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Effect of Nerve Degeneration by 6-Hydroxydopamine on Catecholamine-Stimulated Adenosine 3′,5′-Monophosphate Formation in Rat Cerebral Cortex

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SUMMARY

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Intraventricular injection of 6-hydroxydopamine leads to two types of alterations in the effect of norepinephrine on the cyclic 3′,5′-AMP content of slices of rat cerebral cortex: an early-developing, presynaptic effect and a late-developing, postsynaptic effect. The early effect is attributed to the destruction of adrenergic nerve terminals by 6-hydroxydopamine and the resultant loss of presynaptic catecholamine uptake sites. This conclusion is based on three primary observations: (a) the potentiation of the effect of a threshold concentration of norepinephrine (1 μM) by 6-hydroxydopamine followed approximately the same time course as the loss of ability of the slices to accumulate [^{3}H]norepinephrine; (b) cocaine, which inhibits the presynaptic accumulation of [^{3}H]norepinephrine, potentiated the effects of low concentrations of norepinephrine in slices from control rats but did not alter the effect of norepinephrine in slices from 6-hydroxydopamine-treated animals; (c) the EC_{50} for norepinephrine was reduced from 5.2 to 1.7 μM by treatment with 6-hydroxydopamine, but the EC_{50} for isoproterenol (which is not accumulated presynaptically) was not altered. Also, cocaine did not potentiate the increase in cyclic AMP content induced by isoproterenol in slices from control animals. The increase in the effect of high concentrations (30 μM) of norepinephrine did not occur until 96 hr after treatment of the rats with 6-hydroxydopamine. This late-developing increase in responsiveness would appear to be a postsynaptic phenomenon, since it occurs during a time span (72–96 hr) when there is no further change in inhibition of the uptake of [^{3}H]norepinephrine. Furthermore, there is an increase in responsiveness to isoproterenol as well as to norepinephrine. Adenosine also causes an increase in the cyclic AMP content of rat cortex slices, but its effects are not altered by prior treatment with 6-hydroxydopamine.

INTRODUCTION

In slices of rat cerebral cortex catecholamines can interact with two different types of adrenergic receptors (similar to *alpha* and *beta* receptors) to cause an increase in the cellular content of adenosine 3′,5′-monophosphate (1). Adenosine can also elevate the cAMP[2] content of rat cerebral cortex,

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[2] The abbreviation used is: cAMP, adenosine (cyclic) 3′,5′-monophosphate.

and when slices are incubated in the presence of both adenosine and catecholamines a greater than additive response is observed (2).

The role of cAMP in the function of the brain is not clear, but there is evidence that catecholamines can alter the electrical properties of both peripheral (3) and central nervous system (4) neurons by a mechanism involving an increase in their cAMP content. There is evidence that glial cells also may contain adenylate cyclase systems that are activated by catecholamines (5–8). Thus changes in the cAMP content of brain slices, elicited by catecholamines, could be the result of changes in both neurons and glia. Irrespective of the type of cell involved, it seems reasonable that these cells should lie in close proximity to adrenergic nerve endings if changes in cAMP content are to be brought about by neurophysiological events.

In order to examine further the properties of the catecholamine-sensitive adenylate cyclase of rat cerebral cortex, the effects of agents which alter the function of adrenergic neurons or receptors have been studied to determine whether specific alterations at the level of adenylate cyclase can be defined. 6-Hydroxydopamine causes a rather specific destruction of adrenergic nerve terminals and a resultant supersentitivity of adrenergically innervated tissue to exogenous norepinephrine (see ref. 9). Adrenergic nerve section also results in supersensitivity of the innervated organ to exogenous catecholamines (10, 11). Weiss (12) observed an increased response of adenylate cyclase to catecholamines in homogenates of rat pineal glands following ablation of the superior cervical ganglion. There have been two brief reports (13, 14) indicating that in brain slices prepared from 6-hydroxydopamine-treated rats cAMP levels show enhanced responsiveness to exogenous norepinephrine. These observations indicate that destruction of adrenergic nerves can alter the properties of adenylate cyclase and suggest that such changes might be related to the supersensitivity phenomenon. We report here further evidence in support of this suggestion.

METHODS AND MATERIALS

Administration of 6-hydroxydopamine. Male Sprague-Dawley rats (200–220 g) were anesthetized with 0.4 ml of a 1:10 dilution of fentanyl-droperidol (Innovar-Vet) injected subcutaneously. A polyethylene cannula was placed into the lateral ventricle according to the method of de Balbian Verster *et al.* (15). Each animal received 0.1 ml of a penicillin G solution (500,000 units/ml) as prophylaxis against bacterial infection. Twenty-four hours after the surgery, when the rats had completely recovered from the anesthesia, they received an intraventricular injection of 250 μg of 6-hydroxydopamine (free base) in 20 μl of an artificial cerebrospinal fluid described by Merlis (16) containing ascorbic acid (1 mg/ml). A second injection was given 24 hr later. In some experiments (as noted) the dose of 6-hydroxydopamine was reduced to 2 × 100 μg or 2 × 50 μg. Animals which served as controls received two injections of ascorbic acid in Merlis' solution.

Measurement of accumulation of [^{14}C]-cAMP. Animals were killed by decapitation 96 hr after the first injection of 6-hydroxydopamine unless otherwise indicated. The cerebral cortex was dissected and sliced with a McIlwain tissue chopper into sections 0.26 × 0.26 × 1.0 mm thick.

Accumulation of [^{14}C]cAMP was determined according to the procedure of Shimizu *et al.* (18) with minor modifications. This method involves a 60-min incubation of the tissue with [^{14}C]adenine, during which time [^{14}C]adenine is converted within the slices to [^{14}C]ATP. The tissue is then washed and incubated for an additional 30 min with the experimental drugs. The conversion to [^{14}C]-cAMP is determined by isolation of cAMP and ATP from 5% trichloracetic acid extracts of the slices with the use of Dowex 50 cation exchange column chromatography and Whatman ET81 anion exchange paper chromatography. Results are expressed as percentage of conversion to [^{14}C]cAMP. Such values represent (cpm of cAMP × 100)/(cpm of ATP + cAMP), an expression which normalizes the data for variations in the degree of labeling of cellular ATP. In order to justify the use of this assay procedure, we carried out extensive preliminary

experiments wherein comparison of the results from the assay of Shimizu *et al.* (17) was made with results from the measurement of cAMP by the isotope dilution-binding assay of Gilman (18). Both assays were performed on the same samples. From such a comparison (also see ref. 1) it was concluded that either method provides essentially the same measure of changes in cAMP content. The [^{14}C]cAMP assay of Shimizu *et al.* was used because in our laboratory it was more reproducible than direct measurement of cAMP.

Enzyme assays. Adenylate cyclase activity was determined by a modification (19) of the method of Krishna *et al.* (20). Phosphodiesterase activity was measured by the method of O'Dea *et al.* (21).

Uptake of [^{3}H]norepinephrine. The uptake of [^{3}H]norepinephrine by slices (chopped tissue) of cerebral cortex in treated and control animals was determined by methods previously described (22).

Materials. 6-Hydroxydopamine hydrobromide was purchased from Regis Chemical Company. *l*-Norepinephrine *d*-bitartrate and *dl*-isoproterenol hydrochloride were obtained from Sigma Chemical Company. Adenosine was purchased from Calbiochem. [8-^{3}H]Adenosine 3′,5′-monophosphate (14.3 Ci/mmole), [8-^{14}C]adenine (58 mCi/mmole), and [8-^{3}H]adenosine triphosphate (17 Ci/mmole) were purchased from Schwarz/Mann. Propranolol hydrochloride and phentolamine mesylate (Regitine) were obtained from Ayerst Laboratories and Ciba, respectively. Whatman ET81 anion exchange paper and Amberlite SB-2 ion exchange paper were supplied by Reeve Angel. Innovar-Vet was obtained from Pitman-Moore; each milliliter contains 0.4 mg of fentanyl and 20 mg of droperidol with 1.8 mg of methylparaben, 0.2 mg of propylparaben, and lactic acid to adjust the pH to 3.1 ± 0.4.

RESULTS

All rats receiving 500 μg of 6-hydroxydopamine in a single intraventricular injection died within 4 hr. The rats tolerated this total amount of drug if it was divided into two injections of 250 μg administered 24 hr apart. Four hours after the first injection all rats became quiescent and would remain prone when left undisturbed. However, they were quite sensitive to auditory and tactile stimuli, to which they reacted by jumping violently. The treatment also caused loss of appetite, which resulted in a 10–15% decrease in body weight within 24 hr. Injections of smaller doses of 6-hydroxydopamine caused a smaller weight loss although the reactions to sensory stimuli were still exaggerated. Upon decapitation, the bodies of treated animals immediately lost muscle tone and did not exhibit the typical kicking reflex. These signs were considered characteristic of effective treatment with 6-hydroxydopamine; thus treated animals that did not demonstrate these symptoms were not used in the study.

Experiments were performed to determine whether the accumulation of cAMP elicited by norepinephrine in cortical slices would be modified by preliminary treatment of the animals with 6-hydroxydopamine. The dose-response relationship and time course of the effect of the drug are shown in Figs. 1 and 2. Concentrations of 1 μM and 30 μM norepinephrine were chosen, since the former is near threshold and the latter is maximally effective (see Fig. 4). From Fig. 1 it can be seen that the magnitude of the effects of both concentrations of norepinephrine increased as the dose of 6-hydroxydopamine was increased. Of particular interest was the observation (Fig. 2) that the change in effect at the two concentrations of norepinephrine followed different time courses. The response to 1 μM norepinephrine was enhanced within 24 hr after treatment with 6-hydroxydopamine (Fig. 3), and by 48–72 hours it had reached a plateau. In contrast, the magnitude of the response to 30 μM norepinephrine was almost identical in treated and control animals up to 72 hr, whereupon a 2-fold increase in effect occurred within the next 24 hr in 6-hydroxydopamine-treated rats. The further increase in the response to 1.0 μM norepinephrine which occurred between 72 and 96 hr could be related to this late-developing increase in responsiveness observed with 30 μM norepinephrine.

Figure 3 shows the time course of the

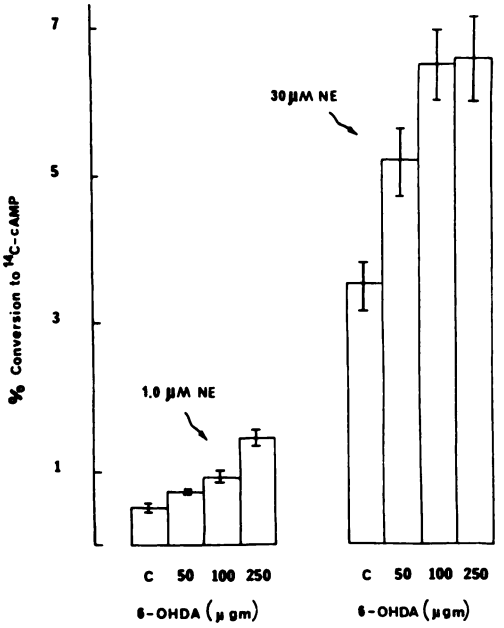

FIG. 1. *Effect of norepinephrine (NE) (1.0 and 30 μM) on [^{14}C]cAMP formation in slices of cerebral cortex from rats treated with increasing dosages of 6-hydroxydopamine (6-OHDA)*

Each value represents the mean ± standard error of single determinations on four treated rats and eight control (C) rats. Each dose was injected two times, 24 hr apart, and the rats were killed 96 hr after the first injection.

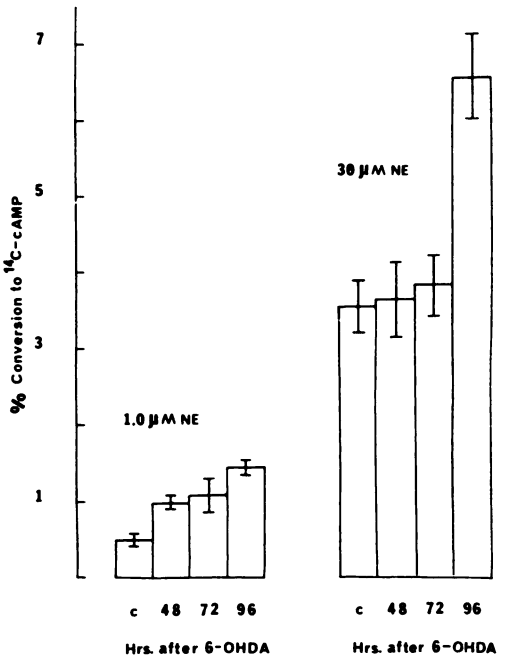

FIG. 2. *Effect of norepinephrine (NE) (1.0 and 30 μM) on [^{14}C]cAMP formation in slices of rat cerebral cortex as a function of time after treatment with two doses of 250 μg of 6-hydroxydopamine (6-OHDA)*

Each value represents the mean ± standard error of single determinations on four treated rats and eight control (C) rats. The times indicated are the hours after the first of two injections of 6-hydroxydopamine.

effect of two 250-μg doses of 6-hydroxydopamine on the accumulation of [^{3}H]norepinephrine in chopped cerebral cortex. The inhibition produced by 6-hydroxydopamine (54%) was nearly maximal by 72 hr and remained essentially unchanged through 96 hr. For comparison, the 6-hydroxydopamine-induced change in percentage conversion to [^{14}C]cAMP at 1.0 μM norepinephrine is also plotted in Fig. 3. There is a good correlation (coefficient = 0.91) between the percentage inhibition of [^{3}H]norepinephrine accumulation and the increased sensitivity to 1.0 μM norepinephrine up to 72 hr.

The effect of 6-hydroxydopamine treatment on the accumulation of [^{14}C]cAMP in the presence of various concentrations of norepinephrine is shown in Fig. 4. Also shown is the effect of cocaine added simultaneously with norepinephrine to the incubation mixtures. The absolute values for percentage conversion to [^{14}C]cAMP are shown in Fig. 4A, whereas Fig. 4B depicts the results as a percentage of the effect of 30 μM norepinephrine. The data in this figure illustrate four important points: (a) treatment with 6-hydroxydopamine reduced the EC_{50} for norepinephrine from 5.2 to 1.7 μM (Fig. 4B); (b) 6-hydroxydopamine increased the maximal response to norepinephrine about 2-fold (Fig. 4A; also see Figs. 1 and 2); (c) cocaine reduced the EC_{50} for norepinephrine from 5.2 to 2.5 μM but did not significantly change the maximal response; and (d) cocaine had no significant effect on the response to norepinephrine of slices from 6-hydroxydopamine-treated animals.

Similar experiments, carried out using *dl*-isoproterenol as the adenylate cyclase

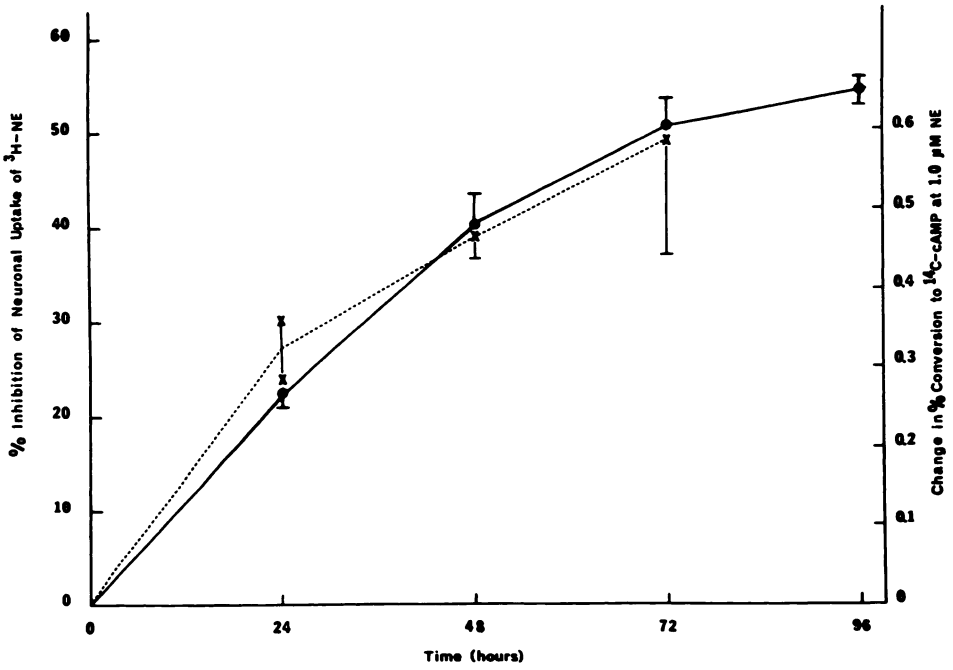

FIG. 3. *Comparison of effects of two 250-μg doses of 6-hydroxydopamine on accumulation of [^{3}H]norepinephrine (^{3}H-NE) in chopped rat cerebral cortex tissue and on formation of [^{14}C]cAMP elicited by 1.0 μM norepinephrine.*

The percentage inhibition of uptake of 0.1 μM [^{3}H]norepinephrine (●——●) was calculated as

$$\frac{R_c - R_i}{R_c - R_0} \times 100$$

where R_c is equal to the tissue to medium ratio (T/M) in tissue from vehicle-treated control rats, R_i is T/M in tissue from 6-hydroxydopamine-treated rats, and R_0 is T/M from the tissue of untreated control rats incubated at 0°. T/M was calculated as (disintegrations per minute per gram of tissue, wet weight)/(disintegrations per minute per milliliter of incubation medium.) Each value is the result of triplicate determinations on the tissues from three animals and thus represents the mean ± standard error of nine determinations. The abscissa indicates the number of hours between the first injection of 6-hydroxydopamine and the initiation of the uptake experiment. The animals used at 24 hr received only one injection of 6-hydroxydopamine. The change in percentage conversion to [^{14}C]cAMP elicited by 1.0 μM norepinephrine (×- - -×) is shown at 48 and 72 hr as the mean ± standard error of the values obtained from three treated animals, and at 24 hr as duplicate determinations on tissue from a single rat. The percentage conversion to [^{14}C]cAMP elicited by 1.0 μM norepinephrine in slices from three untreated control rats was subtracted from each experimental value.

agonist, are shown in Fig. 5. Treatment with 6-hydroxydopamine did not significantly change the EC_{50} for isoproterenol (Fig. 5B), although the maximal effect was increased 2-fold (Fig. 5A). In contrast to its effect on the response to norepinephrine, cocaine had no effect on the sensitivity of the slices to isoproterenol.

The responsiveness of the cerebral cortex slices to adenosine was not significantly altered by treatment with 6-hydroxydopamine (Fig. 6). The response to histamine (100 μM), a weak adenylate cyclase agonist in rat cerebral cortex, was only slightly increased (not shown). Basal levels of [^{14}C]-cAMP were not altered by treatment of animals with 6-hydroxydopamine. Basal values ranged from 0.25 to 0.50, expressed as percentage conversion to [^{14}C]cAMP.

Figure 7 shows the effect of increasing

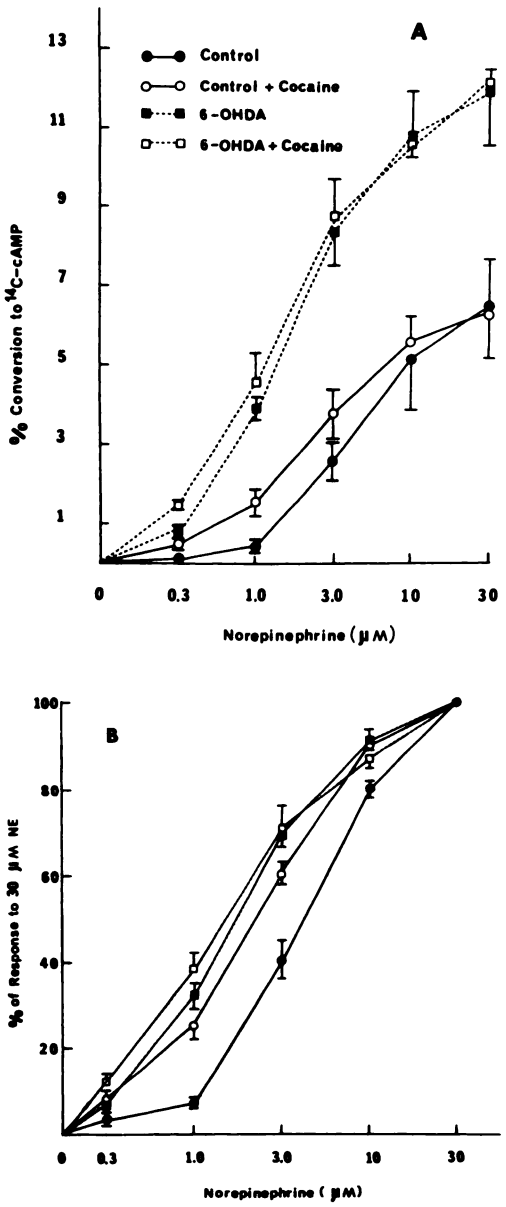

Fig. 4 *Effect of various concentrations of norepinephrine (NE) on [^{14}C]cAMP formation in slices of cerebral cortex from control rats and rats treated with two doses of 250 μg of 6-hydroxydopamine (6-OHDA).*

A. Percentage conversion to [^{14}C]cAMP. The percentage conversion in the absence of norepinephrine was subtracted from each individual value. Each point is the mean ± standard error for three separate determinations. In each experiment, tissue from two control rats was pooled and concentration-effect curves for norepinephrine in the presence and absence of cocaine (10 μM) were determined. The same procedure was carried out with the pooled tissue from two 6-hydroxydopamine treated rats. B. Percentage of response to 30 μM norepinephrine. For each experiment, the percentage conversion to [^{14}C]cAMP at each lower concentration of norepinephrine was expressed as a percentage of the value obtained at 30 μM norepinephrine. Each point is the mean ± standard error for three separate determinations. In this and all subsequent experiments animals were killed 96 hr after the first of two injections of 250 μg of 6-hydroxydopamine given 24 hr apart.

concentrations (0.1–30 μM) of either propranolol (Fig. 7A) or phentolamine (Fig. 7B) on the response of 6-hydroxydopamine-treated and control preparations to 30 μM norepinephrine. In each case it is clear that, compared to controls, the percentage inhibition produced by these antagonists was not altered by treatment with 6-hydroxydopamine.

Prior treatment with 6-hydroxydopamine did not cause a significant change in basal or NaF-stimulated adenylate cyclase activity as measured in homogenates of rat cerebral cortex (Table 1). There was a small (25%), but statistically significant, decrease in the activity of cAMP phosphodiesterase activity in the same homogenates, as measured for both the low K_m enzyme (1 μM cAMP) and the high K_m enzyme (100 μM cAMP) (Table 2).

DISCUSSION

The increase in accumulation of cAMP elicited by catecholamines in brain slices after treatment with 6-hydroxydopamine has been observed by others. In the earlier studies there were differences in the magnitude of the response, which appeared to be functions of the concentration of norepinephrine or the time between the injection of 6-hydroxydopamine and measurement of its effect. Weiss and Strada (13) reported that 4 weeks after the injection of two 250-μg doses of 6-hydroxydopamine slices of rat cerebral cortex were more responsive to 5 μM norepinephrine than were controls but that 50 μM norepinephrine elicited about the same response in both control and treated preparations. Palmer (14) showed that treat-

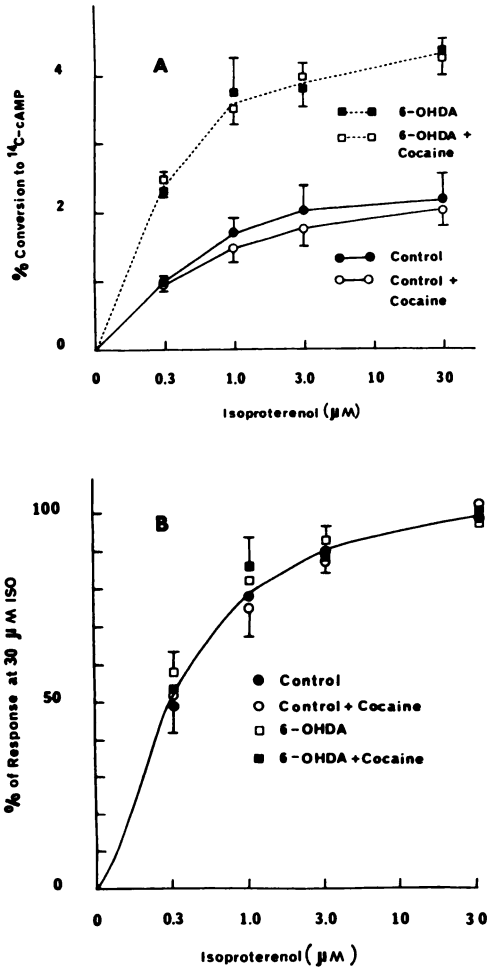

FIG. 5. *Effect of various concentrations of isoproterenol (ISO) on [^{14}C]cAMP formation in slices of cerebral cortex from control rats and rats treated with two 250-μg doses of 6-hydroxydopamine (6-OHDA).*

Experimental conditions were as described in Fig. 4. A. Percentage conversion to [^{14}C]cAMP. B. Data from Fig. 5A expressed as a percentage of the effect of 30 μM isoproterenol.

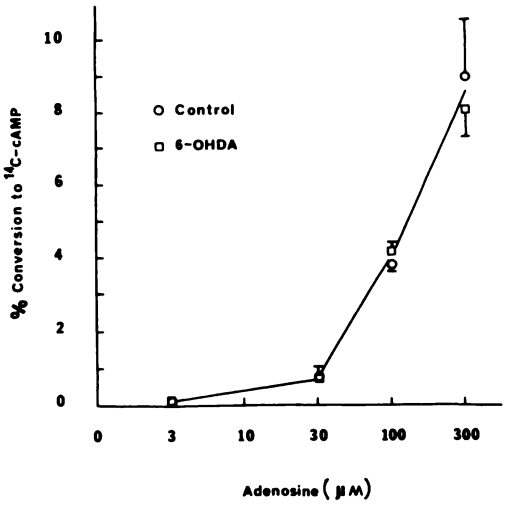

FIG. 6. *Effect of various concentrations of adenosine on [^{14}C]cAMP formation in slices of cerebral cortex from control rats and rats treated with two 250-μg doses of 6-hydroxydopamine (6-OHDA)*

For experimental conditions, see Fig. 4.

ment of rats with two 250-μg doses of 6-hydroxydopamine caused a 2-fold increase in the response of slices to 10 μM norepinephrine, but observed no significant difference in the response to 1 μM norepinephrine when the measurements were made 1 week after the second injection of 6-hydroxydopamine. The results presented in this report extend these earlier observations and provide the basis for a hypothesis which partially explains the effects of 6-hydroxydopamine.

Intraventricular injection of 6-hydroxydopamine causes two types of alterations which influence the responsiveness of adenylate cyclase to exogenous catecholamines: an early-developing, presynaptic effect and a late-developing, postsynaptic effect.

Uptake of norepinephrine into the presynaptic nerve terminal has been shown to be a predominant factor in the regulation of its concentration in the immediate vicinity of the postsynaptic receptors in the peripheral nervous system, and the same is thought to be true in the central nervous system (23, 24). There is considerable evidence that 6-hydroxydopamine causes an irreversible and selective degeneration of norepinephrine-containing nerve terminals in the central as well as the peripheral nervous system (9, 25–27). The early-developing effect of 6-hydroxydopamine primarily influences the sensitivity of the slices to low concentrations of exogenous norepinephrine and is attributed to the destruction of nerve terminals and the resultant loss of presynaptic amine uptake sites. Three sets of observations support this conclusion. (a) The potentiation of the effect of a thresh-

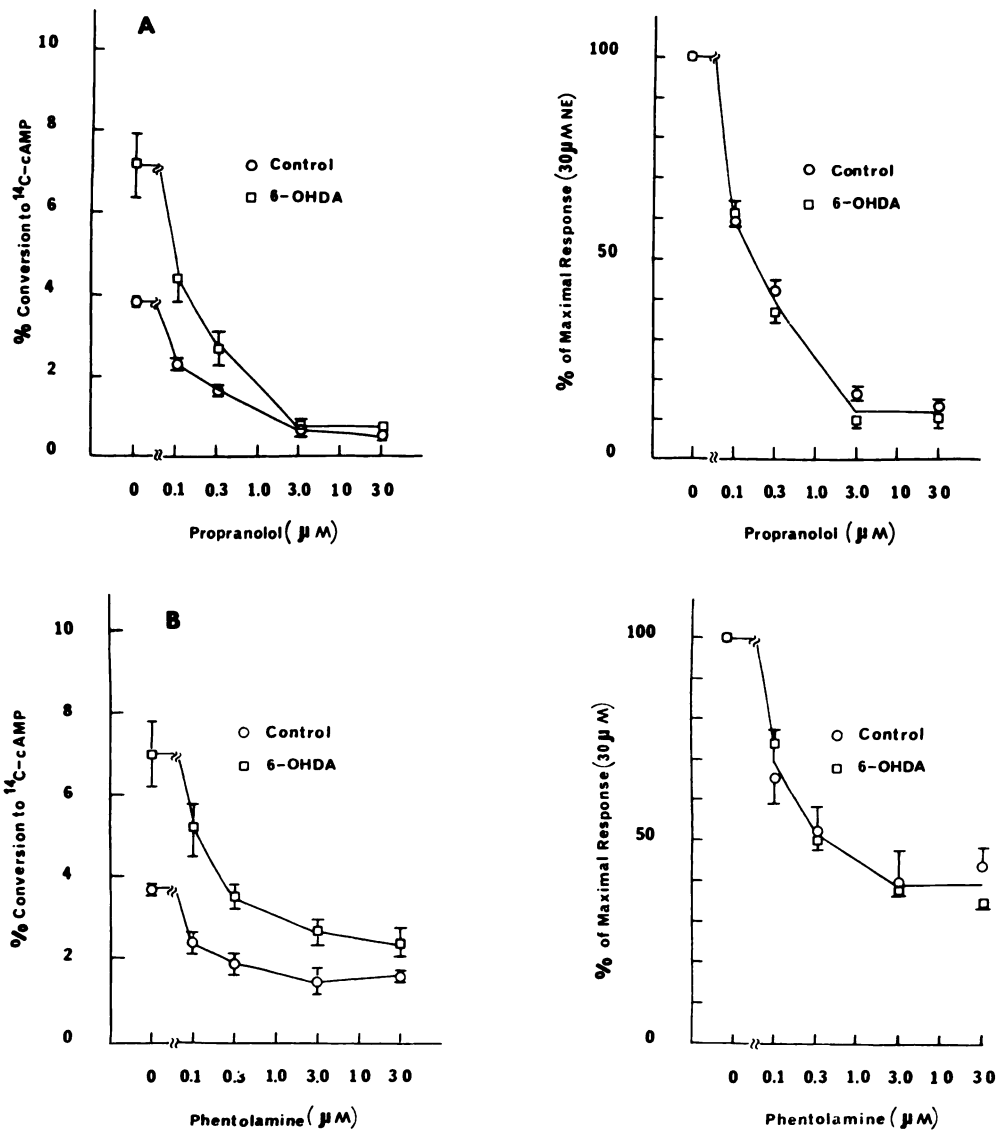

FIG. 7. *Effects of propranolol and phentolamine on response to 30 μM norepinephrine of slices from control rats and rats treated with two 250-μg doses of 6-hydroxydopamine (6-OHDA).*

A. The left-hand panel shows the effect of propranolol, expressed as percentage conversion to [^{14}C]-cAMP. In the right-hand panel the same data are expressed as a percentage of the response to 30 μM norepinephrine. B. The experimental design was the same as in Fig. 7A, except that phentolamine was the inhibitor. For both Fig. 7A and B each point represents the mean ± standard error for duplicate determinations on tissue from each of three rats ($N = 6$).

old concentration of norepinephrine (1 μM) by 6-hydroxydopamine follows approximately the same time course (up to 72 hr) as the inhibition of accumulation of [^{3}H]-norepinephrine. At a concentration of 1 μM, norepinephrine is accumulated predominantly by the high-affinity neuronal uptake system (uptake$_1$) (28). (b) Cocaine potentiated the effect of low (1–3 μM) but not high (30 μM) concentrations of norepinephrine in slices from control animals. It is known that cocaine effectively inhibits

TABLE 1

Adenylate cyclase activity in homogenates of cerebral cortex from control and 6-hydroxydopamine-treated rats

Treated rats received two 250-μg doses of 6-hydroxydopamine. The conversion of [^{3}H]ATP (0.4 mM) to [^{3}H]cAMP during a 10-min incubation was measured (for details, see ref. 19). Each value represents the mean ± standard error of determinations from three animals. The concentration of sodium fluoride was 10 mM.

Rats	Basal	NaF
	pmoles [^{3}H]cAMP/ mg protein/min	*pmoles [^{3}H]cAMP/ mg protein/min*
Control	377 ± 36	530 ± 44
Treated	303 ± 27	491 ± 23

TABLE 2

Phosphodiesterase activity in homogenates of cerebral cortex from control and 6-hydroxydopamine-treated rats

Treated rats received two 250-μg doses of 6-hydroxydopamine. In essence, the assay involves the conversion of [^{3}H]cAMP to [^{3}H]5′-AMP, then to [^{3}H]adenosine, which is isolated on Amberlite SB-2 ion exchange paper (21). Activity at 1.0 μM cAMP is due primarily to the action of the low K_m enzyme, while activity at 100 μM cAMP is due primarily to the high K_m enzyme. Each value represents the mean ± standard error of determinations from three animals.

Rats	1.0 μM cAMP	100 μM cAMP
	pmoles [^{3}H]adenosine/mg protein/min	*nmoles [^{3}H]adenosine/mg protein/min*
Control	662.4 ± 42.4	22.0 ± 1.3
Treated	491.9 ± 22.3	17.7 ± 0.1
	$p < 0.02$	$p < 0.02$

uptake$_1$ (29). However, high concentrations (e.g., 30 μM) of norepinephrine are accumulated predominantly by a low-affinity uptake system (uptake$_2$), which is not effectively inhibited by cocaine (30). Cocaine did not significantly potentiate the effect of 1–3 μM norepinephrine on the formation of [^{14}C]-cAMP in slices from 6-hydroxydopamine-treated rats. Even though the maximal inhibition of [^{3}H]norepinephrine uptake by 6-hydroxydopamine was only 54%, this is the fraction of neuronal uptake important for the activation of adenylate cyclase, since cAMP content was not increased further by cocaine. (c) The EC_{50} for norepinephrine was reduced by treatment with 6-hydroxydopamine, but the EC_{50} for isoproterenol was not affected. Nor did cocaine potentiate the effect of isoproterenol. This is consistent with observations (31, 32) that isoproterenol does not interact effectively with the high-affinity amine uptake system.

Treatment with 6-hydroxydopamine also results in a late-developing alteration which primarily affects the maximal responsiveness of the adenylate cyclase system and is not *directly* related to destruction of adrenergic nerves. We have termed it a postsynaptic effect, since it occurs during a time span (72–96 hr) when there is no further change in the degree of inhibition of the uptake of [^{3}H]norepinephrine and because there is an increase in responsiveness to isoproterenol which cannot be explained by an alteration in the presynaptic amine uptake process.

We have not examined the effects of 6-hydroxydopamine at times later than 96 hr. However, the results of Huang, Ho, and Daly[3] indicate that the response to 100 μM norepinephrine is increased no more than 100–150% between 5 and 20 days after a single injection of 250 μg of 6-hydroxydopamine.

Because we chose to measure the accumulation of cAMP and did not determine the rate of synthesis directly, the increase in maximal accumulation could have resulted from a decrease in phosphodiesterase activity. However, direct measurement of both phosphodiesterase and adenylate cyclase showed only minor changes in total enzyme activities. Furthermore, no increase in the maximal effect of, or the sensitivity to, adenosine was observed. Since adenosine and norepinephrine interact synergistically in rat cortex slices, it is probable that they act on the same cells. Thus the postsynaptic alterations induced by 6-hydroxydopamine would appear to be related more to the adrenergic receptors than to a general increase in adenylate cyclase activity.

[3] M. Huang, A. K. S. Ho, and J. W. Daly, personal communication.

Since two different types of adrenergic receptors appear to mediate the effect of norepinephrine on the cAMP content of rat cerebral cortex slices (1), experiments were carried out to determine whether both types of receptors are involved in the proposed postsynaptic effect of 6-hydroxydopamine. It is clear from the results in Fig. 7 that if an increase in receptors did occur it did not result in a change in the proportion of the two types, since both propranolol and phentolamine caused the same percentage inhibition of the effect of norepinephrine in slices from control and treated animals.

The results of this study and others (12–14) suggest that adrenergic nerves exert a negative influence on the responsiveness of adenylate cyclase to exogenous catecholamines. It is clear from the results presented here that the potency of exogenous norepinephrine was affected by the presence or absence of the presynaptic amine uptake system. However, a mechanism to explain the proposed postsynaptic effect of 6-hydroxydopamine is more difficult to formulate. Of interest in this regard are the results of Weiss (12), who reported that in rats surgical destruction of the adrenergic innervation of the pineal gland led to an increase in the responsiveness of pineal adenylate cyclase to high concentrations (100 μM) of norepinephrine. In that study (12) adenylate cyclase activity was measured directly in homogenates, using exogenous, labeled ATP as substrate for the enzyme reaction. Thus destruction of adrenergic nerves might have led to an alteration in the adenylate cyclase system per se. Certainly such results cannot be explained by direct involvement of the amine uptake system. In an analogous manner it is possible that destruction of adrenergic neurons by 6-hydroxydopamine could account indirectly for the proposed postsynaptic effect of this compound. If the tonic release of norepinephrine by adrenergic neurons exerted a negative influence on the responsiveness of adenylate cyclase to catecholamines, then destruction of the neurons by 6-hydroxydopamine would reduce the release of norepinephrine and could account for the slow development of a supersensitive state of the enzyme system. Although such a mechanism is highly speculative, it does lead to a testable prediction; i.e., any manipulation which reduces the tonic release of norepinephrine from nerve endings should lead to a similar postsynaptic increase in responsiveness. We are currently investigating the effect of reserpine and α-methyl-*p*-tyrosine in this regard.

Supersensitivity to sympathomimetic amines in organs innervated with peripheral adrenergic nerves has been studied extensively. Trendelenburg (10, 11) has discussed this phenomenon in terms of two components, a presynaptic alteration induced by postganglionic nerve section and a postsynaptic alteration induced by either post- or preganglionic nerve section. There appear to be marked similarities in the effects of 6-hydroxydopamine observed in this report and the effects of postganglionic nerve section in the peripheral nervous system.

It would be of interest to know the cellular localization of the catecholamine-responsive adenylate cyclase of rat cerebral cortex. There is convincing evidence of catecholamine-sensitive adenylate cyclases in both central (4) and peripheral (3) nervous system neurons. There also is evidence to suggest that glial cells contain a catecholamine-sensitive enzyme (5–8). The results presented here do not address this problem directly, but they do indicate that whatever the type of cell or cells involved, they must exist within the sphere of influence of the amine uptake process in adrenergic nerve endings.

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