Enhancement of Dopamine-Stimulated Adenylate Cyclase Activity in Rat Caudate after Lesions in Substantia Nigra: Evidence for Denervation Supersensitivity

dopamine receptor/nigro-striatal pathway/Parkinson’s disease/cyclic AMP

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ABSTRACT Unilateral radiofrequency lesions or chemical lesions with 6-hydroxydopamine were produced in the substantia nigra of rat brain in order to destroy dopaminergic innervations to caudate nucleus and thereby to produce functional denervation supersensitivity. Both types of lesions resulted in enhanced stimulation of caudate adenylate cyclase (EC 4.6.1.1) activity by dopamine at all dopamine concentrations tested, with more marked enhancement at the lower concentrations. Response to another dopamine agonist, 1-(3,4-dihydroxybenzyl)-4-(2-pyrimidinyl)piperazine (S584) was also enhanced. 6-Hydroxydopamine lesions resulted in selective enhancement of the dopamine-stimulated component of adenylate cyclase, whereas radiofrequency lesions resulted also in a marked decrease in basal activity. It is postulated that the basal activity of caudate represents primarily an adenylate cyclase distinct from that stimulated by dopamine and destroyed only by the less selective radiofrequency lesion. The enhancement of dopamine-sensitive adenylate cyclase after lesions serves as indirect evidence for a significant role of this system in the transmitter function of dopamine and indicates, furthermore, that it is directly involved in dopamine receptor supersensitivity in vivo produced by denervation.

The catecholamine neurotransmitters regulate a variety of metabolic processes by enhancing the formation of adenosine 3',5'-cyclic monophosphate (cyclic AMP), generally by way of β-adrenergic receptors (1), but in the central nervous system in some instances also by way of α-receptors (2, 3). Recent reports have demonstrated the presence of dopamine-stimulated adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] in retina (4, 5) and in brain caudate nucleus, nucleus acumbens, olfactory tubercle, and cerebral cortex (6-11).

A second messenger function for cyclic AMP in noradrenergic transmission from locus coeruleus to cerebellar Purkinje cell (12) and in dopaminergic transmission from interneurons in sympathetic ganglia (13) has been proposed. However, this role of cyclic AMP in Purkinje cells has been disputed (14), and the involvement of cyclic AMP in central dopaminergic transmission has yet to be established.

Denervation-supersensitivity is a phenomenon involving functional alteration in synaptic process. Various studies have shown that sympathetic denervation of adrenergically innervated organs results in supersensitivity to catecholamines (15, 16). Two separate components have been postulated in the development of such supersensitivity: an acute component due to presynaptic degeneration and decreased presynaptic re-uptake of catecholamines, and a more chronic or gradually developing component involving increased sensitivity or response at the postsynaptic receptor level. Of possible relevance to the mechanisms underlying the second component is the finding that superior cervical ganglionectomy results, after four weeks, in increased NaF-stimulated and norepinephrine-stimulated adenylate cyclase in homogenates of pineal gland (17). In addition, other recent studies have suggested that intraventricular administration of 6-hydroxydopamine (and the presynaptic functional denervation of neuronal catecholamine receptors that results) produces increased sensitivity (17) or increased maximal response of brain slices to norepinephrine in vitro (18-20).

We have recently reported preliminary studies indicating a chronic alteration of adenylate cyclase activity in the rat caudate after radiofrequency lesions in the substantia nigra (21). The present paper describes the effects of both radiofrequency and 6-hydroxydopamine lesions on adenylate cyclase activity in caudate nucleus. This study represents an attempt to evaluate the increased postsynaptic dopamine receptor sensitivity seen after denervation of a dopaminergic brain system with respect to dopamine-stimulated adenylate cyclase.

MATERIALS AND METHODS

Lesions. Unilateral radiofrequency or 6-hydroxydopamine lesions were placed in the substantia nigra of male Sprague-Dawley (Holtsman) rats by standard surgical and stereotaxic techniques. For the chemical lesions, 8 μg of 6-hydroxydopamine in 4 μl of an isotonic saline solution containing 0.2 mg/ml of ascorbic acid was delivered through an acutely implanted cannula at a rate of 1 μl/min. All animals were of the same weight (240-250 g) at the time of surgery. After full recovery from surgery, effectiveness of the lesion was confirmed by observations of rotational behavior induced by systemic injections of apomorphine or Piribedil (ET-495). All animals used in this study showed consistent rotational behavior. The animals were then killed (10-36 days after lesion for the animals with radiofrequency lesions and 100-120 days after lesion for animals with 6-hydroxydopamine lesions). For each experimental animal killed, a control animal without a lesion (from the same shipment as the experimental animal and maintained in identical fashion for the same period of time) was also killed. Left and right caudates were removed within 2-3 min from both experimental and control animals.
level of mesencephalic and ventral tectable lesion were adjacent structures with all-glass hormone to Tris-maleate buffer (pH 7.4) by placing 2.5 mM 324 10 M 8 mM 324 10 M 8 mM Isopropylnorepinephrine, 10 M 3884 Medical Type Cyclase 8.4 frequency 7.8 + dopamine 75 7.2 + of control No lesion Right-side lesion

<table>
<thead>
<tr>
<th>Additions to assay</th>
<th>pmol of cyclic AMP/mg of protein per 2.5 min</th>
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<tbody>
<tr>
<td>Basal</td>
<td>302 ± 73</td>
</tr>
<tr>
<td>Dopamine, 10 M</td>
<td>772 ± 88</td>
</tr>
<tr>
<td>Apomorphine, 10 M</td>
<td>620 ± 102</td>
</tr>
<tr>
<td>S584, 10 M</td>
<td>664 ± 53</td>
</tr>
<tr>
<td>Dopamine, 10 M + pimozone, 1 M</td>
<td>335 ± 104</td>
</tr>
<tr>
<td>Dopamine, 10 M + fluphenazine, 10 M</td>
<td>344 ± 76</td>
</tr>
<tr>
<td>Dopamine, 10 M + propranolol, 10 M</td>
<td>785 ± 74</td>
</tr>
<tr>
<td>S584, 10 M + fluphenazine, 10 M</td>
<td>385 ± 36</td>
</tr>
<tr>
<td>Isopropylnorepinephrine, 10 M</td>
<td>351 ± 38</td>
</tr>
<tr>
<td>NaF, 8 M</td>
<td>324 ± 46</td>
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Assays were done as described in the text. Each value is an average of four experiments ± SE.

and transferred directly to homogenizing medium for adenylate cyclase assay. Portions of caudate were saved for dopamine estimation.

The remainder of the brain stem and midbrains from the animals with radiofrequency lesions were then fixed in formalin. Frozen sections cut at 40 μm were prepared and stained with cresyl violet for histological verification of the lesions. In general, the lesions in the animals responsive to apomorphine were modest in size (<0.5 mm in width and 1.0 mm in height) and limited to substantia nigra and immediately adjacent structures (cerebral peduncle, zona incerta). In most of these animals, the fullest extent was in the lateral portion of the substantia nigra, at about the anterior-posterior level of the ventral tegmental decussation. Animals not responsive to apomorphine were found either to have no detectable lesion at all or to have massive damage to the entire mesencephalic and ventral thalamic region.

**Adenylate Cyclase Assay.** Fresh caudates, immediately after removal from the brain, were gently homogenized by hand in all-glass homogenizers at 0–4°C in medium containing 2 mM Tris-maleate buffer (pH 7.4) plus 0.8 mM EGTA at a dilution of 1:75 (wt/vol). Aliquots of 50 μl each of homogenate were then incubated in a shaking-water bath at 30°C for 2.5 min in a final volume of 200 μl of incubation medium containing 80 mM Tris-maleate buffer (pH 7.4), 10 mM theophylline, 2 mM MgSO4, 0.5 mM ATP, and appropriate hormone or other test substances. The reaction was terminated by placing assay tubes in a boiling-water bath for 2.5 min.

**TABLE 2. Dopamine content of caudate nucleus from normal rats and rats with lesions of the substantia nigra**

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>μg of dopamine/g of tissue (wt weight)</th>
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<tbody>
<tr>
<td></td>
<td>No lesion</td>
</tr>
<tr>
<td></td>
<td>Left caudate</td>
</tr>
<tr>
<td>6-Hydroxy-dopamine</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td>Radiofrequency</td>
<td>8.4 ± 0.5</td>
</tr>
</tbody>
</table>

Each value is an average of 5 experiments ± SE.

**RESULTS**

**General Characteristics of Dopamine-Stimulated Adenylate Cy clase.** Dopamine (10 M) caused a 2-fold or greater stimulation of adenylate cyclase activity of normal rat caudate nucleus (Table 1). Isopropynorphinephrine, a potent stimulator of β-adrenergic receptor systems, and NaF, a non-specific stimulator of adenylate cyclase in many tissues including whole rat brain, showed no effect on caudate enzyme under these assay conditions. In addition to dopamine, both apomorphine, a dopamine receptor stimulator, and 1-(3,4-dihydroxybenzyl)-4-(2-pyrimidinyl) piperazin (S584) stimulated the caudate cyclase system (Table 1). S584 is a metabolite of Piribedil (ET 495) (23), an antiparkinson drug with dopamine-receptor stimulating properties in vivo (24). We
have presented evidence elsewhere that Pribedil, although itself without direct effect on caudate adenylate cyclase, does activate cyclase and stimulates cyclic AMP levels when incubated with intact caudate slices in vitro (8, 25). Miller and Iverson have also reported the stimulatory effect of SS84 on caudate cyclase (26). The stimulatory effect of dopamine and SS84 was blocked by neuroleptic drugs such as fluphenazine, chlorpromazine, pimozide, and haloperidol, but not by β-adrenergic receptor blocking agents such as propranolol and sotalol (representative data are summarized in Table 1). Stimulation by apomorphine and blockade by neuroleptic agents have been reported as specific properties of dopamine-stimulated, central adenylate cyclase systems (5, 6, 8, 10).

**Effect of 6-Hydroxydopamine and Radiofrequency Lesions on Dopamine Content of Caudate Nucleus.** The dopamine content of ipsilateral caudate nucleus was decreased significantly, as compared to the contralateral side, after either 6-hydroxydopamine or radiofrequency lesions in the nigrostriatal pathway (Table 2). In these studies the chemically produced lesion with 6-hydroxydopamine was clearly the more effective procedure, even though only about 14% of dopamine remained after the radiofrequency lesion.

**Effects of Radiofrequency Lesions in the Substantia Nigra on Adenylate Cyclase Activity of Caudate Nucleus.** As shown in Table 3, basal adenylate cyclase activity of the left caudate from animals with radiofrequency lesions is about the same as those of left and right caudate from animals (control) without lesions. However, the basal activity is significantly decreased (Table 3) in ipsilateral caudate of animals with lesions. Dopamine or SS84 caused a 2- to 3-fold stimulation of adenylate cyclase in both caudates of animals without lesions and contralateral caudate of animals with lesions. However, in the ipsilateral caudate the stimulation of enzyme by dopamine or SS84 was more than 7-fold at low concentration, suggesting a supersensitivity in the dopamine receptor after lesions were made in the substantia nigra. NaF and isopropylnorepinephrine were without effect in both groups of animals. The absolute increment in adenylate cyclase due to 100 μM, 1 μM dopamine, or 0.1 μM SS84 was also significantly increased after lesion (Table 3), suggesting increase in total receptor activity as well as in sensitivity (see also below and Discussion).

**Effects of 6-Hydroxydopamine Lesions in the Substantia Nigra on Adenylate Cyclase Activity of Caudate Nucleus.** The results obtained with 6-hydroxydopamine lesions in the substantia nigra are shown in Fig. 1. Unlike the radiofrequency lesions, no difference in basal activity of adenylate cyclase was observed. However, a supersensitivity to low concentrations of dopamine was clearly seen in the ipsilateral caudate as compared to the contralateral caudate of lesioned animals or left and right caudate of control animals (Fig. 1). The differences in enzyme activity of right and left caudate at 0.1 μM, 1 μM, and 100 μM dopamine concentration are each statistically significant (Fig. 1B). As shown for control animals and for those with radiofrequency lesions, isopropylnorepinephrine and NaF were also without effect in animals with 6-hydroxydopamine lesions (data not shown). The results presented in Fig. 1B indicate no change in basal activity after 6-hydroxydopamine lesions but a significantly enhanced stimulation of adenylate cyclase by catecholamines. This finding suggests a relation-

![Fig. 1. Influence of 6-hydroxydopamine lesions on adenylate cyclase activity. Substantia nigra lesions on the right side and adenylate cyclase assayed of caudate nucleus were carried out as described in the text. R, Right caudate (○); L, left caudate (○). (A), Control animals; (B), lesioned animals. Each point represents an average of four replicate experiments ± SE.](image)
procedure to result in appreciable loss in activity of rat caudate. It may also be noted that the specific activity (both basal and with dopamine) of control rat caudate adenylate cyclase reported in this study is greater than (more than twice) that reported by other investigators (9, 26), probably due to very rapid removal and assay of tissue.

The enhanced dopamine response of adenylate cyclase from denervated caudate extends also to another dopamine agonist, SS54, the active catechol metabolite of Piribedil (ET495, Trivastal) (8, 25, 26). On the other hand, this enhanced response does not appear to be associated with transformation to or addition of a less selective catecholamine receptor, since isopropyl noradrenephrine is still inactive after denervation. Isopropyl noradrenephrine is typically a β-agonist, although in primate (but not rodent) caudate and retina adenylate cyclase systems it is also a dopamine-receptor agonist (effect blocked by neuroleptic drugs but not by propranolol) (7, 8).

The rat caudate nucleus contains not only very high dopamine-stimulated adenylate cyclase activity, but also much higher basal (control) activity than does the surrounding area, cerebral cortex, or whole brain. This basal activity of caudate is probably not due to release of endogenous dopamine since it is not inhibited by homogenization in the presence of potent dopamine-blocking agents under conditions that prevent the effect of added dopamine (unpublished studies) and since it is not altered by complete depletion of dopamine due to 6-hydroxydopamine lesions, shown in the present study. Nevertheless, this basal activity was decreased to about one-third the control value by relatively small radiofrequency lesions (Table 3). These results suggest that the major portion of the basal adenylate cyclase activity of caudate is anatomically distinct from the postsynaptic dopamine-sensitive cyclase system that is enhanced by both types of lesions.

Enhancement of dopamine-stimulated adenylate cyclase by denervation may possibly be mediated by release of the postsynaptic neuron from an inhibited or restrained state normally present and possibly due directly or indirectly to an action of cyclic AMP and/or dopamine. The component of supersensitivity involving loss of presynaptic sites for re-uptake of catecholamine is presumed to be not operative in this study since that component would be destroyed by the extremely hypotonic conditions used for homogenization.) Several possible alterations in the adenylate cyclase system might result in enhancement of dopamine response, including: (i) relative increase in catalytic component of adenylate cyclase (with or without pre-existing receptor excess), (ii) increase in both dopamine-receptor and catalytic components of adenylate cyclase, (iii) selective increase in the dopamine-receptor component, and (iv) increase in efficacy of "coupling" of dopamine-receptor activation to stimulation of the catalytic component. The findings reported here of markedly enhanced sensitivity to dopamine, together with more moderate increase in maximal stimulation by dopamine, are against possibility i and are in favor of ii or more particularly iii or iv. In contrast to previously reported studies of pineal adenylate cyclase, where denervation was found to result in increased response to fluoride ion (18), we have no such independent measure of catalytic activity available as yet for the caudate system. Also, since, as discussed earlier, we believe that the major portion of the basal activity in caudate may be due to a separate dopamine-independent cyclase system, lack of enhancement of this activity after 6-hydroxydopamine lesion provides evidence neither for nor against an increase in the catalytic subunit of cyclase. Further studies will be needed to more completely evaluate the nature of the response of cyclase to denervation. The present data permit us to conclude, however, that sensitivity and, to a lesser extent, total capacity of the dopamine receptor component of the caudate adenylate cyclase system increase after denervation. This finding indicates that adenylate cyclase is directly involved in the phenomenon of denervation supersensitivity in a major central dopaminergic neuronal pathway. Also, it provides indirect support for the hypothesis that cyclic AMP plays a role in catecholamine-mediated synaptic events.

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