Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(dopamine uptake sites/mazindol autoradiography/Parkinson disease/squirrel monkey/neurodegeneration)

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ABSTRACT The meperidine analogue derivative 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces nigrostriatal fiber damage and severe parkinsonism in humans and animals. MPTP-induced parkinsonism has been proposed as a model of Parkinson disease, but doubts have been raised about whether the patterns of nigrostriatal fiber loss are similar. We report here observations on [3H]mazindol monoamine (principally dopamine) uptake-site binding in the striatum of monkeys (Saimiri sciureus) exposed to low doses of MPTP. We show that this treatment produces a pattern of nigrostriatal degeneration characteristic of that seen in Parkinson disease, in which there is greater depletion of dopaminergic markers in the putamen than in the caudate nucleus, especially posteriorly. Moreover, within the regions of diminished uptake-site binding in the MPTP-treated monkeys, there is differential preservation of binding in striosomes relative to the surrounding matrix. We suggest that both regional and striosome/matrix patterns of nigrostriatal depletion are key features of MPTP-induced neurodegeneration and that both patterns may provide clues to the mechanisms underlying neurodegeneration in Parkinson disease as well.

Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a behavioral state in humans and other primates that strongly resembles idiopathic Parkinson disease (1, 2). MPTP-induced parkinsonism is highly responsive to L-dopa therapy, and in the brains of MPTP-treated primates (including one human) it has been shown that there is a concomitant loss of dopamine-containing neurons of the nigrostriatal tract, also a hallmark of pathology in Parkinson disease (3–8). These characteristics suggest that MPTP-induced parkinsonism may be similar to the idiopathic disease and have motivated a large body of work aimed at understanding the molecular mechanisms underlying MPTP-induced neurotoxicity (8–11).

A key feature of the neurodegeneration in Parkinson disease is a characteristic gradient of dopamine loss in the striatum, with greater defects in the putamen than in the caudate nucleus, and greater defects posteriorly than anteriorly in the putamen (4–7, 12). This pattern has not been found consistently in MPTP-treated monkeys (13–16). Instead, MPTP is capable of depleting dopaminergic markers from nearly the entire caudate nucleus as well as from the putamen, leaving only the ventral striatum (nucleus accumbens and olfactory tubercle) and the adjoining ventral caudoputamen relatively spared. This more global deficit has been interpreted as suggesting that MPTP-induced parkinsonism, for all its similarities to Parkinson disease, may not be a suitable model for the disease (14, 15, 17). This is a fundamentally important issue, because the MPTP model not only implicates environmental toxins as possible etiologic factors in Parkinson disease (11) but also, if correct, could greatly enhance efforts to understand the molecular basis of cell death in Parkinson disease (18).

To address this issue experimentally, we treated monkeys with low doses of MPTP in an effort to bring about intermediate levels of nigrostriatal fiber loss. We monitored monoamine uptake-site binding with [3H]mazindol as a postmortem marker for monoaminergic terminals (19). We looked for gradients of reduced [3H]mazindol binding, and we also used the binding distributions to determine whether dopamine-containing fibers innervating the two main neurochemical compartments of the primate striatum, the striosomes and matrix (20, 21), were differently affected as suggested for MPTP intoxication in dogs (22, 23).

METHODS

MPTP Treatment and Behavioral Observations. Six adult feral squirrel monkeys (Saimiri sciureus) were given single subcutaneous injections of MPTP at the following doses: 1.5 mg/kg (S13 and S14), 2 mg/kg (S11 and S12), and 2.5 mg/kg (S7 and S8) (see Table 1). The behavior of each animal was observed, and total body movements were measured in 1-hr sessions with a photocell activity monitor capable of sensing body movements with an amplitude greater than one body width. Four to 29 sessions were given prior to administration of MPTP, and 5–10 afterwards (see Table 1).

Tissue Preparation. Ten days after the treatment, these animals and three untreated control squirrel monkeys were deeply anesthetized with sodium pentobarbital (12 mg/kg), and their brains were removed, quickly cut into blocks, and frozen in powdered dry ice. Serial transverse sections 10–15 μm thick were cut on a cryostat, thaw-mounted onto gelatin-coated slides, air dried, and stored in 20 sets of regularly spaced sections at −20°C until use.

[3H]Mazindol Binding. Binding incubations were performed as described previously (19, 24) on two complete sets of sections for each brain and on additional control and trial sections. Frozen sections were brought to room temperature, were preincubated for 5 min at 4°C in 50 mM Tris-HCl buffer, pH 7.9, containing 120 mM NaCl and 5 mM KCl to wash off endogenous ligand, and were incubated for 40 min with 15 nM [3H]mazindol (22.7 Ci/mmol; Du Pont NEN; 1 Ci = 37 GBq) in 50 mM Tris-HCl buffer containing 300 mM NaCl, 5 mM

Abbreviation: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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KCl, and 0.3 μM desipramine to block the norepinephrine transporter. After incubation, the sections were washed in the incubation buffer (two times for 3 min) and ice-cold water (10 sec) and dried under a cool air stream. Nonspecific binding was determined for control sections incubated in the presence of 30 μM benzotropine. The standard sets of sections were apposed to 3H-sensitive film (LKB Ultrashot) for 2 weeks; films with trial sections were exposed for shorter and longer times. Tritium standards (3H Micro-scales, Amersham) and nonspecific binding control sections were apposed to each film. To permit within-case analysis, films with sections at different anteroposterior levels from each control and each MPTP-treated monkey were prepared. To permit cross-case analysis, sets of sections from different MPTP-treated monkeys were applied to the same films (along with control sections and sections from control monkeys). Sections and films were treated as nearly identically as possible.

**Densitometry.** Densitometric analysis was performed with a Biocom 200 Image Analyzer (Les Ulis, France). For analysis of binding levels in the striatal matrix, optical density measurements were made at 12 sites per section, avoiding striosomes where these could be identified as such. For each monkey, measurements were made in five sections (10 hemispheres) per rostrocaudal level (rostral, middle, and caudal, see Fig. 3A), with occasional exceptions where tissue was missing. Optical density values were converted to nCi/mg tissue equivalent with the aid of autoradiographic standards on the films. Values for right and left sides were pooled.

**Fig. 1.** [3H]Mazindol binding autoradiograms illustrating patterns of partial depletion of monoamine uptake sites in the squirrel monkey striatum induced by low-dose MPTP treatment (A, B, C) relative to control levels of binding in an untreated squirrel monkey (A', B', C'). Binding appears white. Sections in A–C show anterior, middle, and posterior striatal levels from Sq11 (2.0 mg of MPTP per kg, 10-day survival). Sections in A'–C' show approximately matched levels from control Sq10. Sections were processed as similarly as possible; those shown in A, B, and C were incubated together and were exposed on the same films; sections in A', B', and C' were incubated together; all films were exposed for 15 days. Note in B and C that the greatest loss of binding in the MPTP-treated monkey occurs posteriorly and laterally, but that patches of spared binding (examples at asterisks) occur in regions of otherwise diminished binding. Loss of binding in the anterior striatum (A) is smaller, but patterns of heightened binding nevertheless are visible in a background of slightly reduced binding. Patches of reduced binding appear in the medial and ventral caudate nucleus in both the control and MPTP-treated monkeys (examples at arrowheads). CN, caudate nucleus; P, putamen; IC, internal capsule; NA, nucleus accumbens. (Scale bar indicates 1 mm.)
Drawings at the top of Fig. 3 show schematically the 12 sites sampled in representative rostral, middle, and caudal sections. The tail of the caudate nucleus was not analyzed. Additional densitometric measurements of patches of heightened binding visible in the striatum were also made and were analyzed separately. Values from three control monkeys (Sq4, -6, and -10) were pooled, but values from different MPTP-treated monkeys were not pooled because the front of diminished binding observed (see below) reached different levels in different monkeys.

RESULTS

Two striking patterns of partial loss of [3H]mazindol binding occurred in the caudate-putamen of the MPTP-treated monkeys (Fig. 1). The first was a systematic topography of diminished binding in which the putamen was more affected than the caudate nucleus, and posterior striatal levels were more affected than anterior levels. These lateral-to-medial and posterior-to-anterior gradients are illustrated in Fig. 1 for Sq11, which received MPTP at 2.0 mg/kg. Binding in the ventral caudate nucleus and putamen was least affected at all levels. In the control monkeys, marked gradients in binding were not observed in the caudate nucleus and putamen, but binding in the ventral striatum was lower than that in the dorsal striatum.

The second pattern of binding found in the MPTP-treated brains was a relative sparing of [3H]mazindol signal in striosomes. As shown in Fig. 1, in the striatal regions exhibiting decreases in binding, there were scattered focal zones in which much higher binding was present. These patches of heightened binding lined up with striosomes identified in adjacent sections stained for butyrylcholinesterase activity (Fig. 2). In ventral districts, where considerable [3H]mazindol binding persisted, striosomes could often be recognized as zones of reduced labeling in the MPTP-treated monkeys, as they can be in control monkeys (Fig. 1 and refs. 24 and 26).

To make quantitative estimates of the reduced binding found after MPTP treatment, we carried out densitometry on films from all animals. Fig. 3 summarizes the values for Sq11, based on samplings of the matrix compartment in relation to mean values for the control monkeys, also from samples taken from extrastriosomal matrix insofar as it could be identified. The quantitative data demonstrate relative sparing of [3H]mazindol binding in the most medial, ventral, and rostral parts of the striatum, and they show that the gradients of loss of binding were systematic. Densitometry on the patches of relatively spared [3H]mazindol binding suggested that binding in striosomes reached a maximum of 100% enhancement relative to adjoining matrix in the caudate nucleus and a maximum of 160% enhancement relative to matrix in the putamen.

There were marked individual differences in the extent of the reduced [3H]mazindol binding found in the striatum of the six MPTP-treated monkeys. In one monkey (Sq8, given 2.5 mg/kg), there was nearly total depletion of [3H]mazindol binding in the caudate-putamen, even rostrally. This pattern resembled the patterns reported for experiments in which large or repeated doses of MPTP were given (13–16). In a second monkey (Sq14, given 1.5 mg/kg), the MPTP treatment was nearly ineffective in reducing [3H]mazindol binding sites except in the posterior putamen. In the four remaining animals, caudolateral-to-rostromedial gradients of diminished binding were prominent, and patches of relatively preserved binding in striosomes appeared in the regions of partial loss in all but the most severely affected of these four monkeys (Sq13, given 1.5 mg/kg). In this monkey, binding was reduced nearly to background levels except far anteriorly, and even there binding in both striosomes and matrix was reduced relative to that in the controls. The forward and medial progression of the loss of binding varied from monkey to monkey, as did the relative degree of reduced binding in the dorsal third of the putamen (very marked in Sq8, Sq12, and Sq13; less marked in Sq7 and Sq11). The prominence of striosomal sparing also varied (especially marked in Sq7 and Sq11, less pronounced in others). Despite such individual differences, the general topographic and compartmental patterns of loss in the monkeys were remarkably consistent.

The relation between the dose of MPTP administered and the severity of loss of [3H]mazindol binding was not clear, because the next-to-most severe and the least severe depletions occurred in monkeys given the same dose of MPTP (Sq13 and Sq14, respectively, 1.5 mg/kg). The behavioral measurements (Table 1) showed marked loss of total body movements in all monkeys, with remaining activity of 2–29%.

All monkeys also showed marked bradykinesia. Of the two monkeys showing the smallest diminished total body movements relative to controls, one (Sq14) had the least affected [3H]mazindol binding. The second (Sq11) showed intermediate loss but considerable sparing of binding in the putaminal matrix except in an oblique central band (Fig. 1).

DISCUSSION

These findings demonstrate that MPTP-induced loss of monamine uptake-site binding in the primate caudate nucleus and

![Fig. 2. Photographs of two serial sections demonstrating that patches of spared [3H]mazindol binding in the MPTP-treated squirrel monkey striatum (A) correspond to striosomes identified by butyrylthiocholinesterase histochemistry (B). A shows five prominent patches of binding (asterisks) in the putamen of Sq11. Corresponding zones in B appear as sites with butyrylcholinesterase-rich rims (see asterisks), which mark striosomes in this part of the striatum (25). (Scale bar indicates 1 mm.)](image-url)
suggests that the patterns of partial loss in the other four monkeys represented intermediate phases preceding complete destruction of nigrostriatal terminals. This point is particularly important when comparing the MPTP-induced changes to those in Parkinson disease, as dopaminergic cell loss in the idiopathic disease is also nearly always partial (3, 28, 29).

Although the predominance of putaminal loss of \(^{3}H\)mazindol binding we saw in the MPTP-treated monkeys is similar to the predominant putaminal loss of dopamine described for Parkinson disease brains (4–7, 12), we did not see a reverse (rostral most, caudal least) gradient of depletion in the caudate nucleus, as reported for Parkinson disease brains by some groups (4, 12). The lateral-to-medial gradient of uptake-site loss we found in the striatum does parallel findings for Parkinson disease (6). The fields of diminished \(^{3}H\)mazindol binding in the putamen and lateral caudate nucleus seemed related, as did the relative striosome/matrix binding in the two nuclei: often patches of strong binding in striosomes appeared just on either side of the internal capsule.

There was remarkably clear evidence for relative preservation of uptake-site binding in striosomes located in otherwise clearly affected striatal regions in these MPTP-treated monkeys. In some severely-depleted regions, most of the remaining binding was in striosomes. This pattern suggests that the striosomes, like the medial caudate nucleus and anterior putamen, are differently, and perhaps less rapidly, affected than the matrix and remaining parts of the putamen and caudate nucleus. Ultimately, however, binding in the striosomes can also be depleted, as shown in our own most severely affected monkeys and by findings in other studies (13, 14). The differences in loss of binding between striosomes and matrix thus do not seem to reflect differences in ultimate susceptibility of the two striatal compartments but, rather, differences in the rate of onset or progression of the MPTP-induced pathology.

It is not known whether such a differential striosome-matrix pattern of vulnerability occurs in the striatum in Parkinson disease. A compartmental pattern of nigrostriatal damage has been reported for MPTP-treated dogs, in which marked fiber degeneration was seen in the matrix but not in striosomes at 8- to 14-day survival times (22, 23). Furthermore, after chronic MPTP treatment and extended (15-week) survival times, differential up-regulation of \(\mu\) opiate receptor ligand binding has been found in the striosomal system in monkeys (27). Clearly, a key feature to look for in early-stage neuropathology in Parkinson disease will be differential vulnerability of the striosome and matrix compartments.

The pronounced neurochemical gradients and compartmentalization in the striatum (21, 28), or the corresponding nonuniform neurochemistry of the midbrain dopaminergic cell groups (18, 28, 29), or both could contribute to the patterns of differential vulnerability reported here. For example, it has been proposed that differences in monoamine uptake-site concentration in different regions of the normal striatum could result in lower rates of uptake of the toxic metabolites of MPTP or MPTP-like substances in some regions (such as ventral striatum) than in others and, consequently, lower relative rates of nigrostriatal terminal damage (24, 30). Our findings confirm relatively low levels of uptake-site binding in the ventral striatum of normal monkeys and show a relative sparing of uptake-site binding in the ventral striatum of MPTP-treated monkeys. However, the topography of binding in the MPTP-treated monkeys did not support such an inverse correlation for the dorsal striatum. First, we did not observe pronounced gradients of binding in the controls that would predict the particular vulnerability of the posterior putamen (Fig. 2). Second, the most dramatic instances of striosomal preservation of binding were in the putamen and lateral caudate nucleus of the MPTP-treated monkeys, particularly caudally, whereas the relatively re-
duced [\(^{3}H\)]mazindol binding in striosomes of the normal primate striatum tends to occur medially and anteriorly (24, 26). In fact, in one of our control monkeys (Sq10), dorsolateral striosomes even appeared as regions of slightly (4–10%) elevated binding (Fig. 1).

The variations in individual susceptibility seen in this study have implications for the hypothesis that an environmental agent, possibly a toxin, could precipitate Parkinson disease. We found that single subcutaneous injections of MPTP at the same or similar doses can produce a range of neurodegenerative effects from nearly complete to very mild loss of [\(^{3}H\)]mazindol binding. We also noted individual differences in the severity of behavioral symptoms shown by the monkeys. Such differences are also characteristics of primates treated with MPTP for prolonged periods (16, 31, 32), and they are reminiscent of the variability in symptoms and neurodegeneration in Parkinson disease. We did not determine the origins of such variability, but our findings may have direct bearing on the complexity of epidemiological data on the incidence of parkinsonian disorders. One potentially important variable, age, was unknown for the feral monkeys we used.

Our findings clearly raise the possibility that low-dose exposure to neurotoxins in humans might produce a gradual loss of dopaminergic nigrostriatal fibers following the gradients and compartmental patterns we describe here. The preferential early loss of nigrostriatal markers in the striatal matrix, especially in the sensorimotor part of the putamen and lateral caudate nucleus, is consistent with early motor symptoms of MPTP-induced parkinsonism, also a feature of Parkinson disease. Nearly all of the major projections to the primate striatum that originate in somatic sensory and motor cortex terminate in the putamen and adjoining lateral margin of the caudate nucleus (33, 34). The low-dose MPTP-treated monkey may provide a new model for studying the differential vulnerability of the putamen and the extrastriosomal matrix with respect to their dopaminergic innervations and the functional effects of this vulnerability. The pattern of loss we found further suggests that functions of the basal ganglia relying on nigrostriatal modulation would increasingly involve the medial caudate nucleus and the striosomal system in neurotoxin-induced parkinsonism. These striatal subdivisions receive incoming information from parts of the prefrontal association cortex, but they do not receive a major sensorimotor cortical input (refs. 35–37; F. M. Ebben and A.M.G., unpublished observations). Such an imbalance between dopamine-denervated sensorimotor striatum and dopamine-innervated frontolimbic-associated striatum could help to account for some of the selective behavioral deficits that occur during the evolution of parkinsonian syndromes.

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Table 1. Motor activity before and after MPTP treatment

<table>
<thead>
<tr>
<th>Monkey</th>
<th>MPTP dose, mg/kg</th>
<th>Body movements per min (number of sessions)</th>
<th>% of pretreatment activity after MPTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Sq13</td>
<td>1.5</td>
<td>28.82 ± 2.87 (21)</td>
<td>2.67 ± 0.36 (10)</td>
</tr>
<tr>
<td>Sq14</td>
<td>1.5</td>
<td>24.81 ± 2.95 (27)</td>
<td>6.14 ± 1.11 (10)</td>
</tr>
<tr>
<td>Sq11</td>
<td>2.0</td>
<td>15.38 ± 0.87 (29)</td>
<td>4.47 ± 1.26 (10)</td>
</tr>
<tr>
<td>Sq12</td>
<td>2.0</td>
<td>26.92 ± 2.77 (21)</td>
<td>2.98 ± 0.76 (10)</td>
</tr>
<tr>
<td>Sq7</td>
<td>2.5</td>
<td>46.99 ± 3.21 (4)</td>
<td>1.19 ± 0.27 (5)</td>
</tr>
<tr>
<td>Sq8</td>
<td>2.5</td>
<td>34.53 ± 3.98 (5)</td>
<td>0.69 ± 0.44 (5)</td>
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</tbody>
</table>

Body movements are reported as mean ± SEM.