intriguing, albeit indirect, evidence that excitotoxicity might play a role in PD. For example, oxidative stress may be an important factor in the death of dopaminergic nigral neurons. The oxidative damage and death of dopamine neurons associated with the toxin, methamphetamine, can be blocked with an NMDA antagonist *(46)*. A mitochondrial bioenergetic defect may also be central to the etiology of PD. For reasons discussed next, NMDA antagonists also protect against the neurotoxic effects of mitochondrial poisons, including the active metabolite of the dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) *(47)*. The fact that NMDA receptor antagonists can prevent neuronal death induced by mechanisms that are believed to be relevant to the pathogenesis of PD suggests that excitotoxicity may be a factor in the neurodegeneration characteristic of this disorder.

Glutamate and Bioenergetic Defects—"A Dangerous Liaison"

It has been suggested that excitotoxicity may play a role in the pathophysiology of numerous neurologic diseases. However, thus far, a direct role for endogenous glutamate-mediated toxicity has been demonstrated only in hypoxic/ischemic brain damage, in which a large increase in extracellular glutamate and a concomitant depression of the uptake (inactivation) system seem to be responsible for neuronal death *(48,49)*. A direct toxic action of glutamate in the pathophysiology of chronic, neurodegenerative disorders, such as PD, seems less likely. The brain is remarkably resistant to very high concentrations of glutamate; in addition, unlike hypoxia/ischemia, no consistent evidence of changes in the extracellular levels or in the inactivation mechanisms of glutamate, capable *per se* of overcoming the adaptive defenses of the brain, has ever been provided for PD.

Thus, the normal brain possesses the instruments necessary to deal with a potential neurotoxin like glutamate. But what happens if, for any reason, neuronal energy production is impaired? A possible answer comes from a re-examination of the properties of the NMDA receptor. As noted previously, at normal resting membrane potential, the receptor ion channel is blocked by extracellular Mg^{2+}. The blockade by Mg^{2+} is voltage-dependent, such that the degree of blockade is reduced as a neuron becomes depolarized for any reason. When a neuron is depolarized and Mg^{2+} block is consequently relieved, binding of glutamate and glycine leads to a large Ca^{2+} influx.

The ability to maintain membrane polarity depends on functional ion pumps, in particular, the Na^+/K^+ ATPase *(50)*. The Na^+/K^+ ATPase, in turn, depends on an adequate supply of ATP, more than 90% of which is derived from mitochondrial oxidative metabolism *(51)*. Impaired mitochondrial function depletes ATP, disrupts Na^+/K^+ ATPase activity, and causes depolarization *(51)*. It ensues that, in this circumstance of depolarization, even nontoxic levels of glutamate may become lethal *(52)*.

This has led to formulation of the "weak" or "indirect" excitotoxic hypothesis *(53,54)*, which is based on the concept that any process that impairs a neuron's ability to maintain normal membrane potential can be expected to enhance its vulnerability to glutamate. Substantial evidence has been provided in support

of this hypothesis. In neuronal culture, inhibition of oxidative phosphorylation by cyanide, or Na^+/K^+ ATPase by ouabain, causes innocuous concentrations of glutamate to become toxic, whereas in chick retina, hypoglycemia or sodium cyanide produces "excitotoxic" lesions. In both cases, NMDA antagonists prevent neuronal death *(55–57)*. Furthermore, intrastriatal injection of amino-oxyacetic acid, which reduces ATP levels by inhibiting the malate–aspartate shunt, results in excitotoxic lesions that are prevented by NMDA antagonists *(58)*. Similarly, inhibition of complex II of the mitochondrial respiratory chain, induced by intrastriatal injection of malonate, causes a neuronal lesion that is excitotoxic in character and blocked by NMDA antagonists *(59,60)*.

An important feature of the interaction between excitotoxicity and bioenergetic defects is that they are synergistic *(61)*. That is, in the setting of a mild metabolic disturbance, nontoxic concentrations of glutamate and other agonists produce widespread, severe damage. This indicates that when neuronal mitochondria are not functioning optimally, neurons are sensitized to the toxic effects of glutamate. Thus, excitotoxic cell death can occur even in the absence of abnormally elevated glutamate levels, and NMDA receptors seem to be central to the synergistic interaction between glutamate and bioenergetic defects. This relationship between excitotoxicity and bioenergetics may play a role in the pathophysiology of a variety of neurodegenerative diseases *(62)* and may be particularly relevant to PD pathophysiology.

Neuronal Energy Metabolism Involvement in PD

Although significant progress has been made in understanding the pathophysiology of PD, the most important question is still unanswered: What is the *primum movens* of the degeneration of nigral dopaminergic neurons? An accidental contribution came in the early 1980s, when numerous young adults presented with a PD-like syndrome. Extensive investigation demonstrated that this phenomenon was caused by the unintentional self-administration of MPTP, a product of the illicit synthesis of meperidine analogs *(63,64)*. MPTP, which has since been widely used to reproduce a clinical and neuropathological picture of PD in primates *(65)*, freely crosses the blood–brain barrier. Once in the brain, this "protoxin" is oxidized to 1-methyl-4-phenylpyridinium (MPP^+), the active toxin, by monoamine oxidase type B. MPP^+ is then taken up selectively by nigral neurons, via the dopamine transporter, and further accumulated in mitochondria, where it can reach concentrations in the millimolar range *(66–71)*. Ultimately, toxicity results from inhibition of mitochondrial respiration, as a consequence of MPP^+ binding to the rotenone-sensitive site of complex I (NADH:ubiquinone oxidoreductase), the proximal enzyme of the mitochondrial electron transport chain *(72,73)* (Fig. 2). In the brain, MPP^+ has an affinity for complex I in the low millimolar range *(74,75)*. The same phenomenon occurs in human blood platelets, which seem to possess their own active transport system for MPP^+ *(76–79)*. Interestingly, complex I inhibitors, including MPP^+, have been reported to induce apoptosis in dopaminergic PC-12 cells *(80)*.

Complex I is the largest and most complicated of the protein complexes in the inner membrane of mitochondria. In mammals, it is composed of more than 40 polypeptides, seven of which are mitochondrially encoded, and it is functionally organized in different subunits and operational entities *(81–83)*. The identification of complex I as the target of MPP^+ toxicity has prompted extensive investigation of bioenergetic mechanisms in PD. In fact, a complex I defect in the substantia nigra of PD patients has been reported by several authors *(84–86)*. This alteration, which is limited to the nigra and does not involve, at least to the same extent, other components of the respiratory chain, seems to be specific for PD. But what causes the reduction in nigral complex I activity? Mitochondrial DNA (mtDNA) is vulnerable to mutagenic insults because it lacks a protein coat and repair mechanisms. Although

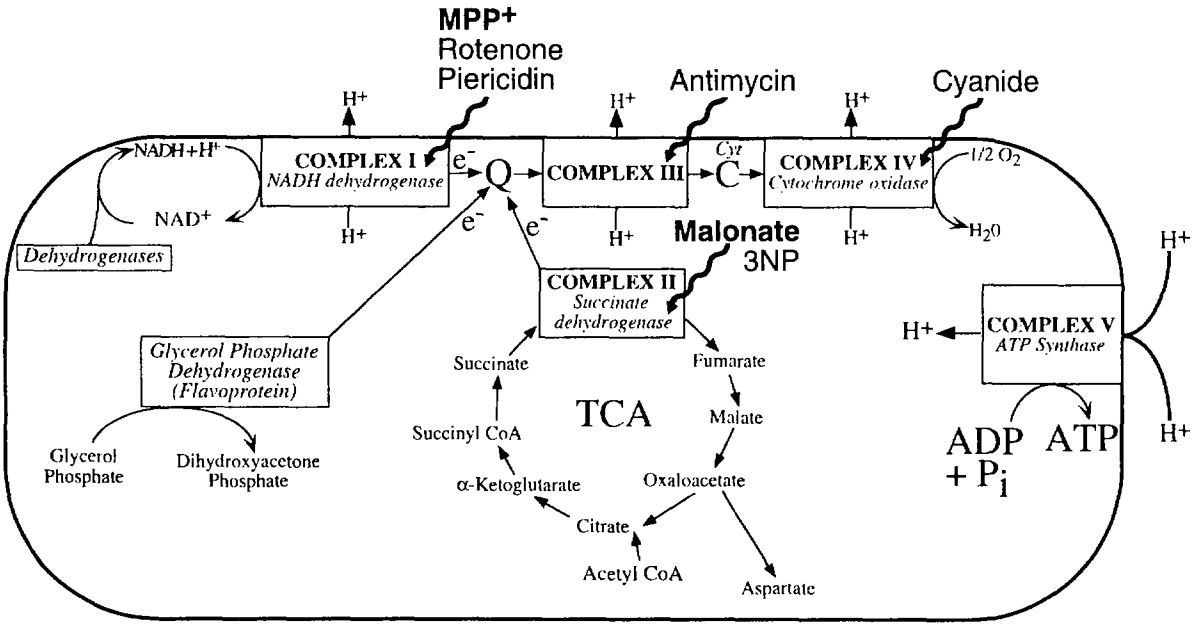

Fig. 2. Schematic representation of the mitochondrial electron transport chain demonstrating the sites at which MPP^+ and malonate exert their toxic actions.

mutations in mtDNA have been proposed to explain the complex I deficit, no consistent evidence of deletions or mutations in brain mtDNA has been provided in PD *(87)*.

Instead, oxidative damage to mtDNA, or to complex I itself, might explain the complex I defect. Using 8-hydroxy-2'-deoxyguanosine as a marker for oxidative damage to human brain DNA, it has been shown that during aging mtDNA accumulates 10-fold more damage than nuclear DNA. Moreover, in subjects over age 70, there is 15-fold more damage in mtDNA *(88)*. Complex I, being the largest and most complicated of the electron-transferring complexes, is highly vulnerable to oxidative damage *(89)*. In addition, partial inhibition of complex I leads to free radical formation that may cause irreversible damage to the enzyme complex *(90)*.

Oxidative Stress in PD Pathophysiology

In recent years, mounting evidence has suggested that oxidative stress may play a central role in the pathophysiology of PD *(37,91)*. The substantia nigra is exposed to a high degree of oxidative stress as a consequence of formation of cytotoxic oxygen radicals. The activities of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis, and monoamine oxidase, which catabolyzes catecholamines, cause the formation of H_2O_2 as a normal byproduct *(37)*. Auto-oxidation of dopamine, which leads to the production of melanin, also yields H_2O_2 *(92)*. H_2O_2, toxic *per se*, slowly decomposes to •OH, the most reactive free radical. This nonenzymatic reaction is accelerated in the presence of iron (particularly when it is in the free, ferrous form, Fe^{2+}), which is abundant physiologically in the pars compacta of substantia nigra. That iron can be toxic has been confirmed by the fact that, in rats, it causes selective damage to nigral neurons when injected locally. Also, the iron chelator desferrioxamine can prevent the toxic effect of 6-hydroxydopamine on nigral dopaminergic neurons *(93,94)*. In fact, abnormally elevated levels of iron have been reported in the substantia nigra of PD patients, but opposite

changes in ferritin, the protein that binds Fe^{2+}, have been found *(95–99)*. Thus, there is disagreement as to whether this increase is ascribable to higher levels of free or ferritin-bound iron. It is noteworthy, however, that similar increases in brain concentrations of iron have been described in other neurodegenerative diseases, such as multiple system atrophy, Huntington's disease, Alzheimer's disease, and progressive sopranuclear palsy *(100–102)*. Thus, the increased iron levels may represent a consequence, rather than the cause of the degenerative process observed in PD.

The tripeptide glutathione and the enzyme superoxide dismutase (SOD), along with other enzymes, such as catalase and glutathione peroxidase, are responsible for scavenging cytotoxic free radicals. Glutathione, in its reduced form (GSH), intervenes as electron donor in the reduction of H_2O_2 to H_2O and O_2 catalyzed by glutathione peroxidase. As a result, it is oxidized to GSSG (oxidized glutathione), then rapidly reduced back to GSH by glutathione reductase. GSH can also scavenge singlet oxygen and •OH nonenzymatically. SOD exists in three forms, a cytosolic copper–zinc associated form (CuZnSOD), a mitochondrial manganese-associated form (MnSOD), and an extracellular form of CuZnSOD. All three SODs reduce the possibility of •OH formation by transforming $•O_2^-$ into H_2O_2 *(37,103,104)*. Decreased levels of total glutathione (GSH + GSSG) and GSH have been reported in the substantia nigra of PD patients *(105–107)*, whereas both mitochondrial and cytosolic SOD were found to be increased *(108,109)*. Since an excess of H_2O_2 can interfere with the reduction of GSSG to GSH, increased production of free radicals may account for the reduction in GSH. The same increase in free radicals may be responsible for the increase of SOD, whose activity is substrate-dependent. In contrast to the changes in iron levels, these alterations appear to be specific to PD. However, whether they represent a preexisting condition or a specific response to the pathological process is still a matter for debate.

Another mechanism that could account for reduced nigral GSH levels is mitochondrial dysfunction. Treatment of hepatic cells *in vitro* with mitochondrial poisons causes a rapid loss of both GSH and GSSG *(110)*. In addition, intracerebral microinjection of the mitochondrial inhibitor, malonate, depletes GSH by 40% within 4 h (Halpern and Greenamyre, unpublished data). Thus, the loss of GSH reported in PD might be secondary to the complex I defect in this disorder.

In summary, it is apparent that there are several potential sources of oxidative stress to dopaminergic neurons. These include a partial complex I defect, byproducts of catecholamine catabolism, an increase in total iron content, and depletion of GSH. Is there any evidence that these mechanisms actually play a role in the pathogenesis of PD? Postmortem studies of PD brains show that in the substantia nigra, there is an increase in the levels of malondialdehyde, a late-stage marker of lipid peroxidation *(164)*. Lipid hydroperoxides, an earlier and more specific marker for lipid peroxidation are also increased by 11-fold in the substantia nigra *(165)*. Thus, postmortem studies support the notion that oxidative damage is central to the neurodegeneration of PD.

Unified Hypothesis of PD Pathogenesis

Seemingly disparate processes, including a mitochondrial (bioenergetic) defect, excitotoxicity, excessive free radical formation, and depletion of GSH have been implicated in the pathogenesis of PD. Despite the different nature of these mechanisms, they are related, and it is possible to envision a scheme in which any one of these processes might lead to all of the others (Fig. 3).

Mitochondrial defects can lead indirectly to NMDA receptor-mediated excitotoxicity, and activation of NMDA receptors can lead to mitochondrial dysfunction. Thus, a feedforward cycle of NMDA receptor activation and mitochondrial dysfunction can be envisaged. In this scheme, the initiating event could be at the level of the mitochondrion, or the NMDA recep-

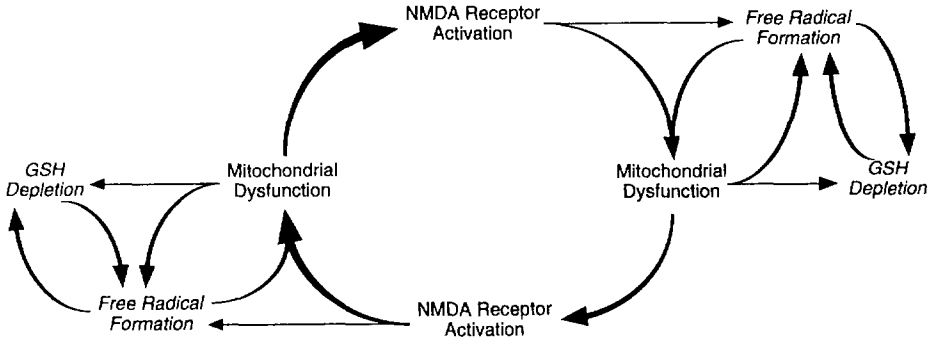

Fig. 3. A unified hypothesis of PD pathogenesis. The central portion of the diagram shows a feed-forward cycle in which NMDA receptor activation causes mitochondrial dysfunction and impairment of mitochondrial function sensitizes to NMDA receptor-mediated toxicity *(see text)*. This cycle of damage could be initiated at the level of the NMDA receptor or the mitochondrion, or both. Both NMDA receptor activation and mitochondrial impairment generate free radicals that, in turn, damage mitochondria and deplete GSH. As shown in the diagram, all of these events could also begin with excessive free radical formation or a defect in GSH homeostasis. Both NMDA receptor activation and mitochondrial dysfunction can cause apoptotic or necrotic neuronal death, depending on the intensity and chronicity of the insult.

tor. Either way, once the cycle is set in motion, an escalating pattern of neuronal damage and death may ensue. A consequence of both NMDA receptor activation and mitochondrial dysfunction is generation of free radicals which, in turn, may lead to depletion of GSH.

It is also possible to suggest that excessive levels of free radicals, possibly secondary to a loss of GSH, could initiate this cascade. Free radicals damage the components of the mitochondrial electron transport chain and cause both decreased GSH and increased free radical production. Impaired mitochondrial function leads to markedly enhanced vulnerability to glutamate excitotoxicity, especially that mediated by NMDA receptors.

Excitotoxicity, mitochondrial impairment, free radical formation, and depletion of GSH are intimately related. An abnormality in any of these systems may activate, through parallel and serial pathways, a self-perpetuating cycle leading to neuronal damage and death (Fig. 3). A critical aspect of this hypothesis is that glutamate levels need not be abnormally elevated for excitotoxic damage to occur. Importantly, however, this scenario suggests that it may be possible to intervene therapeutically by using NMDA receptor antagonists to break this cycle.

Basal Ganglia Circuitry in PD

Degeneration of dopamine neurons in the substantia nigra pars compacta (SNc) causes dopaminergic denervation of the striatum. Striatal dopamine depletion is followed by a cascade of complex alterations in the activity of basal ganglia nuclei, which results ultimately in the development of PD symptomatology. As a neurotransmitter, glutamate plays a pivotal role in basal ganglia circuitry, and it is primarily involved in the PD-related neurochemical alterations.

Functional Anatomy of the Basal Ganglia

The basal ganglia nuclei are currently viewed as components of a complicated circuit that receives, processes, and transmits back to the cortex signals necessary for the proper execution of movements *(65,111)*. The main input nucleus of this circuitry is the striatum, which receives excitatory, glutamatergic projections from virtually all regions of cerebral cortex *(112,113)* and a dense dopaminergic input from SNc *(114)*. Striatal neurons are connected to the

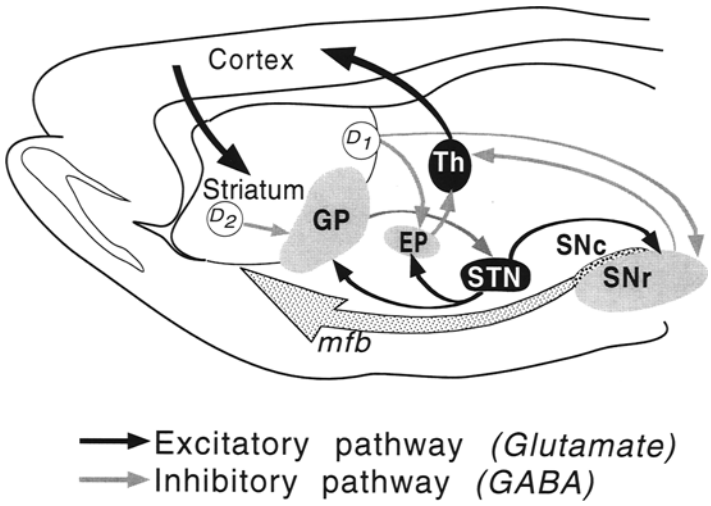

Fig. 4. Highly simplified schematic diagram of rat basal ganglia circuitry. The striatum receives excitatory, glutamatergic projections from virtually all regions of cerebral cortex and a dense dopaminergic input from substantia nigra pars compacta (SNc) via the medial forebrain bundle (mfb). Striatal neurons are connected to the output nuclei of the basal ganglia, the medial segment of globus pallidus (MGP; the rat homolog is entopeduncular nucleus, EP), and the substantia nigra pars reticulata (SNr), by two different pathways: a direct pathway, consisting of direct projections to MGP/EP and SNr, and an indirect pathway, wherein the striatum projects to the lateral segment of the globus pallidus (LGP; the rat homolog is globus pallidus, GP) which, in turn, sends projections to the subthalamic nucleus (STN); STN then projects to MGP/EP and SNr. Both the direct and indirect striatal projections, as well as the pathway from LGP/GP to STN, are GABAergic, therefore inhibitory. The direct pathway is thought to originate from striatal neurons containing GABA and substance P, and expressing predominantly D_1 dopamine receptors, whereas neurons giving rise to the indirect pathway contain GABA and enkephalin and express primarily D_2 receptors. In contrast, the pathways from STN to MGP/EP and SNr are excitatory and glutamatergic. MGP/EP and SNr send GABAergic projections to the ventral anterior and ventral lateral nuclei of the thalamus (Th) which then closes the loop from cortex by sending excitatory (presumably glutamatergic) projections back to the motor cortex.

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Glutamatergic Neurotransmission in the Basal Ganglia

Striatum

The striatum receives dense glutamatergic input from neocortex and thalamus. Although glutamate receptors are found throughout the basal ganglia *(125–127)*, binding studies show that the highest densities are in striatum *(127,128)*. Moreover, it appears that NMDA receptors are selectively enriched in projection neurons *(129)*, which may indicate that the major influence of glutamate on striatal projection neurons is mediated by NMDA receptors. Recent studies also indicate that projection neurons differ from interneurons in terms of the specific NMDAR subunit mRNAs they express *(130)*. Presumably, this leads to distinct physiological and pharmacological properties of NMDA receptors in these populations of cells. Similarly, there is a differential expression of AMPA receptor subunits in projection neurons and interneurons *(131)*. In particular, the GluR1 subunit does not appear to be expressed on projection neurons *(131)*.

Release of glutamate in the striatum seems to be modulated, in part, by nigrostriatal dopaminergic projections, although the molecular and anatomical substrates of this interaction are uncertain. Chronic blockade of D_2 dopamine receptors causes an increase in the levels of both basal extracellular and potassium-releasable glutamate in striatum *(132)*. There is also electrophysiological evidence that striatal dopamine depletion leads to increased spontaneous glutamate release in striatum *(133)*. In addition, excitatory amino acids stimulate striatal acetylcholine release through NMDA, but not AMPA, receptors *(134)*.

Output Nuclei

As shown by anatomical, biochemical, and electrophysiological studies, a fundamental component of basal ganglia circuitry, the STN, is glutamatergic *(135–137)*. Subthalamic neurons show selective glutamate-like immunoreactivity, but do not stain for other transmitters. Also, the nonselective glutamate receptor antagonist kynurenic acid blocks the postsynaptic responses elicited in the SNr by STN stimulation *(137)*. As mentioned previously, STN activity plays a major role in modulating basal ganglia ouput. Electrical stimulation of STN, which mimics overactivity of this nucleus, induces metabolic activation in SNr as well as GP and EP *(138)*. Conversely, STN ablation reduces oxidative metabolism in the same nuclei *(139,140)*.

The pharmacology of glutamatergic synapses in the basal ganglia output nuclei has not been fully characterized. In rodents, AMPA receptors have a higher relative density than NMDA receptors in STN, SNr, GP, and EP *(127)*. Thus, based on receptor densities, it seems that AMPA receptors may have a larger role than NMDA receptors in motor-related synaptic transmission in MGP/EP and SNr. This is further supported by the fact that intrapallidal infusion of the AMPA/kainate receptor antagonist NBQX reduces the firing rate of GP neurons significantly, whereas no effect is observed when the NMDA antagonist MK-801 is injected *(141)*. The cellular localization of AMPA and NMDA receptor subunits in the basal ganglia output nuclei has not yet been defined.

To summarize, the striatum receives a massive glutamatergic input and, at least in rodents, NMDA receptors may be the predominant glutamate receptor in terms of motor-related activity. Downstream, postsynaptic to the STN, it appears that AMPA receptors play a more important role than NMDA receptors.

Glutamatergic Neurotransmission in PD

Dopaminergic denervation of the striatum, reproduced in animal models of PD, causes complex changes in the functional circuitry of the basal ganglia (Fig. 5). Striatal GABAergic neurons projecting directly to MGP/EP and SNr become underactive, thus causing a disin-

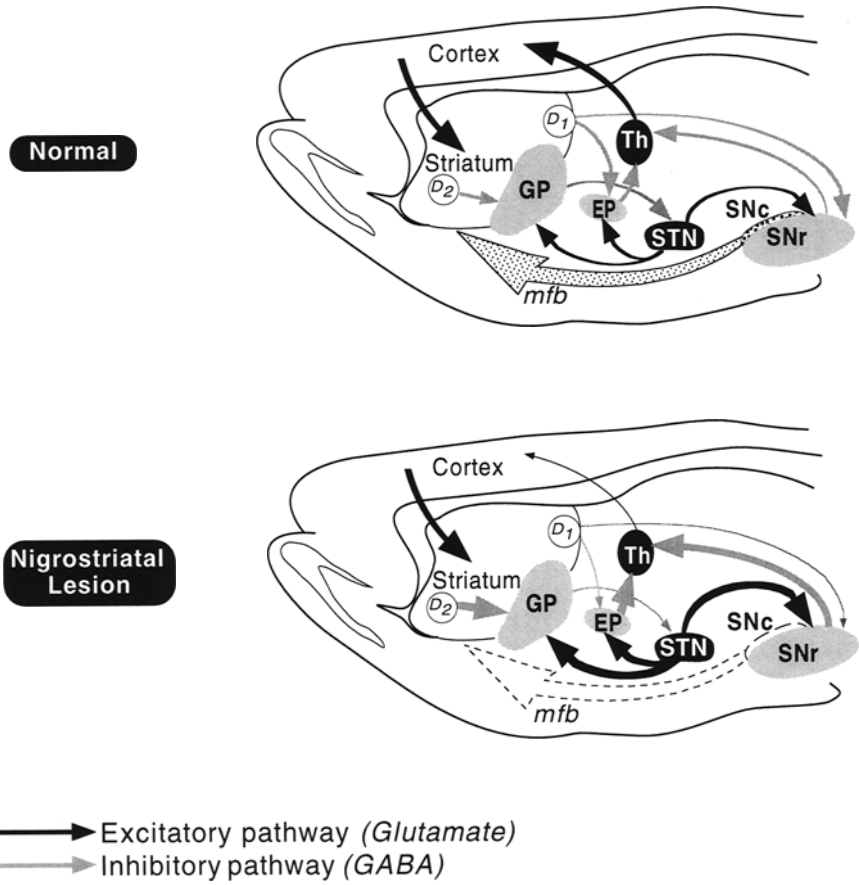

Fig. 5. Changes in basal ganglia circuitry after nigrostriatal dopamine depletion. **(A)** Normal functional anatomy. **(B)** After nigrostriatal denervation, striatal GABAergic neurons projecting directly to MGP/EP and SNr become underactive, thus causing a disinhibition of these GABAergic nuclei. In addition, striatal GABAergic neurons projecting to LGP/GP become overactive. Therefore, the firing rate of the LGP/GP GABAergic projection to STN decreases, leading to overactivity of STN which, via its glutamatergic projections, overstimulates MGP/EP and SNr. Thus, in the setting of nigrostriatal dopamine depletion, the activity of the GABAergic output nuclei is increased by two simultaneous, but distinct mechanisms: reduced GABAergic inhibition from the striatum and increased excitatory input from the STN. As a result of the overactivity of the GABAergic projections from MGP/EP and SNr, neurons of the ventrolateral thalamus (Th) that project to cortex become underactive. This reduction in the outflow from the motor thalamus to the motor cortex is believed to underlie many of the clinical manifestations of PD.

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Based on this scheme, it can be anticipated that there might be regulatory changes in basal ganglia glutamate receptors. As noted above, after striatal dopamine depletion there is electrophysiological evidence for increased spontaneous release of glutamate in striatum. Further evidence for enhanced glutamatergic activity under conditions of reduced dopaminergic tone comes from studies by Yamamoto and colleagues, that have shown that chronic D_2 receptor blockade or dopaminergic denervation causes elevated extracellular levels of basal and potassium-releasable glutamate in striatum *(132* and personal communication). In a rodent model of unilateral DA denervation, there is a downregulation of striatal NMDA receptors, but not AMPA receptors *(142)*. These results complement the work of Klockgether and Turski, which showed that microinjection of NMDA, but not AMPA, into the anterior striatum produces signs of parkinsonism in rats *(143)*.

Downstream from the striatum, overactivity of the excitatory glutamatergic projections from STN to the basal ganglia output nuclei appears to play a major role in the pathophysiology of parkinsonian motor signs *(144–148)*. Using a rodent model, it has been shown that striatal dopaminergic denervation also leads to metabolic activation of the basal ganglia output nuclei, presumably because of increased excitatory input from STN *(142)*. Consistent with this interpretation, selective lesions of STN reduce metabolic activity in these nuclei *(139,140)*. Moreover, in dopamine-depleted rats, STN lesions normalize metabolic activity in the basal ganglia output nuclei (Blandini and Greenamyre, unpublished results). In the rodent model of unilateral DA depletion, there is a selective downregulation of AMPA receptors, but not NMDA receptors, in the basal ganglia output nuclei, consistent with the hypothesized importance of AMPA receptors in these regions. These results are also consonant with the work of Klockgether and Turski *(143)* showing that microinjection of AMPA, but not NMDA, into the basal ganglia output nuclei induces parkinsonism in rats.

In conclusion, it appears that AMPA and NMDA receptor systems in the basal ganglia regulate differently in response to dopaminergic denervation of the striatum. Whether there is the same relationship between striatal NMDA receptors and output nuclei AMPA receptors in the primate brain remains to be investigated. The molecular basis of this glutamate receptor regulation is currently being studied.

Therapeutic Potential of Glutamate Receptor Antagonists in PD

Neuroprotection

As noted above, NMDA antagonists can prevent neuronal death induced by several distinct mechanisms that are thought to be relevant to the pathogenesis of PD. NMDA antagonists protect the substantia nigra from the bioenergetic and oxidative stress of MPP^+ toxicity *(149)* and provide almost complete protection to the nigrostriatal pathway against the oxidative damage caused by metamphetamine *(150)*. Furthermore, the noncompetitive NMDA antagonist MK-801, the competitive antagonist LY274614, and the glycine site antagonist 7-chlorokynurenate, but not the AMPA antagonist NBQX, block the toxicity of a mitochondrial poison in vivo *(151)*. In addition, the depletion of GSH that is induced by mitochondrial dysfunction is attenuated by systemic administration of an NMDA antagonist (Halpern and Greenamyre, unpublished data). Thus, NMDA antagonists might act as neuroprotective agents by retarding the damage and death of dopaminergic neurons, and thereby slowing disease progression. In this regard, the recent report by Rajput and colleagues *(152)* that the weak NMDA channel blocker, amantadine, is associated with improved survival is particularly intriguing. This study needs to be replicated, and the potential mechanisms for this effect need to be better defined. Yet, such preliminary results raise the hope that long-term use of well-tolerated glutamate receptor antagonists may provide neuroprotection and retard disease progression.

Symptomatic Treatment

The regulatory changes in basal ganglia glutamate neurotransmission that occur in models of PD raise the possibility that manipulation of the glutamate system may have antiparkinsonian effects. For example, the central importance of the glutamatergic STN was demonstrated in parkinsonian monkeys when it was shown that STN ablation ameliorated signs of parkinsonism *(148)*. Furthermore, Benabid and colleagues have recently demonstrated that high-frequency electrical stimulation of STN, by means of electrodes implanted directly in the STN, induces dramatic amelioration of symptomatology in PD patients, presumably by interrupting normal STN output *(153)*.

It is also possible to prevent the functional consequences of STN overactivity by antagonizing glutamatergic neurotransmission pharmacologically. In parkinsonian monkeys, stereotactic administration of the nonselective glutamate antagonist, kynurenate, into the MGP reverses parkinsonian signs in a reversible, dose-dependent fashion *(154)*. Similarly, stereotactic microinjections of glutamate antagonists into the EP (the rodent homolog of MGP), SNr, or STN have shown antiparkinsonian effects in reserpinized, dopamine-depleted rats; such an effect was not observed when the same drugs were injected into the striatum *(155,156)*. In contrast, using reserpinized mice, Carlson and her colleagues have consistently demonstrated that intrastriatal injection of NMDA antagonists reverses reserpine-induced akinesia *(157)*. Similarly, Schimidt and coworkers have shown that intrastriatal administration of NMDA antagonists reduces haloperidol-induced catalepsy *(158)*. The origin of such discrepancy is not clear, although it may be dependent on the specific model employed.

Systemic aministration of the non-NMDA antagonist, NBQX, or the competitive NMDA antagonist, CPP, especially when coadministered with threshold doses of L-DOPA, has proven able to ameliorate parkinsonian symptoms in animal models of PD *(155,159)*. Similarly, the clinical response to coadministration of L-DOPA and remacemide, a noncompetitive NMDA receptor blocker with anticonvulsant properties, has been shown to be better than L-DOPA alone *(160)*. Finally, it is noteworthy that three drugs currently used in clinical practice for PD, amantadine, memantine, and budipine, have recently been recognized as NMDA receptor antagonists *(23,162–164)*.

Conclusions

Glutamate plays a pivotal role in the pathophysiology of PD. As a neurotransmitter, it is involved in the functional modifications that affect basal ganglia circuitry in response to dopaminergic denervation of the striatum. Glutamatergic pathways into the striatum and from the STN to the basal ganglia output nuclei become overactive. As a result, there are regulatory changes in both AMPA and NMDA receptors in specific loci in this circuit. Several lines of evidence indicate that this altered glutamatergic neurotransmission plays a central role in the motor symptoms of PD. As a neurotoxin, glutamate may intervene indirectly in the pathogenesis of PD. Dopaminergic neurons of the SNc are normally under considerable metabolic and oxidative stress. The mitochondrial dysfunction and loss of GSH that are characteristic of PD may render nigral neurons particularly susceptible to the excitotoxic effects of glutamate. An understanding of the role of glutamate, both as neurotransmitter and neurotoxin, in the pathophysiology of PD may lead to the development of novel therapeutic approaches to PD.

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