MOLECULAR PHARMACOLOGY, 12, 639-648

Stimulation by Dopamine of Adenosine Cyclic 3',5'-Monophosphate Formation in Rat Caudate Nucleus: Effect of Lesions of the Nigro-neostriatal Pathway

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(Received August 25, 1975)

SUMMARY

KRUEGER, BRUCE K., FORN, JAVIER, WALTERS, JUDITH R., ROTH, ROBERT H. & GREENGARD, PAUL (1976) Stimulation by dopamine of adenosine cyclic 3',5'-monophosphate formation in rat caudate nucleus: effect of lesions of the nigro-neostriatal pathway. *Mol. Pharmacol.*, 12, 639-648.

Stimulation by dopamine of cyclic 3',5'-AMP formation in slices and homogenates of caudate nucleus was studied in rats that exhibited apomorphine-induced contralateral circling following unilateral lesions of the nigro-neostriatal system. Electrothermic lesions or 6-hydroxydopamine injections were made in the ascending dopaminergic pathway, and resulted in 93-95% depletion of endogenous dopamine in the ipsilateral caudate nucleus. An increase in the ability of submaximal concentrations of dopamine to stimulate cyclic AMP formation was observed, 3-30 days after placement of the lesions, in slices prepared from the caudate nucleus on the lesioned side. In contrast to the results obtained with slices, the stimulation by dopamine of adenylate cyclase activity in homogenates of caudate nucleus was the same on the lesioned as on the control side. The data suggest that contralateral circling, induced by dopamine agonists in animals with unilateral lesions in the nigro-neostriatal pathway, may be due to an increased ability of the dopamine agonists to stimulate cyclic AMP formation on the lesioned side. This increased ability of dopamine agonists to raise cyclic AMP levels appears to result primarily from the lesion-induced destruction of presynaptic dopamine-containing nerve terminals in the caudate nucleus rather than from a change in dopamine-sensitive adenylate cyclase in the postsynaptic cells. The stimulation by dopamine of cyclic AMP formation in slices of caudate nucleus may be a useful biochemical model for the study of denervation supersensitivity in the nigro-neostriatal pathway.

This work was supported by United States Public Health Service Grants NS-08440, MH-17387, and MH-14092, and by a grant from Hoffmann-La Roche.

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INTRODUCTION

Considerable evidence has accumulated suggesting that dopamine may function as a neurotransmitter in the mammalian central nervous system. Fluorescence histochemical techniques have revealed several dopaminergic pathways, including the nigro-neostriatal system, in the rat and human brain (1-4). Iontophoretically

applied dopamine depresses the firing rate of neurons in the caudate nucleus (5–7), thus mimicking the effects of electrical stimulation of the nigro-neostriatal pathway (5, 6). Extrapyramidal motor dysfunction associated with Parkinson's disease has been attributed to a depletion of dopamine in the caudate nucleus, resulting from degeneration of dopamine-containing neurons whose cell bodies are located in the substantia nigra (8).

A variety of evidence suggests that either denervation or depletion of endogenous catecholamines results in an increased sensitivity of postsynaptic catecholamine receptors to neurotransmitters (9, 10). *l*-Norepinephrine stimulation of rat pineal adenylate cyclase activity is enhanced by prior superior cervical ganglionectomy (11, 12). Destruction of adrenergic nerve terminals by 6-hydroxydopamine causes an increase in the stimulation by *l*-norepinephrine of cyclic 3′,5′-AMP formation in slices of rat cerebral cortex (13–15). Increased norepinephrine-induced cyclic AMP synthesis is also observed following depletion of brain catecholamines by reserpine (16).

Apparent increases in receptor sensitivity are also observed in dopaminergic systems following denervation. Injection of 6-hydroxydopamine into the caudate nucleus (7) causes an increase in the sensitivity of neurons in the caudate nucleus to iontophoretically applied dopamine, the endogenous neurotransmitter in the nigro-neostriatal system. In rats and mice with unilateral lesions of the nigro-neostriatal system, administration of apomorphine or L-dopa results in circling in a direction contralateral to the side of the lesion (17–20); the rate of contralateral circling behavior has been shown to correlate with the degree of depletion of dopamine in the caudate nucleus on the lesioned side (19). It has been suggested that this circling behavior is the result of a supersensitivity of dopamine receptors on the denervated side caused by the loss of dopaminergic input (17–19).

Recent evidence suggests that dopamine may exert its physiological effects in mammalian superior cervical sympathetic ganglia and in the caudate nucleus by increasing intracellular levels of cyclic AMP. In these tissues, dopamine raises cyclic AMP levels (21–23), and cyclic AMP mimics the electrophysiological effects of dopamine (7, 24). A dopamine-sensitive adenylate cyclase has been found in homogenates of the ganglion (21) and caudate nucleus (25). Moreover, studies in many laboratories have provided considerable evidence suggesting that the dopamine-binding portion of the dopamine-sensitive adenylate cyclase, observed in homogenates of caudate nucleus as well as of other brain regions, may be the dopamine receptor (25–34).

In view of the possible association between the dopamine receptor and dopamine-sensitive adenylate cyclase, it might be expected that lesions of the nigro-neostriatal system would alter the activity of this enzyme in homogenates of the caudate nucleus. Several groups of workers have investigated this possibility. One group has reported an increase in sensitivity to dopamine of adenylate cyclase in homogenates from the ipsilateral side of rats with unilateral 6-hydroxydopamine or radiofrequency lesions in the substantia nigra (35). However, other groups of investigators, in either published (36) or unpublished[3] studies, have been unable to show a change in dopamine-sensitive adenylate cyclase activity in rats and mice exhibiting turning behavior due to lesions of the nigro-neostriatal system. In the present study we have investigated the effects of electrothermic and 6-hydroxydopamine-induced lesions of the nigro-neostriatal pathway on dopamine-stimulated formation of cyclic AMP, not only in homogenates but also in slices of rat caudate nucleus.

MATERIALS AND METHODS

Placement of lesions in nigro-neostriatal pathway. Male Sprague-Dawley rats, 220–280 g, from Charles River Breeding Laboratories, were anesthetized with chloral hydrate (400 mg/kg intraperitoneally).

[3] J. W. Kebabian, U. Ungerstedt, B. J. Hoffer, G. R. Siggins, and P. Greengard, unpublished observations; M. Goldstein and U. Ungerstedt, personal communication; L. L. Iversen, personal communication.

Unilateral electrothermic lesions were placed in the nigro-neostriatal pathway at a point corresponding to section A 3180 μ according to König and Klippel (37), essentially as described by Hökfelt and Ungerstedt (38) and modified by Walters *et al.* (39). The animals were killed 3–30 days later. Upon the death of each rat the caudate nuclei were removed for assay of endogenous dopamine and/or cyclic AMP formation *in vitro*. The remaining brain tissue was fixed in buffered formalin, sectioned, and stained with cresyl violet for histological verification of the location and extent of the lesion.

6-Hydroxydopamine lesions were made in the nigro-neostriatal pathway just anterior to the substantia nigra. The injection cannula was lowered to a point just above the ascending dopaminergic bundle, 0.2 mm dorsal to the position used for electrothermic lesioning, and 8 μg of 6-hydroxydopamine in a total volume of 4 μl of vehicle (0.2 M ascorbic acid in 0.9% NaCl) were then injected at a rate of 2 μl/min. Minimal nonspecific damage to the tissue in the vicinity of the injection cannula tip was found under the experimental conditions used.

As a measure of the effectiveness of the lesions, dopamine was determined by a modification of the method of Laverty and Taylor (40) as described previously (41).

Changes in motor behavior following administration of apomorphine were studied in rats which had received unilateral lesions in the nigro-neostriatal pathway. Animals (10–20 days after lesioning) were placed in translucent white plastic buckets with transparent covers, and the absence of spontaneous turning behavior was verified. Animals then received apomorphine (0.3–3 mg/kg intraperitoneally in 0.2 ml of distilled H_2O). The number of net turns per minute was subsequently recorded for a 30-min period following apomorphine administration.

Measurement of cyclic AMP levels in slices. For measurement of cyclic AMP levels in slices of caudate nucleus, tissue was prepared and incubated as described previously (22) with modification. Caudate nuclei (caudate-putamen, excluding globus pallidus) from 7–10 rats were pooled and placed in Krebs-Ringer-bicarbonate buffer (containing NaCl, 124 mM; KCl, 5.0 mM; $NaHCO_3$, 26 mM; $CaCl_2$, 0.8 mM; $MgCl_2$, 1.3 mM; KH_2PO_4, 1.2 mM; and glucose, 10 mM, pH 7.4, equilibrated with 95% O_2–5% CO_2) at room temperature. Tissues from control and lesioned sides were prepared and assayed for cyclic AMP separately. Slices were prepared on a McIlwain tissue chopper, using a 0.26-mm excursion. The tissue was chopped twice, the second time in a direction perpendicular to the first. The resulting slices were suspended in Krebs-Ringer-bicarbonate buffer (50 mg of tissue per milliliter) at 37°. The slices were allowed to settle, and the supernatant was removed. Fresh Krebs-Ringer-bicarbonate buffer was added, and the tissue was resuspended and incubated for 60 min. Then 0.2-ml aliquots of the suspension of slices were added to tubes containing 3-isobutyl-1-methylxanthine (a phosphodiesterase inhibitor; final concentration, 1 mM) and various concentrations of dopamine or other test substances in a volume of 0.1 ml of Krebs-Ringer-bicarbonate buffer so that the final volume was 0.3 ml. For each experimental condition, three to six replicate assay tubes were incubated. Each tube was flushed with 95% O_2–5% CO_2, sealed, and incubated for 15 min. The incubation was terminated by placing the tubes in a boiling water bath for 9 min. The tubes were centrifuged at 2000 × *g* for 5 min, and duplicate 0.05- or 0.10-ml aliquots of the supernatant were assayed for cyclic AMP by the method of Brown *et al.* (42). The remaining material was dissolved in 0.1 N NaOH for protein determination by the method of Lowry *et al.* (43). Within each experiment, cyclic AMP concentrations were calculated as a percentage of the basal level. The results obtained in two to five separate experiments were combined, and the data reported represent the means ± standard errors for 8–16 replicate samples for each experimental condition.

Measurement of adenylate cyclase activity in homogenates. Dopamine-sensitive adenylate cyclase was assayed as described previously (25) with minor modifi-

cation. Caudate nuclei from control and lesioned sides were homogenized separately in 100 volumes of 4 mM Tris-Cl–4 mM EGTA,[4] pH 7.4, using 12 manual up-and-down strokes in a loosely fitting glass-Teflon tissue grinder. Aliquots (50 μl) of this homogenate were added to the standard assay mixture (final volume, 0.5 ml) for measurement of adenylate cyclase activity. This assay mixture contained (final concentrations) Tris-maleate, 80 mM; $MgCl_2$, 2.0 mM; EGTA, 0.6 mM; 3-isobutyl-1-methylxanthine, 1.0 mM; ATP, 0.5 mM; and the indicated concentrations of dopamine, pH 7.4. The assay mixture was incubated in the absence of ATP for 20 min at 0° and then for 1 min at 30°. The adenylate cyclase reaction was then initiated by the addition of 0.1 ml of ATP. The reaction was allowed to proceed for 3 min at 30° and was terminated by boiling for 2 min. For each experimental condition, samples were incubated in triplicate, and duplicate 50-μl aliquots of each sample were assayed for cyclic AMP by the method of Brown *et al.* (42).

Materials. Dopamine HCl, *l*-isoproterenol HCl, and *l*-norepinephrine HCl were obtained from Sigma. 3-Isobutyl-1-methylxanthine and 6-hydroxydopamine were purchased from Aldrich. Cyclic [*G*-^{3}H]AMP (25–30 Ci/mmole) was obtained from New England Nuclear. All other reagents were of the highest grade commercially available.

RESULTS

Determination of effectiveness of lesions. Rats which received either unilateral electrothermic lesions or unilateral 6-hydroxydopamine lesions in the nigro-neostriatal pathway were found to have 93–95% depletion of endogenous dopamine in the ipsilateral caudate nucleus 10 days after placement of the lesions (Table 1). This effect occurred rapidly: rats with unilateral electrothermic lesions in the nigro-neostriatal pathway were found to have 94% depletion of endogenous dopamine on the ipsilateral side 3 days after placement of the lesions (data not shown).

[4] The abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid.

TABLE 1

Effect of lesions of nigro-neostriatal system on dopamine content of caudate nucleus

The dopamine concentration was determined in caudate nuclei of rats that had received either unilateral electrothermic lesions (n = 12) or unilateral 6-hydroxydopamine injections (n = 3) in the nigro-neostriatal pathway. Dopamine measurements were made 10 days after lesioning.

Side	Dopamine content	
	Electrothermic lesion	6-Hydroxydopamine lesion
	μg/g tissue	
Control	10.0 ± 0.7	10.5 ± 0.4
Lesioned	0.7 ± 0.2	0.5 ± 0.3

The electrothermic lesion was found to be spherical, approximately 1.0 mm in diameter, and encompassed the ascending dopaminergic pathway dorsal to the medial forebrain bundle (44). It also included part of the medial forebrain bundle, part of the fields of Forel, and parts of the medial edge of the crus cerebrae and subthalamic nuclei. In each experiment with slices of caudate nucleus, tissue from 7–10 animals was pooled; in no experiment was more than one animal found, by subsequent histological analysis, to have either an incomplete lesion or a lesion that resulted in destruction of an area greater than that described above.

Effects of lesions on dopamine- and apomorphine-induced cyclic AMP formation in slices. Since denervation supersensitivity, as measured behaviorally, is nearly maximal within 10–15 days after placement of lesions (17), we studied the effect of dopamine on cyclic AMP levels in slices of caudate nucleus from rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10–15 days earlier (Fig. 1A). In slices of caudate nucleus prepared from the control side, a half-maximal increase in cyclic AMP content was caused by about 40 μM dopamine. [In a previous study (22), half-maximal stimulation of cyclic AMP levels was observed with 60 μM dopamine.] In slices prepared from the lesioned side, 5 μM dopamine caused a half-maximal increase in cyclic AMP levels. An increase in sensitivity to dopamine was also observed using

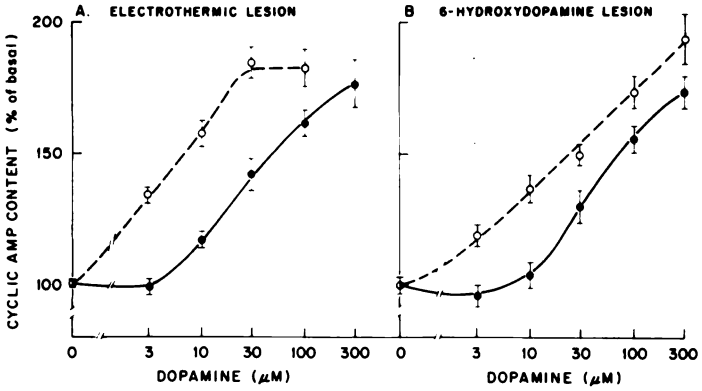

FIG. 1. *Effect of various concentrations of dopamine on cyclic AMP content of slices of caudate nucleus from control (●) and lesioned (○) sides of rats which had received unilateral lesions in the nigro-neostriatal pathway 10–15 days earlier*

Cyclic AMP levels were calculated as a percentage of the basal level of cyclic AMP observed in the absence of added dopamine, and are expressed as means ± standard errors for 10–15 samples. A. Electrothermic lesion. Basal levels of cyclic AMP were 10.7 ± 0.5 and 10.8 ± 0.4 pmoles/mg of protein (n = 15) on the control and lesioned sides, respectively. B. 6-Hydroxydopamine lesion. Basal levels of cyclic AMP were 10.7 ± 0.5 and 11.0 ± 0.3 pmoles/mg of protein (n = 10) on the control and lesioned sides, respectively.

rats with 6-hydroxydopamine lesions in the nigro-neostriatal pathway (Fig. 1B). Although basal levels of cyclic AMP varied slightly from one experiment to another and from the control to the lesioned side, no consistent effect of lesioning on basal levels of cyclic AMP was observed following either electrothermic or 6-hydroxydopamine lesions in the nigro-neostriatal pathway.

The effect of lesioning on apomorphine-induced cyclic AMP formation was variable. In four of nine experiments, using animals that had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10–15 days earlier, submaximal concentrations of apomorphine caused greater increases in cyclic AMP levels on the lesioned side than on the control side. However, in the remaining five experiments, no effect of electrothermic lesions was observed on the apomorphine-induced increase in cyclic AMP content of caudate nucleus slices. The reason for the poor reproducibility of the effect of lesions on apomorphine stimulation of cyclic AMP levels *in vitro*, in contrast to the more consistent apomorphine-induced circling behavior in lesioned animals, is not known.

Contralateral circling behavior is observed, following administration of dopaminergic agonists to rats and mice, as early as 3 days after unilateral lesioning of the nigro-neostriatal pathway (17, 19). Therefore the effect of various concentrations of dopamine on cyclic AMP levels was examined in slices of caudate nucleus from rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 3 days earlier (Fig. 2). In slices from the control side, half-maximal stimulation was obtained with 50–60 μM dopamine; in contrast, in slices prepared from the caudate nucleus on the lesioned side, half-maximal stimulation was seen with about 10–12 μM dopamine. An increased elevation of cyclic AMP levels in the caudate nucleus by submaximal concentrations of dopamine was also observed on the lesioned side in rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 30 days earlier (data not shown). The increase in sensitivity to dopamine at 30 days was similar in magnitude to that observed in rats used 10–15 days after placement of lesions (Fig. 1A).

Effect of lesions on dopamine-sensitive adenylate cyclase activity in homogenates. Dopamine-sensitive adenylate cyclase was assayed in homogenates of caudate nucleus from rats which had received unilat-

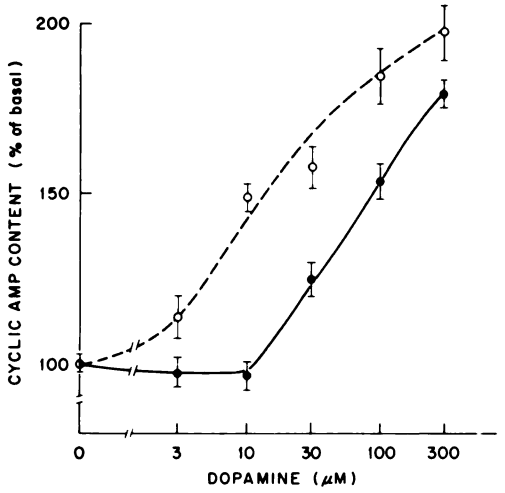

FIG. 2. *Effect of various concentrations of dopamine on cyclic AMP content of slices of caudate nucleus from control (●) and lesioned (○) sides of rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 3 days earlier*

Cyclic AMP levels were calculated as a percentage of the basal level of cyclic AMP observed in the absence of added dopamine, and are expressed as means ± standard errors for 10 samples. Basal levels of cyclic AMP were 9.7 ± 0.4 and 8.7 ± 0.3 pmoles/mg of protein on the control and lesioned sides, respectively.

eral lesions in the nigro-neostriatal pathway 16–20 days earlier. The effects of electrothermic lesions (Fig. 3A) and of 6-hydroxydopamine-induced lesions (Fig. 3B) in the nigro-neostriatal pathway were studied. In both cases only rats which exhibited contralateral circling behavior in response to apomorphine were used.[5] Half-maximal stimulation of adenylate cyclase activity was observed with 3–4 μM dopamine in homogenates of caudate nuclei from control as well as from lesioned sides. This K_a value for dopamine is in agreement with previously published values (4–5 μM, refs. 25, 27, 29, 35) for dopamine-sensitive adenylate cyclase. No effect of the lesions was detected on the basal adenylate cyclase activity observed in the absence of dopamine.

In a separate series of experiments, dopamine-sensitive adenylate cyclase was assayed in homogenates of caudate nucleus from rats that had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10 days earlier and that exhibited contralateral circling in response to apomorphine. Moreover, using a sensitive radioenzymatic assay which allowed determination of endogenous dopamine in the same caudate nucleus homogenates used for adenylate cyclase assays, 95% depletion of endogenous dopamine was found in the ipsilateral caudate nuclei. Half-maximal stimulation of adenylate cyclase activity was observed with 3–4 μM dopamine on both control and lesioned sides.[6]

Effect of lesions on l-isoproterenol- and l-norepinephrine-induced cyclic AMP formation in slices. In a previous study (22) it was found that *l*-isoproterenol increased cyclic AMP levels in slices of caudate nucleus from normal rats, apparently through activation of *beta* adrenergic receptors, which were pharmacologically distinguishable from dopamine receptors in the same tissue. A half-maximal increase in cyclic AMP levels occurred with 0.03 μM *l*-isoproterenol. The effect of submaximal and supramaximal concentrations of *l*-isoproterenol on cyclic AMP levels in slices of caudate nucleus from rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10 days earlier is shown in Table 2. There was no significant effect of the lesion on either the sensitivity to *l*-isoproterenol or the maximal stimulation by *l*-isoproterenol. Thus destruction of dopaminergic nerve terminals in the caudate nu-

[5] Rats which had received unilateral lesions 16–20 days previously were tested for turning behavior in response to administration of apomorphine as described in MATERIALS AND METHODS. These tests were conducted at least 24 hr before the animals were killed for adenylate cyclase assays. Two forms of behavioral response were observed. About 50% of the rats exhibited circling, contralateral to the side of the lesion, in response to 0.3–1 mg/kg of apomorphine. The turning began about 10 min after injection, and the animals circled at a rate of about 9–12 turns/min for at least 20–30 min. Rats exhibiting this form of behavior were used for the adenylate cyclase assays described above. The remaining rats displayed pronounced contralateral posturing in response to 1–3 mg/kg of apomorphine, and also exhibited increased gnawing and sniffing directed toward the contralateral side.

[6] P. Copeland, B. K. Krueger, R. H. Roth, and P. Greengard, unpublished observations.

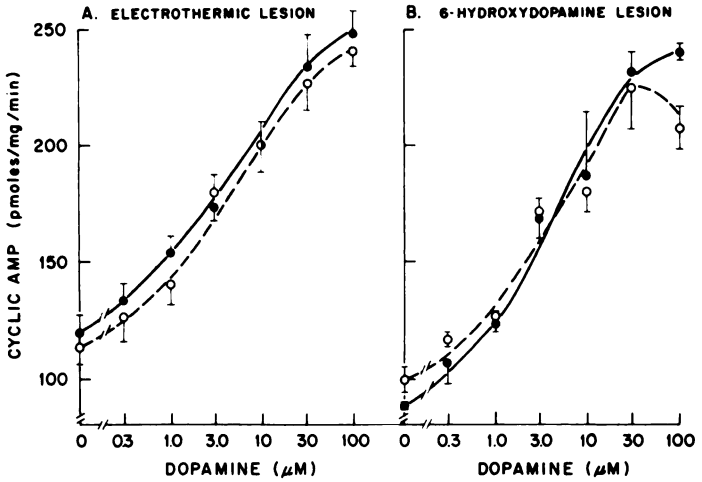

FIG. 3. *Effect of various concentrations of dopamine on adenylate cyclase activity in homogenates of caudate nucleus from control (●) and lesioned (○) sides of rats which had received unilateral lesions in the nigro-neostriatal pathway 16–20 days earlier*

A. Electrothermic lesion. Adenylate cyclase activity was measured on triplicate samples from each of three rats; each value represents the mean ± standard error for the nine samples. B. 6-Hydroxydopamine lesion. Adenylate cyclase activity was measured on triplicate samples from each of two rats; each value represents the mean ± standard error for the six samples.

TABLE 2

Effect of l-isoproterenol on cyclic AMP content of caudate nucleus slices

Cyclic AMP levels were measured in slices of caudate nucleus from rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10 days prior to death. Data were calculated as a percentage of the basal cyclic AMP level in the absence of *l*-isoproterenol, and are expressed as means ± standard errors for 8 samples. Basal levels of cyclic AMP were 10.8 ± 0.5 and 10.3 ± 0.3 pmoles/mg of protein for the control and lesioned sides, respectively.

Addition	Cyclic AMP content	
	Control side	Lesioned side
	% basal	
None	100 ± 7	100 ± 4
0.01 μM *l*-Isoproterenol	170 ± 14	154 ± 9
1.0 μM *l*-Isoproterenol	352 ± 13	331 ± 15

cleus appeared to have no effect on stimulation of the *beta* adrenergic receptors by *l*-isoproterenol.

It was also found previously (22) that *l*-norepinephrine increased cyclic AMP levels in slices of caudate nucleus from normal rats. This effect was attributed to the stimulation by *l*-norepinephrine of dopamine receptors and of *beta* adrenergic receptors in the same tissue. The effect of various concentrations of *l*-norepinephrine on cyclic AMP levels in slices of caudate nucleus from rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10 days earlier is shown in Fig. 4. In slices from the control side, half-maximal stimulation was observed with 30 μM *l*-norepinephrine, in agreement with previous observations using normal rats (22). In slices prepared from the lesioned side, 4 μM *l*-norepinephrine caused a half-maximal increase in cyclic AMP levels. No effect of lesioning was observed on the maximal level of cyclic AMP attainable with 100 μM *l*-norepinephrine. Cocaine (100 μM) caused a lowering of the apparent K_a for *l*-norepinephrine from 30 μM to 4 μM in slices prepared from the control side, but had no effect on the apparent K_a of 4 μM for *l*-norepinephrine in slices from the lesioned side (data not shown).

DISCUSSION

A variety of studies have indicated that lesions of the nigro-neostriatal pathway cause supersensitivity of the dopaminergic system in the caudate nucleus. This conclusion is based primarily on behavioral (17–20) and electrophysiological (7) studies

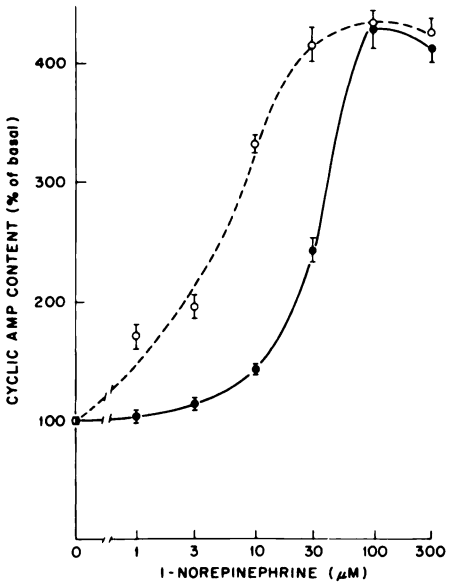

FIG. 4. *Effect of various concentrations of l-norepinephrine on cyclic AMP content of slices of caudate nucleus from control (●) and lesioned (○) sides of rats which had received unilateral electrothermic lesions in the nigro-neostriatal pathway 10 days earlier*

Cyclic AMP levels were calculated as a percentage of the basal level of cyclic AMP observed in the absence of added *l*-norepinephrine and are expressed as means ± standard errors for 16 samples. Basal levels of cyclic AMP were 13.3 ± 0.9 and 13.4 ± 0.8 pmoles/mg of protein on the control and lesioned sides, respectively.

of the effects of exogenously administered dopamine or dopaminergic agonists. In the present studies, experiments were performed to test whether this functional supersensitivity might be reflected in a change in the cyclic AMP system in the caudate nucleus. Such a functional supersensitivity could be due to one or more of the following mechanisms: (a) an increase in the affinity for dopamine of the postsynaptic dopamine receptors; (b) an increase in the number of postsynaptic dopamine receptors; (c) an increase in the ability of the agonist to reach the receptors as a result of the destruction of presynaptic nerve terminals; or (d) an alteration at a point subsequent to receptor stimulation, e.g., a decrease in phosphodiesterase activity or an increase in cyclic AMP-dependent protein phosphorylation.

In the present studies it was found that lesions in the nigro-neostriatal pathway resulted in a decrease in the concentration of dopamine required to cause a half-maximal increase in cyclic AMP formation in slices prepared from the caudate nucleus on the lesioned side. Moreover, the concentration of dopamine causing a half-maximal increase in cyclic AMP formation in slices prepared from denervated caudate nucleus was similar to the corresponding value in homogenates of both normal and denervated caudate nucleus. These observations are compatible with any of the following interpretations. If it is assumed that the properties of the dopamine-sensitive adenylate cyclase studied in the present investigation were not affected by homogenization, then the increased ability of dopamine to stimulate cyclic AMP formation in slices (Figs. 1 and 2), but not in homogenates (Fig. 3), of caudate nucleus following denervation suggests that the supersensitivity observed in behavioral and electrophysiological studies need not be due to an increased sensitivity or an increased number of postsynaptic dopamine receptors. Rather, the results would suggest that lesions of the nigro-neostriatal pathway and the resulting destruction of presynaptic dopaminergic nerve terminals may result in an increased accessibility of postsynaptic receptors to exogenously applied dopamine. This may be due to the lesion-induced destruction of presynaptic catecholamine uptake sites. [However, both cocaine (10–1000 μM) and benztropine (1–100 μM), potent inhibitors of dopamine uptake (45), were unable to potentiate the stimulation by dopamine of cyclic AMP formation (22).[7]] Another conceivable "presynaptic" explanation for the present results, but one for which there is no precedent or direct experimental evidence, is that the destruction of presynaptic dopamine-containing nerve endings, produced by denervation, results in the elimination of a structural barrier which in normal slices limits the rate of diffusion of exogenous dopamine to the postsynaptic receptors. The removal either of presynap-

[7] B. K. Krueger and P. Greengard, unpublished observations.

tic neuronal uptake sites or of such a structural barrier, during homogenization under hypotonic conditions, could explain the observation that lesions in the nigro-neostriatal pathway do not result in an increase in dopamine-sensitive adenylate cyclase activity in homogenates. Alternatively, the present results can also be explained if it is assumed that denervation causes an increase in the sensitivity to dopamine of the adenylate cyclase in the postsynaptic membrane and that this effect is mimicked by homogenization in a hypotonic medium.

The *l*-isoproterenol-induced increase in cyclic AMP and most of the *l*-norepinephrine-induced increase in cyclic AMP are mediated through activation of *beta* adrenergic receptors (22). Unfortunately, almost no information exists about either the cellular localization or the physiological role of *beta* adrenergic receptors in the caudate nucleus. Therefore it would be premature to attempt to interpret the *l*-norepinephrine data obtained in the present study, which indicate that lesions of the nigro-neostriatal pathway cause an increase in the sensitivity of the cyclic AMP-generating system to this catecholamine (Fig. 4).

The results of the present study suggest that denervation supersensitivity, as indicated by apomorphine-induced contralateral circling, may be due to destruction of presynaptic dopamine-containing nerve terminals. Other investigators have reported that either lesions in the nigro-neostriatal pathway (35) or prolonged neuroleptic treatment (46) can cause an increase of dopamine-sensitive adenylate cyclase activity in the caudate nucleus.[8] It would appear, therefore, that modifications of either pre- or postsynaptic elements, or both, may, depending on the experimental conditions, contribute to functional supersensitivity of the nigro-neostriatal system.

[8] The present results, which fail to show an effect of denervation on dopamine-sensitive adenylate cyclase activity in homogenates of caudate nucleus, and similar results obtained by others[3] (36), appear to be in conflict with the results of Mishra *et al.* (35). A collaborative study is in progress with Dr. M. H. Makman in an effort to clarify the basis for the differences in results.

Denervation supersensitivity, as indicated by apomorphine-induced contralateral circling, develops rapidly after placement of lesions, being detectable within 3 days and reaching a maximal level within 20 days (17, 19). Similarly, as found in the present study, the increased ability of submaximal concentrations of dopamine to stimulate cyclic AMP formation in slices of caudate nucleus is detectable within 3 days and is maximal within 10–15 days after placement of lesions in the nigro-neostriatal pathway. Thus this increased sensitivity to dopamine observed in slices may be a meaningful biochemical correlate of the denervation supersensitivity observed *in vivo*. Finally, the demonstration that the increase in dopamine receptor sensitivity, as indicated by contralateral circling, is associated with an increased sensitivity of the cyclic AMP-generating system provides further support for the hypothesis (25) that cyclic AMP mediates the postsynaptic actions of dopamine in the caudate nucleus.

ACKNOWLEDGMENTS

We thank Janice Abele and Frank Wilson for excellent technical assistance.

REFERENCES

1. Andén, N.-E., Carlsson, A., Dahlström, A., Fuxe, K., Hillarp, N.-A. & Larsson, K. (1964) *Life Sci.*, 3, 523–530.
2. Ungerstedt, U. (1971) *Acta Physiol. Scand.*, Suppl. 367, 1–48.
3. Olson, L., Nystrom, B. & Seiger, A. (1973) *Brain Res.*, 63, 231–247.
4. Hökfelt, T., Ljungdahl, A., Fuxe, K. & Johansson, O. (1974) *Science*, 184, 177–179.
5. Bloom, F. E., Costa, E. & Salmoiraghi, G. C. (1965) *J. Pharmacol. Exp. Ther.*, 150, 244–252.
6. Connor, J. D. (1970) *J. Physiol. (Lond.)*, 208, 691–703.
7. Siggins, G. A., Hoffer, B. J. & Ungerstedt, U. (1974) *Life Sci.*, 15, 779–792.
8. Hornykiewicz, O. (1966) *Pharmacol. Rev.*, 18, 925–964.
9. Trendelenburg, U. (1966) *Pharmacol. Rev.*, 18, 629–640.
10. Creese, I. & Iversen, S. D. (1975) in *Pre- and Postsynaptic Receptors*, Vol. 3, *Modern Pharmacology-Toxicology* (Usdin, E. & Bunney, W. E., Jr., eds.), pp. 171–189, Marcel Dekker, New York.

11. Weiss, B. & Costa, E. (1967) *Science*, **156**, 1750–1752.
12. Romero, J. A. & Axelrod, J. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, **72**, 1661–1665.
13. Palmer, G. D. (1972) *Neuropharmacology*, **11**, 145–149.
14. Kalisker, A., Rutledge, C. O. & Perkins, J. P. (1973) *Mol. Pharmacol.*, **9**, 619–629.
15. Huang, M., Ho, A. K. S. & Daly, J. W. (1973) *Mol. Pharmacol.*, **9**, 711–717.
16. Dismukes, K. & Daly, J. W. (1974) *Mol. Pharmacol.*, **10**, 933–940.
17. Ungerstedt, U. (1971) *Acta Physiol. Scand.*, Suppl. 367, 69–93.
18. Von Voigtlander, P. F. & Moore, K. E. (1973) *Neuropharmacology*, **12**, 451–462.
19. Thornburg, J. E. & Moore, K. E. (1975) *J. Pharmacol. Exp. Ther.*, **192**, 42–49.
20. Costall, B. & Naylor, R. J. (1975) *Psychopharmacologia*, **41**, 57–64.
21. Kebabian, J. W. & Greengard, P. (1971) *Science*, **174**, 1346–1349.
22. Forn, J., Krueger, B. K. & Greengard, P. (1974) *Science*, **186**, 1118–1120.
23. Wilkening, D. & Makman, M. H. (1975) *Brain Res.*, **92**, 522–528.
24. McAfee, D. A. & Greengard, P. (1972) *Science*, **178**, 310–312.
25. Kebabian, J. W., Petzold, G. L. & Greengard, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 2145–2149.
26. Sheppard, H. & Burghardt, C. R. (1974) *Mol. Pharmacol.*, **10**, 721–726.
27. Miller, R., Horn, A., Iversen, L. L. & Pinder, R. (1974) *Nature*, **250**, 238–241.
28. Makman, M. H., Brown, J. H. & Mishra, R. K. (1975) in *Adv. Cyclic Nucleotide Res.*, **5**, 661–679.
29. Clement-Cormier, Y. C., Kebabian, J. W., Petzold, G. L. & Greengard, P. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 1113–1117.
30. Horn, A. S., Cuello, A. C. & Miller, R. J. (1974) *J. Neurochem.*, **22**, 265–270.
31. Mishra, R. K., Demirjian, C., Katzman, R. & Makman, M. H. (1975) *Brain Res.*, **96**, 395–399.
32. Miller, R. J., Horn, A. S. & Iversen, L. L. (1974) *Mol. Pharmacol.*, **10**, 759–766.
33. Karobath, M. & Leitich, H. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 2915–2918.
34. Iversen, L. L. (1975) *Science*, **188**, 1084–1089.
35. Mishra, R. K., Gardner, E. L., Katzman, R. & Makman, M. H. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 3883–3887.
36. Von Voigtlander, P. F., Boukma, S. J. & Moore, K. E. (1973) *Neuropharmacology*, **12**, 1081–1086.
37. König, J. F. R. & Klippel, R. A. (1963) *The Rat Brain: a Stereotaxic Atlas*, Krieger, Huntington, New York.
38. Hökfelt, T. & Ungerstedt, U. (1969) *Acta Physiol. Scand.*, **76**, 415–426.
39. Walters, J. R., Roth, R. H. & Aghajanian, G. K. (1973) *J. Pharmacol. Exp. Ther.*, **186**, 630–639.
40. Laverty, R. & Taylor, K. M. (1968) *Anal. Biochem.*, **22**, 269–279.
41. Walters, J. R. & Roth, R. H. (1972) *Biochem. Pharmacol.*, **21**, 2111–2121.
42. Brown, B. L., Albano, J. D. M., Ekins, R. P. & Sgherzi, A. M. (1971) *Biochem. J.*, **121**, 561–562.
43. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
44. Jacobowitz, D. M. & Palkovits, M. (1974) *J. Comp. Neurol.*, **157**, 13–22.
45. Coyle, J. T. & Snyder, S. H. (1969) *Science*, **166**, 899–901.
46. Iwatsubo, K. & Clouet, D. H. (1975) *Biochem. Pharmacol.*, **24**, 1499–1503.