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Effect of Long-Term Changes in Sympathetic Nervous Activity on the *Beta*-Adrenergic Receptor-Adenylate Cyclase Complex of Rat Pineal Gland

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SUMMARY

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The effect of sympathetic input on *beta*-adrenergic receptors and adenylate cyclase activity of rat pineal gland was determined by surgically, chemically, or physiologically reducing the sympathetic input to the gland. Bilaterally decentralizing the superior cervical ganglia of blinded rats increased the density of *beta*-adrenergic receptors as measured by the binding of [^{3}H]dihydroalprenolol, but did not change the affinity of the receptors for the ligand. The increased density of receptors following ganglionic decentralization was accompanied by an increase in norepinephrine-sensitive adenylate cyclase activity. An increase in the density of pineal gland *beta*-adrenergic receptors was also observed in rats that were chemically sympathectomized by administration of guanethidine or reserpine. In pineal glands isolated from rats treated with guanethidine as neonates, the accumulation of cyclic AMP in response to norepinephrine was greater than that found in glands from rats treated with 0.9% NaCl solution. Similarly, in pineal glands of rats treated with repeated doses of reserpine, there was an increase in norepinephrine-sensitive adenylate cyclase activity as compared with that found in the glands from 0.9% NaCl-treated control animals. Maintaining rats in constant light, which physiologically reduces the sympathetic input to the pineal gland, also caused an increased density of *beta*-receptors in this gland. These experiments support the hypothesis that the increased responsiveness of catecholamine-sensitive adenylate cyclase seen in adrenergically innervated tissue following reduced sympathetic input is due to an increased density of *beta*-adrenergic receptors.

INTRODUCTION

In a given tissue the responsiveness of the adenylate cyclase system to catecholamine agonists is modified by the prior degree of sympathetic input to the tissue. Reducing sympathetic input surgically (1–3), chemically (4–6), or physiologically (1, 7) produces a supersensitive response of adenylate cyclase to norepinephrine. Conversely, chronic overexposure of the *beta*-adrenergic receptor-adenylate cyclase complex to *beta*-adrenergic agonists, produced either by administering these agonists themselves (3, 5, 8) or by administering drugs that block the reuptake of catecholamines into sympathetic nerve endings (9), produces a subsensitive response of the enzyme to catecholamines.

Recently, with the use of high-affinity receptor ligands, such as DHA,[2] it has been demonstrated that *beta*-adrenergic supersensitivity produced by chemical or physiological manipulations is associated with an increase in density but not affinity of *beta*-adrenergic receptors for the ligands (10–13). In tissues that show a subsensitive response of adenylate cyclase to *beta*-adrenergic agonists, there is a corresponding decrease in the number of *beta*-adrenergic receptor binding sites (9, 10, 13–15). However, reports of changes in receptor density following surgical reduction in adrenergic input have been limited to one study which showed a 25% increase in DHA binding in iris following denervation (16).

Since the sympathetic input to the pineal gland can be readily manipulated by altering the lighting conditions (17) or by removing the superior cervical ganglia (18),

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[2] The abbreviation used is: DHA, dihydroalprenolol.

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and since this gland had a high density of *beta*-receptors (19) and a high activity of the catecholamine-sensitive adenylate cyclase system (20), we examined the effects of altered sympathetic activity on the *beta*-adrenergic receptor-adenylate cyclase complex of this gland.

METHODS

Animals. Male and pregnant female Sprague-Dawley rats were purchased from Zivic-Miller Laboratories, Allison Park, Pa. All animals were housed under standard laboratory conditions in temperature-controlled rooms and, except for those kept in constant light, were subjected to a 12-hr alternating light-dark cycle.

Surgical preparation of the animals (bilateral decentralization or superior cervical ganglionectomy or blinding by bilateral enucleation) was performed by Zivic-Miller Laboratories. Surgically sympathectomized rats were 4 weeks old at the time of operation. These animals were killed 4–6 weeks after surgery.

Drug treatment. Blinded 10-week-old male rats were treated with reserpine (Sandril; Lilly and Company, Indianapolis, Ind.), 1 μmole/kg per day, i.p., for 3 days. Control rats received injections of 0.9% NaCl solution. The animals were decapitated 24 hr after the last dose. Male and female neonatal rats were treated with guanethidine sulfate, 170 μmoles/kg per day, s.c., 5 days/week for 3 weeks beginning on the 7th day after birth. This treatment produces a permanent chemical sympathectomy (21). Control rats received 0.9% NaCl solution, 10 ml/kg, according to the same schedule. These rats were killed at 2 months of age for the measurement of cyclic AMP and at 8 months for the receptor binding studies.

Assay of beta-adrenergic receptors. Pineal glands were homogenized with a Brinkman Polytron (setting 4.5 for 15 sec) in Tris buffer (50 mM), pH 8.0, containing 3 mM $MgCl_2$. *Beta*-adrenergic receptors were estimated by measuring the binding at various concentrations of [^{3}H]DHA (0.5 nM to 40.5 nM) as described by Lefkowitz *et al.* (22), modified for the pineal gland (19). Specific DHA binding is defined as the total DHA binding minus the nonspecific binding, determined in the presence of 20 μM *l*-propranolol. Scatchard analysis (23) of the DHA binding data was used to estimate the apparent dissociation constants (K_d) of DHA for the *beta*-adrenergic receptors and the maximal density of these receptors.

Assay of cyclic AMP. Rats maintained in a 12-hr light-dark cycle were decapitated approximately 6 hr after the start of the light period, and the norepinephrine-stimulated accumulation of cyclic AMP was determined in cultured whole pineal glands as previously described (24).

Assay of adenylate cyclase activity. Adenylate cyclase activity of pineal gland homogenates was determined by a modification (20) of the method of Krishna *et al.* (25), using an ATP-regenerating system (26).

Protein assay. Protein was measured by the method of Lowry *et al.* (27), using bovine serum albumin as the standard.

MATERIALS

[^{3}H]DHA was purchased from New England Nuclear Corporation, Boston, Mass., and [8-^{14}C]ATP-disodium from Schwarz/Mann Company, Orangeburg, N. Y. ATP-disodium, cyclic AMP, and *l*-arterenol bitartrate (norepinephrine) were obtained from Sigma Chemical Company, St. Louis, Mo.; creatine phosphate disodium and creatine kinase were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. *l*-Propranolol HCl was generously donated by Ayerst Laboratories, New York, N. Y.; guanethidine sulfate, by Ciba-Geigy Company, Summit, N. J.

RESULTS

Effect of light exposure on beta-adrenergic receptors. The influence of light exposure on *beta*-adrenergic receptors in pineal glands is shown in Fig. 1. In animals kept in constant light for 4 weeks (Fig. 1A), the binding of DHA was significantly ($p < 0.05$) greater than that found in rats that had been blinded for 4 weeks. A Scatchard analysis of these data (Fig. 1B) shows that maximal binding of DHA in the light-exposed rats was approximately twice that in the blinded animals (1470 fmoles bound per milligram of protein in light-exposed rats as compared with 670 fmoles bound per milligram of protein in blinded rats). The apparent K_d values for DHA binding were not significantly different in the two groups (approximately 5 nM).

Effect of surgical sympathectomy on beta-adrenergic receptors. Since variations in the lighting conditions alter adrenergic receptor density in the pineal gland, blinded rats were used to study the influence of surgical sympathectomy on the *beta*-adrenergic receptors in this gland. Table 1 shows that the binding of DHA (20 nM) was slightly (not significantly) greater in pineal glands from rats whose superior cervical ganglia had been removed than in the glands of neuronally intact animals. However, DHA binding was significantly greater ($p < 0.005$) in the pineal glands of rats whose superior cervical ganglia were surgically decentralized. Further studies (Table 2) showed that the maximal density of the *beta*-receptors (maximal binding) in pineal glands of rats whose ganglia were decentralized was approximately 50% greater than that of intact glands. No differences were found in the K_d values for DHA between glands from decentralized and sham-operated control animals. This increase in the number of *beta*-adrenergic receptors was apparent when the data were calculated on the basis of protein content (Table 2) or on the basis of maximal number of receptors per pineal gland; i.e., there were 96 ± 6 fmoles of DHA bound per pineal gland in sham-operated rats and 130 ± 8 fmoles of DHA bound per pineal gland in rats whose ganglia had been decentralized ($p < 0.005$).

Effect of surgical sympathectomy on adenylate cyclase activity. To determine whether the increased density of *beta*-receptors in decentralized pineal glands was associated with a concomitant increase in norepinephrine-sensitive adenylate cyclase activity, the activity of this enzyme was measured in pineal glands from blinded rats subjected to superior cervical decentralization or sham operations. Figure 2 shows that the basal adenylate cyclase activity was unchanged by bilateral superior cervical ganglion decentralization. Norepinephrine produced a significant ($p < 0.001$) increase in enzyme activity in

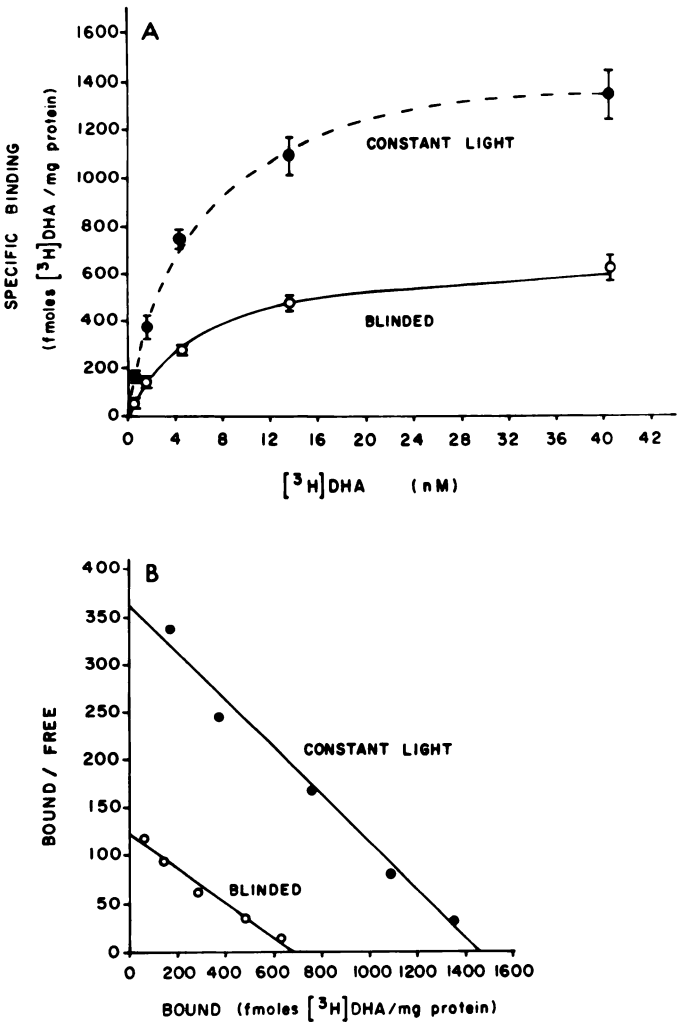

FIG. 1. *Effect of light exposure on the specific binding of DHA in rat pineal gland*

Rats were either blinded or sham-operated at 4 weeks of age. All animals were kept in constant light for an additional 6 weeks at which time the pineal glands were removed and assayed for DHA binding as described under Methods.

A. The specific binding of DHA as a function of the concentration of DHA. Each point represents the mean ±standard error of the mean of six to eight experiments. Binding was significantly greater ($p < 0.05$) at each concentration of DHA in the glands from animals kept in constant light as compared with those from blinded rats (Student's t-test).

B. Scatchard analysis of the data. The maximal DHA binding in pineal glands of blinded rats was 670 ± 50 fmoles/mg of protein, and the maximal DHA binding in pineal glands of animals kept in constant light was 1470 ± 80 fmoles/mg of protein ($p < 0.001$). In both cases the apparent K_d values were approximately 5 nM.

both the decentralized and control groups; however, the response to norepinephrine in the pineal glands from the decentralized rats was approximately twice that of the control animals ($p < 0.01$).

Effect of chemical sympathectomy on the beta-adrenergic receptor-adenylate cyclase complex. Two models of chemical sympathectomy were used to modify sympathetic input to the pineal gland: (a) treatment of neonatal rats with guanethidine, which produces a permanent sympathectomy (21) and (b) treatment of adult rats

TABLE 1

Effect of surgical sympathectomy on the binding of [3H]DHA in rat pineal gland

Four-week-old rats were blinded and either sham-operated or surgically sympathectomized by bilateral superior cervical ganglionectomy or by bilateral decentralization. The animals were killed at 10 weeks of age and the binding of [3H]DHA (20 nM) was measured in whole pineal gland homogenates as described under Methods. Values given for specific binding are means ± standard error of the mean.

Surgical treatment	Specific binding	No. of experiments
	fmoles DHA bound/ mg protein	
Sham-operated	430 ± 60	6
Ganglionectomized	590 ± 70	4
Decentralized	750 ± 30[a]	4

[a] $p < 0.005$ compared with sham-operated control rats.

with reserpine, which depletes norepinephrine stores in sympathetic nerve endings (28). At all concentrations of DHA studied, the pineal glands from the guanethidine-treated rats showed greater specific binding of DHA as compared with that found in the glands of the control rats (Fig. 3A). Analysis of these data by the method of Scatchard (Fig. 3B) indicated that guanethidine treatment did not change the apparent affinity (K_d = 5 nM) of the receptors for the DHA in the two groups of animals. However, there was almost a 2-fold increase in the maximal number of receptors in pineal glands of guanethidine-treated rats as compared with that of the NaCl-treated controls.

The effect of guanethidine treatment on norepinephrine-stimulated adenylate cyclase was estimated by measuring the increase in the content of cyclic AMP in cultured pineal glands (Fig. 4). The basal concentrations of cyclic AMP in cultured pineal glands from control and guanethidine-treated rats were similar. Supramaximal concentrations of norepinephrine (50 μM) produced approximately a 15-fold increase in the concentration of cyclic AMP in pineal glands from control animals but approximately a 45-fold increase in cyclic AMP in pineal glands from guanethidine-treated rats. Thus, the norepinephrine-stimulated increase in the concentration of cyclic AMP was approximately 3 times greater in cul-

TABLE 2

Effect of bilateral superior cervical ganglion decentralization on beta-adrenergic receptors in pineal glands of blinded rats

Four-week-old rats were sham-operated or surgically sympathectomized by bilateral superior cervical ganglion decentralization. The animals were killed at 10 weeks of age and the binding of DHA (0.5–40.5 nM) was measured as described under Methods. Maximal binding and K_d values were calculated from Scatchard analyses of the binding data. Values given are means ± standard error of the mean of eight experiments.

Surgical treatment	Maximal binding	K_d
	fmoles DHA bound/mg protein	*nM*
Sham-operated	700 ± 50	6.3 ± 0.7
Decentralized	1040 ± 60[a]	6.5 ± 1.1

[a] $p < 0.001$ compared with sham-operated animals.

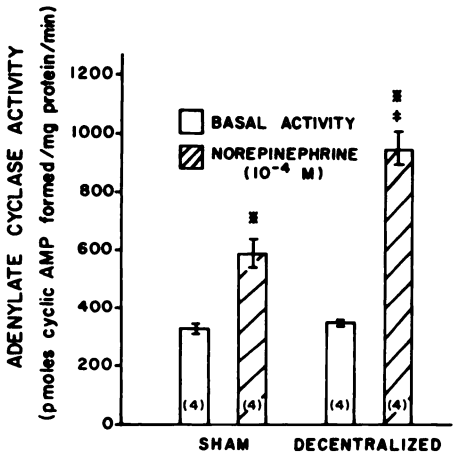

FIG. 2. *Effect of superior cervical ganglion decentralization on norepinephrine-sensitive adenylate cyclase activity in pineal glands of blinded rats*

All rats were blinded at 4 weeks of age. Bilateral decentralization of the superior cervical ganglia or sham operations were also performed when rats were 4 weeks old. Adenylate cyclase activity was measured in pineal gland homogenates when rats were 10 weeks old. Samples were assayed in the presence and absence of *l*-norepinephrine (10^{-4} M). Bars represent the mean adenylate cyclase activity of the number of experiments shown in parentheses. Vertical brackets indicate the standard error of the mean.

* $p < 0.005$ (Student's t-test for paired data) compared with values obtained in the absence of norepinephrine.

‡ $p < 0.05$ (unpaired *t*-test) compared with values from corresponding sham-operated rats.

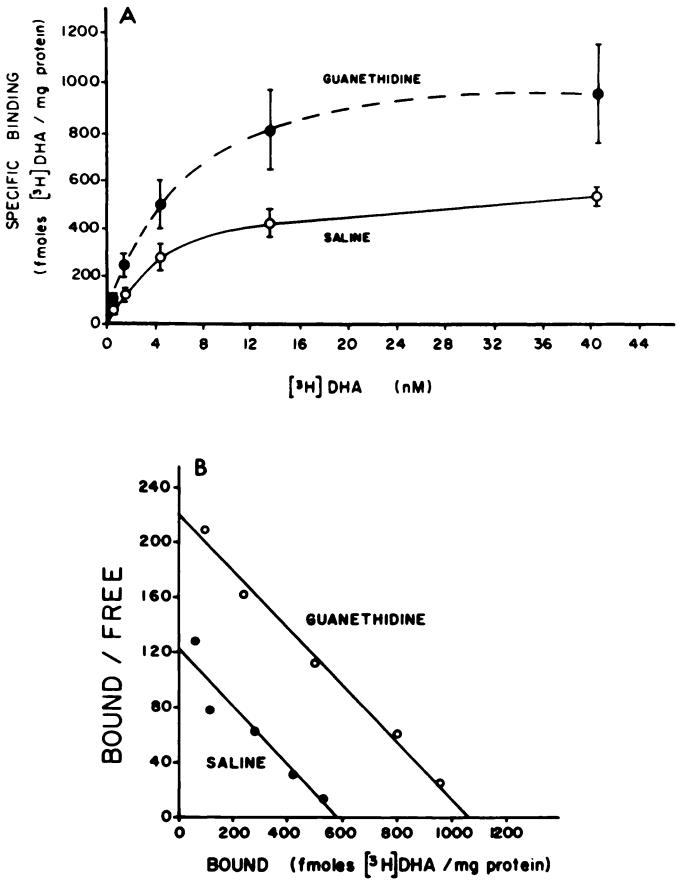

FIG. 3. *Effect of guanethidine-induced chemical sympathectomy on the specific binding of DHA in rat pineal glands*

Rats were treated as neonates with guanethidine or 0.9% NaCl as described under Methods. Rats were killed when 8 months old, pineal glands were removed, and the binding of DHA in whole homogenates was measured as described under Methods. Each point represents the mean ± standard error of the mean of results obtained in three experiments (A). K_d and maximal DHA binding values were obtained from a linear regression analysis of the Scatchard plot (B). The K_d values were as follows: control, 4.7 nM; guanethidine, 4.8 nM. Maximal DHA binding values were as follows: control, 580 fmoles/mg of protein; guanethidine, 1100 fmoles/mg of protein.

tured pineal glands from the sympathectomized treated rats than in those from the control animals.

The effect of reserpine-induced chemical sympathectomy on the *beta*-receptor-adenylate cyclase complex in the pineal gland was examined by measuring the specific binding of a saturating concentration of DHA (20 nM) in pineal homogenates from control (NaCl-treated) and reserpine-treated rats. All rats were blinded when they were 10 weeks of age. The binding of DHA was significantly greater ($p < 0.05$) in the pineal glands of reserpine-treated rats than in those of control animals (Fig. 5).

Figure 6 shows that, although norepinephrine significantly increased adenylate cyclase activity in pineal gland homogenates of both groups of rats, the norepinephrine-stimulated adenylate cyclase activity of reserpine-treated rats was significantly greater ($p < 0.01$) than that of NaCl-treated animals. The basal adenylate cyclase activities were similar in both groups.

DISCUSSION

The present results lend further support for the hypothesis made more than a decade ago (1) that long-term alterations in sympathetic activity can modify the density of *beta*-adrenergic receptors and the responsiveness of the adenylate cyclase system to catecholamines in the target tissue. Using the pineal gland as a model, we found that decreasing its sympathetic input by several surgical, chemical, and physiological techniques resulted in an increased density of *beta*-adrenergic receptors and an increased sensitivity of adenylate cyclase to norepinephrine.

For example, exposing rats to constant light, which decreases adrenergic input to the pineal gland (17), increased the number, but not the affinity, of *beta*-receptors in the glands as compared with those of blinded rats. This finding is in agreement with previous results showing that exposure of rats to light results in an increased binding of DHA in pineal glands (10, 19). By using blinded rats to eliminate the variable influence of light exposure on the *beta*-adrenergic receptor-adenylate cyclase complex, we found that surgically decreasing sympathetic input significantly elevated the density of the *beta*-receptors in the gland; however, there was no change in the affinity of the receptors for DHA.

Superior cervical ganglionectomy was not as effective as decentralization of the ganglia in increasing the binding of DHA. This may have been due to the degeneration of the adrenergic nerve endings in the pineal glands of the ganglionectomized rats. Parfitt and Klein (29) demonstrated that the presence of intact presynaptic adre-

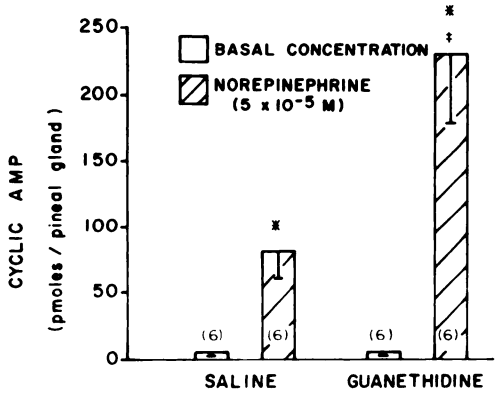

FIG. 4. *Effect of guanethidine-induced chemical sympathectomy on the norepinephrine-induced elevation of cyclic AMP in rat pineal glands*

Animals were treated as described under Methods and decapitated at 8 weeks of age. The pineal glands were removed, and the elevation of cyclic AMP in whole pineal glands in response to *l*-norepinephrine (50 μM) was determined. Each value represents the mean ± standard error of the mean of six experiments.

* $p < 0.001$ (paired *t*-test) compared with values obtained in the absence of norepinephrine.

‡ $p < 0.01$ (unpaired *t*-test) compared with values from corresponding 0.9% NaCl-treated rats.

nergic terminals in decentralized pineal glands reduces sympathetic stimulation of postjunctional receptors by taking up circulating catecholamines and thus enhances the development of postsynaptic supersensitivity (apparently, the rat pineal gland lies outside the blood-brain barrier).

Chemical sympathectomy produced changes in DHA binding similar to those produced by surgical sympathectomy. That is, guanethidine treatment of neonatal rats produced a 2-fold elevation in the number of *beta*-receptors in the pineal gland but did not change the affinity of the receptors for the DHA. Similarly, repeated administration of reserpine also increased DHA binding by increasing the number, but not the affinity, of *beta*-receptors in the pineal gland. Previous studies have shown that guanethidine treatment increases DHA binding in heart (12) and that reserpine treatment increases DHA binding in several areas of rat brain (13, 19).

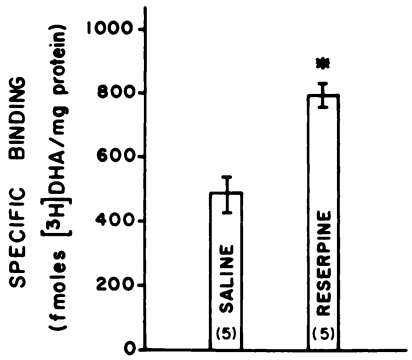

FIG. 5. *Effect of reserpine treatment on beta-adrenergic receptors in pineal glands of blinded rats*

Rats were treated at 10 weeks of age with reserpine (1 μmole/kg per day for 3 days) or 0.9% NaCl. Twenty-four hours later the pineal glands were removed and specific binding of DHA (20 nM) was determined in pineal gland homogenates. Each bar represents the mean of five experiments. Vertical brackets indicate the standard error of the mean.

* $p < 0.05$ compared with values obtained from 0.9% NaCl-treated rats.

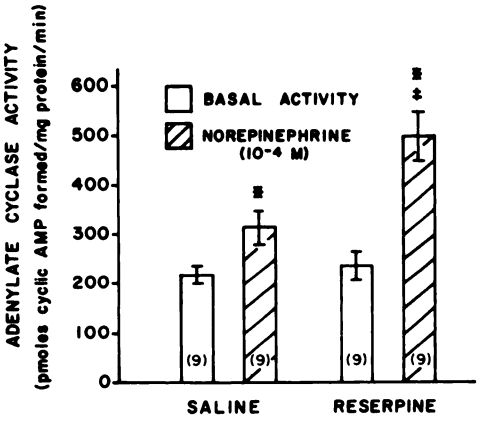

FIG. 6. *Effect of reserpine treatment on norepinephrine-sensitive adenylate cyclase activity in pineal glands of blinded rats*

Blinded rats were treated at 10 weeks of age with 0.9% NaCl or reserpine (1 μmole/kg per day for 3 days). Twenty-four hours later the pineal glands were homogenized and assayed for adenylate cyclase activity in the absence and presence of norepinephrine (10^{-4} M). Bars represent the mean adenylate cyclase activity of the number of experiments shown in parentheses. Vertical brackets indicate the standard error of the mean.

* $p < 0.001$ (paired *t*-test) compared with values obtained in the absence of norepinephrine.

‡ $p < 0.01$ (unpaired *t*-test) compared with corresponding values obtained in 0.9% NaCl-treated rats.

To correlate the observed changes in *beta*-receptor density following surgical and chemical sympathectomy of the pineal gland with a functional biochemical parameter, we measured the responsiveness of the catecholamine-sensitive adenylate cycylase system in the pineal glands following these sympathectomy procedures. The response of the enzyme to norepinephrine was enhanced in pineal glands from rats in which the superior cervical ganglion had been decentralized, as well as in the glands from rats treated with guanethidine and reserpine. These results are in accord with earlier studies showing an enhanced responsiveness of adenylate cyclase to norepinephrine in pineal glands from rats in which sympathetic input to the gland was reduced by constant exposure to light or by superior cervical ganglionectomy (1).

This increased number of *beta*-adrenergic receptors that followed a reduction in adrenergic input to the pineal gland not only correlated with an increased elevation of cyclic AMP but was also associated functionally with a markedly greater induction of serotonin *N*-acetyltransferase (5), the enzyme activated by cyclic AMP (30) and which is responsible for catalyzing the formation of the antigonadotropic principle melatonin (31, 32). Apparently, the increased functional capacity of the pineal gland following a long-term reduction in sympathetic input is caused by a decreased exposure of the gland to norepinephrine which results in an increased number of *beta*-adrenergic receptors.

In conclusion, alterations in adrenergic input produce opposite effects on the *beta*-adrenergic receptor-linked

adenylate cyclase system depending upon whether these alterations are acute or persistent (32). On the one hand, acute electrical stimulation of adrenergic fibers innervating the pineal gland (33), through the release of norepinephrine, causes a rapid, readily reversible activation of postjunctional *beta*-adrenergic receptors (19), leading to an increased activity of adenylate cyclase (20) and to an increased intracellular concentration of cyclic AMP (24). On the other hand, chronic stimulation of sympathetic input apparently produces a reduction in the density of *beta*-adrenergic receptors and a decreased responsiveness of adenylate cyclase to catecholamines. These alterations provide the biochemical means by which a tissue can adjust its biochemical response to norepinephrine in the face of a prolonged increase or diminution in sympathetic activity.

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