Dopaminergic Neurons Reduced to Silence by Oxidative Stress: An Early Step in the Death Cascade in Parkinson's Disease?

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Parkinson's disease (PD) is a frequent neurodegenerative disorder primarily of sporadic origin, but a small proportion of cases are inherited (*1*, *2*). The most important pathological feature of the disease is the death of brainstem dopaminergic (DA) neurons in the substantia nigra (SN) (*3*). The loss of these neurons leads to a deficit in striatal dopamine, which is responsible for the motor symptoms characteristic of the disease. The etiology of PD remains unknown, but the molecular mechanisms that underlie the pathology are beginning to be understood. In particular, cellular perturbations resulting from mitochondrial dysfunction (*4*, *5*) and increased oxidative stress (*5*–*7*) appear to play a crucial role in the demise of DA neurons.

Several lines of evidence suggest that mitochondria are key effectors of DA cell death in PD: (i) Mitochondrial complex I activity is reduced in autopsy brains and in platelets of patients with PD (*4*). (ii) The complex I inhibitor 1-methyl-4-phenylpyridinium (MPP^+) selectively kills DA neurons in the SN in mice and nonhuman primates after systemic administration of its precursor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (*5*). (iii) Accidental self-administration of MPTP in humans leads to a neurodegenerative condition that is almost indistinguishable from PD (*8*). (iv) Other complex I inhibitors, such as the plant toxins rotenone and annonacin, are also toxic to SN DA neurons (*9*–*11*). The crucial role of oxidative stress in neuronal death in PD is suggested primarily by data showing that the neurons that are less vulnerable to PD are those that are better equipped to combat reactive oxygen species (ROS)–mediated insults (*6*, *12*). Adding support to this hypothesis, transgenic mice in which the activity of Cu/Zn-superoxide dismutase (SOD), a superoxide-scavenging enzyme, is increased are resistant to MPTP (*5*), whereas mice without this enzyme or glutathione peroxidase, a scavenger of H_2O_2, are more susceptible to the toxin (*13*). Furthermore, the *DJ-1* gene, which encodes a protein that protects against the deleterious effects of oxidative stress, particularly in mitochondria, is mutated in rare forms of familial PD (*2*).

The mechanisms that link mitochondrial dysfunction to oxidative stress and neuronal death have not yet been totally elucidated. Different candidate mechanisms have been proposed. In particular, it has been suggested that MPTP-induced neurodegeneration may involve the release of cytochrome c from dysfunctional mitochondria to the cytoplasm by a ROS-driven mechanism involving the proapoptotic protein Bax (*14*). Accordingly, Bax ablation prevents DA cell degeneration in MPTP-intoxicated mice (*15*). Moreover, it is believed that a reduction in the activity of the proteasome, a proteolytic enzyme complex reported to be dysfunctional in certain forms of inherited PD (*2*), may increase the susceptibility of DA neurons to low-level oxidative stress produced by partial inhibition of complex I activity (*16*). A study that implicates K_{ATP} channels as a key target of mitochondrial dysfunction and oxidative stress (*17*) provides an important piece of the puzzle.

Adenosine triphosphate–sensitive K (K_{ATP}) channels are multimeric proteins composed of inwardly rectifying pore-forming subunits (Kir6.2 in brain neurons) and regulatory, sulfonylurea receptor subunits (*18*). K_{ATP} channels open in response to ATP depletion. This causes K^+ efflux and membrane hyperpolarization, which lowers the electrical activity of the cell and energy consumption, thereby linking metabolic state to excitability (*19*, *20*). K_{ATP} channels play a key role in the physiological function of DA neurons because they control dopamine release in the striatum (*21*). They may also be essential for their survival because ROS, which are involved in the pathophysiology of PD, also operate as physiological activators of K_{ATP} channels in DA neurons (*21*).

The demonstration that K_{ATP} channels are instrumental in DA cell death caused by impairment of mitochondrial function comes from a key study showing that genetic invalidation of the channel pore-forming subunit Kir6.2 protects these neurons from chronic, low-dose administration of MPTP (*17*). The invalidation of K_{ATP} channels not only protected SN DA cell bodies but also their striatal terminals, which is of interest if we consider that restoring dopamine release remains the main objective of a neuroprotective treatment in PD. This result might seem paradoxical, however, with regard to another study showing that hippocampal neurons in *Kir6.2*$^{-/-}$ mice are more vulnerable to acute ischemic attacks (*22*), but the effects of K_{ATP} channel activation might be diametrically opposed, depending on the duration and severity of the metabolic insult.

Patch-clamp recordings established that complex I inhibition with MPP^+ or rotenone activates K_{ATP} channels in SN DA neurons from control mice, making these neurons progressively hyperpolarized and functionally silent. At variance, SN DA neurons from *Kir6.2*$^{-/-}$ mice remain normally active in the presence of these inhibitors (*17*), leading to the conclusion that the activity of these neurons may also control their survival. Accordingly, the concentration-dependent increase in K_{ATP} channel conductance produced by MPP^+ in control SN DA neurons (*17*) was almost superimposable on its ability to kill selectively these neurons in mesencephalic cultures (*23*). This observation is consistent with previous findings showing that selective DA cell death can be prevented by inducing a moderate influx of calcium through the activation of specific subtypes of voltage-gated channels in another model system of mesencephalic cultures (*24*–*26*). Interestingly, a SOD mimetic prevented K_{ATP} chan-

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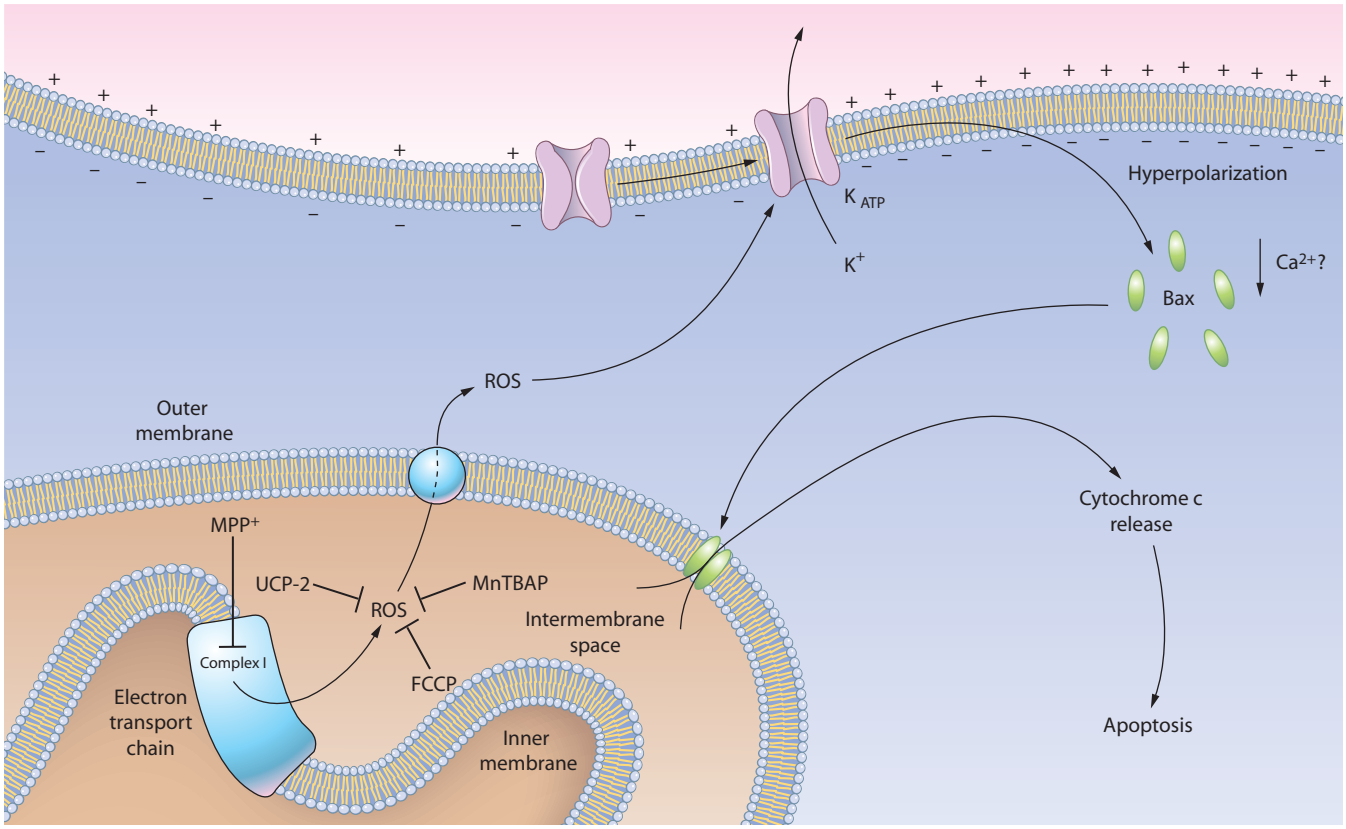
nel–mediated hyperpolarization produced by complex I inhibition (*17*) in agreement with studies showing that mitochondrial ROS operate as activators of K_{ATP} channels in DA neurons when ATP levels are not affected or are only moderately affected (*21*).

This finding also indicates that ROS, and not a drop in ATP concentrations, are the initial death signal for DA neurons (*17*). We can only speculate on the nature of the downstream events that link K_{ATP} channel opening to neuronal death. The data from Liss and colleagues (*17*) and other studies (*24*–*26*) point to the crucial need for DA neurons to maintain a certain level of electrical activity to survive. Putcha and colleagues (*27*) suggest that an increase in excitability may serve to prevent the relocation of the proapoptotic molecule Bax from the cytosol to the mitochondria. One may therefore speculate that hyperpolarization due to K_{ATP} channel activation may facilitate Bax recruitment to mitochondria, thereby activating the apoptotic pathway after complex I inhibition (*14*, *28*).



DA neurons	MPP+	UCP-2 abundance	ROS concentration	K_{ATP} activity	Electrical activity	Neuronal survival
SN	+	+	+++	+++	-	-
VTA	+	++	+	+/-	++	++

Fig. 1. Schematic representation of the molecular mechanisms that may underlie the selective vulnerability of SN DA neurons to mitochondrial dysfunction and oxidative stress. Mitochondrial complex I inhibition by MPP^+ results in K_{ATP} channel activation, leading to chronic hyperpolarization and subsequently to the death of SN DA neurons (*17*). Lowering oxidative stress with the SOD mimetic Mn(III)tetrakis(4-benzoic acid)porphrin chloride (MnTBAP) or by mild mitochondrial uncoupling with low concentrations of carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP) prevents neuronal hyperpolarization, which indicates that ROS produced as a consequence of complex I inhibition activate K_{ATP} channels. Bax is a putative target of the death signal produced by hyperpolarization (*14*, *27*), possibly via a mechanism that involves a reduction in intracellular calcium levels (*25*, *27*). Once activated, Bax translocates to the mitochondria where it triggers the release of cytochrome c into the cytoplasm, leading to neuronal death by apoptosis (*14*). Note that mitochondrial ROS, which activate K_{ATP} channels, may also serve to increase the pool of cytochrome c that is releasable from the mitochondrial intermembrane space (*14*). The invalidation of Kir6.2, the pore-forming unit of K_{ATP} channels in DA neurons, confers resistance to complex I inhibition because ROS no longer induce hyperpolarization (*17*). VTA DA neurons are probably intrinsically resistant to complex I inhibition because of a mild constitutive uncoupling of the mitochondria in these neurons, leading to reduced ROS production and less activation of K_{ATP} channels. The uncoupling protein UCP-2 may play a key role in this effect (*30*). Note that ROS, but not ATP, are the active regulators of K_{ATP} channels in the proposed model because the metabolic insult caused by complex I inhibition is moderate (*17*).

A key characteristic of PD, which is also observed in mice or monkeys treated with MPTP and in humans after accidental contact with the toxin, is the greater vulnerability of SN DA neurons relative to neighboring ventral tegmental area (VTA) DA neurons (*6*, *8*, *29*). Patch-clamp recordings demonstrate that the concentrations of complex I inhibitors that cause SN DA neurons to become functionally silent do not affect the activity of VTA DA neurons, which suggests that different electrophysiological responses may account for the differential vulnerability of these two populations of neurons. Single-cell reverse transcription polymerase chain reaction (RT-PCR) analysis rules out, however, that the observed differences result from the presence of K_{ATP} channels with distinct subunit composition (*17*). Instead, VTA DA neurons may have a lower sensitivity for K_{ATP} channel activation because of an intrinsic capacity to buffer mitochondrial ROS due to partial constitutive uncoupling of their respiratory chain (*17*, *30*). In support of this view, transcripts encoding the brain uncoupling protein–2 (UCP-2) are more abundant in VTA than in SN DA neurons, and mild pharmacological uncoupling can totally restore the activity of SN DA neurons in midbrain slices during complex I inhibition, similar to what is observed with a SOD mimetic (*17*). Furthermore, transgenic mice that overexpressed UCP-2 were less sensitive to MPTP and produced less ROS in vivo relative to wild-type littermates, whereas the inverse was true in *UCP-2* knockout mice (*30*). Finally, one may assume that the consequence of mild constitutive uncoupling on K_{ATP} channel activation in VTA DA neurons may be reinforced by the fact that these neurons also possess, intrinsically and in their proximal environment, a better antioxidant system to handle oxidative stress (*6*, *12*). In particular, the highest density of glial cells that express glutathione peroxidase is found in the VTA (*6*), and basal levels of glutathione peroxiduse and catalase activities are more elevated in this nucleus than in the SN (*12*). A schematic representation of the mechanisms that may be responsible for the selective vulnerability of SN DA neurons to mitochondrial dysfunction and oxidative stress is given in Fig. 1.

The possibility that ROS-mediated activation of K_{ATP} channels (and subsequent reduction in electrical activity and excitability) participates in DA cell loss in PD is attractive. The expression of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine, is induced by electrical activity (*31*) but is markedly decreased in a population of morphologically intact DA neurons that are prone to degenerate in the brain of PD subjects (*32*), which suggests that these neurons might become progressively hyperpolarized before they die. These neurons are probably under intense oxidative stress for two reasons: (i) They all contain neuromelanin (*32*), a pigment produced by nonenzymatic oxidation of cytosolic (nonvesicular) dopamine through a process that generates highly reactive free radicals (*33*, *34*); and (ii) neuromelanin has the capacity to store and release large amounts of iron (*35*), a transition metal that is increased in the SN of PD patients (*6*) and serves as a catalyst for hydroxyl radical production (*7*). This suggests that ROS generated as a direct or indirect consequence of dopamine catabolism may also contribute to the activation of K_{ATP} channels in the diseased neurons. Finally, the hypothesis that DA neurons may become progressively hyperpolarized in the course of their demise is evocative of the protective effects of the depolarizing agent nicotine in the MPTP model of PD (*36*) and may account for the fact that cigarette smokers present a lower risk of developing the disease (*37*).

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