Sympathetic Innervation in the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Primate Model of Parkinson’s Disease

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ABSTRACT

Cardiac sympathetic denervation occurs commonly in Parkinson’s disease. This study explored whether analogous denervation occurs in primates with Parkinsonism from systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). 6-[18F]Fluorodopamine positron emission tomographic scanning and plasma levels of catecholamines and their deaminated metabolites were used to assess sympathetic and adrenomedullary function in rhesus monkeys, in the untreated state (n = 3), 2 weeks after a series of four MPTP injections, before establishment of Parkinsonism (acute phase, n = 1); a month later, after four more MPTP doses, associated with severe Parkinsonism (subacute phase, n = 1); or more than 2 years from the last dose (remote phase, n = 3), with persistent severe Parkinsonism. A positive control received i.v. 6-hydroxydopamine 1 week before 6-[18F]fluorodopamine scanning. Acute MPTP treatment increased cardiac 6-[18F]fluorodopamine-derived radioactivity, whereas 6-hydroxydopamine markedly decreased cardiac radioactivity, despite similarly low plasma levels of catecholamines and metabolites after either treatment. Subacutely, plasma catecholamines remained decreased, but now with myocardial 6-[18F]fluorodopamine-derived radioactivity also decreased. Remotely, MPTP-treated monkeys had lower plasma catecholamines and higher myocardial 6-[18F]fluorodopamine-derived radioactivity than did untreated animals. The results indicate that in nonhuman primates, systemic MPTP administration produces multiphasic effects on peripheral catecholamine systems, with nearly complete recovery by 2 years. MPTP- and 6-hydroxydopamine-induced changes differ markedly, probably from ganglionic or preganglionic neurotoxicity with the former and more severe cardiac sympathetic neurotoxicity with the latter. Because of multiphasic sympathetic and adrenomedullary effects, without cardioselective sympathetic denervation at any time, the primate MPTP model does not mimic the changes in peripheral catecholamine systems that characterize the human disease.

Parkinson’s disease features cardiac sympathetic denervation, as evidenced by decreased myocardial concentrations of radioactivity after administration of the sympathoneural imaging agents 123I-metaiodobenzylguanidine (Satoh et al., 1997; Braune et al., 1998, 1999; Yoshita et al., 1998; Satoh et al., 1999; Druschky et al., 2000; Ohmura, 2000), [N-methyl-11C]meta-hydroxyephedrine (Berding et al., 2003), and 6-[18F]fluorodopamine (Goldstein et al., 2000); virtually absent entry of norepinephrine, the sympathetic neurotransmitter, and dihydroxyphenylalanine and dihydroxyphenylglycol, indices of norepinephrine synthesis and turnover, into the cardiac venous drainage (Goldstein et al., 2000). Myocardial tissue obtained at autopsy of patients with Parkinson’s disease contains decreased tyrosine hydroxylase immunoreactivity, a marker of cardiac sympathetic denervation (Orimo et al., 2001, 2002).

The sympathetic denervation in Parkinson’s disease seems to be relatively selective for the heart, because 123I-metaiodobenzylguanidine- and 6-[18F]fluorodopamine-derived radioactivity remain unchanged in most other body organs (Goldstein et al., 2000; Reinhardt et al., 2000; Taki et al., 2000), and levels of norepinephrine in antecubital venous plasma are normal (Senard et al., 1993; Goldstein et al., 2002).

The ability of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to produce Parkinsonism and nigrostriatal neurotoxicity has supported the concept of Parkinson’s disease resulting from exposure to an environmental toxin. In contrast with extensive literature about central neural dopaminergic neurotoxicity by systemically administered MPTP, relatively little is known about possible toxicity to peripheral catecholamine-producing cells. MPTP treatment has been reported not to deplete adrenomedullary catecholamines (Stoddard et al., 1994). Plasma levels of the norepinephrine metabolite methoxyhydroxyphenylglycol fall relatively little (Bankiewicz et al., 1986). On the other hand, MPTP-treated mice have decreased cardiac accumulation of 123I-metaiodo-

ABBREVIATIONS: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.
Materials and Methods

Subjects. The animal research protocol was approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke. A total of six adult male rhesus monkeys (Macaca mulatta; body mass 4-6 kg) were studied. Animals were housed singly with a 12-h light/dark cycle and fed Purina monkey chow twice daily with water ad libitum.

MPTP-Induced Parkinsonism. Two monkeys had 6-\textsuperscript{18}F-fluorodopamine scanning before drug treatment; three had the scanning after chronic Parkinsonism produced in the remote past by systemic MPTP, with no injection in the past 2 years; one had the scanning before MPTP administration, 2 weeks after a series of four doses, when the animal was not yet Parkinsonian, and again about a month later, after another four doses of MPTP, when the animal had severe Parkinsonism; and one had the scanning before and 1 week after systemic administration of 6-hydroxydopamine at a previously identified sympathetic dose (Goldstein et al., 1991). Administration of MPTP i.v. produces bilateral damage to the substantia nigra pars compacta and a bilateral Parkinsonian syndrome. A Parkinsonian rating scale was used to quantify the clinical status of the monkeys. The scale includes ratings of 10 Parkinsonian features (tremor, posture, locomotion, hypokinesia, bradykinesia, balance, fine and gross motor skills, startle response, and freezing) and drug-related side effects (hypermobility, psychological disturbance, vomiting, and diarrhea). Scores on a 40-point scale were used to classify the monkeys as stage 1 to stage 4, with stage 1 representing mild Parkinsonism, and stage 4 severe bilateral Parkinsonism. All the MPTP-treated monkeys in this study had stage 4 bilateral Parkinsonism. Administration of 6-hydroxydopamine i.v. does not produce Parkinsonian features, because of the blood-brain barrier for catecholamines.

6-\textsuperscript{18}F-Fluorodopamine Scanning. MPTP-treated animals and controls underwent cardiac sympathetic neuroimaging by 6-\textsuperscript{18}F-fluorodopamine positron emission tomographic scanning, in a manner similar to that applied in humans (Goldstein et al., 1997a). Briefly, the animal, while under monitored general anesthesia with isoflurane and artificially ventilated, was positioned in an Advance scanner (General Electric, Milwaukie, WI), with the thorax or head in the gantry. 6-\textsuperscript{18}F-Fluorodopamine (dose in most cases 0.25 mCi) dissolved in approximately 10 ml of normal saline was infused intrave-
left ventricular myocardium could not be distinguished from that in the chamber, due to both decreased uptake and accelerated loss of radioactivity in the tissue (Fig. 5). There was also decreased radioactivity in sympathetically innervated structures in the head. The curve relating myocardial 6-[18F]fluorodopamine-derived radioactivity with time differed clearly between this animal and another tested before and after MPTP-induced Parkinsonism, because although the two animals had similarly decreased radioactivity immediately after 6-[18F]fluorodopamine administration, subsequently the MPTP-treated monkey had a decreased rate of loss of the radioactivity, whereas the 6-hydroxydopamine-treated monkey had an increased rate of loss. Thus, by 25 min after initiation of the injection of 6-[18F]fluorodopamine, the myocardial radioactivity concentration in the MPTP-treated monkey was almost 4 times that in the 6-hydroxydopamine-treated monkey.

Plasma levels of norepinephrine, epinephrine, dihydroxyphenylglycol, and dihydroxyphenylacetic acid were all much lower in the acute and subacute phases after MPTP than in untreated monkeys (Fig. 4), the values similar to those after 6-hydroxydopamine treatment. In contrast, in the remote phase, plasma levels of these catechols were only slightly lower than in untreated animals.

**Discussion**

In this study, unexpectedly, MPTP-treated monkeys with severe, chronic Parkinsonism had about the same amount of cardiac 6-[18F]fluorodopamine-derived radioactivity as untreated monkeys. Even in the animal with evidence for loss of cardiac sympathetic innervation subacutely after eight MPTP injections over several weeks, the loss was quantitative, not qualitative. The findings therefore did not mimic those in Parkinson's disease in humans, where most patients have evidence for loss of cardiac sympathetic nerves (Goldstein et al., 2000).

In complete contrast, a monkey treated with 6-hydroxydopamine as a positive control had markedly decreased 6-[18F]fluorodopamine-derived radioactivity throughout the left ventricular myocardium. The two neurotoxins therefore exerted quite different effects on cardiac sympathetic innervation.

Another unexpected finding was that, compared with values in untreated animals, the monkey that received MPTP treatment within a few weeks before 6-[18F]fluorodopamine scanning had very low plasma levels of catecholamines and their deaminated metabolites as did untreated monkeys. Even in the animal with evidence for loss of cardiac sympathetic innervation subacutely after eight MPTP injections over several weeks, the loss was quantitative, not qualitative. The findings therefore did not mimic those in Parkinson's disease in humans, where most patients have evidence for loss of cardiac sympathetic nerves (Goldstein et al., 2000).

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healthy volunteers and in anesthetized dogs during i.v. infu-
sion of trimethaphan to block ganglionic neurotransmission
(Goldstein et al., 1990, 1993, 1997b). The results, and simi-
larities to those during ganglion blockade in other studies,
lead us to propose early neurotoxic effects of MPTP at the
level of preganglionic or ganglionic neurotransmission, re-
sulting in decreased exocytotic release of norepinephrine
from intact cardiac sympathetic terminals. By an analogous
mechanism, MPTP would decrease adrenomedullary secre-
tion concurrently, resulting in low plasma epinephrine levels.

After repeated administration of MPTP over several weeks
in one monkey, plasma levels of catechols remained very low,
whereas cardiac 6-[18F]fluorodopamine-derived radioactivity fell
to subnormal levels. We interpret this pattern in terms of
a subacute phase involving actual loss of cardiac sympathetic
terminals. By an analogous mechanism, MPTP would decrease adrenomedullary secretion concurrently, resulting in low plasma epinephrine levels.

Over the course of many months or years from the last
administration of MPTP, sympathetic and adrenomedullary
cells seem to recover or regrow, so that cardiac 6-[18F]fluoro-
dopamine-derived radioactivity and plasma levels of cate-
cholamines eventually approximately normalize. Slightly
increased cardiac 6-[18F]fluorodopamine-derived radioactivity
and slightly decreased plasma levels of catechols in this
phase might reflect a residual preganglionic lesion, corre-
sponding to chronic Parkinsonism. The finding of long-term
recovery of peripheral catecholamine-producing cells fits
with evidence for recovery of behavioral and central dopami-
ergic function in primates by a year after MPTP injection
(Elsworth et al., 2000).

6-Hydroxydopamine rapidly destroys sympathetic nerve
terminals, as confirmed by virtually undetectable 6-[18F]flu-
orodopamine-derived radioactivity at a time that would cor-
respond to the acute phase after MPTP injection. Because
day they are catecholamines, neither 6-hydroxydopamine nor
6-[18F]fluorodopamine penetrates the blood-brain barrier. In
fact, as demonstrated in the present study, 6-\(^{18}\)F\)fluorodopamine scanning reveals the central nervous system by negative contrast. After sympatholysis by 6-hydroxydopamine, not only was the peak myocardial concentration of 6-\(^{18}\)F\)fluorodopamine-derived radioactivity decreased but also the radioactivity declined further, rapidly, to even lower levels, confirming previous reports in nonprimate laboratory animals (Chang et al., 1990; Goldstein et al., 1991). In marked contrast, as noted above, after MPTP cardiac 6-\(^{18}\)F\)fluorodopamine-derived radioactivity declined quite slowly. If lack of entry of 6-hydroxydopamine into the brain explained the difference in time-activity curves for cardiac 6-\(^{18}\)F\)fluorodopamine-derived radioactivity, then one would deduce that MPTP decreases exocytotic release from sympathetic nerves because of a neurotoxic effect in the central nervous system. Whether MPTP destroys brainstem catecholaminergic neurons descending to spinal preganglionic neurons remains unknown.

The cardiac sympathetic neuroimaging and plasma catecholamine findings in the primate MPTP model differed importantly from those reported previously in clinical Parkinson’s disease. In Parkinson’s disease, loss of cardiac sympathetic innervation is an early finding (Druschky et al., 2000; Omura, 2000; Reinhardt et al., 2000; Takatsu et al., 2000), without evidence for a premonitory phase involving a preganglionic lesion; sympathetic denervation is relatively selective for the heart, with normal plasma levels of catecholamines (Goldstein et al., 2000; Takatsu et al., 2000; Taki et al., 2000); and cardiac sympathetic denervation progresses over time, with loss of terminals in the left ventricular free wall or apex occurring faster than loss in the basal anterosepal myocardium (Li et al., 2002). Finally, although after heart transplantation some cardiac sympathetic reinnervation can occur (Kaye et al., 1993), no report to date has noted evidence for recovery of cardiac sympathetic innervation in humans with Parkinson’s disease.

In patients with MPTP-induced Parkinsonism, the movement disorder can worsen over years, associated with further loss of 6-\(^{18}\)F\)fluorodopa-derived radioactivity in the nigrostriatal system (Vingerhoets et al., 1994). This might reflect prolonged neurotoxicity, rather than simply an additive effect of aging (Cordes et al., 1994). Whether patients with MPTP-induced Parkinsonism have evidence for cardiac sympathetic denervation remains unknown.

Neither MPTP nor 6-hydroxydopamine provides a completely satisfactory animal model of peripheral catecholaminergic dysfunction in Parkinson’s disease. Systemic administration of MPTP is inadequate, because in the acute phase there is a preganglionic lesion resulting in low plasma levels of catecholamines and increased retention of catecholamines; in the subacute phase there may be decreased cardiac neuronal uptake of catecholamines but if so this occurs without accelerated loss; in the chronic phase there is recovery. Systemic administration of 6-hydroxydopamine also is inadequate, because the blood brain barrier for catecholamines prevents sufficient entry of the neurotoxin into the central nervous system to produce Parkinsonism.

This study involved only a very small number of animals. In designing the experiment, we had to take into account not only statistical power but also the expense and scarcity of 6-\(^{18}\)F\)fluorodopamine scanning, competition with other clinical and preclinical research protocols involving 6-\(^{18}\)F\)fluorodopamine scanning, and financial and ethical limitations on treating and maintaining primates with MPTP-induced severe Parkinsonism. These considerations led to an extraordinarily constrained experiment, consisting mainly of demonstrations, with inadequate numbers for statistically meaningful comparisons.

In summary, nonhuman primates treated with MPTP seem to undergo phases of peripheral catecholaminergic dysfunction. First to develop, over the course of a few weeks from a single injection, is a preganglionic lesion, resulting in decreased exocytotic release of catecholamines from intact cells and if anything increased retention of cardiac 6-\(^{18}\)F\)fluorodopamine-derived radioactivity. Over the course of months of repeated treatment, some loss of cardiac sympathetic terminals takes place, with persistence of the preganglionic lesion, resulting in a noticeable but not total loss of cardiac 6-\(^{18}\)F\)fluorodopamine-derived radioactivity and continued low plasma levels of catecholamines. In a long-term recovery phase, both cardiac 6-\(^{18}\)F\)fluorodopamine-derived radioactivity and plasma levels of catecholamines revert toward normal. The acute phase corresponds with behavioral signs of early Parkinsonism, the subacute and remote phases with established Parkinsonism.

The field of neurotoxin-induced Parkinsonism, as a model of the clinical condition, would benefit from identification of a substance that destroys cardiac sympathetic noradrenergic nerves and also penetrates the blood-brain barrier to destroy nigrostriatal dopamine cells as early effects.

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**References**


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