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Supersensitivity in Rat Cerebral Cortex: Pre- and Postsynaptic Effects of 6-Hydroxydopamine at Noradrenergic Synapses

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SUMMARY

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The intraventricular administration of 6-hydroxydopamine resulted in the destruction of noradrenergic nerve terminals in the rat cerebral cortex and a 90% decrease in norepinephrine content. The time courses of the effects of this treatment on catecholamine-stimulated cyclic 3',5'-AMP accumulation in slices of cerebral cortex and on *beta* adrenergic receptors were determined to investigate the mechanisms underlying supersensitivity in this system. The EC_{50} for norepinephrine stimulation of cyclic AMP accumulation decreased following 6-hydroxydopamine administration. This decrease was apparent within 1 day and was probably due to the loss of the presynaptic uptake system for norepinephrine. The density of *beta* adrenergic receptors, as determined by measuring the binding of the high-affinity *beta* adrenergic receptor antagonist [^{125}I]iodohydroxybenzylpindolol, and maximal levels of catecholamine-stimulated cyclic AMP accumulation increased with a slower time course, reaching peak levels approximately 16 days after treatment with 6-hydroxydopamine. The maximal increase in the density of *beta* adrenergic receptors was 50%, while maximal levels of cyclic AMP accumulation in treated rats were approximately twice those measured in control animals. The K_D of [^{125}I]iodohydroxybenzylpindolol for *beta* adrenergic receptors and the EC_{50} of isoproterenol for stimulation of cyclic AMP accumulation were unchanged following 6-hydroxydopamine administration, suggesting that the intrinsic properties of the postsynaptic receptor were not affected by denervation. The administration of 6-hydroxydopamine did not affect fluoride-sensitive adenylate cyclase activity. The results are consistent with the idea that the supersensitivity to catecholamines that occurs in the cerebral cortex following 6-hydroxydopamine administration had both pre- and postsynaptic components. The slowly developing postsynaptic component can be at least partially explained by an increase in the density of *beta* adrenergic receptors.

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INTRODUCTION

Removal of the neuronal input to an organ or nerve often results in an enhanced response following application of

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an appropriate transmitter (1). The most detailed investigation of this phenomenon has involved studies of the vertebrate neuromuscular junction (2, 3), but the phenomenon has also been observed in electrophysiological studies of the cardiac ganglion of the mud puppy (4) and of parasympathetic ganglion cells in frog heart (5). Behavioral studies (6) and biochemical investigations carried out with preparations of the mammalian central nervous system (7–11) and of dog heart (12) have also provided support for the idea that denervation supersensitivity is a widespread phenomenon.

A variety of cellular mechanisms have been shown to be involved in the supersensitivity that occurs following denervation. Studies of vertebrate skeletal muscle have shown that the increase in sensitivity involves a change in the number and distribution of receptors (13, 14). In addition to an increase in the sensitivity of the tissue to acetylcholine and in the number of receptors, there is a marked loss of acetylcholinesterase activity (15, 16). On the other hand, a decrease in the resting membrane potential and a consequent increase in excitability have been reported in studies of postjunctional supersensitivity in the guinea pig vas deferens as well as in other preparations of smooth muscle (17, 18).

Denervation of adrenergically innervated tissues leads to a supersensitive response to exogenously applied catecholamines (19). This phenomenon, which has been extensively characterized in the peripheral sympathetic nervous system, includes at least two components. First, the loss of the presynaptic nerve terminal and its associated uptake system (20) results in a decreased capacity to inactivate catecholamines and in an increased concentration of amine at the postsynaptic receptor site. The pharmacological result of this alteration is a shift to the left of the dose-response curve for norepinephrine. The second phenomenon that occurs following denervation of sympathetically innervated organs develops with a slower time course and appears to reflect alterations at the level of the postsynaptic cell.

The intraventricular administration of 6-OHDA[4] results in the selective destruction of norepinephrine- and dopamine-containing nerve terminals in the central nervous system (see ref. 21). Thus 6-OHDA is a useful agent for investigating the influence of presynaptic noradrenergic input on the properties of postsynaptic neurons. An increase in cyclic 3′,5′-AMP production in the cerebral cortex of rats in response to catecholamines has been described following the administration of 6-OHDA (9–11). This increased responsiveness had both presynaptic and postsynaptic components. The potency of norepinephrine for the stimulation of cyclic AMP synthesis increased rapidly in parallel with the loss of presynaptic endings and the associated system for the reuptake of norepinephrine. In addition, a slowly developing increase in the accumulation of cyclic AMP occurred in response to maximally effective concentrations of catecholamines.

Recently, *beta* adrenergic receptors have been directly identified in membrane fractions from the mammalian cerebral cortex, using radiolabeled antagonists (22–25). Thus, it is now possible to measure several of the components of the *beta* adrenergic receptor–adenylate cyclase system. In the present report, experiments are described that define the temporal relationship between the loss of presynaptic nerve terminals, changes in the catecholamine-responsive cyclic AMP-generating system, and the density of *beta* adrenergic receptors. On the basis of results obtained in preliminary experiments, we have recently suggested (26) that an increased density of *beta* adrenergic receptors in the cerebral cortices of rats treated with 6-OHDA contributes to the increased cyclic AMP accumulation observed in response to a maximally stimulating concentration of isoproterenol. However, to demonstrate clearly that the increases in receptor density are related to the increased biochemical response, the time course of the increase in *beta* receptor concentration must be correlated with the time course of the increased maximal cyclic AMP accu-

[4] The abbreviations used are: 6-OHDA, 6-hydroxydopamine; IHYP, [^{125}I]iodohydroxybenzylpindolol.

mulation, and not with other phenomena that occur in this system as a consequence of treatment with 6-OHDA.

METHODS

Animals and tissue preparation. Male Sprague-Dawley rats (5–6 weeks old) from a colony maintained in our animal care facility were injected intraventricularly (27, 28) on each of 2 successive days with 200 μg (free base) of 6-OHDA dissolved in 20 μl of 0.9% sodium chloride. A burr hole was made 2.5 mm caudal and 1.5 mm lateral to the crossing of the coronal and sagittal sutures. Drug was injected through a 26-gauge needle 3.5–4 mm beneath the surface of the cranium. Sodium ascorbate (1 mg/ml, pH 5) was added to prevent oxidation of the 6-OHDA. Rats were killed by decapitation at various times from 1 to 30 days after the first injection. The entire cerebral cortex from each rat was dissected free of midbrain structures, sliced (1×0.26×0.26 mm) with a McIlwain tissue chopper, and resuspended in 10 ml of oxygenated (95% O_2–5% CO_2) Krebs-Ringer buffer (29, 30). Aliquots of each cortex were assayed for cyclic AMP accumulation in the presence of various concentrations of norepinephrine or isoproterenol. A portion of each resuspended cortex was homogenized in 15 ml of 0.32 M sucrose–10 mM Tris, pH 7.5, using a motor-driven Teflon-glass homogenizer. The homogenates were centrifuged at 20,000 × *g* for 10 min, and the resulting pellets were resuspended (200 ml/g of tissue, wet weight) in 0.9% NaCl–10 mM Tris, pH 7.5, for use in binding studies.

Accumulation of cyclic AMP in tissue slices. Cyclic AMP accumulation was determined in slices after labeling of ATP stores with [^{3}H]adenine (29, 30). The slices were suspended in oxygenated Krebs-Ringer buffer and incubated in a covered, shaking water bath at 37° under an atmosphere of 95% O_2–5% CO_2. After a 20-min incubation, the slices were sedimented by centrifugation and resuspended in buffer containing 2.5 μCi/ml of [^{3}H]adenine (specific activity, 31.7 Ci/mmol). Slices were incubated for 30 min, washed three times by centrifugation, and resuspended in Krebs-Ringer buffer containing 1 μM pargyline, 1 mM sodium ascorbate, and 1 mM 3-isobutyl-1-methylxanthine. Samples (2.5 ml) of resuspended labeled slices were then incubated for 40 min in the presence of various concentrations of (–)-norepinephrine or (−)-isoproterenol. The reaction was terminated by the addition of trichloroacetic acid (final concentration, 5%). Cyclic AMP (0.33 mg) was added as a carrier, and [^{3}H]ATP and [^{3}H]cyclic AMP were purified by sequential Dowex and alumina column chromatography (29). Results are expressed as the percentage of [^{3}H]ATP converted to [^{3}H]cyclic AMP. In some experiments, half of the sliced tissue was first incubated with [^{3}H]adenine and [^{3}H]cyclic AMP accumulation was determined as described above. The remaining half of the tissue was incubated without [^{3}H]adenine, and then basal and isoproterenol-stimulated cyclic AMP accumulation was measured by the protein binding assay of Gilman (31). Comparable results were obtained by the two methods. The prior labeling method was routinely employed because in our laboratory it was more reproducible than the direct measurement of cyclic AMP levels.

Beta adrenergic receptor assay. Hydroxybenzylpindolol (32) was iodinated, and IHYP was purified to theoretical specific activity (2.2 Ci/μmole) as previously described (33, 34). An aliquot (150 μl) of a crude membrane preparation (approximately 60 μg of protein) was incubated with IHYP and 100 μM phentolamine in new disposable polypropylene test tubes (Falcon 2018) in a final volume of 0.250 ml (23). Phentolamine decreased nonspecific binding [defined here as binding in the presence of 0.3 μM (±)-propranolol] without affecting specific binding [defined as the difference between total binding and nonspecific binding (23)]. Specific binding typically represented 75–85% of total binding. Samples were incubated for 30 min at 37°. To terminate the reaction, 10 ml of 0.9% NaCl, 10 mM Tris, pH 7.5, were added to each assay tube and the samples were rapidly filtered using Gelman type AE glass fiber filters. Each filter was washed with an additional 10 ml of buffer at 37°. Radioactivity was determined by liquid scintillation spectrometry at an ef-

ficiency for ^{125}I of approximately 70% (34).

The K_D and density of binding sites for IHYP were determined by measuring the amount of IHYP bound at different concentrations of ligand ranging from 38 to 450 pM. The data were then analyzed as described by Scatchard (35). The K_D of IHYP is not affected by treatment with 6-OHDA (26), and it is therefore possible to quantitate the effect of 6-OHDA by determining the amount of IHYP bound at an arbitrary concentration of ligand. In practice, experiments were carried out at a concentration of IHYP of 0.1 nM, which is approximately equal to its K_D. The effect of 6-OHDA is shown in absolute terms (Fig. 2) when complete saturation curves were obtained, and as percentage change when only a single concentration of IHYP was used (Fig. 1B).

In previous experiments (26) the density of binding sites and the K_D of IHYP were determined by adding varying amounts of IHYP of low specific activity (100–200 Ci/mole) to a constant amount of IHYP of high specific activity (2.2 Ci/μmole) and then analyzing thz data by the method of Scatchard (35). The low-specific-activity IHYP was purified using high-performance liquid chromatography (34). IHYP was eluted from a micro C-18 column (36-inch) in a single peak with a retention time of 15 min. The high-specific-activity ligand was purified by paper chromatography (33, 34).

In spite of the fact that the low-specific-activity IHYP eluted from the liquid chromatography column as a single peak, it was discovered during the course of recent studies that IHYP was partially inactivated by the column procedure. Thus, Scatchard analysis carried out using low-specific-activity IHYP gave erroneously high values for the K_D of IHYP and for the density of *beta* adrenergic receptors (23, 26). The values determined with high-specific-activity ligand were 5–10 times lower than those determined with low-specific-activity ligand. Despite the quantitative error introduced by the partial inactivation of the low-specific-activity IHYP, the results obtained with the two methods are qualitatively identical. Specifically, the increase in receptor density previously observed with low-specific-activity IHYP has been confirmed in the current study with high-specific-activity IHYP. All values for the K_D of IHYP and for the density of binding sites reported in this communication were determined using only high-specific-activity IHYP.

Additional methods. Adenylate cyclase activity was determined in the presence of 4 mM NaF by the method of Krishna *et al.* (36) as previously described (34). Protein was determined by the method of Lowry *et al.* (37), using bovine serum albumin as a standard. Norepinephrine levels were determined in homogenates by the method of Coyle and Henry (38) as modified by Nelson and Molinoff (39).

Materials. Hydroxybenzylpindolol was a gift from Dr. D. Hauser, Sandoz Pharmaceuticals. Regis Chemical Corporation was the source of 6-hydroxydopamine hydrobromide. Phentolamine mesylate was obtained from Ciba, and (±)-propranolol, from Ayerst. [^{3}H]Adenine, [^{3}H]cyclic AMP, and Na^{125}I were obtained from New England Nuclear. Pargyline was a gift from Dr. R. E. Singiser, Abbott Laboratories. (−)-norepinephrine HCl, cyclic AMP, ATP, (−)-isoproterenol (+)-bitartrate, and protein kinase (beef heart) were obtained from Sigma; crystalline bovine serum albumin and ascorbic acid, from Baker Chemical Company; and 3-isobutyl-1-methylxanthine, from Aldrich Chemical Company.

RESULTS

Norepinephrine levels. Twenty-four hours following an intraventricular injection of 6-OHDA, there was a 50% decrease in the norepinephrine content of the rat cerebral cortex (Fig. 1A). Norepinephrine levels fell to approximately 30% of control values 48 hr after the first of two injections. There were small additional decreases after the first 48 hr, until, 30 days following 6-OHDA administration the content in the cortex was less than 10% of that in control animals. The depletion of norepinephrine reflects the rapid destruction of norepinephrine-containing nerve endings in the cerebral cortex following 6-OHDA administration (21).

Binding of IHYP to beta adrenergic

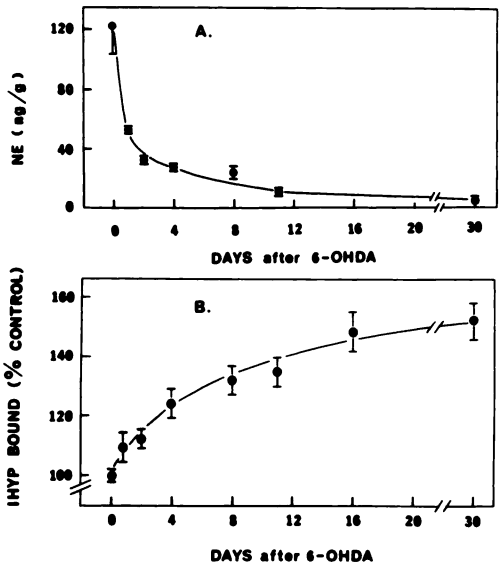

FIG. 1. *Time course of effect of 6-OHDA treatment on norepinephrine (NE) levels and density of beta adrenergic receptors in rat cerebral cortex*

A. Rats were injected intraventricularly with 200 μg (free base) of 6-OHDA on each of 2 successive days, and norepinephrine levels were measured at various times after the first injection. Results are expressed as nanograms of norepinephrine per gram of tissue, wet weight. Each point is the mean ± standard error of 6–27 animals.

B. The densities of IHYP binding sites were determined at various times after the first 6-OHDA injection and are expressed as a percentage of binding in control cortices in the same experiments. The amount of IHYP specifically bound (in femtomoles per milligram of protein) was measured at a concentration of IHYP approximately equal to its K_D (actual concentrations used varied from 110 to 190 pM). Control values ranged from 21.4 ± 0.6 to 44.1 ± 1.3 fmoles/mg of protein. The data represent eight separate experiments, each of which included sham-treated rats. Each experimental point represents the mean ± standard error of values from 6–33 animals.

receptors. We have previously shown (23) that the binding of IHYP to particulate fractions of rat cerebral cortex has properties similar to those which would be expected of binding to *beta* adrenergic receptors *in vitro*. The binding is saturable, reversible, of high affinity, and is stereospecifically inhibited by *beta* adrenergic receptor ligands (23).

The amount of IHYP bound was determined in cortices of control and 6-OHDA-treated animals at various concentrations of IHYP (Fig. 2). The administration of 6-OHDA led to an increase in the amount of IHYP bound. The same data were analyzed by the method of Scatchard (35) (Fig. 2, inset). The results are consistent with the existence of only a single class of high-affinity binding site. The slopes of the lines show that the K_D of IHYP is the same in control and treated animals. In the experiments shown in Fig. 2 the density of sites was increased by 50%.

In typical experiments the amount of specifically bound IHYP (in femtomoles per milligram of protein) was measured at a ligand concentration approximately equal to the K_D of IHYP (0.1 nM) in membrane preparations from cortices of control rats and rats that had been treated with 6-OHDA. The concentration of *beta* adrenergic receptors in the cerebral cortices of treated animals was not significantly different from that in sham-treated controls 1 or 2 days following treatment with 6-OHDA (Fig. 1B). Receptor density increased on subsequent days, reaching a maximal level of approximately 150% of control values after 16 days. The effect of 6-OHDA persisted for at least 100 days (data not shown). The increases seen at 4 days and at all subsequent times were highly significant ($p < .01$) when compared with controls according to Dunnett's multiple-comparison test for comparing several experimental values with a single control group (40). Combined results obtained in 10 separate experiments are shown in Fig. 1B. Each experiment included sham-treated controls and rats that had been treated 1–30 days earlier. Because of variations in the concentration of IHYP used in each assay (110–190, pM), the density of *beta* adrenergic receptors measured in control (sham-treated) groups varied from experiment to experiment (21.4–44.1 fmoles/mg of protein). The data from treated animals are expressed as a percentage of the mean density measured in cortices from groups of control animals that were assayed on the same day. This means of expressing the data was necessary because of the small changes that were observed. Complete time course stud-

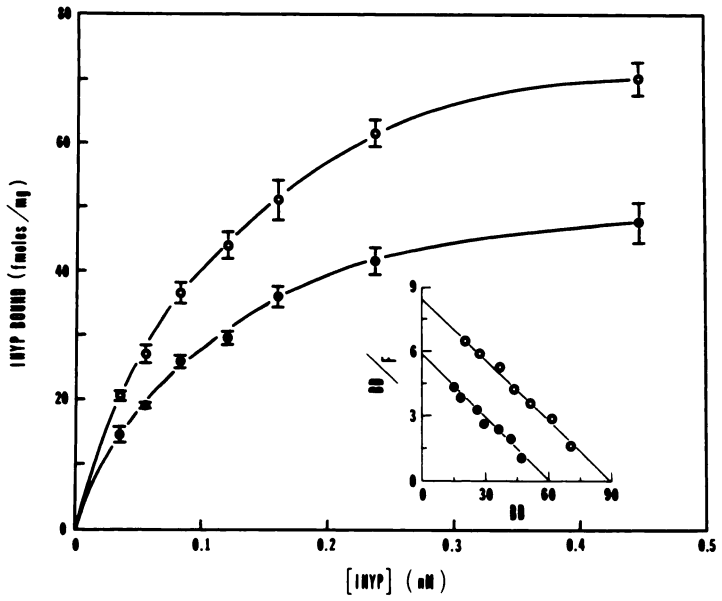

FIG. 2. *Saturation of IHYP binding sites in membranes from cortices of control and 6-ODHA-treated rats*
The amount of IHYP specifically bound (in femtomoles per milligram of protein) on the ordinate is plotted against the nanomolar concentration of IHYP on the abscissa. Each point represents the mean ± standard error of separate saturation curves determined in duplicate on membrane fractions from six sham-treated (●) or 6-OHDA-treated (○) rats 16 days after the first of two injections. The inset shows the same data plotted by the method of Scatchard (35). The abscissa is the amount of IHYP specifically bound (in femtomoles per milligram), and the ordinate (in units of 10^{-4} liters/mg) is the amount of bound IHYP divided by the concentration of free IHYP. The K_D values determined from the slopes of the lines are 102 pM for control and 107 pM for 6-OHDA-treated rats. The intercepts on the abscissa give values for the maximum number of binding sites. In this experiment there were 59.7 fmoles of IHYP binding sites per milligram of protein in membranes from controls and 89.7 fmoles/mg in membranes from 6-OHDA-treated rats.

ies were carried out (days 1-11) on three separate occasions, yielding qualitatively identical results. In these experiments the density of receptors was either unchanged or slightly increased on days 1 and 2. There were significant increases in density only on day 4 and thereafter.

Cyclic AMP accumulation in brain slices. Cyclic AMP accumulation was determined by the pre-labeling technique of Shimizu *et al.* (30) and by the protein binding assay of Gilman (31). Similar results were obtained with both assays (Fig. 3). With either assay cyclic AMP accumulation, measured in the presence of 30 μM (−)-isoproterenol, was greater in slices from 6-OHDA-treated animals than from control animals.

The accumulation of cyclic AMP in the presence of norepinephrine was measured in slices of cerebral cortex (Fig. 4). The *alpha* adrenergic receptor antagonist phenotlamine (3 μM) was included in these incubations so that only *beta* adrenergic receptor-mediated synthesis of cyclic AMP would be measured. Brain slices prepared from animals that had been treated with 6-OHDA showed a 1.5-fold increase in the amount of cyclic AMP synthesized in the presence of a maximally effective concentration of norepinephrine 4 days after the first injection of 6-OHDA. The maximal increase in cyclic AMP accumulation was approximately 2-fold (Fig. 4). An increase in the potency of norepinephrine for stimulation of cyclic AMP accumulation also occurred following treatment with 6-OHDA (Fig. 4). This change displayed a more rapid time course than did the enhanced response to a maximally effective concentration of norepinephrine. In control animals, the concentration of norepi-

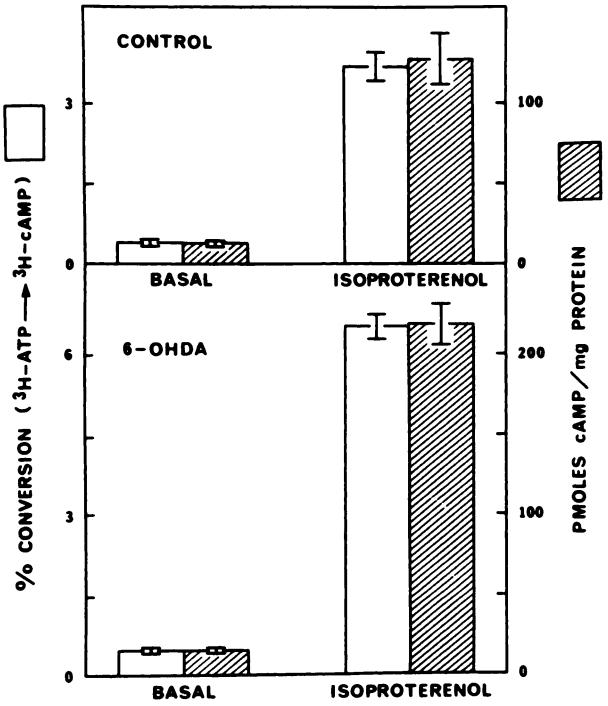

FIG. 3. *Accumulation of cyclic AMP in slices from cerebral cortices of sham-treated rats or rats treated with 6-OHDA*

Rats received the first injection 8 days prior to being killed. Cyclic AMP accumulation was measured (see METHODS) as the percentage of [^{3}H]ATP converted to cyclic [^{3}H]AMP (open bars) or as picomoles of cyclic AMP per milligram of protein (hatched bars). Each bar represents the mean ± standard error of determinations on six animals.

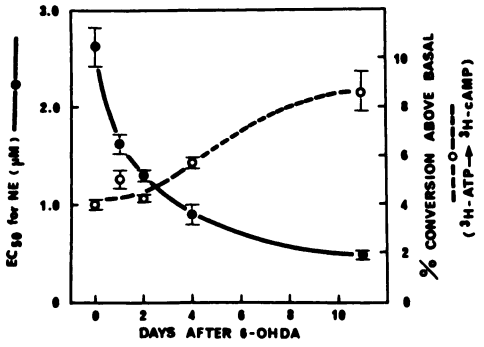

FIG. 4. *Effect of 6-OHDA on norepinephrine-stimulated cyclic AMP accumulation in slices of rat cerebral cortex*

The accumulation of cyclic AMP in response to various concentrations of norepinephrine (NE) was measured after labeling of tissue ATP pools with [^{3}H]adenine. Rats were treated with 6-OHDA as described in METHODS 1, 2, 4, or 11 days prior to being killed, and the data for each individual rat were plotted as described by Eadie (41) and Hofstee (42), yielding values for EC_{50} and maximal percentage conversion. Each point represents the mean ± standard error of values from three or four rats.

nephrine producing a half-maximal increase in cyclic AMP production was 2.6 μM. The EC_{50} for cyclic AMP synthesis was 1.6 μM 1 day after the first injection of 6-OHDA and appeared to reach a minimal value of approximately 0.5 μM by 11 days after the first injection of 6-OHDA. The time course of the change in the EC_{50} for norepinephrine was similar to that observed for the depletion of norepinephrine levels following 6-OHDA treatment (Fig. 1A). The observed loss of endogenous norepinephrine reflects the destruction of noradrenergic terminals, and it is likely that the increased potency of norepinephrine is a consequence of the loss of the uptake system for norepinephrine that is associated with presynaptic noradrenergic terminals.

Cyclic AMP accumulation in response to a maximally effective concentration (30 μM) of (−)-isoproterenol also increased following 6-OHDA treatment (Fig. 5). This increase followed a time course similar to

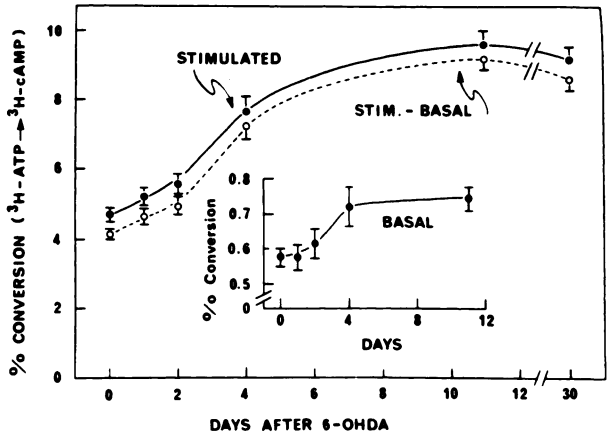

FIG. 5. *Time course of effect of 6-OHDA on cyclic AMP accumulation in slices of rat cerebral cortex in the presence of a maximally stimulating concentration of (−)-isoproterenol*

Rats were treated as described in METHODS 1-30 days prior to being killed, and cyclic AMP accumulation was measured. The percentage conversion of [^{3}H]ATP to cyclic [^{3}H]AMP in the presence of 30 μM isoproterenol (●) and in the presence of 30 μM isoproterenol minus the basal percentage conversion (○) is shown. The inset shows the effect of 6-OHDA treatment on basal cyclic AMP accumulation. Each point in the figure and inset represents the mean ± standard error of 13 or 14 animals.

that of the increased cyclic AMP accumulation that occurred in response to a maximally effective concentration of norepinephrine (Fig. 4) and to the increase in the density of *beta* adrenergic receptors (Fig. 1B). Isoproterenol has a low affinity for the presynaptic uptake system (43), and the EC_{50} value of isoproterenol was not changed by treatment with 6-OHDA.

Basal levels of cyclic AMP accumulation as determined by the pre-labeling assay were consistently greater than control values at times longer than 2 days after the administration of 6-OHDA (Fig. 5, inset). Experiments were performed to determine whether 6-OHDA administration led to changes in the amount of adenylate cyclase present in the cerebral cortex. The synthesis of cyclic AMP in the presence of 4 mM NaF was determined in a resuspended membrane pellet obtained by centrifugation at 20,000 × *g* for 10 min. Fluoride-sensitve adenylate cyclase activity (picomoles per milligram of protein per minute) was 329 ± 6 in control animals, 318 ± 7 in rats 4 days after the first 6-OHDA injection, and 323 ± 6 in rats 10 days after 6-OHDA (N = 12 for each group). The increases in basal and catecholamine-stimulated levels of cyclic AMP accumulation therefore do not appear to reflect an increase in total adenylate cyclase activity.

DISCUSSION

The catecholamine-responsive cyclic AMP-generating system is affected in a number of tissues by alterations of the normal neuronal input. Thus, reduction of norepinephrine stores following the administration of reserpine (44–46), chemical sympathectomy with 6-OHDA (9–11), or denervation of adrenergically innervated tissues (7, 12) resulted in enhanced synthesis of cyclic AMP in response to maximally effective concentrations of catecholamines. The molecular mechanisms responsible for these increases in cyclic AMP synthesis have not been completely defined. Recent reports from this and other laboratories have suggested that changes in the density of *beta* adrenergic receptors are sometimes involved in regulating the accumulation of cyclic AMP induced by adrenergic agonists (26, 47–50). For example, an increase in the density of *beta* adrenergic receptors was found to occur along with the enhanced response to maximally effective concentrations of isoproterenol that is observed in the rat cerebral cortex 1 week after the intraventricular administration of 6-OHDA (26). In the present study the

roles of pre- and postsynaptic elements of 6-OHDA-induced supersensitivity and the relationship between receptor concentration and postsynaptic responsiveness have been assessed by defining the temporal sequence of the loss of presynaptic nerve terminals and of the development of changes in *beta* adrenergic receptors and catecholamine-stimulated cyclic AMP accumulation.

Following treatment with 6-OHDA, norepinephrine content decreased to 50% of control levels within 24 hr and to 30% after 48 hr (Fig. 1A). The EC_{50} for norepinephrine-induced cyclic AMP accumulation decreased with a similar time course (Fig. 4). Thus 1 day after 6-OHDA administration the EC_{50} for cyclic AMP synthesis was 62% of that seen in control animals. The similarity in time courses of the decrease in norepinephrine levels and the decrease in the EC_{50} for norepinephrine-stimulated cyclic AMP synthesis is consistent with the idea that it is the loss of the uptake system for norepinephrine that is primarily responsible for the change in the apparent affinity of norepinephrine for the stimulation of cyclic AMP synthesis. Kalisker *et al.* (10) reached the same conclusion in studies of the effect of cocaine on the EC_{50} value of norepinephrine for cyclic AMP synthesis following the administration of 6-OHDA to rats. In the present studies no change in the potency of (−)-isoproterenol was observed in slices from treated rats. This result can be explained by the fact that isoproterenol is a poor substrate for the uptake system (43).

Following the intraventricular administration of 6-OHDA, there is an increase in the amount of cyclic AMP that accumulates in response to a maximally effective concentration of either isoproterenol or norepinephrine. The enhanced response occurred following a 2–3-day lag, in contrast to the rapid decrease in norepinephrine levels and the EC_{50} for norepinephrine stimulation of cyclic AMP accumulation. The biochemical basis for the enhanced response of the postsynaptic *beta* adrenergic receptor–adenylate cyclase system was investigated by following the time course of the changes in several individual constituents of this system. Basal levels of the conversion of [^{3}H]ATP to cyclic [^{3}H]AMP were consistently increased after 6-OHDA administration. This increase followed a time course similar to that of the increase in isoproterenol- and norepinephrine-stimulated cyclic AMP accumulation. When basal levels of endogenous cyclic AMP were determined using a protein binding assay, a small increase was seen in some but not all experiments following treatment with 6-OHDA. On the other hand, Kalisker *et al.* (10) and Huang *et al.* (11) have reported that following the intraventricular administration of 6-OHDA there was no change in the basal level of conversion of [^{3}H]ATP to cyclic [^{3}H]AMP in slices of cerebral cortex. The difference from our results may be due to the routine use of a phosphodiesterase inhibitor in the current studies. We have previously suggested that the increase in basal levels of cyclic AMP accumulation might be due to an increase in the amount of adenylate cyclase activity (26). However, fluoride-stimulated enzyme activity was unchanged in homogenates following treatment with 6-OHDA. Thus it is unlikely that a significant increase in total adenylate cyclase occurred as a consequence of 6-OHDA administration.

There are several factors that complicate the interpretation of studies of adrenergic receptors in the mammalian central nervous system. *Beta* adrenergic receptors may be associated with glia as well as with neurons (51–53). Although it is possible that the drug has effects on glia, it is more likely that the observed changes are due to effects of 6-OHDA on neurons. Radioautographic or histofluorescent (54) techniques may allow an analysis of the cellular distribution of the increase in the density of *beta* adrenergic receptors after 6-OHDA treatment. Second, stimulation of *alpha* adrenergic receptors as well as *beta* adrenergic receptors leads to an increase in cyclic AMP synthesis in the brain. This factor is unlikely to complicate the results of this study, because IHYP and isoproterenol interact specifically with *beta* adrenergic receptors.

We have previously reported that 6-OHDA treatment does not affect the affinity of IHYP for *beta* adrenergic receptors

in rat cerebral cortex (26). On the other hand, a significant increase was found in the density of *beta* adrenergic receptors 8 days following treatment with 6-OHDA (26). The time course of this increase (Fig. 1B) was similar to the time course of the increase in cyclic AMP production in response to maximally effective concentrations of isoproterenol or norepinephrine (Figs. 4 and 5). However, the magnitude of the increase in cyclic AMP accumulation was substantially greater than the increase in receptor density. This difference could be attributable to the fact that binding studies are carried out with membranes prepared from homogenates while cyclic AMP accumulation is determined in slices. We have previously (26) tried to quantitate IHYP binding in slices, but the low percentage of specific binding (30–50%) would make it difficult to detect changes of the magnitude observed in the current studies. On the other hand, hormone-sensitive adenylate cyclase activity is difficult to measure in cell-free preparations of brain tissue (55).

One simple explanation of the difference in magnitude of the increases is that factors in addition to changes in *beta* adrenergic receptor density and the loss of presynaptic terminals affect the regulation of the responsiveness of this system. For example, there was no change in adenylate cyclase activity, but an increase in a small but functionally important pool of adenylate cyclase molecules could contribute to this enhanced responsiveness. In view of the difference in the tissue preparations used, a definitive conclusion cannot be drawn at this time.

In a parallel study the effects of denervation have been investigated in the rat cerebral cortex during ontogeny (56). After treatment of neonatal rats with 6-OHDA, a 45–75% increase in cyclic AMP accumulation and a 40–60% increase in the density of *beta* adrenergic receptors were observed. These increases were apparent as early as postnatal day 6. Although the administration of 6-OHDA to newborn rats led to an increase in receptor density, there was no change in the time course of development of receptors. As in the present studies, there was no change in fluoride-sensitive cyclase activity.

Kalisker *et al.* (10) reported that phosphodiesterase activity increased by approximately 25% following the administration of 6-OHDA to adult rats. In the present experiments, assays of cyclic AMP accumulation were carried out in the presence of an inhibitor of phosphodiesterase. However, a change in degradative capacity could have contributed to the changes in cyclic AMP accumulation that were observed.

Significant increases in the density of *beta* adrenergic receptors did not occur until at least 3 days after 6-OHDA administration, although norepinephrine levels were markedly reduced within 24 hr. A half-maximal decrease in norepinephrine levels was seen at 1 day, whereas a half-maximal increase in the density of receptors did not occur until 4 days following 6-OHDA administration. The time course of the maximal increases in norepinephrine- and isoproterenol-stimulated cyclic AMP accumulation was similar to that of the change in receptor density. This, together with the long-term nature of the changes, suggests that a new steady-state level of receptors is achieved and that this increased level of receptors contributes to the increased postsynaptic sensitivity of the receptor-cyclase system.

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REFERENCES

1. Cannon, W. B. & Rosenblueth, A. (1949) *The Supersensitivity of Denervated Structures*, Macmillan, New York.
2. Axelsson, J. & Thesleff, S. (1959) *J. Physiol. (Lond.)*, 147, 178–193.
3. Miledi, R. (1960) *J. Physiol. (Lond.)*, 151, 1–23.
4. Roper, S. (1976) *J. Physiol. (Lond.)*, 254, 455–473.
5. Kuffler, S. W., Dennis, M. J. & Harris, A. J. (1971) *Proc. Soc. Lond., Ser. B, Biol. Sci.*, 177, 555–563.
6. Ungerstedt, U. (1971) *Acta Physiol. Scand. Suppl.*, 367, 69–93.
7. Weiss, B. & Strada, S. J. (1972) *Adv. Cyclic Nucleotide Res.*, 1, 357–374.
8. Mishra, R. K., Gardner, E. L., Katzman, R. & Makman, M. H. (1974) *Proc. Natl. Acad. Sci. U. S. A.* 71, 3883–3887.

9. Palmer, G. C. (1972) *Neuropharmacology*, 11, 145–149.
10. Kalisker, A., Rutledge, C. O. & Perkins, J. P. (1973) *Mol. Pharmacol.*, 9, 619–629.
11. Huang, M., Ho, A. K. S. & Daly, J. W. (1973) *Mol. Pharmacol.*, 9, 711–717.
12. Palmer, G. C., Spurgeon, H. A. & Priola, D. V. (1975) *J. Cyclic Nucleotide Res.*, 1, 89–95.
13. Miledi, R. & Potter, L. T. (1971) *Nature*, 233, 599–603.
14. Berg, D. K., Kelley, R. B., Sargent, P. B., Williamson, P. & Hall, Z. W. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, 69, 147–151.
15. Hall, Z. W. (1973) *J. Neurobiol.*, 4, 343–361.
16. McConnell, M. G. & Simpson, L. L. (1976) *J. Pharmacol. Exp. Ther.*, 198, 507–517.
17. Fleming, W. W., McPhillips, J. J. & Westfall, D. P. (1973) *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.*, 68, 56–119.
18. Fleming, W. W. & Westfall, D. P. (1975) *J. Pharmacol. Exp. Ther.*, 192, 381–389.
19. Trendelenburg, U. (1966) *Pharmacol. Rev.*, 18, 629–640.
20. Iversen, L. L. (1967) *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*, Cambridge University Press, Cambridge, England.
21. Kostrzewa, R. M. & Jacobowitz, D. M. (1974) *Pharmacol. Rev.*, 26, 199–288.
22. Alexander, R. W., Davis, J. N. & Lefkowitz, R. J. (1975) *Nature*, 258, 437–440.
23. Sporn, J. R. & Molinoff, P. B. (1976) *J. Cyclic Nucleotide Res.*, 2, 149–161.
24. Bylund, D. B. & Snyder, S. H. (1976) *Mol. Pharmacol.*, 12, 568–580.
25. Davis, J. N. & Lefkowitz, R. J. (1976) *Brain Res.*, 113, 214–218.
26. Sporn, J. R., Harden, T. K., Wolfe, B. B. & Molinoff, P. B. (1976) *Science*, 194, 624–626.
27. Uretsky, N. J. & Iversen, L. L. (1970) *J. Neurochem.*, 17, 269–278.
28. Noble, E. P., Wurtman, R. J. & Axelrod, J. (1967) *Life Sci.*, 6, 281–291.
29. Perkins, J. P. & Moore, M. M. (1973) *J. Pharmacol. Exp. Ther.*, 185, 371–378.
30. Shimizu, H., Daly, J. W. & Creveling, C. R. (1969) *J. Neurochem.*, 16, 1609–1619.
31. Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. U. S. A.*, 67, 305–312.
32. Aurbach, G. D., Fedak, S. A., Woodard, C. J., Palmer, J. S., Hauser, D. & Troxler, F. (1974) *Science*, 186, 1223–1224.
33. Maguire, M. E., Wiklund, R. A., Anderson, H. J. & Gilman, A. G. (1976) *J. Biol. Chem.*, 251, 1221–1231.
34. Harden, T. K., Wolfe, B. B. & Molinoff, P. B. (1976) *Mol. Pharmacol.*, 12, 1–15.
35. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.*, 51, 660–672.
36. Krishna, G., Weiss, B. & Brodie, B. B. (1968) *J. Pharmacol. Exp. Ther.*, 163, 379–385.
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, 193, 265–275.
38. Coyle, J. T. & Henry, D. (1973) *J. Neurochem.*, 21, 61–67.
39. Nelson, D. L. & Molinoff, P. B. (1976) *J. Pharmacol. Exp. Ther.*, 196, 346–359.
40. Dunnett, C. W. (1964) *Biometrics*, 20, 482–491.
41. Eadie, G. S. (1952) *Science*, 116, 688.
42. Hofstee, B. H. J. (1952) *Science*, 116, 329–331.
43. Burgen, A. S. V. & Iversen, L. L. (1965) *Br. J. Pharmacol.*, 25, 34–49.
44. Palmer, G. C., Sulser, F. & Robison, G. A. (1973) *Neuropharmacology*, 12, 327–337.
45. Williams, B. J. & Pirch, J. H. (1974) *Brain Res.*, 68, 227–234.
46. Dismukes, K. & Daly, J. W. (1974) *Mol. Pharmacol.*, 10, 933–940.
47. Kebabian, J. W., Zatz, M., Romero, J. A. & Axelrod, J. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 3735–3739.
48. Wolfe, B. B., Harden, T. K. & Molinoff, P. B. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, 73, 1343–1347.
49. Mukherjee, C., Caron, M. G. & Lefkowitz, R. J. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 1945–1949.
50. Harden, T. K., Wolfe, B. B., Sporn, J. R., Perkins, J. P. & Molinoff, P. B. (1977) *Brain Res.*, 125, 99–108.
51. Gilman, A. G. & Nirenberg, M. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, 68, 2165–2168.
52. Schultz, J., Hamprecht, B. & Daly, J. W. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, 69, 1266–1270.
53. Clark, R. B. & Perkins, J. P. (1971) *Proc. Natl. Acad. Sci., U. S. A.*, 68, 2757–2760.
54. Melamed, E., Lahav, M. & Atlas, D. (1976) *Nature*, 261, 420–422.
55. Daly, J. (1975) in *Handbook of Psychopharmacology* (Iversen, L. L., Iversen, S. D. & Snyder, S. H., eds.), Vol. 5, p. 47, Plenum, New York.
56. Harden, T. K., Wolfe, B. B., Sporn, J. R., Poulos, B. K. & Molinoff, P. B. (1977) *J. Pharmacol. Exp. Ther.*, in press.