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Altered Responsiveness of Cerebral *Beta* Adrenoceptors Assessed by Adenosine Cyclic 3',5'-Monophosphate Formation and [^{3}H]Propranolol Binding

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SUMMARY

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The chronic treatment of neonate chicks with reserpine or 6-hydroxydopamine severely depleted cerebral catecholamines and resulted in an increased responsiveness of *beta* adrenoceptor-mediated cyclic 3',5'-AMP formation in the cerebral hemispheres both *in vivo* and in slices *in vitro*. Conversely, treatment of chicks with isoproterenol suspended in glycerol trioleate, to effect slow release of the catecholamine, induced marked densensitization of the cyclic AMP response. The enhanced response to isoproterenol was already observed *in vivo* 12 hr after a single injection of reserpine and was maintained for at least 72 hr. Densensitization was fully developed 3-6 hr after chronic isoproterenol, but normal responses were again observed by 18 hr. The altered responsiveness was specific for *beta* adrenoceptor agonists both *in vivo* and in cerebral slices *in vitro*. The responses to histamine and adenosine were not significantly altered. However, incubation of slices in the presence of the phosphodiesterase inhibitor Ro 20-1724 at least partially reversed the hyporesponsiveness induced by chronic isoproterenol without influencing the enhanced responses seen in reserpine-treated chicks. On the other hand, the activity of adenylate cyclase and phosphodiesterase in homogenates of cerebral hemispheres was similar in all groups. Despite an increased responsiveness of *beta* adrenoceptor-mediated cyclic AMP formation after reserpine, there was no change in the characteristics of binding of the specific ligand [^{3}H]propranolol to cerebral membranes from these animals. However, the densensitized cyclic AMP response induced by chronic isoproterenol was accompanied by a 30% reduction in the maximum binding of [^{3}H]propranolol but not in the binding affinity of the ligand to cerebral membranes. It is proposed that cerebral *beta* adrenoceptors can adjust their responsiveness in relation to the availability of catecholamines, and possible mechanisms are discussed.

INTRODUCTION

There is growing evidence for the concept that cells can regulate their responsiveness to hormones or neurotransmitters, and it is now recognized that this phenomenon may represent an important homeostatic mechanism in cellular communication (1). For example, it has been shown that the sensitivity of adrenergically innervated organs to catecholamines is enhanced by denervation and sup-

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pressed by overexposure to catecholamines (2, 3). Indeed, Axelrod and his colleagues have shown compensatory increases or decreases in the responsiveness of adenylate cyclase in the pineal to catecholamines (4), and in recent experiments these workers have provided evidence that these changes may reflect alterations in *beta* adrenoceptor binding sites (5).

In the central nervous system there is also good evidence to suggest that postsynaptic adrenoceptors can exhibit hyperresponsiveness to exogenous agonists following destruction of catecholamine-containing nerve terminals with 6-hydroxydopamine or after reserpine-induced depletion of cerebral catecholamines. These supersensitive responses have been assessed electrophysiologically (6) and behaviorally (7, 8). Moreover, investigation of catecholamine-stimulated cyclic 3',5'-AMP formation in cerebral slices has indicated that these altered responses may result from events close to the receptor itself (9, 10), and recent work has indeed revealed an increase in cerebral *beta* adrenoceptor binding sites following 6-hydroxydopamine administration to rats (11).

The present experiments were undertaken to manipulate pharmacologically the responsiveness of catecholamine-stimulated cyclic AMP formation in chick cerebral hemispheres. Previous experiments from this laboratory have demonstrated that this species exhibits a particularly marked cyclic AMP response to catecholamines that appears to be mediated solely by a *beta* adrenoceptor (12). Moreover, by virtue of the incomplete development of the blood-brain barrier in the young chick, experiments can be performed both *in vivo* and *in vitro*. Further analysis of the altered receptor responsiveness has been performed by examination of the characteristics of binding of a specific *beta* adrenoceptor ligand, [^{3}H]propranolol (13, 14), to cerebral membranes. Some of this work has been communicated to the British Pharmacological Society (15).

MATERIALS AND METHODS

All experiments were performed on 2–5-day-old male Ranger chicks. Reserpine was administered daily for 2 days (unless otherwise stated) at a dose of 2.5 mg/kg subcutaneously. 6-Hydroxydopamine was injected intracerebroventricularly at a dose of 60 μg in 10 μl of 0.9% NaCl containing 1 mg/ml of ascorbic acid on 2 successive days. In the experiments examining the effect of chronic isoproterenol treatment, (−)-isoproterenol was suspended in glycerol trioleate and administered subcutaneously (150 μmoles/kg) in two doses at 12-hr intervals to effect slow release of the catecholamine. Control chicks were treated with appropriate vehicle in each case. Unless otherwise stated, chicks were killed 24 hr after the last reserpine injection, 96 hr after the last 6-hydroxydopamine injection, or 6 hr after isoproterenol treatment. In the experiments *in vivo*, chicks were injected intravenously with (−)-isoproterenol or histamine and the cerebral hemispheres were removed by freeze-blowing (16) 2 min later. Previous experiments (17) have demonstrated peak responses to these amines at this time. Cerebral tissue was homogenized in 10 volumes of 80% ethanol at −5°, and following centrifugation, the supernatants were taken to dryness at 40°, dissolved in distilled water, and purified by column chromatography (18). Cyclic AMP was then assayed by a protein binding saturation assay (19). Experiments *in vitro* were performed on 0.37-mm-thick slices of cerebral hemispheres that had been incubated for 60 min in Krebs-Ringer-bicarbonate buffer containing 10 mM glucose. The slices were then transferred to fresh medium (10 mg of tissue per 5 ml of medium) in the presence and absence of the potent phosphodiesterase inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone -Ro 20-1724) and exposed to the appropriate agonist for 15 min. After this time slices were harvested and homogenized in ethanol, and cyclic AMP was determined as above. Peak responses to catecholamines, histamine, and adenosine were shown to occur after 10–25 min of incubation. Protein was estimated by the method of Lowry *et al.* (20).

For the measurement of adenylate cyclase activity, 100–200 mg of cerebral tissue were gently homogenized in 25 vol-

umes of 2 mM Tris-maleate buffer, pH 7.4. The enzyme assay system contained 80 mM Tris-maleate (pH 7.4), 10 mM $MgSO_4$, 10 mM aminophylline, and 50 μl of the homogenate (200–500 μg of protein). Reactions were initiated by the addition of ATP (final concentration, 1 mM), and incubations were normally carried out for 3 min at 30°. The reaction was terminated by placing the tubes in a boiling water bath for 3 min; after cooling, neutral alumina (10 mg) was added to each tube and the samples were stirred. Preliminary experiments had shown that alumina efficiently removed interfering noncyclic nucleotides. After centrifugation, samples of the supernatant fluid were assayed for cyclic AMP as above. Standard curves in the binding assay were appropriately supplemented with supernatant fluid taken from zero-incubation-time samples. Cyclic 3',5'-nucleotide phosphodiesterase activity of crude homogenates was assayed by the isotopic method of Thompson and Appleman (21) with recent modifications (22).

Cerebral membranes for [^{3}H]propranolol binding were routinely prepared as follows. Cerebral tissue was homogenized in 20 volumes of ice-cold 0.32 M sucrose, using a motor-driven Teflon-glass homogenizer, and the homogenate was centrifuged at 1000 × *g* for 15 min. The supernatant was then centrifuged at 48,000 × *g* for 60 min, and the resultant pellet was resuspended in ice-cold buffer containing 50 mM Tris-HCl and 15 mM $MgCl_2$, pH 7.8. In the binding assay, membrane suspensions (0.3–0.6 mg of protein) were incubated at 23° with [^{3}H]propranolol (1–40 nM) and the appropriate concentration of drug in 50 mM Tris-HCl buffer (pH 7.8) containing 15 mM $MgCl_2$ in a final volume of 250 μl. After 15 min the samples were rapidly diluted with 1 ml of ice-cold buffer and filtered under reduced pressure through Whatman glass fiber discs (GF/B or GF/C), and the filters were washed twice with 6 ml of buffer (filtering and washing took less than 5 sec). The filters were dried at room temperature and then shaken with 5 ml of Triton X-100–toluene scintillator, and the radioactivity was determined by liquid scintillation counting. In every experiment nonspecific binding was determined by measuring the radioactivity obtained when incubations were carried out in the presence of 200 μM (−)-isoproterenol, and specific binding was defined as the difference between total binding and nonspecific binding. Specific binding of 15 nM [^{3}H]propranolol reached equilibrium in 2 min at 23° and normally constituted 50–70% of the total binding.

Norepinephrine, dopamine, and 5-hydroxytryptamine were extracted from chick cerebral tissue according to Shellenberger and Gordon (23) and assayed spectrofluorometrically (24, 25).

(±)-4*n*-[^{3}H]Propranolol (21–26 Ci/mmole) and [8-^{3}H]adenosine cyclic 3',5'-monophosphate (25–30 Ci/mmole) were obtained from the Radiochemical Centre, Amersham.

(−)-Isoproterenol HCl and 6-hydroxydopamine were obtained from Aldrich. (−)-Norepinephrine HCl was purchased from Sigma; adenosine, from Boehringer; and histamine acid phosphate, from British Drug Houses. (−)-Propranolol was kindly donated by ICI Pharmaceuticals. All other reagents were of the highest grade commercially available.

RESULTS

Effects of reserpine or 6-hydroxydopamine on cerebral biogenic amines. Treatment of chicks with reserpine resulted in profound depletion of cerebral norepinephrine, dopamine, and 5-hydroxytryptamine (Table 1). On the other hand, the intracerebroventricular injection of 6-hydroxydopamine, although severely influencing the catecholamines, did not affect the concentration of 5-hydroxytryptamine in chick cerebral tissue (Table 1). This emphasizes the selective neurotoxic effect of this agent on catecholamine-containing nerve terminals previously reported in mammalian brain (26).

Effects of reserpine, 6-hydroxydopamine, or chronic isoproterenol treatment on isoproterenol- and histamine-induced cyclic AMP formation in vivo. In confirmation of previous results from this laboratory (17), the intravenous administration of isoproterenol or histamine resulted in a

TABLE 1

Effect of reserpine and 6-hydroxydopamine on chick cerebral norepinephrine, dopamine, and 5-hydroxytryptamine content

The results represent the means ± standard errors of at least five separate experiments.

Treatment	Time after last injection	Norepinephrine	Dopamine	5-Hydroxytryptamine
	days	*ng/g*	*ng/g*	*ng/g*
Vehicle only		456 ± 12	354 ± 19	965 ± 31
Reserpine, 2 × 2.5 mg/kg	1	63 ± 4[a]	183 ± 24[a]	476 ± 8[a]
	3	200 ± 3[a]	256 ± 11[a]	694 ± 29[a]
6-Hydroxydopamine, 2 × 60 μg	2	151 ± 32[a]	220 ± 21[a]	871 ± 29
	4	104 ± 20[a]	192 ± 16[a]	909 ± 31

[a] $p < 0.01$.

marked increase of cyclic AMP in the cerebral hemispheres of control chicks (Fig. 1). Moreover, there was significant enhancement of the response to isoproterenol, but not to histamine, in chicks treated chronically with reserpine or 6-hydroxydopamine. Conversely, after chicks had been treated with isoproterenol suspended in glycerol to effect slow release of the catecholamine, subsequent intravenous injection of the *beta* agonist barely stimulated cyclic AMP formation over basal levels. However, histamine produced a response similar to that induced in control chicks.

An enhanced response to isoproterenol was already observed *in vivo* 12 hr after a single injection of reserpine (Fig. 2), and although this effect was reduced after 36 hr, it still remained significantly above control chicks at 72 hr. Following chronic treatment with isoproterenol, the cyclic AMP response to a subsequent acute injection of this agonist was rapidly reduced (Fig. 2). This marked desensitization was short-lived, however; normal responses to isoproterenol were observed 18 hr after the chronic treatment.

Effect of reserpine or chronic isoproterenol treatment on biogenic amine-stimulated cyclic AMP formation in slices. A further examination of this altered responsiveness to isoproterenol was made *in vitro*. As reported earlier from this laboratory, a maximally effective concentration of isoproterenol (10 μM) induced a 30-fold increase in cyclic AMP in chick cerebral hemisphere slices incubated *in vitro*. Slices prepared from reserpine-treated chicks displayed a significantly increased responsiveness to this *beta* agonist,

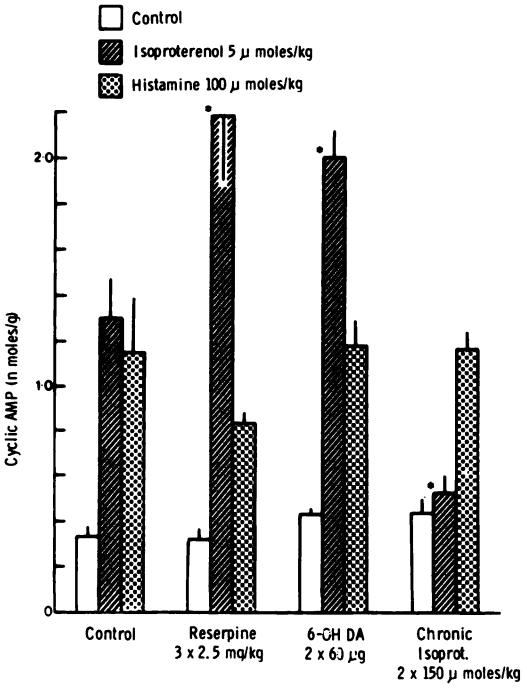

FIG. 1. *Effects of reserpine, 6-hydroxydopamine (6-OH DA), and chronic isoproterenol treatments on cyclic AMP accumulation induced in vivo in chick cerebral hemispheres by isoproterenol or histamine*

Chicks received injections of either vehicle or drugs at the doses indicated. Twenty-four hours after the last reserpine injection, 96 hr after 6-hydroxydopamine, or 6 hr after chronic isoproterenol, they were injected intravenously with isoproterenol or histamine, and the cerebral hemispheres were removed by freeze-blowing 2 min later. Each value is the mean ± standard error of five separate experiments.

* $p < 0.05$ compared with control.

whereas slices prepared from chicks treated chronically with isoproterenol were considerably less responsive than

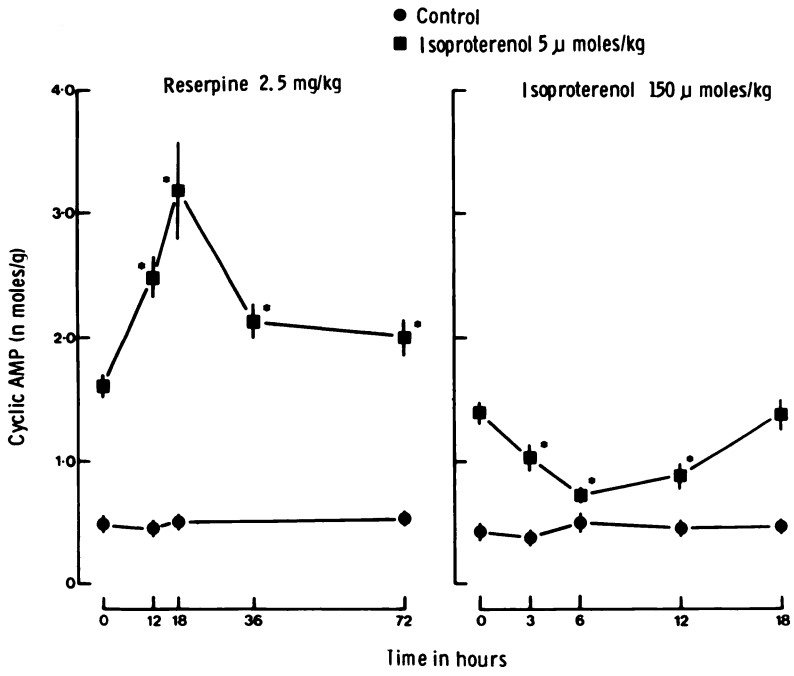

FIG. 2. *Time course of changes in responsiveness to isoproterenol on cyclic AMP accumulation in vivo*
Chicks were treated with a single injection of reserpine (2.5 mg/kg) or isoproterenol (150 μmoles/kg in glycerol), and at the times indicated were injected intravenously with isoproterenol (5 μmoles/kg) and killed 2 min later by freeze-blowing. Values are means ± standard errors of four experiments.
* $p < 0.05$ compared with chicks not previously treated with reserpine or isoproterenol.

control slices (Fig. 3). The effects of histamine and adenosine on the accumulation of the cyclic nucleotide were similar in all groups of chicks. The ability of the *beta* adrenoceptor-blocking drug (−)-propranolol to suppress isoproterenol-stimulated cyclic AMP formation *in vitro* was remarkably similar in control, hyper-, and hyporesponsive cerebral tissue (Table 2). However, when the slices were incubated in the presence of the potent phosphodiesterase inhibitor Ro 20-1724, although the increased responsiveness to isoproterenol was still evident in the reserpine-treated group, the densensitization induced by chronic isoproterenol treatment was reversed to the extent that cyclic AMP accumulations now were not significantly different from those of control chicks (Fig. 4).

Effects of reserpine or chronic isoproterenol treatment on adenylate cyclase and phosphodiesterase activity in homogenates. Adenylate cyclase activity was assayed in homogenates of cerebral hemispheres from chicks that had been chronically treated with reserpine or isoproterenol. Neither basal activity nor the activity stimulated by sodium fluoride or by 5'-guanyl-ylimidodiphosphate was significantly altered by these treatments (Fig. 5). Moreover, the activity of cyclic AMP phosphodiesterase was similar in all groups whether incubations were carried out in the presence or absence of the calcium chelator 1,2-di(2-aminoethoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid. The following data were obtained: control, 2.08 ± 0.21 (n = 5); reserpine (2 × 2.5 mg/kg), 2.35 ± 0.19 (n = 5); chronic isoproterenol (2 × 150 μmoles/kg), 1.81 ± 0.20 (n = 5) nmoles/mg of protein per minute.

Effect of reserpine or chronic isoproterenol treatment on specific binding of [^{3}H]propranolol to cerebral membranes. Previous experiments (13, 14) demonstrated that [^{3}H]propranolol binding sites on chick cerebral membranes possess characteristics associated with true *beta* adrenoceptors. In the present experiments, although enhanced isoproterenol-stimulated

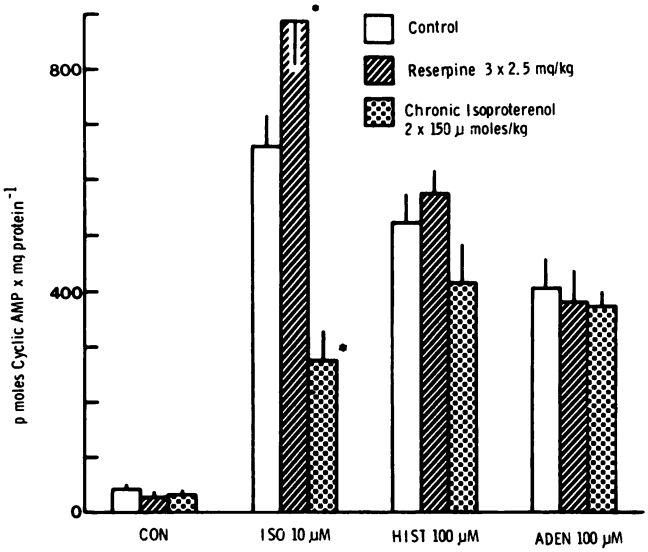

FIG. 3. *Accumulation of cyclic AMP induced by isoproterenol (ISO), histamine (HIST), and adenosine (ADEN) in cerebral hemisphere slices prepared from chicks treated with reserpine or chronic isoproterenol*

Chicks were killed 24 hr after the last reserpine injection or 6 hr after the last chronic isoproterenol injection. Values are means ± standard errors of five experiments.

* $p < 0.05$ compared with chicks not previously treated with reserpine or isoproterenol.

TABLE 2

Effect of (−)-propranolol on (−)-isoproterenol-stimulated cyclic AMP accumulation in chick cerebral hemisphere slices

Groups of five chicks were treated with reserpine (2.5 mg/kg) 2 days and 1 day before death or with (−)-isoproterenol (150 μmoles/kg, suspended in glycerol trioleate) 12 hr and 6 hr before death. Slices were incubated with isoproterenol in the presence and absence of propranolol for 15 min. Values shown are the means ± standard errors of five experiments.

Addition	Cyclic AMP		
	Control	Reserpine	Chronic isoproterenol
	pmoles/mg protein		
None	20 ± 1.4	24 ± 2.1	28 ± 1.1
(−)-Isoproterenol, 1 μM	310 ± 29	514 ± 72	124 ± 19
(−)-Propranolol, 1 μM	18 ± 1	17 ± 2	12 ± 2
(−)-Propranolol, 0.01 μM; isoproterenol, 1 μM	284 ± 25	488 ± 31	98 ± 8
(−)-Propranolol, 0.1 μM; isoproterenol, 1 μM	79 ± 14	120 ± 19	49 ± 4
(−)-Propranolol, 1 μM; isoproterenol, 1 μM	17 ± 3	18 ± 2	15 ± 2

cyclic AMP formation was observed in slices prepared from reserpine-treated chicks, the specific binding of [^{3}H]propranolol to receptor sites was identical with that seen in the control vehicle-treated group (Fig. 6). 6-Hydroxydopamine-treated chicks also exhibited an unchanged *beta* adrenoceptor binding site population (data not shown). However, in chicks treated chronically with isoproterenol, the severe depression of *beta* adrenoceptor-mediated cyclic AMP formation was accompanied by a 30% reduction in the maximum binding of [^{3}H]propranolol to cerebral membranes (Fig. 7). Scatchard plots of these data (Fig. 8) indicated a fall

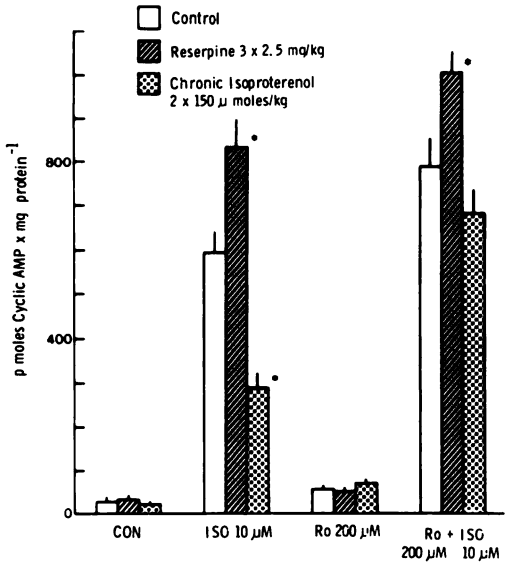

FIG. 4. *Effect of Ro 20-1724 on response of chick cerebral hemisphere slices to isoproterenol*

Chicks were killed 24 hr after the last reserpine injection or 6 hr after the last chronic isoproterenol injection. Slices were incubated with isoproterenol (ISO) in the presence and absence of Ro 20-1724 (Ro) for 15 min. Values are the means ± standard errors of five experiments.

* $p < 0.05$ compared with chicks not previously treated with reserpine or isoproterenol.

in the total number of receptors without a significant change in the binding affinity of the ligand.

DISCUSSION

There are now a number of reports describing altered responsiveness of hormonal or neurotransmitter-stimulated cyclic AMP formation in tissues or cultured cells. Although this phenomenon appears to be common to a number of tissues, the underlying mechanism is apparently not the same in all tissues. There is some evidence that an alteration in certain forms of phosphodiesterase (27, 28) or a change in the intracellular distribution of cyclic AMP, leading to a different rate of breakdown of the cyclic nucleotide (29), may be related to the altered responses. Other work has implicated the generation of inhibitors of adenylate cyclase (30), an alteration in the efficiency of coupling of the receptor and catalytic components of the enzyme complex (31), or a change in the number of plasma membrane receptors for their specific hormones or neurotransmitters (5, 32).

In the present experiments, drug treatments that resulted in chronic changes in the degree of stimulation of cerebral *beta* adrenoceptors induced opposite alterations in the responsiveness of *beta* adrenoceptor-mediated cyclic AMP formation. Thus the chronic depletion of cerebral catecholamines by 6-hydroxydopamine or reserpine resulted in hyperresponsiveness of the cyclic AMP system, whereas the chronic administration of isoproterenol markedly suppressed the response *in vivo* and *in vitro*. In some ways the alterations in responsiveness exhibited common features. First, they were both agonist-specific. The sensitivity to neither histamine nor adenosine was changed by the drug treatments. Moreover, the onset of the al-

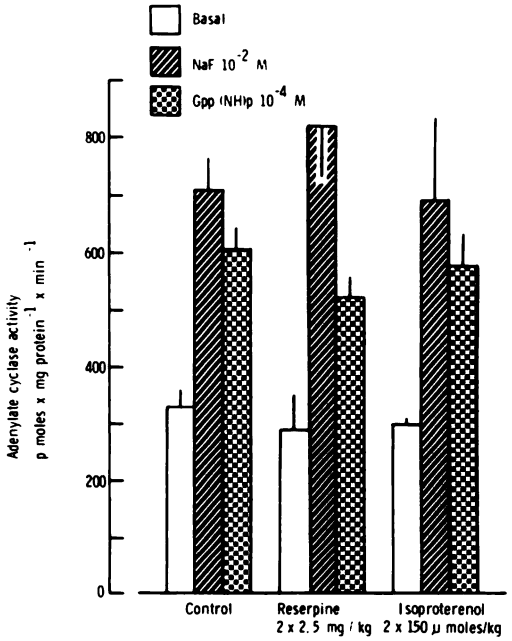

FIG. 5. *Adenylate cyclase activity in homogenates of cerebral hemispheres of chicks treated with reserpine or chronic isoproterenol*

Chicks were killed 24 hr after the last reserpine injection and 6 hr after the last chronic isoproterenol injection. Values are the means ± standard errors of five experiments. Gpp(NH)p, 5'-guanylylimidodiphosphate.

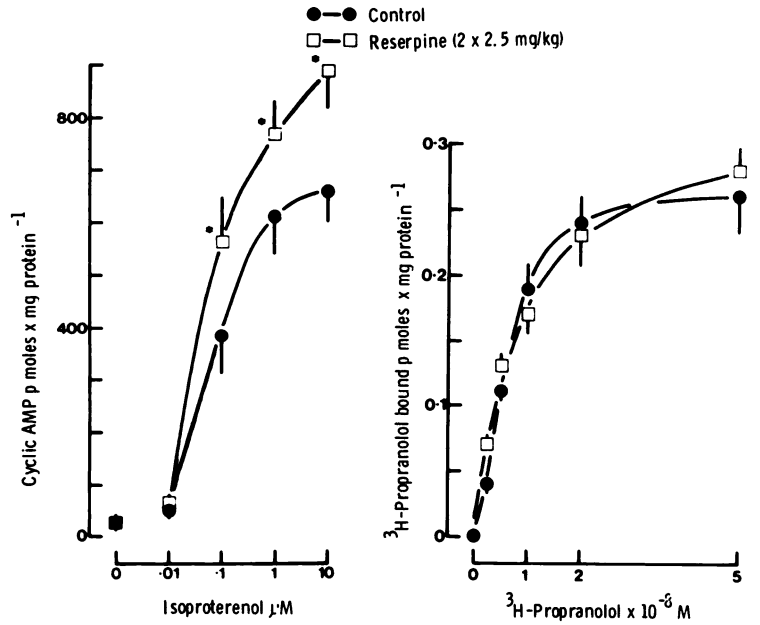

FIG. 6. *Isoproterenol-stimulated cyclic AMP accumulation in cerebral hemisphere slices and specific [³H]propranolol binding in cerebral membranes from control or reserpine-treated chicks*

Chicks were killed 24 hr after the final reserpine injection, and each point represents the mean ± standard error of five determinations.

* $p < 0.05$ compared with control.

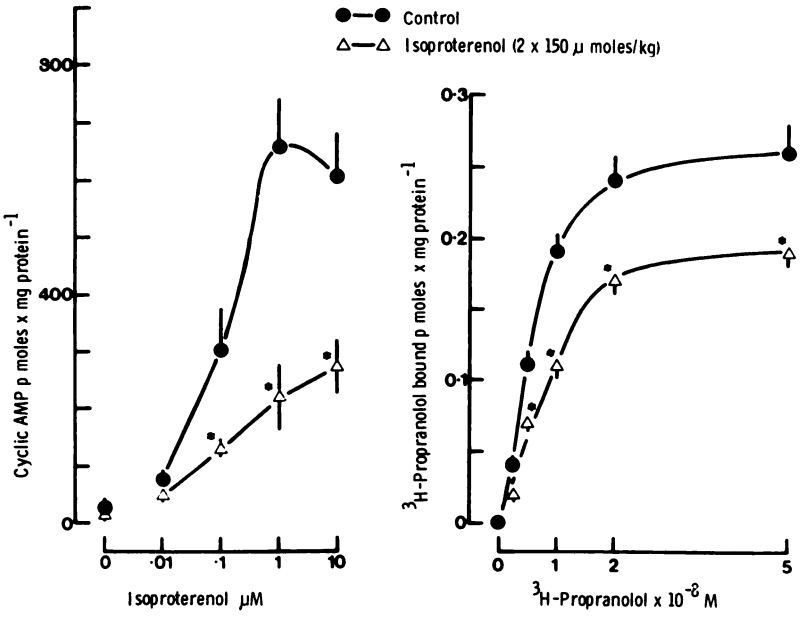

FIG. 7. *Isoproterenol-stimulated cyclic AMP accumulation in cerebral hemisphere slices and specific [³H]propranolol binding to cerebral membranes prepared from chicks chronically treated with isoproterenol*

Chicks were killed 6 hr after the last isoproterenol injection, and each point represents the mean ± standard error of at least five determinations.

* $p < 0.05$ compared with control.

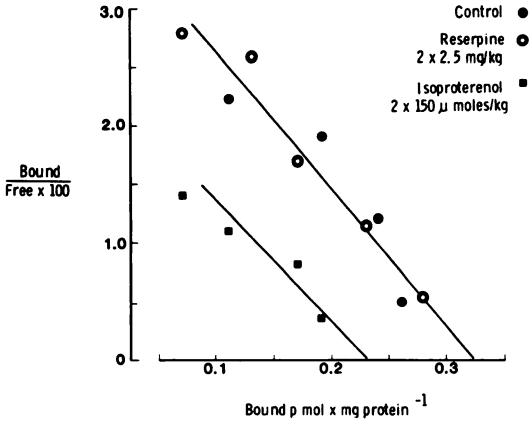

FIG. 8. *Scatchard analysis of [³H]propranolol binding to control and reserpine- or isoproterenol-treated chicks*

Points have been plotted from data in Figs. 6 and 7. Control, K_d 8.5 nM, B_{max} 0.318 pmole/mg of protein; reserpine, K_d 8.4 nM, B_{max} 0.325 pmole/mg of protein; chronic isoproterenol, K_d 8.9 nM, B_{max} 0.228 pmole/mg of protein.

tered states of responsiveness was relatively rapid (3–12 hr) and readily reversible, and in both cases the maximal accumulation of cyclic AMP was modified without any marked change in the apparent K_m of the response to isoproterenol. The nature of these changes makes it unlikely that they could be due to alterations in catecholamine uptake at nerve endings or, in the case of subsensitivity, to residual receptor-bound isoproterenol being carried over into the assays *in vitro*. Under these circumstances there would be shifts in the affinity of *beta* adrenoceptor-mediated cyclic AMP formation rather than alteration of the maximal response.

Since all the experiments were carried out in whole-cell systems, it is possible that changes in phosphodiesterase activity may be related to the altered responses. This seems improbable in the case of reserpine-induced hyperresponsiveness, since the effect still persisted in the presence of the potent phosphodiesterase inhibitor Ro 20-1724. However, the desensitization induced by chronic isoproterenol was at least partially reversed by this compound, suggesting that if Ro 20-1724 acts as a phosphodiesterase inhibitor in this system, an increased activity of this enzyme may be related to the densensitization. However, since the changes in sensitivity were quite agonist-specific, a selective increase in phosphodiesterase within a compartment associated only with the *beta* adrenoceptor–adenylate cyclase complex is indicated. On the other hand, there is evidence that Ro 20-1724 can inhibit the uptake of adenosine in brain slices (33), and this nucleoside has been reported to restore cyclic AMP responses to biogenic amines in acutely densensitized guinea pig cerebral cortex slices (34). It is possible, therefore, that the ability of Ro 20-1724 to reverse the hyporesponsiveness of chick cerebral tissue induced by chronic isoproterenol may be unrelated to phosphodiesterase inhibition. In any case the activity of phosphodiesterase in homogenates was similar in all groups studied, as was the activity of adenylate cyclase under basal conditions or after fluoride and guanine nucleotide stimulation.

There is now considerable evidence that the total number or accessibility of specific receptors for several hormones can be altered by manipulating the hormone concentration in contact with the target cells (1). Indeed, recent work in pineal and frog erythrocytes (5, 32) has established that alterations in *beta* adrenoceptor sensitivity are associated with changes in receptor binding sites. In the present experiments, a significant loss of [³H]propranolol binding sites in membranes prepared from chicks that had received chronic isoproterenol suggests that a similar phenomenon can occur within the central nervous system. However, the fall in receptor density was clearly less than the loss of the cyclic AMP response to isoproterenol *in vitro*. Sporn *et al.* (11) have recently demonstrated that 6-hydroxydopamine-treated rats exhibit an 80% increase in the maximal accumulation of cyclic AMP induced by isoproterenol in cerebral cortex slices, but that the *beta* adrenoceptor binding sites were increased by only 30%. These disparities may in part explain why there was no significant increase in receptors accompanying the modest 20–30% elevation in cyclic AMP response seen in reserpine-treated chicks *in vitro*. Charness *et*

al. (35) have recently reported similar discrepancies between enhanced isoproterenol-stimulated adenylate cyclase in rat reticulocytes, and work from this laboratory has shown that there are marked differences between the number of binding sites and the intrinsic activity of *beta* adrenoceptor-mediated cyclic AMP formation in different areas of chick brain (14, 36). These observations emphasize the intervening steps between receptor occupancy and stimulation of adenylate cyclase and suggest that alterations may also occur in the efficiency of "coupling" of receptor and catalytic sites of this enzyme.

There have been some recent reports demonstrating a reduced responsiveness of catecholamine-stimulated cyclic AMP formation in rat cerebral tissue following the chronic administration of tricyclic antidepressants and monoamine oxidase inhibitors (37, 38). However, until the degree of redundancy of cerebral adrenoceptors and, indeed, their definitive cellular localization is known, it will be difficult to relate these changes to the therapeutic action of these drugs. Nevertheless, the present studies suggest that cerebral *beta* adrenoceptors, whether associated with neurons or glia, are at least postsynaptic and within the influence of noradrenergic nerve endings. Moreover, the results stress the adaptability of cerebral *beta* adrenoceptors, and future work is being directed toward an understanding of the mechanisms of altered neurotransmitter receptor function.

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