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

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Received March 20, 2003; accepted April 24, 2003

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 adenomedullary 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 adenomedullary 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; Braun et al., 1998; Yoshida 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-

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-[18F]fluorodopamine scanning before drug treatment; three had the scanning after chronic Parkinsonism produced in the remote past by systemic MPTP, with no injection within 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 sympatholytic 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 (hyperkinesia, 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-[18F]Fluorodopamine Scanning. MPTP-treated animals and controls underwent cardiac sympathetic neuroimaging by 6-[18F]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, Milwaukee, WI), with the thorax or head in the gantry. 6-[18F]Fluorodopamine (dose in most cases 0.25 mCi) dissolved in approximately 10 ml of normal saline was infused intravascularly at a constant rate for 3 min. Dynamic 3-dimensional thoracic scanning was performed for at least 30 min, followed by a static 3-dimensional imaging of the head for 15 min.

Results

Untreated monkeys had intense 6-[18F]fluorodopamine-derived radioactivity in the left ventricular myocardium, as well as the liver, spleen, nasopharyngeal mucosa, thyroid gland, and submandibular and parotid salivary glands (Fig. 1; Table 1). Parkinsonian monkeys treated with MPTP in the remote past had myocardial concentrations 6-[18F]fluorodopamine-derived radioactivity that did not differ from those in untreated monkeys (Fig. 2).

In the monkey studied before MPTP administration, after the last of a series of four MPTP injections, before establishment of Parkinsonism (acute phase), and about a month later after another series of four MPTP injections, when the animal was severely Parkinsonian (subacute phase), myocardial 6-[18F]fluorodopamine-derived radioactivity was increased in the acute phase and decreased in the subacute phase (Figs. 1 and 3). Analogous increases in radioactivity were also noted in sympathetically innervated structures of the head (Fig. 1, middle). Plasma levels of catecholamines and their deaminated metabolites were decreased at both time points (Fig. 4). In the monkey treated with a single dose of 6-hydroxydopamine, 6-[18F]fluorodopamine-derived radioactivity in the heart decreased markedly in the acute phase and returned to control levels in the subacute phase (Fig. 4). The extent and severity of MPTP neurotoxicity are well known to vary substantially across species and strains (Luthman and Sundstrom, 1990). Although MPTP exposure unquestionably evokes Parkinsonism in humans and other primates (Langston et al., 1983; Langston and Ballard, 1983; Ballard et al., 1985; Bankiewicz et al., 1986; Skirboll et al., 1990; Eberling et al., 1997), whether in primates MPTP produces cardioselective sympathetic denervation as in Parkinson’s disease has been unknown. This study used 6-[18F]fluorodopamine positron emission tomographic scanning and plasma levels of catecholamines and their deaminated metabolites to assess cardiac and overall sympathetic innervation in rhesus monkeys with severe Parkinsonism from systemic MPTP injection. The results were compared with those in untreated animals or in a positive control monkey treated with 6-hydroxydopamine, which is well known to abolish sympathetic terminal innervation and markedly reduce cardiac 6-[18F]fluorodopamine-derived radioactivity (Goldstein et al., 1991).
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.

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart</th>
<th>Liver</th>
<th>Salivary</th>
<th>Thyroid</th>
<th>NP</th>
<th>LV Chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (2)</td>
<td>2,565</td>
<td>1,850</td>
<td>1,164</td>
<td>463</td>
<td>801</td>
<td>1,155</td>
</tr>
<tr>
<td>MPTP acute (1)</td>
<td>4,355</td>
<td>3,787</td>
<td>1,253</td>
<td>480</td>
<td>809</td>
<td>2,337</td>
</tr>
<tr>
<td>MPTP subacute (1)</td>
<td>1,859</td>
<td>1,342</td>
<td></td>
<td>847</td>
<td>1,164</td>
<td>1,018</td>
</tr>
<tr>
<td>MPTP remote (3)</td>
<td>3,128</td>
<td>3,350</td>
<td>1,205</td>
<td>485</td>
<td>722</td>
<td>878</td>
</tr>
<tr>
<td>6-OHDA (1)</td>
<td>1,129</td>
<td>643</td>
<td></td>
<td>746</td>
<td>306</td>
<td>607</td>
</tr>
</tbody>
</table>

LV, left ventricle; NP, nasopharyngeal mucosa; 6-OHDA, 6-hydroxydopamine.

### Discussion

In this study, unexpectedly, MPTP-treated monkeys with severe, chronic Parkinsonism had about the same amount of cardiac 6-[^18F]fluorodopamine-derived radioactivity and concurrently measured 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).

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, yet increased cardiac 6-[^18F]fluorodopamine-derived radioactivity. Curves relating cardiac 6-[^18F]fluorodopamine-derived radioactivity with time indicated slower loss of radioactivity in MPTP-treated animals. A quite similar pattern of low plasma norepinephrine levels, increased cardiac 6-[^18F]fluorodopamine-derived radioactivity, and slowed loss of radioactivity occurs in...
healthy volunteers and in anesthetized dogs during i.v. infusion of trimethaphan to block ganglionic neurotransmission (Goldstein et al., 1990, 1993, 1997b). The results, and similarities 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, resulting in decreased exocytotic release of norepinephrine from intact cardiac sympathetic terminals. By an analogous mechanism, MPTP would decrease adrenomedullary secretion 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]fluorodopamine-derived radioactivity and plasma levels of catecholamines 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, corresponding to chronic Parkinsonism. The finding of long-term recovery of peripheral catecholamine-producing cells fits with evidence for recovery of behavioral and central dopaminergic 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]fluorodopamine-derived radioactivity at a time that would correspond to the acute phase after MPTP injection. Because they are catecholamines, neither 6-hydroxydopamine nor 6-[18F]fluorodopamine penetrates the blood-brain barrier. In
fact, as demonstrated in the present study, 6-[18F]fluorodopamine scanning reveals the central nervous system by neg-
ative contrast. After sympatholysis by 6-hydroxydopamine, not only was the peak myocardial concentration of 6-[18F]flu-
orodopamine-derived radioactivity decreased but also the ra-
doactivity declined further, rapidly, to even lower levels, con-
fiming previous reports in nonprimate laboratory ani-
imals (Chang et al., 1990; Goldstein et al., 1991). In marked
contrast, as noted above, after MPTP cardiac 6-[18F]fluoro-
dopamine-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-[18F]fluoro-
dopamine-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 neu-
rons descending to spinal preganglionic neurons remains
unknown.

The cardiac sympathetic neuroimaging and plasma cate-
cholamine findings in the primate MPTP model differed im-
portantly from those reported previously in clinical Parkin-
son’s disease. In Parkinson’s disease, loss of cardiac sympa-
thetic innervation is an early finding (Druschky et al.,
2000; Ohmura, 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 cate-
cholamines (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 ven-
tricular free wall or apex occurring faster than loss in the
basal anteroseptal myocardium (Li et al., 2002). Finally,
although after heart transplantation some cardiac sympa-
thetic 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 move-
dment disorder can worsen over years, associated with further
loss of 6-[18F]fluorodopa-derived radioactivity in the nigro-
striatal system (Vingerhoets et al., 1994). This might reflect
prolonged neurotoxicity, rather than simply an additive ef-
fect of aging (Cordes et al., 1994). Whether patients with
MPTP-induced Parkinsonism have evidence for cardiac sym-
pathetic denervation remains unknown.

Neither MPTP nor 6-hydroxydopamine provides a com-
pletely satisfactory animal model of peripheral catecholami-
nergic dysfunction in Parkinson’s disease. Systemic admin-
istration 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 neu-
ronal uptake of catecholamines but if so this occurs without
accelerated loss; in the chronic phase there is recovery. Sys-
temic administration of 6-hydroxydopamine also is inade-
quate, 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-[18F]fluorodopamine scanning, competition with other clin-
cal and preclinical research protocols involving 6-[18F]flu-
orodopamine scanning, and financial and ethical limitations
on treating and maintaining primates with MPTP-induced severe Parkinsonism. These considerations led to an extraor-
dinarily constrained experiment, consisting mainly of demo-
strations, with inadequate numbers for statistically mean-
ingful comparisons.

In summary, nonhuman primates treated with MPTP
seem to undergo phases of peripheral catecholaminergic dys-
function. 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-[18F]fluoro-
dopamine-derived radioactivity. Over the course of months of
repeated treatment, some loss of cardiac sympathetic termi-

dals takes place, with persistence of the preganglionic lesion,
resulting in a noticeable but not total loss of cardiac
6-[18F]fluorodopamine-derived radioactivity and continued
low plasma levels of catecholamines. In a long-term recovery
phase, both cardiac 6-[18F]fluorodopamine-derived radioac-
tivity 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.

Acknowledgments

We gratefully acknowledge the assistance of Cindy Prevost and
the National Institutes of Health Positron Emission Tomography
Department.

References


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