Journal of Bioenergetics and Biomembranes, Vol. 36, No. 4, August 2004 (© 2004)

MPTP as a Mitochondrial Neurotoxic Model of Parkinson's Disease

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Received March 24, 2004; accepted May 7, 2004

1-Methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent neurotoxin extensively used to model Parkinson's disease (PD). A cascade of deleterous events, in which mitochondria play a pivotal role, drives MPTP neurotoxicity. How mitochondria are affected by MPTP and how their defect contributes to the demise of dopaminergic neurons in this model of PD are discussed in this review.

KEY WORDS: MPTP; Parkinson's disease; neurodegeneration; mitochondria; oxidative stress; ATP depletion; programmed cell death.

1-Methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a by-product of the chemical synthesis of a meperidine analog with potent heroin-like effects (Ziering *et al*., 1947). MPTP can induce a parkinsonian syndrome in humans and nonhuman primates almost indistinguishable from Parkinson's disease (PD) on both clinical and neuropathological standpoints (Langston and Irwin, 1986). Over the years, MPTP has been used in a host of different animal species, especially in mice (Heiklla *et al*., 1989), to recapitulate the hallmark of PD cellular pathology, namely the degeneration of the nigrostriatal dopaminergic pathway (Dauer and Przedborski, 2003). Although the MPTP model departs from PD on several significant aspects, it continues to be regarded as the best experimental model of this common neurodegenerative disease. With respect to PD, enthusiasm for the MPTP model is driven by the belief that unraveling the MPTP neurotoxic process in animals may provide hints into the mechanisms responsible for the demise of dopaminergic neurons in human PD.

Various key cellular and molecular components underlying the MPTP neurotoxic process have been reviewed in details in the following references (Dauer and Przedborski, 2003; Przedborski and Vila, 2003) and will thus not be discussed here. Instead, the focus of this minireview will be devoted to the role of the mitochondria in the deleterious effects of the parkinsonian toxin MPTP.

FIRST STEP FIRST

MPTP is a protoxin whose toxicokinetics is a complex, multistep process (Dauer and Przedborski, 2003). As indicated by its octanol/water partition coefficient of 15.6 (Riachi *et al*., 1989), MPTP is a highly lipophilic molecule, which is able to readily permeate lipid bilayer membranes. It is therefore not surprising to observe that MPTP crosses the blood-brain barrier in a matter of seconds after its systemic administration (Markey *et al*., 1984). Once in the brain, it is rapidly converted into 1-methyl-4-phenylpyridinium (MPP^+), the actual neurotoxin (Heikkila *et al*., 1984). This critical transformation of MPTP into MPP^+ is a two-step process. First, MPTP undergoes a two-electron oxidation, catalyzed by monoamine oxidase B (MAO-B), yielding the intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) (Chiba *et al*., 1984). Given the discrete cellular distribution of MAO-B in the brain (Kitahama *et al*., 1991), it is believed that the conversion of MPTP to $MPDP^+$ occurs specifically in glial and serotonergic cells, and not in dopaminergic neurons. $MPDP^+$ is an unstable molecule which readily undergoes spontaneous

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disproportionation to MPP^+ and MPTP (Chiba *et al*., 1985; Peterson *et al*., 1985).

Once formed, MPP^+ is presumably released from glial and serotonergic cells into the extracellular space prior to entering dopaminergic neurons. Yet, MPP^+ has an octanol/water partition coefficient of 0.09 (Riachi *et al*., 1989), which indicates that, while being a lipophilic cation, MPP^+ is far less lipophilic than MPTP. Thus, unlike MPTP, MPP^+ is most likely unable to easily diffuse across cellular lipid bilayer membranes. Instead, it is to be expected that the release of MPP^+ from its intracellular sites of formation and entry into adjacent neurons depend on specialized carriers. Consistent with this view is the fact that MPP^+ access to dopaminergic neurons relies on the plasma membrane dopamine transporter (Bezard *et al*., 1999; Javitch *et al*., 1985).

MITOCHONDRIAL ACCUMULATION

Once inside neurons, MPP^+ rapidly accumulates in the mitochondrial matrix (Ramsay *et al*., 1986; Ramsay and Singer, 1986). Initially, it was thought that MPP^+ gains access to the mitochondrial matrix through a carrier (Ramsay *et al*., 1986; Ramsay and Singer, 1986). However, it is now well established that MPP^+ is passively transported (Davey *et al*., 1992; Hoppel *et al*., 1987) by a mechanism relying entirely upon the large mitochondrial transmembrane potential gradient ($\Delta\psi$) of -150 to -170 mV (Aiuchi *et al*., 1988; Davey *et al*., 1992; Hoppel *et al*., 1987; Ramsay *et al*., 1986; Ramsay and Singer, 1986).

Like with other lipophilic cations (Rottenberg, 1984), the higher the concentrations of intramitochondrial MPP^+, the lower the $\Delta\psi$ and, consequently, the slower the uptake of extramitochondrial MPP^+ (Davey *et al*., 1992; Hoppel *et al*., 1987). The demonstration that the ion-pairing agent tetraphenylboron anion increases both the rate and the extent of MPP^+ uptake in isolated mitochondria (Aiuchi *et al*., 1988; Davey *et al*., 1992; Hoppel *et al*., 1987) further supports this concept. As discussed below, MPP^+ inhibits mitochondrial respiration, which likely also contributes to the loss of the $\Delta\psi$ gradient and to the dampening of the mitochondrial uptake of MPP^+. It is thus not surprising that the accumulation of MPP^+ by energized mitochondria behaves as a saturable phenomenon in the presence of high extramitochondrial concentrations of MPP^+ (e.g., >10 mM) (Ramsay and Singer, 1986) and appears to reach a steady state after a few minutes (Davey *et al*., 1992; Ramsay *et al*., 1986). This apparent steady state persists until mitochondrial suspension becomes anaerobic or $\Delta\psi$ is collapsed by the addition of an uncoupler agent such as carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (Ramsay *et al*., 1986). Remarkably, energized mitochondria incubated with 0.5 mM MPP^+ reach matrix concentrations of more than 24 mM after only 10 min (Ramsay and Singer, 1986). This fast and avid uptake suggests that most, if not all, of the cytosolic MPP^+ would eventually accumulate in the mitochondrial matrix after the systemic injection of MPTP.

INTRAMITOCHONDRIAL MPP^+

It is well established that intramitochondrial MPP^+ inhibits oxidative phosphorylation (Nicklas *et al*., 1985; Singer *et al*., 1987). Intramitochondrial MPP^+ also appears to inhibit the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase (Mizuno *et al*., 1987a). Although both mitochondrial metabolic alterations may contribute to MPP^+ cytotoxicity, attention has been paid almost exclusively to the action of MPP^+ on the respiratory chain.

It is well documented that MPP^+ impairs, in a dose- and time-dependent manner, the ADP-stimulated oxygen consumption (State 3) in intact mitochondria supported by the NADH-linked substrates glutamate and malate (Mizuno *et al*., 1987b; Nicklas *et al*., 1985). MPP^+ is, however, ineffective in inhibiting the oxygen consumption in mitochondria supported by succinate (Mizuno *et al*., 1987b; Nicklas *et al*., 1985). Furthermore, MPP^+ prevents the binding of the classical Complex I inhibitor [^{14}C]-rotenone to electron transport particles (Ramsay *et al*., 1991a). Collectively these findings indicate that MPP^+, like rotenone and piericidin A, impairs mitochondrial respiration by inhibiting the multi-subunit enzyme Complex I (i.e., NADH-ubiquinone oxidoreductase) of the mitochondrial electron transport chain. This straightforward interpretation is supported by the electron spin resonance demonstration that MPP^+ does actually bind to Complex I and blocks the terminal step of electron transfer from the highest potential iron–sulfur cluster of Complex I called N2 to ubiquinone (Ramsay *et al*., 1987).

The use of several MPP^+ analogs and cationic inhibitors has demonstrated that MPP^+ binds at two distinct sites within the mitochondrial electron transport chain region comprised between N2 and ubiquinone (Gluck *et al*., 1994; Miyoshi *et al*., 1997, 1998; Ramsay *et al*., 1989, 1991b; Ramsay and Singer, 1992). These studies have also demonstrated that the occupation of both sites appears to be required for complete inhibition of NADH oxidation. The binding of MPP^+ to the first, more *hydrophilic* site seems to primarily affect the functional coupling between the PSST and the ND1 subunit of Complex I and to account

for only 40% of the MPP^+-induced reduction in NADH oxidation (Schuler and Casida, 2001). The binding of MPP^+ to the second, more *hydrophobic* site seems quite potent in blocking Complex I enzymatic activity (Schuler and Casida, 2001). Yet, the exact location of this second binding site in Complex I remains to be determined. Nonetheless, the importance of the binding to PSST, but not to the ND1 subunit in the inhibition of Complex I-mediated NADH oxidation (Schuler *et al*., 1999; Schule and Casida, 2001), suggests that the MPP^+ *hydrophobic* site must also be situated somewhere in the PSST subunit. This *hydrophobic* site appears not to exist for other typical Complex I inhibitors such as rotenone and piericidin A (Schuler and Casida, 2001). Accordingly, while MPP^+ binds to Complex I, as do rotenone and piericidin A (Gluck *et al*., 1994; Krueger *et al*., 1993), it may not bind to exactly the same Complex I subunit or subunit part as these two other Complex I inhibitors. Also worth noting is the fact that MPP^+, compared to rotenone and piericidin A, is a far weaker inhibitor of Complex I, which may explain why millimolar concentrations of MPP^+ are needed to inhibit NADH-oxidation in electron transport particles (Hoppel *et al*., 1987).

CONSEQUENCES OF MPP^+-INDUCED COMPLEX I INHIBITION

In response to MPP^+ binding to Complex I, the flow of electrons along the respiratory chain is hampered in both dose- and time-dependent manners (Hasegawa *et al*., 1990; Nicklas *et al*., 1985; Vyas *et al*., 1986). The importance of the inhibition of Complex I in the MPTP-induced neurotoxicity in vivo is supported by the demonstration that strategies aimed at stimulating oxidative phosphorylation via by-passing the blockade of Complex I not only improve mitochondrial respiration but also mitigate dopaminergic neurodegeneration in mice (Tieu *et al*., 2003).

The current hypothesis on MPTP cytotoxicity posits that one of the main contributors to cell death is the impaired synthesis of ATP resulting from the inhibition of Complex I by MPP^+. Relevant to this view is the fact that MPP^+ indeed causes a rapid and profound depletion of cellular ATP levels in isolated hepatocytes (Di Monte *et al*., 1986), in brain synaptosomal preparations (Scotcher *et al*., 1990), and in whole mouse brain tissues (Chan *et al*., 1991). It appears, however, that Complex I activity should be reduced by more that 50% to cause significant ATP depletion in nonsynaptic brain mitochondria (Davey and Clark, 1996). Furthermore, in vivo MPTP causes only a transient 20% reduction in mouse striatal and midbrain ATP levels (Chan *et al*., 1991). These facts argue against MPP^+-related ATP deficits being the sole factor underlying MPTP-induced cell death.

Another consequence of Complex I inhibition by MPP^+ is an increased production of reactive oxygen species (ROS). It was shown that incubation of MPTP with brain mitochondria resulted in an oxygen-dependent formation of ROS (Rossetti *et al*., 1988). It was also shown that incubation of MPP^+ with bovine heart submitochondrial particles causes a production of superoxide radicals when MPP^+ is used at the concentrations expected to be found inside neurons after MPTP systemic administration (Hasegawa *et al*., 1990). In this study, the authors also demonstrate that the degree of Complex I inhibition is proportional to the amount of superoxide radical produced (Hasegawa *et al*., 1990). Because modulations of key mitochondrial ROS scavengers, such as manganese superoxide dismutase, affect MPTP-induced neurotoxicity in mice (Andreassen *et al*., 2001; Klivenyi *et al*., 1998), it is reasonable to assert that MPP^+-related ROS production also contributes to MPTP-induced cell death.

CONCLUSION

As discussed above, ATP depletion and ROS overproduction appear to occur soon after MPTP injection, subjecting the intoxicated cells, early on, to an energy crisis and oxidative stress. However, the time course of these perturbations reviewed in the following reference (Przedborski and Vila, 2003) appears to correlate poorly with the time course of neuronal death in vivo (Jackson-Lewis *et al*., 1995). What this meta-analysis is suggesting is that only a few neurons are probably succumbing to the early combined effects of ATP depletion and ROS overproduction. Instead, mounting evidence discussed in the following references (Dauer and Przedborski, 2003; Przedborski and Vila, 2003) indicates that rather than killing the cells, alterations in ATP synthesis and ROS production are pivotal in triggering cell-death-related molecular pathways which, once activated, rapidly lead to the demise of the intoxicated neurons.

Interestingly enough, among these latter molecular pathways, it appears that the mitochondrial-dependent programmed cell death machinery plays a critical role (Vila *et al*., 2001). As illustrated in Fig. 1, it is thus plausible that the death of neurons caused by MPTP results from a circular cascade of deleterious events starting at the mitochondria by the alteration of the oxidative phosphorylation and finishing also at the mitochondria by the activation of the programmed cell death machinery. Whether the whole circuit depicted above is entirely orchestrated

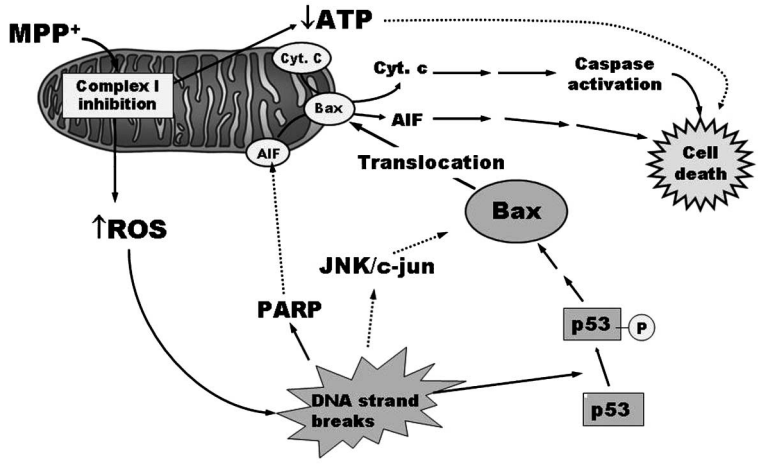

Fig. 1. Illustration *of the proposed circular nature of the MPP$^+$-mediated cell death cascade* MPP$^+$ enters in the mitochondrion and binds to Complex I, whereby it inhibits ATP synthesis and stimulates ROS production. These two initial events lead to a host of cellular perturbations such as DNA damage, which, in turn, trigger a variety of cell-death-related pathways. These include activations of p53 by phosphorylation (p53-*p*) and JNK/c-Jun, which lead to Bax induction and translocation to the mitochondria. DNA damage also stimulates poly(ADP-ribose) polymerase (PARP) activity. Bax translocation and PARP activation promote the translocation of cytochrome *c* and apoptosis-inducing factor (AIF) from the mitochondria to the cytosol. Once in the cytosol, cytochrome c participates in a caspase-dependent cell death process, while AIF participates in a caspase-independent cell death process, both of which are not necessary mutually exclusive. Solid arrow, known mechanism; dashed arrow, speculated mechanism.

at the level of the mitochondria or whether it also involves perturbations that arise in the cytosol (e.g., protein nitration, cyclooxygenase-2 induction) and the nucleus (e.g., DNA damage, PARP activation) of the intoxicated cells is the focus of several ongoing studies in our laboratory.

ACKNOWLEDGMENTS

The authors thank Mr Matthew Lucas for his assistance in preparing this article. The authors are supported by NIH/NINDS Grant Nos. R29 NS37345, RO1 NS38586 and NS42269, P50 NS38370, and P01 NS11766-27A1, NIH/NIA Grant RO1 AG021617-01, the U.S. Department of Defense Grant (DAMD 17-99-1-9471, DAMD 17-03-1, and DAMD17-03-1-0428), the Lowenstein Foundation, the Lillian Goldman Charitable Trust, the Parkinson's Disease Foundation, the American Parkinson Disease Association, and the MDA/Wings Over Wall Street.

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