Tyrosine administration increases striatal dopamine release in rats with partial nigrostriatal lesions

(dihydroxyphenylacetic acid/homovanillic acid/striatum)

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ABSTRACT Partial, unilateral nigrostriatal lesions of varying severity were produced in rats by injecting graded doses of 6-hydroxypidamino into the substantia nigra. Formation of the dopamine metabolites dihydroxyphenylacetic acid and homovanillic acid in each surviving nigrostriatal neuron (estimated by the ratios of dihydroxyphenylacetic acid to dopamine and homovanillic acid to dopamine in the striatum) increased significantly when dopamine concentrations in striata containing lesions had been reduced to 25% or less of control values, but remained unchanged in rats with less severe lesions. These findings suggest that, in rats with severe damage of nigrostriatal dopaminergic neurons, surviving neurons increase their firing rates and accelerate dopamine synthesis and release. In rats that had lesions and enhanced striatal dopamine release, but not in rats with less lesions (i.e., which reduced ipsilateral dopamine concentrations by less than 75%), administration of tyrosine (250 mg/kg) caused further significant increase in formation of dihydroxyphenylacetic acid and homovanillic acid. These findings provide further evidence that tyrosine availability can enhance dopamine synthesis in and release from nigrostriatal neurons if the firing rates of these neurons are accelerated.

The limiting step in catecholamine biosynthesis is hydroxylation of the amino acid precursor tyrosine to 3,4-dihydroxyphenylalanine (dopa); this process is catalyzed by the enzyme tyrosine hydroxylase (1). Evidence suggests that the rates of tyrosine hydroxylation and subsequent dopamine or norepinephrine release can depend on the availability of tyrosine to catecholaminergic neurons: tyrosine administration accelerates brain dopa accumulation in rats given benserase, an inhibit of central aromatic L-amino acid decarboxylase (2,3), and increases brain levels of methoxyhydroxyphenylethlyglycol sulfate in spontaneously hypertensive rats (4) or in animals subjected to cold stress (5). Dopamine synthesis in and release from nigrostriatal neurons are also accelerated after tyrosine administration in animals treated with haloperidol (6), a drug that blocks central dopamine receptors and increases the firing rates of nigrostriatal neurons (7), but not in control or probenecid-treated rats. These observations raise the possibility that in catecholaminergic brain neurons, tyrosine's effects on neurotransmitter synthesis and release may vary with neuronal activity, becoming important when neurons are firing frequently but not when they are relatively quiescent (8).

The present studies examine the relationship between apparent firing rates of nigrostriatal neurons and the precursor dependence of dopamine release, by testing the effects of exogenous tyrosine on accumulation of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (dopac) and homovanillic acid (HVA) in striata of rats with partial, unilateral nigrostriatal lesions. This animal model was chosen on the basis of evidence that when the number of functioning nigrostriatal dopaminergic neurons is reduced, the remaining neurons become hyperactive. Agid et al. (9) reported increases in the conversion of [3H]tyrosine to [3H]dopamine in the striatum after partial ipsilateral destruction of nigrostriatal dopaminergic neurons by intranigral injections of 6-hydroxydopamine (6-OH-dopamine). Similarly, we noted increases in the striatal dopa accumulation among rats with lesions receiving a central decarboxylase inhibitor (unpublished data). In corpora striata from parkinsonian brains, analyzed post mortem, the ratio of HVA to dopamine is higher than in control brains (10–13), also suggesting accelerated dopamine synthesis in and release from surviving striatal dopaminergic neurons. If tyrosine could accelerate dopamine synthesis and release only in hyperactive nigro-neostriatal neurons, then an effect of exogenous tyrosine might be observed in striata that contained lesions, but not in control, contralateral striata from the same animals.

METHODS

Male Sprague-Dawley rats (150–200 g; Charles River Breeding Laboratories) had free access to Big Red Rat Chow and tap water and were exposed to light from 8 a.m. to 8 p.m. (Vita Lite, 300 μW/cm²; DuroTest, North Bergen, NJ). Partial, unilateral nigrostriatal lesions of varying severity were produced by the following procedure. Rats anesthetized with sodium pentobarbital (Nembutal) were placed in a Kopf stereotaxic device. Then 8, 5, 4, 2, 1, or 0.5 μg of 6-OH-dopamine (in 4 μl of saline containing 0.2 mg of ascorbic acid per ml) was injected stereotaxically into the right anteromedial substantia nigra, level A 2400, −2.6 mm dorsoventral, 1.6 mm mediolateral, according to the atlas of König and Klippel (14). Rats were decapitated 2 weeks after lesions were produced; brains were removed and the corpora striata were dissected, frozen on dry ice, and then homogenized in 20 vol of 0.1 M perchloric acid. Dopamine, dopac, and the dopamine metabolites dopac and HVA were measured in deproteinized aliquots by reverse-phase high-performance liquid chromatography with electrochemical detection (15, 16). In experiments on tyrosine, 5 or 8 μg of 6-OH-dopamine was injected into the substantia nigra, and 2 weeks later animals received the highly soluble methylester form of tyrosine (250 mg/kg intraperitoneally, dissolved in saline; Aldrich). Administration of this agent increases brain tyrosine concentrations (4). Additional groups of rats, injected

Abbreviations: HVA, homovanillic acid; 1-dopa, 1-3,4-dihydroxyphenylalanine; dopac, 3,4-dihydroxyphenylacetic acid; 6-OH-dopamine, 6-hydroxydopamine.

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intranigrally with 8 μg of 6-OH-dopamine, subsequently received tyrosine methylester (100 mg/kg) or another large neutral amino acid, valine (500 mg/kg, dissolved in saline), intraperitoneally. Control rats were injected with saline. Animals were decapitated 1 hr after injection, and striatal dopamine, dopa, dopac, and HVA levels were measured. Tyrosine levels in the rest of the brain were estimated fluorimetrically (17).

RESULTS

Striatal Concentrations of Dopamine and Its Metabolites After Lesions. Striatal dopamine levels in rats injected with 6-OH-dopamine reflect the extent of destruction of the nigrostriatal system (18). In these studies, intranigral injection of various doses (0.5–8.0 μg) of 6-OH-dopamine produced a wide range of dose-dependent decreases in striatal dopamine concentrations ipsilateral to the lesion. The higher the 6-OH-dopamine dose used, the greater were the reductions in striatal dopamine concentrations, compared with those in the contralateral, unlesioned striata. Animals with lesions could be divided into three groups based on the severity of the reduction in striatal dopamine ipsilateral to the lesion (Table 1). The ratio of dopac or HVA concentration to the dopamine concentration (dopac/dopamine or HVA/dopamine) indicates the dopamine release from nigrostriatal neurons in any striatum. In striata contralateral to the lesion, dopamine, dopac, and HVA concentrations, as well as the ratios dopac/dopamine and HVA/dopamine, remained unchanged in all groups with lesions and were similar to those observed in striata of control animals without lesions (Table 1). Hence, the intact, contralateral striata could serve as control tissues for the effects of unilateral nigrostriatal lesions on dopamine release. In rats with partial nigrostriatal lesions of only mild or moderate severity (as reflected by dopamine levels 25% or more of controls), the striatal dopac/dopamine and HVA/dopamine ratios did not change ipsilateral to the lesions. However, in rats whose striatal dopamine levels were less than 25% of controls, these ratios increased significantly (Table 1).

To provide a clearer index of dopamine release from surviving nigrostriatal neurons, we expressed the ratios dopac/dopamine and HVA/dopamine in striata containing lesions as percentages of the same ratios in the control side in each individual animal. When dopamine release rates per neuron on the sides with and without lesions are equal, this percentage should be close to 100%; it will be higher if dopac and HVA formation per dopaminergic neuron increases in the striatum containing lesions. This percentage was significantly increased only in rats with severe nigrostriatal lesions (striatal dopamine ipsilateral to the lesion reduced to less than 25% of control) and not in rats with mild or moderate lesions (Table 1).

Effect of Tyrosine Injection on Striatal Dopac and HVA Formation in Rats with Lesions. Unilateral intranigral injections of 8 μg of 6-OH-dopamine markedly reduced dopamine levels in the striata containing lesions to less than 25% of those observed in the contralateral sides without lesions. Striatal dopamine concentrations after intranigral injections of 5 μg of 6-OH-dopamine were moderately reduced (to 25–50% of those on the sides without lesions). In both groups with lesions, the extent of reduction in striatal dopamine was very similar in animals receiving tyrosine, valine, and saline (Table 2).

Tyrosine administration significantly elevated brain tyrosine concentrations, whereas valine lowered brain tyrosine (Table 2). Neither amino acid affected dopamine levels in control striata without lesions (Table 2). Dopa was undetectable in striatal tissues obtained from all groups of rats (limit of sensitivity in the chromatographic assay used was approximately 0.1 ng/mg).

In saline-treated control rats with partial nigrostriatal lesions of moderate severity (intranigral injection of 5 μg of 6-OH-dopamine), the ratios of dopac (or HVA) to dopamine in striata

<table>
<thead>
<tr>
<th>Group</th>
<th>Dopamine, ng/mg wet weight</th>
<th>Dopac, ng/mg wet weight</th>
<th>HVA, ng/mg wet weight</th>
<th>Dopac/dopamine</th>
<th>HVA/dopamine</th>
<th>Dopac/dopamine lesions + dopac/dopamine control, %</th>
<th>HVA/dopamine lesions + HVA/dopamine control, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, no lesions (n = 7)</td>
<td>8.35 ± 0.66</td>
<td>0.56 ± 0.09</td>
<td>0.60 ± 0.05</td>
<td>0.065 ± 0.011</td>
<td>0.075 ± 0.006</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dopamine = 51–100% of control sides (n = 9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side with lesions</td>
<td>7.11 ± 0.74</td>
<td>0.50 ± 0.05</td>
<td>0.50 ± 0.04</td>
<td>0.069 ± 0.007</td>
<td>0.070 ± 0.007</td>
<td>113 ± 3</td>
<td>99 ± 7</td>
</tr>
<tr>
<td>Contralateral</td>
<td>8.66 ± 0.44</td>
<td>0.48 ± 0.06</td>
<td>0.57 ± 0.05</td>
<td>0.055 ± 0.010</td>
<td>0.068 ± 0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine = 25–50% of control sides (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side with lesions</td>
<td>3.62 ± 0.50*</td>
<td>0.30 ± 0.05</td>
<td>0.33 ± 0.03</td>
<td>0.072 ± 0.012</td>
<td>0.078 ± 0.070</td>
<td>130 ± 25</td>
<td>119 ± 8</td>
</tr>
<tr>
<td>Contralateral</td>
<td>8.64 ± 0.65</td>
<td>0.48 ± 0.07</td>
<td>0.54 ± 0.04</td>
<td>0.056 ± 0.007</td>
<td>0.063 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine = 0–25% of control sides (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side with lesions</td>
<td>0.74 ± 0.20*</td>
<td>0.12 ± 0.04*</td>
<td>0.09 ± 0.03*</td>
<td>0.176 ± 0.051*</td>
<td>0.131 ± 0.024*</td>
<td>274 ± 28*</td>
<td>230 ± 24*</td>
</tr>
<tr>
<td>Contralateral</td>
<td>9.35 ± 0.64</td>
<td>0.52 ± 0.04</td>
<td>0.46 ± 0.02</td>
<td>0.060 ± 0.005</td>
<td>0.053 ± 0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rats that had been injected with 6-OH-dopamine (0.5–8 μg) into the right substantia nigra were divided into three groups with mild, moderate, or severe partial, unilateral nigrostriatal lesions, according to dopamine levels remaining in the striata with lesions (compared to those in contralateral sides without lesions). Data are given as means ± SEM.

* P < 0.01, differs from controls with no lesions and from contralateral sides.

† P < 0.01, differs from corresponding value in group with lesions with striatal dopamine levels reduced to 51–100% of control sides (one-way analysis of variance followed by Scheffe's test).

‡ Ratio in controls with no lesions theoretically equals 100%.
Table 2. Effect of tyrosine administration on brain tyrosine levels and on striatal dopamine concentrations in rats with moderate and severe nigrostriatal lesions

<table>
<thead>
<tr>
<th>Lesion severity</th>
<th>Group</th>
<th>Striatal dopamine concentrations</th>
<th>Brain tyrosine, ( \mu g/g )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control side, ( \text{ng/mg} )</td>
<td>Side with lesions, ( \text{ng/mg} )</td>
</tr>
<tr>
<td>Moderate (5 ( \mu g ) of 6-OH-dopamine)</td>
<td>(( n = 6 ))</td>
<td>8.04 ± 1.15</td>
<td>2.81 ± 0.53*</td>
</tr>
<tr>
<td></td>
<td>Tyrosine, 250 mg/kg (( n = 8 ))</td>
<td>7.86 ± 0.92</td>
<td>2.91 ± 0.47*</td>
</tr>
<tr>
<td>Severe (8 ( \mu g ) of 6-OH-dopamine)</td>
<td>Control (( n = 22 ))</td>
<td>7.80 ± 0.72</td>
<td>0.98 ± 0.29*</td>
</tr>
<tr>
<td></td>
<td>Tyrosine, 100 mg/kg (( n = 11 ))</td>
<td>8.16 ± 1.30</td>
<td>1.02 ± 0.24*</td>
</tr>
<tr>
<td></td>
<td>Tyrosine, 250 mg/kg (( n = 19 ))</td>
<td>7.71 ± 1.26</td>
<td>0.65 ± 0.21*</td>
</tr>
<tr>
<td></td>
<td>Valine, 500 mg/kg (( n = 7 ))</td>
<td>8.05 ± 0.96</td>
<td>0.77 ± 0.17*</td>
</tr>
</tbody>
</table>

Rats were injected unilaterally with 5 or 8 \( \mu g \) of 6-OH-dopamine into the right substantia nigra. Tyrosine (methyl ester form) or valine was injected intraperitoneally and rats were decapitated 1 hr later. Control rats with lesions were injected with saline. Data are given as means ± SEM. Data were analyzed by one-way analysis of variance followed by Scheffe’s test.

* \( P < 0.001 \), differs from corresponding control side.

† \( P < 0.01 \), differs from corresponding control side and from values on sides with lesions of rats injected with 5 \( \mu g \) of 6-OH-dopamine.

†† \( P < 0.01 \), differs from control value in same group.

with lesions were the same as those in the sides without lesions. Ratios of dopac (or HVA) to dopamine in the sides with lesions, expressed for each animal as percentages of the same ratios in the contralateral sides, were close to 100%, indicating that dopamine release from surviving striatal dopaminergic neurons had remained unaltered (Fig. 1). In these animals, the dopac/dopamine and HVA/dopamine ratios did not change significantly after tyrosine administration (Fig. 1). As noted in the initial experiments (Table 1), these ratios were elevated in striata with lesions of rats with more severe nigrostriatal lesions (Fig. 1). In such animals, tyrosine administration (250 mg/kg) produced further significant increases in striatal dopac/dopamine and HVA/dopamine ratios over those seen in control rats with severe lesions (by approximately 50% and 70%, respectively; Fig. 1). These ratios were not significantly affected by a lower dose of tyrosine (100 mg/kg) or by valine (500 mg/kg).

![Graph showing effect of exogenous tyrosine on striatal dopamine release by surviving neurons after partial, unilateral nigrostriatal lesions.](image-url)

**Fig. 1.** Effect of exogenous tyrosine on striatal dopamine release by surviving neurons after partial, unilateral nigrostriatal lesions. Injections of 5 and 8 \( \mu g \) of 6-OH-dopamine into the right substantia nigra reduced ipsilateral striatal dopamine concentrations to 25–50% and to less than 25%, respectively, compared to those in sides without lesions. Rats were injected with amino acids as shown and were killed 1 hr later. The ratios of dopac and HVA concentrations to that of dopamine (expressed as percentages of the same ratios on the control sides) provide indices for the release of dopamine per nigrostriatal neuron. In control rats, these ratios were increased after severe nigrostriatal lesions (different from value of controls with moderate lesions, \( P < 0.01 \)). In rats with severe nigrostriatal lesions, these ratios were further increased by 250 \( \mu g \) of tyrosine per kg (different from ratio of controls with severe lesions, \( P < 0.05 \)). Columns represent means ± SEM. Table 2 shows numbers of animals and brain tyrosine concentrations. Data were analyzed by analysis of variance followed by Scheffe’s test.
DISCUSSION

These data show that, in an individual animal, tyrosine administration can either accelerate striatal dopamine release or fail to do so, depending on the apparent firing rates of nigrostriatal dopaminergic neurons. In rats with severe, unilateral nigrostriatal lesions (striatal dopamine levels reduced to 25% or less of controls), the ratios of the dopamine metabolites dopac or HVA to dopamine were significantly increased in striata ipsilateral to the lesions and were increased further by tyrosine administration. However, in rats with less severe unilateral lesions, the metabolite/dopamine ratios in striata with lesions were the same as on the control sides with no lesions and were unaffected by tyrosine administration. These findings support the hypothesis (8) that the firing rate of a nigrostriatal dopaminergic neuron controls the extent to which the neuron's dopamine synthesis and release rates are affected by changes in precursor (i.e., tyrosine) availability. Corresponding results were obtained in studies using other treatments that accelerate the firing rates of dopaminergic neurons; tyrosine administration increased dopamine release in striata of haloperidol-pretreated rats (6) and in striatal and hypothalamic tissues of chronically reserpinized rats (19), but not in control rats. Similar mechanisms may operate in cholinergic (20–22), noradrenergic (4, 5), and serotonergic (23) neurons.

In studies using electrical stimulation of the substantia nigra, when the firing rates of nigrostriatal neurons were accelerated, striatal dopac and HVA levels increased but dopamine concentrations were unchanged (24, 25). Hence, changes in striatal dopac and HVA levels may reflect alterations in dopamine release and in the firing rates of nigrostriatal dopaminergic neurons. Assuming that dopamine is distributed equally among striatal dopaminergic neurons, the ratios of dopac or HVA levels to dopamine levels in the striatum indicate dopamine release per nigrostriatal neuron and thus measure the average firing rates of these neurons. In rats with partial, unilateral nigrostriatal lesions of varying severity, the dopac/dopamine or HVA/dopamine ratios (and, by inference, dopamine release from surviving dopaminergic neurons) increase in the striata with lesions, but only when more than 75% of the nigrostriatal dopaminergic neurons have been destroyed. One possible mechanism causing these increases may be related to a decrease in the inhibitory action of the γ-aminobutyric-acid (GABA)-ergic striatonigral feedback system, resulting from reduced dopamine availability to postsynaptic receptors in the striatum with lesions (26, 27).

The present data indicate that striatal dopaminergic neurons that survive after severe, partial nigrostriatal lesions do not reach the limit of their capacity to increase dopamine synthesis and release rates and that they can be influenced by external manipulations like administration of tyrosine. This effect was observed after administration of 250 mg of tyrosine per kg, which tripled brain tyrosine concentrations. A smaller dose (100 mg/kg) induced mild, insignificant increases in HVA/dopamine and did not affect dopac/dopamine ratios in striata with lesions, although brain tyrosine levels increased significantly (Table 2 and Fig. 1). A similar dose of tyrosine produces only mild reductions in arterial blood pressure, but larger doses (above 200 mg/kg) maximally decrease blood pressure and increase brain methoxyhydroxyphenylethylglycol sulfate in spontaneously hypertensive rats (4); these higher doses also reduce serum prolactin and elevate striatal and hypothalamic dopac or HVA levels in chronically reserpinized rats (19). Tyrosine doses of 300–1000 mg/kg enhance dopa accumulation (after blockade of dopa decarboxylase) in different regions of the rat brain (28).

Tyrosine's effect on striatal dopamine release in rats with severe, partial nigrostriatal lesions seems to be specific. In rats with similar lesions given valine (also a large neutral amino acid, but not a precursor for dopamine synthesis), striatal dopamine release did not increase, suggesting that tyrosine's action is not due to a nonspecific effect of amino acid administration. It may be argued that the observed effects of exogenous tyrosine could result from its partial hydroxylation to dopa in peripheral tissues (such as the adrenals or the sympathetic nervous system), decarboxylation to tyramine peripherally or in brain (29, 30), or even by simply displacing dopamine from striatal dopaminergic neurons. However, dopa was undetectable in striatal tissues even after tyrosine administration. If tyramine is indeed formed from administered tyrosine in peripheral tissues, it probably could not cross the blood–brain barrier in appreciable amounts (31).

More critically, if tyrosine's action on nigrostriatal neurons was mediated through such mechanisms, striatal dopamine release induced by exogenous tyrosine would increase in rats with moderate, as well as in those with severe, partial nigrostriatal lesions. It is likely that tyrosine's effect on dopamine synthesis and release in striata with severe lesions is primarily due to its hydroxylation by tyrosine hydroxylase within the surviving hyperactive dopaminergic neurons.

The accelerated dopamine synthesis and release rates in surviving nigrostriatal dopaminergic neurons may compensate for loss of the other neurons. Such a mechanism may account for the emergence of clinical manifestations of Parkinson disease only after an extreme reduction in the number of striatal dopaminergic terminals, when compensatory hyperactivity in the remaining neurons is probably no longer adequate (10). These findings suggest that at this advanced stage of nigrostriatal degeneration, the capacity of the hyperactive remaining dopaminergic neurons to enhance dopamine synthesis and release further may become dependent on precursor (i.e., tyrosine) availability. Tyrosine shares its transport mechanism across the blood–brain barrier with other large neutral amino acids, and its entry into brain depends on the ratio of plasma tyrosine concentration to the sum of the plasma levels of these amino acids (32, 33). The therapeutic potency of exogenous L-dopa in parkinsonism (34), methyldopa's antihypertensive effect in spontaneously hypertensive rats, and methyldopa concentrations in brain (35, 36) varies inversely with dietary protein content. In a similar manner, changes in dietary protein content may alter brain tyrosine levels and produce consequent diurnal or day-to-day fluctuations (albeit subtle) in the severity of clinical manifestations of Parkinson disease.

L-Dopa is presently the most widely used antiparkinsonian drug, but its initial beneficial effect gradually decreases in many patients under long-term therapy (37, 38); therefore, it is now advocated that dopa should be reserved for the more advanced stages of the disease and that other therapeutic modalities be used in milder cases (39). Tyrosine administration might be helpful in reversing, at least temporarily, the neurological phenomena in the initial clinical stages of parkinsonism by further increasing dopamine synthesis and release rates of the surviving, hyperactive dopaminergic neurons. In addition, chronic dopa administration is associated with various side effects which may be derived partially from its conversion to dopamine by decarboxylase in striatal cells other than the dopaminergic terminals (40). In contrast, striatal tyrosine hydroxylase is localized almost exclusively within dopaminergic terminals (41). It is therefore conceivable that exogenous tyrosine will be hydroxylated in the parkinsonian striatum only in the remaining dopaminergic neurons and that the dopamine released thereafter from these terminals will be available predominantly to dopamine receptors.
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