Suppressive Effect of L-Dopa on Dopamine Cells Remaining in the Ventral Tegmental Area of Rats Previously Exposed to the Neurotoxin 6-Hydroxydopamine

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Summary: Ever since the introduction of levo-3,4-dihydroxyphenylalanine (L-dopa) for the treatment of Parkinson’s disease, there has been concern that it might accelerate the degeneration of dopamine neurones. Using rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the medial forebrain bundle (MFB), we have studied the effect of chronic L-dopa treatment on the survival of dopamine cells which remain in the ventral tegmental area (VTA) ipsilateral to a 6-OHDA lesion. Following lesion surgery, rats were treated with L-dopa and carbidopa administered in the drinking water for 27 weeks. At the end of the treatment period, the number of dopamine cells remaining in each of the lesioned and intact substantia nigra (SN) and VTA were assessed, using tyrosine hydroxylase immunohistochemistry. Chronic L-dopa treatment resulted in an apparent reduction in the number of dopamine neurones remaining in the VTA ipsilateral to the lesion, whereas it had no effect on the number of dopamine cells remaining in the intact SN and VTA. This finding suggests a possible suppressive effect in vivo of L-dopa on dopamine cells in the midbrain of adult animals that have been previously exposed to 6-OHDA. Key Words: L-dopa—Neurotoxicity—Ventral tegmental area (VTA)—Parkinson’s disease.

In theory, L-dopa, dopamine and related compounds could be cytotoxic in man since free radical and quinone metabolites of L-dopa are toxic to C1300 catecholamine and melanoma cell lines in vitro (1,2). Similarly, hydrogen peroxide (a product of normal dopamine metabolism) and 6-hydroxydopamine (6-OHDA, produced by oxidation of dopamine in vitro) can induce cell death (1). There is no evidence, however, that chronic L-dopa treatment is toxic in vivo to healthy dopamine cells in the substantia nigra (SN) of normal animals (3–6).

Whether L-dopa is harmful to dopamine neurones that have previously been exposed to a neurotoxic agent, or that are degenerating is uncertain. This question may be of relevance to Parkinson’s disease, where exposure to an unknown environmental or endogenous neurotoxin may contribute to neuronal death (8). In addition, there is morphological evidence that dopamine cells remaining in the SN of patients with Parkinson’s disease are degenerating (9), and levels of TH mRNA, as revealed by in situ hybridisation, may be reduced (10). Similarly, dopamine cells remaining in the midbrain following an ipsilateral 6-OHDA lesion show reduced TH mRNA levels when assessed several months after the lesion (11).

The only pathological study to specifically ad-
dress the question of L-dopa toxicity in Parkinson’s disease suggested that neuronal loss in the SN was not affected by L-dopa treatment (9). However, as extensive loss of dopamine cells in the SN occurs in symptomatic Parkinson’s disease, a detrimental effect of L-dopa treatment on the small remaining population of nigral neurones might be difficult to detect.

Dopamine cells in the ventral segmental area (VTA) also degenerate in Parkinson’s disease, but to a lesser extent than those in the SN (12). To date, the effects of L-dopa treatment on VTA cells have not been reported in normal animals, in experimental models of Parkinson’s disease, or in Parkinson’s disease itself. Rats with a unilateral 6-OHDA lesion (VTA) also degenerate in Parkinson’s disease, but the effect of L-dopa treatment on the small remaining population of nigral neurones might be difficult to detect.

Movement Disorders, Vol. 8, No. 2, 1993

METHODS

6-OHDA Lesion Surgery

Female Wistar rats (200–250 g) anaesthetised with sodium pentobarbital (50 mg/kg ip) received a unilateral 6-OHDA lesion of the left MFB rostral to the SN [8 μg of 6-OHDA as the base, dissolved in 4 μl ascorbate saline (0.1% w/v), infused at a rate of 1 μl/min, as described previously (13)].

TH Immunohistochemistry

Animals were killed within 3 days of stopping L-dopa and carbidopa intake and perfused with 4% paraformaldehyde in phosphate buffered saline (PBS). Sections (20 μm) through the region of the midbrain containing the SN and VTA were immunostained with an antibody to tyrosine hydroxylase (TH) (rabbit antityrosine hydroxylase, Eugene Tech, NJ, diluted 1:2,000 in PBS containing 1% goat serum (NGS)). TH antibody was applied to the sections for 2 h at room temperature and for 48 h at 4°C. Sections were then rinsed in PBS containing 1% normal goat serum (NGS). ABC vectastain (Vector Labs) was used to visualise bound primary antibody. Sections were then rinsed in PBS and then preincubated in TRIS buffer (0.05 M, pH 7.3) containing the chromogen 3,3′-diaminobenzidine (DAB, 0.05%) for 5 min. Hydrogen peroxide was added (final concentration 0.01%) for 6 min. The reaction was stopped.
by removing the DAB-hydrogen peroxide and rinsing with TRIS buffer. Sections were mounted on gelatin-coated slides. TH-positive cells remaining in the SN and VTA on each side of the brain were counted in a minimum of 5 sections, using the methods of Konigsmark (16).

RESULTS

The numbers of TH-positive cells remaining in the intact and lesioned SN and VTA of the two groups of animals are shown in Table 1. In both groups, the 6-OHDA lesion resulted in >96% loss of TH-positive cells in the SN ipsilateral to the lesion, but TH-positive cell loss in the ipsilateral VTA was less marked (a significant reduction for both SN and VTA, p < 0.001, paired Student’s t-test). In both groups of animals, many of the TH-positive cells remaining in the VTA ipsilateral to the lesion were small and round (diameter of most cells ~12 μm) whereas in the intact side (Fig. 1A) cells of both the small round variety and a larger fusiform type of 15–20 μm in length were found.

L-Dopa and carbidopa treatment for 27 weeks (Group 2) did not affect the number of TH-positive cells in the SN and VTA contralateral to the 6-OHDA lesion [intact side, Table 1; (F(1,15) = 0.07, p = 0.78 for SN; and F(1,15) = 0.28, p = 0.6 for VTA; ANOVA]. It was not possible to establish the effect of L-dopa treatment on cells in the 6-OHDA-lesioned SN as, in all rats, only the occasional TH-positive cell remained in the lesioned SN. A 6-OHDA lesion caused a significant reduction in number of TH-positive cells in the ipsilateral VTA compared with the intact side (Fig. 1B) (Table 1). The number of TH-positive cells remaining in the 6-OHDA-lesioned VTA was further reduced in rats receiving l-dopa and carbidopa treatment for 27 weeks (Group 2), as shown in Fig. 1C and Table 1. Thus, the number of TH-positive cells remaining in the VTA ipsilateral to the lesion of animals receiving a lesion alone (Group 1) ranged from 23% to 65% of the intact side, compared with 10–36% in rats receiving L-dopa treatment after the lesion. Using the intact side as covariate, this effect of l-dopa treatment was significant [F(1,15) = 9.34, p = 0.009 ANOVA; t = 2.78, df = 14, p < 0.05, post hoc Dunnett’s test].

DISCUSSION

This study demonstrates three points. First, dopamine neurones in the SN and VTA are differentially affected by an ipsilateral 6-OHDA lesion. The difference in extent of cell loss may reflect a relative resistance of some cells in the VTA to the toxic effects of 6-OHDA. Variation in the susceptibility of different dopamine systems to 6-OHDA is well documented, although the reasons for this are unknown (17). Similarly, in man, melanised dopamine neurones in SN are preferentially lost in Parkinson’s disease (18), whilst dopamine cell loss in VTA is much less marked than that in the SN (12). Alternatively, the difference could be explained by the relative position within the MFB of dopamine fibres emanating from the SN (compactly placed in the lateral region of the MFB) and from the VTA (more dispersed in the medial portion of the MFB) (19). So, the lesion placed in the MFB might more reliably affect fibres derived from the SN. The second finding, that dopamine neurones in the intact SN and VTA are not reduced in number by chronic l-dopa treatment, confirms previous studies (3–5, 13), indicating that dopamine neurones in the healthy SN and VTA of experimental adult animals are not damaged by chronic treatment with l-dopa.

The third and novel finding is that the number of TH-positive neurones remaining in the VTA ipsilateral to a previous 6-OHDA lesion is reduced following chronic l-dopa treatment, suggesting a suppressive effect of l-dopa on those dopamine cells. At another extreme, this observation might suggest that l-dopa treatment was toxic to cells previously exposed to (but not killed by) the neurotoxin 6-OHDA. The exact nature of this effect of l-dopa has not, however, been revealed by the present results. Thus, TH-immunohistochemistry may be an insensitive method of assessing dopamine cell num-

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TABLE 1. TH-positive cell numbers in sections through the SN and VTA of the intact and 6-OHDA lesioned sides of the midbrain

<table>
<thead>
<tr>
<th>Group</th>
<th>Intact</th>
<th>Lesioned</th>
<th>% Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN VTA</td>
<td>SN VTA</td>
<td>SN VTA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(range)</td>
<td>(range)</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(lesion</td>
<td>132.0</td>
<td>2.4</td>
<td>1.8</td>
</tr>
<tr>
<td>only)</td>
<td>± 12.6</td>
<td>± 1.3</td>
<td>± 4.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>136.0</td>
<td>3.3</td>
<td>2.4</td>
</tr>
<tr>
<td>(lesion</td>
<td>± 6.9</td>
<td>± 1.6</td>
<td>± 2.2</td>
</tr>
<tr>
<td>+ l-dopa)</td>
<td>± 9.9</td>
<td>± 2.2</td>
<td>± 1.6</td>
</tr>
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* p < 0.01, ANCOVA. TH-positive cells in substantia nigra (SN) and ventral tegmental area (VTA) of each side of the midbrain were counted in a minimum of five sections (20 μm) taken through level ~5.8 from bregma (23), yielding mean values (± 1 SEM) for each animal. At this level the SN and VTA are clearly separated by rootlets of the oculomotor nerve. All animals received a unilateral 6-OHDA lesion of the left medial forebrain bundle. For animals in group 2 this was followed by l-dopa and carbidopa treatment for 27 weeks.
FIG. 1. TH immunostaining of sections (20 μm) taken through the midbrain (level -5.8 from bregma (23)). A: The intact VTA (right side) from an animal in group 2 (L-dopa treatment for 27 weeks). The photograph has been reversed so as to be comparable in orientation to (B) and (C). B: The 6-OHDA-lesioned VTA (left side) from an animal in group 1 (no L-dopa). C: The 6-OHDA-lesioned VTA (left side) from an animal in group 2 (L-dopa treatment for 27 weeks). The number of cells remaining in the VTA ipsilateral to the lesion is significantly reduced compared to the intact side [(B) compared with (A)]. There was a further significant reduction in TH-positive cell number remaining in the 6-OHDA lesioned VTA of animals in group 2 compared with group 1 [(C) compared with (B)]. Scale bars, 40 μm.

ber, and the apparent reduction in their number might merely reflect a suppression (possibly temporary) of TH production by these cells, as a result of the chronic L-dopa treatment. If this were the explanation, however, one would have expected a similar effect of L-dopa on the SN/VTA dopamine cells remaining in the nonlesioned side in Group 2. This was not observed. So, it remains possible that L-dopa treatment might have produced a true reduction in the number of TH-positive cells previously exposed to 6-OHDA. Unfortunately, the effect of different doses of L-dopa on the number of TH-positive cells remaining in the 6-OHDA-lesioned VTA has not been assessed. However, there is reason to believe that the duration of L-dopa treatment may be important. In a previous study (13), animals with a unilateral 6-OHDA lesion receiving the same dose of L-dopa and carbidopa treatment as in this study, but for a shorter period of 5 weeks, also showed a reduction in the number of surviving TH-positive cells in the 6-OHDA lesioned VTA compared with untreated rats, but this effect was not quite significant. Those animals (13) were killed 5 weeks after L-dopa treatment was discontinued, suggesting that the effect of L-dopa outlasts the duration of treatment.

A speculative interpretation of the present results might suggest that the effects of earlier 6-OHDA exposure and subsequent chronic L-dopa treatment (with possible production of toxic metabolites) were additive and sufficient to result in further cell death. There is evidence that a 6-OHDA lesion can have a long-lasting suppressive effect on the levels of TH mRNA found in dopamine cells remaining in the SN following a 6-OHDA lesion (11). Similarly, dopamine cells that remain in the SN of patients with Parkinson’s disease may be degenerating, as revealed morphologically (9) and by the fact that they may contain reduced levels of TH mRNA (10).

The cause and mechanism(s) of cell death in Par-
Parkinson's disease are, however, unknown, so whether the suppressive effects of L-dopa observed in the present study (which appeared to be restricted to neurons exposed to 6-OHDA) would also be additive to the mechanism(s) causing cell damage in Parkinson's disease are unknown. Nevertheless, there is evidence that some of the molecular mechanisms involved in Parkinson's disease may be similar to those involved in 6-OHDA- and L-dopa-induced toxicity in vitro. 6-OHDA-induced neurotoxicity, and L-dopa toxicity in vitro, involve production of free radicals, which can damage cell membranes, structural proteins and DNA (20). In Parkinson's disease, there is evidence of accelerated free radical production and increased lipid peroxidation in the SN (21). Furthermore, dopa and dopamine can cause death of cultured dorsal root ganglion cells in the presence of ferric iron via a process of free radical generation and increased lipid peroxidation (22). This latter finding may be of relevance to patients with Parkinson's disease receiving L-dopa, where the SN contains elevated levels of ferric iron and lipid peroxide (21). The observation that L-dopa treatment suppresses TH-positive neurones previously exposed to toxic insult may therefore be relevant to the treatment of patients with Parkinson's disease.

Acknowledgment: This work was supported by Parkinson's Disease Society Research Funds and the Medical Research Council Training Fellowship.

REFERENCES