MOLECULAR PHARMACOLOGY, 19:187-193

Subsensitivity of the *Beta*-Adrenergic Receptor-Linked Adenylate Cyclase System of Rat Pineal Gland following Repeated Treatment with Desmethylimipramine and Nialamide

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Received May 5, 1980; Accepted September 30, 1980

SUMMARY

MOYER, J. A., L. H. GREENBERG, A. FRAZER, AND B. WEISS. Subsensitivity of the *beta*-adrenergic receptor-linked adenylate cyclase system of the rat pineal gland following repeated treatment with desmethylimipramine and nialamide. *Mol. Pharmacol.* **19**:187-193 (1981).

The effects of administering the antidepressant drugs desmethylimipramine and nialamide on the *beta*-adrenergic receptor-adenylate cyclase system of rat pineal gland were studied. The maximal elevation of cyclic AMP in response to varying concentrations of norepinephrine added *in vitro* to whole pineal glands was reduced following repeated but not acute administration of desmethylimipramine. Repeated administration of desmethylimipramine also reduced the norepinephrine-induced stimulation of adenylate cyclase activity in pineal gland homogenates. Neither acute nor repeated doses of desmethylimipramine had any significant effect on cyclic AMP phosphodiesterase activity of pineal gland. The loss of norepinephrine sensitivity of the adenylate cyclase system following repeated doses of desmethylimipramine was accompanied by a decreased density of *beta*-adrenergic receptors, as measured by the specific binding of [^{3}H]dihydroalprenolol. This loss of adrenergic receptors may be related to the action of this drug in blocking the reuptake of norepinephrine into the adrenergic nerve terminals, since the effects of desmethylimipramine were prevented in animals whose pineal glands had been sympathetically denervated by ganglionectomy. Repeated but not acute doses of the monoamine oxidase inhibitor, nialamide, also decreased the binding of [^{3}H]dihydroalprenolol in pineal glands and decreased the accumulation of cyclic AMP in response to intraperitoneal administration of isoproterenol. These findings provide further evidence that excessive noradrenergic input produces a compensatory reduction in the number of postsynaptic *beta*-adrenergic receptors, which, in turn, causes a decreased sensitivity of the norepinephrine-stimulated adenylate cyclase system. They suggest further that the mechanism by which antidepressant drugs exert their clinical effects should be re-evaluated, since following repeated administration they produce a reduced, rather than enhanced, responsiveness to noradrenergic stimuli.

INTRODUCTION

Tricyclic antidepressants are widely known to inhibit the active reuptake of transmitter substances such as norepinephrine. Since the reuptake of norepinephrine appears to be the primary means of terminating the postsynaptic action of this transmitter (1), the tricyclic antidepressants can be expected to enhance noradrenergic responses by increasing the availability of norepinephrine at postjunctional receptor sites. This, in fact, has been demonstrated after their acute administration (1, 2). The other major class of antidepressant drugs, the monoamine oxidase inhibitors, prevents the intracellular enzymatic catabolism of norepinephrine and other monoamines and, consequently, increases the levels of norepinephrine in brain (3). On the basis of these pharmacolog-

This work was supported by United States Public Health Service Grants MH-14654, MH-29094, NS-16242, and MH-30096 and by funds from the Veterans Administration.

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0026-895X/81/020187-07$02.00/0

ical effects, a hypothesis on the genesis of affective illnesses has been proposed. According to this hypothesis, antidepressant drugs are effective in treating depression because they overcome an aminergic deficiency (4).

Although the acute administration of antidepressant drugs enhances noradrenergic responsiveness, several independent lines of evidence indicate that chronic antidepressant therapy can reduce certain types of noradrenergic responses in brain. For example, repeated electroconvulsive shock or repeated administration of tricyclic antidepressants (5) or monoamine oxidase inhibitors (6) reduces the norepinephrine-stimulated production of cyclic AMP in rat cerebral cortex and limbic forebrain. In these studies, noradrenergic responsiveness was measured *in vitro*, using brain slices obtained from drug-treated rats.

Recently, we have extended such observations by measuring noradrenergic responsiveness *in vivo*. The model system selected for study was the rat pineal gland since the gland (a) lies outside the blood-brain barrier and, thus, can respond to exogenously-administered catecholamines; (b) has a high density of *beta*-adrenergic receptors (7); (c) has a highly sensitive, norepinephrine-dependent adenylate cyclase (8) capable of generating cyclic AMP (9); and (d) has an adrenergic receptor-linked adenylate cyclase system that has already been shown to be responsive to chronic alterations in noradrenergic input; that is, reduction of noradrenergic input increases the norepinephrine-induced stimulation of adenylate cyclase (10) and increases the density of *beta*-adrenergic receptors (7). Conversely, increased noradrenergic input, induced by the repeated administration of DMI,[4] abolishes the elevation of cyclic AMP in rat pineal gland produced by exogenously administered norepinephrine or isoproterenol (11) and causes a decrease in the density of *beta*-adrenergic receptors in pineal gland (7, 11–13).

In the present report, these latter studies have been extended in an attempt to clarify the sites and mechanisms by which repeated doses of DMI reduce noradrenergic responsiveness in the pineal gland and to determine whether a clinically effective antidepressant of a different class and mechanism of action, such as the monoamine oxidase inhibitor, nialamide, would also produce noradrenergic subsensitivity after chronic administration.

METHODS

Male Sprague-Dawley rats (200–250 g) were maintained in continuous light throughout all studies, a procedure designed to reduce the diurnal fluctuation in *beta*-adrenergic receptor density and to maintain norepinephrine sensitivity at a supersensitive level of responsiveness (10 , 14). After 2 days of continuous light exposure, rats received injections twice daily of 0.9% NaCl, DMI (40 μmoles/kg, i.p.; 10 mg of free base per kilogram), or nialamide (130 μmoles/kg, i.p.; 40 mg of free base per kilogram) for 5 days (total of nine injections). Another group of rats received injections of drug vehicle twice daily for 4 days followed by a single injection of either DMI (40 μmoles/kg, i.p.) or nialamide (130 μmoles/kg, i.p.) on the 5th day.

Bilateral superior cervical ganglionectomies and sham superior cervical ganglionectomies were performed by the supplier (Zivic Miller, Allison Park, Pa.). Drug treatments were begun 1 week postoperatively. Thus, animals receiving DMI repeatedly were killed 12 days after ganglionectomy. At this time after ganglionectomy there is complete degeneration of the sympathetic innervation to the pineal gland (15), but little or no alteration in *beta*-adrenergic responsiveness (10).

For the organ culture experiments, rats were decapitated 1 hr after the last injection, and pineal glands were prepared by a modification of previously employed techniques (16). The glands were placed in polypropylene tubes containing 0.4 ml of BGJb culture medium (Grand Island Biological Company, Grand Island, N. Y.) containing the essential amino acids, vitamins, and glucose. The glands were incubated for 10 min at 37°, after which varying concentrations of *l*-norepinephrine or *l*-isoproterenol were added in 0.1 ml of medium. Incubation in the presence of drug proceeded for 5 min at 37° and was terminated by the addition of 0.5 ml of ice-cold perchloric acid (final concentration 2.5%) to each tube. The pineal glands were sonicated for 15 sec, the sonicate was centrifuged at 49,000 × *g* for 15 min at 4°, and the resulting supernatant fluid was removed, neutralized with excess $CaCO_3$, and centrifuged at 12,000 × *g* for 10 min at 4°. The cyclic AMP concentration of the neutralized extract was measured by radioimmunoassay (17) using ^{125}I-labeled antigen and antiserum (New England Nuclear Corporation, Boston, Mass.). All unknown samples were assayed in triplicate. Standard solutions of cyclic AMP were prepared in a 2.5% perchloric acid-culture medium solution that had been neutralized with $CaCO_3$.

For *in vivo* studies, 1 hr after the final injection, animals received either 0.1% ascorbic acid (controls) or *l*-isoproterenol (2 μmoles/kg in 0.1% ascorbic acid, i.p.). Rats were decapitated 2.5 min later, the time at which preliminary experiments showed that the isoproterenol-induced increase in cyclic AMP levels in pineal gland were maximal. Pineal glands were removed and frozen within 30 sec to control for a postdecapitation increase in cyclic concentration (18). Cyclic AMP was extracted and the concentrations were determined as described above with the exception that standard solutions of cyclic AMP were prepared in 2.5% perchloric acid that had been neutralized with $CaCO_3$.

The binding of [^{3}H] DHA (New England Nuclear Corporation) was performed by the method of Lefkowitz *et al.* (19), adapted for the pineal gland as previously described (7). Briefly stated, the assay was conducted in 150 μl of 50 mM Tris-HCl buffer (pH 8.0) containing 3 mM $MgCl_2$, pineal gland homogenate (0.4 mg of tissue), and DHA (20 nM for studies utilizing one saturating concentration of DHA and concentrations of 0.6 to 18 nM DHA for Scatchard analyses (20). To determine nonspecific binding, incubation mixtures also contained 20 μM *l*-propranolol. Incubations were conducted at 37° for 10 min and terminated by rapidly diluting each sample with 2 ml of Tris-Mg^{2+} buffer (4°). Bound and free DHA were separated by rapid vacuum filtration of the diluted sam-

[4] The abbreviations used are: DMI, desmethylimipramine; DHA, dihydroalprenolol.

ples through Whatman GF/C glass fiber filters. The filters were rapidly washed with 15 ml of 4° buffer, added to scintillation vials containing 5 ml of Scintiverse (Fisher Scientific Company, King of Prussia, Pa.) and counted for radioactivity.

Adenylate cyclase activity was determined in whole gland homogenates by the method of Krishna *et al.* (21). Phosphodiesterase activity was determined by the firefly luciferin-luciferase technique (22). In these experiments, the rats were killed and the pineal glands were removed 1 hr after the last injection of 0.9% NaCl or antidepressant drug.

The statistical analyses conducted were two-tailed Student's *t*-tests for independent samples.

DMI was obtained from USV Laboratories, Tuckahoe, N. Y., and nialamide from Sigma Chemical Corporation, St. Louis, Mo. All other agents were obtained from general commercial sources.

RESULTS

Effect of DMI treatment on catecholamine-stimulated cyclic AMP accumulation in the pineal gland in vitro. To examine the dose-response relationship of the effects of *beta*-adrenergic agonists on cyclic AMP accumulation following DMI administration, the pineal glands from 0.9% NaCl- or DMI-treated rats were incubated *in vitro* in the presence of varying concentrations of norepinephrine and isoproterenol. Figure 1 shows that norepinephrine produced a concentration-dependent increase in the cyclic AMP content of the pineal glands. No statistically significant differences in the response to norepinephrine were seen between control animals and those rats treated acutely with DMI. However, following repeated doses of DMI, the cyclic AMP response to norepinephrine was significantly reduced. Although the approximate ED_{50} for norepinephrine was similar in all groups, repeated treatment with DMI caused an apparent reduction in the maximal response to the catecholamine. Similar results were obtained using isoproterenol as the *beta*-adrenergic agonist (data not shown).

Effect of repeated administration of DMI on adenylate cyclase and cyclic AMP phosphodiesterase activities in pineal gland. The decreased accumulation of cyclic AMP in pineal glands in response to *beta*-adrenergic agonists following repeated DMI administration may be due to the loss of *beta*-adrenergic receptors and, consequently, to a reduced responsiveness of adenylate cyclase or to a compensatory increase in the activity of cyclic AMP phosphodiesterase. Accordingly, we measured the activities of these two enzymes directly.

The responsiveness of adenylate cyclase to norepinephrine was determined *in vitro* in pineal glands of rats that had been given repeated injections of 0.9% NaCl or DMI (Table 1). Although norepinephrine significantly elevated adenylate cyclase activity in both the 0.9% NaCl- and DMI-treated rats, the activation of adenylate

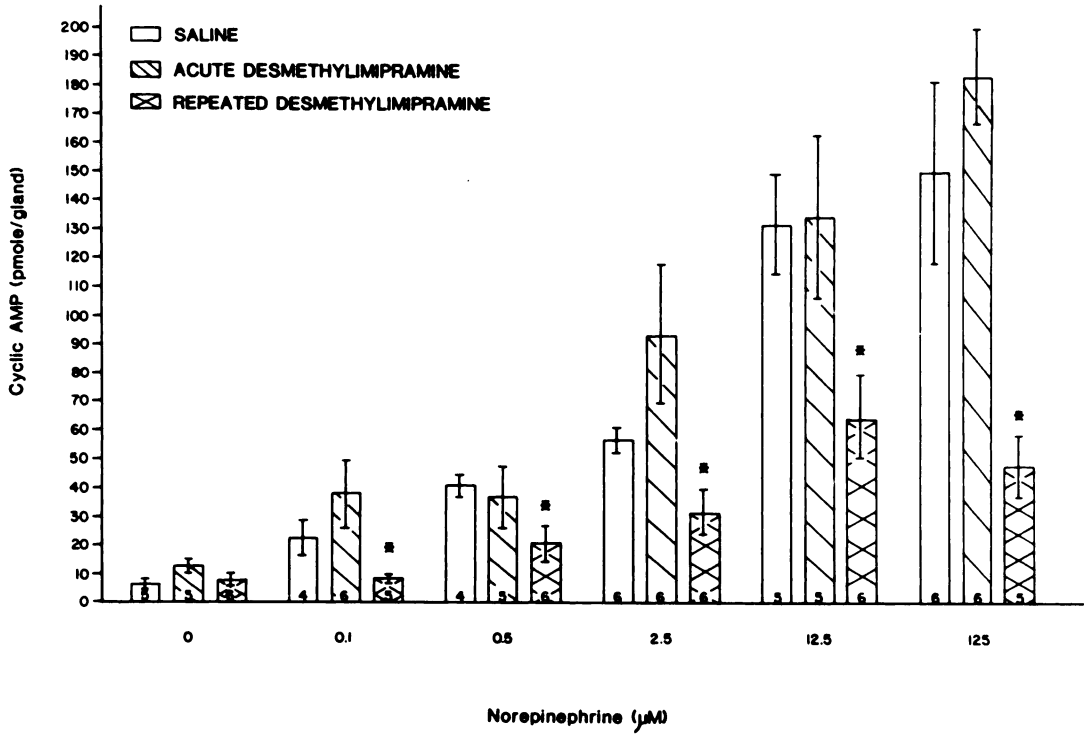

FIG. 1. *Effect of DMI treatment on norepinephrine-stimulated cyclic AMP accumulation in the pineal gland in vitro*

Two groups of rats received either 0.9% NaCl or DMI (40 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). A third group of rats (acute DMI) received eight injections of 0.9% NaCl on days 1–4, followed by a single injection of DMI (40 μmoles/kg, i.p.) on day 5. One hour after the final injection, the rats were decapitated, and whole pineal glands were incubated *in vitro* as described under Methods. Values represent the mean ± standard error of the mean of the number of rats indicated. *Asterisks* indicate values significantly different from those measured in corresponding 0.9% NaCl-treated rats (Student's *t*-test, two-tailed, $p < 0.05$).

TABLE 1

Effect of repeated administration of DMI on norepinephrine-induced stimulation of adenylate cyclase activity in rat pineal glands in vitro

Two groups of rats received either 0.9% NaCl or DMI (40 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). One hour after the final injection, the rats were decapitated and the pineal glands were homogenized and assayed for adenylate cyclase activity in the absence or presence of *l*-norepinephrine (10^{-4} M). Values represent the means ± standard error of the mean of five experiments.

Treatment	Adenylate cyclase activity	
	Basal	Norepinephrine (10^{-4} M)
	pmoles cyclic AMP formed/mg protein/min	
0.9% NaCl	160 ± 20	380 ± 50[a]
DMI	130 ± 10	220 ± 10[a, b]

[a] $p < 0.005$ as compared with corresponding basal values.
[b] $p < 0.025$ as compared with corresponding value in 0.9% NaCl-treated rats.

cyclase by norepinephrine was significantly less in rats given repeated injections of DMI as compared with that produced in 0.9% NaCl-treated control rats. There was no significant difference in the basal adenylate cyclase activity between 0.9% NaCl- and DMI-treated rats.

Most brain tissues, including the pineal gland, have both high and low K_m forms of phosphodiesterase (18). To determine whether DMI altered either of these forms of phosphodiesterase, we measured enzyme activity using a high (400 μM) and low (4 μM) substrate concentration in animals given 0.9% NaCl or treated acutely or repeatedly with DMI. Table 2 shows that there were no significant differences in phosphodiesterase activity between any of the treatment groups at either substrate concentration.

Other experiments showed that DMI added *in vitro*, at concentrations as high as 400 μM, produced little or no inhibition of cyclic AMP phosphodiesterase activity in rat pineal glands (data not shown).

Effect of superior cervical ganglionectomy on DHA binding in DMI-treated rats. To determine whether repeated administration of DMI decreased the density of *beta*-adrenergic receptors in the pineal gland by a direct effect on the postjunctional *beta*-adrenergic receptors or by an indirect effect, such as by inhibiting the reuptake of released transmitter into noradrenergic nerve endings, we examined the effects of DMI in animals whose pineal glands had been sympathetically denervated by bilateral superior cervical ganglionectomy. As is shown in Table 3, repeated doses of DMI produced a significant decrease in DHA binding in pineal glands of sham-operated animals. Superior cervical ganglionectomy itself produced a significant reduction in DHA binding in the pineal glands; however, no further significant reduction was produced by repeated administration of DMI.

Effect of nialamide treatment on the isoproterenol-stimulated accumulation of cyclic AMP in the pineal gland in vivo. Figure 2 shows the effects of the monoamine oxidase inhibitor, nialamide, on *beta*-adrenergic responsiveness in the pineal gland. Rats were treated with either a single or repeated dose of 0.9% NaCl or nialamide. Following this treatment, isoproterenol was administered intraperitoneally, the rats were decapitated, and the concentration of cyclic AMP in pineal gland was measured. In 0.9% NaCl-treated animals, isoproterenol produced greater than a 20-fold increase in the concentration of cyclic AMP. In rats pretreated with an acute dose of nialamide (130 μmoles/kg), the isoproterenol-stimulated increase in cyclic AMP concentration did not differ significantly from that in the 0.9% NaCl-treated rats. However, when rats were given nine repeated doses of nialamide, the elevation of cyclic AMP in response to isoproterenol was significantly reduced. Neither acute nor repeated doses of nialamide significantly changed the basal concentration of cyclic AMP.

Effect of nialamide treatment on DHA binding in the pineal gland. To determine whether the decrease in noradrenergic responsiveness following repeated doses of nialamide was related to an alteration in the density of *beta*-adrenergic receptors, the specific binding of DHA was measured in the pineal glands of rats administered 0.9% NaCl, an acute dose of nialamide, or repeated doses of the drug. DHA binding in the pineal glands of rats given the acute dose of nialamide did not differ significantly from that found in the 0.9% NaCl-treated rats (Table 4). By contrast, DHA binding was significantly

TABLE 2

Effect of DMI on phosphodiesterase activity of rat pineal glands

Rats received either 0.9% NaCl or DMI (40 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). These rats were decapitated 1 hr after the last injection. A third group of rats (acute DMI) received eight injections of 0.9% NaCl on days 1–4, followed by a single injection of DMI (90 μmoles/kg, i.p.) on day 5. These animals were decapitated 15 min after the last injection. This latter dosage regimen results in a concentration of DMI in plasma and pineal gland that is similar to that seen following the repeated administration of DMI (11). Pineal glands were removed, homogenized, and analyzed for phosphodiesterase activity at cyclic AMP substrate concentrations of 4 and 400 μM. Values represent the means ± standard error of the mean for 11 experiments.

Treatment	Cyclic AMP phosphodiesterase activity	
	Cyclic AMP (4 μM)	Cyclic AMP (400 μM)
	nmoles cyclic AMP hydrolyzed/mg protein/min	
0.9% NaCl	0.82 ± 0.23	9.7 ± 1.1
Acute DMI	0.76 ± 0.19	9.8 ± 1.3
Chronic DMI	1.01 ± 0.18	11.3 ± 1.1

TABLE 3

Effect of superior cervical ganglionectomy on binding of DHA in pineal gland following repeated administration of DMI

Superior cervical ganglionectomized and sham-operated animals received either 0.9% NaCl or DMI (40 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). All rats were decapitated 1 hr after the last injection. The pineal glands were removed, homogenized in 50 mM Tris-3 mM Mg^{2+} buffer (pH 8.0), and assayed for specific DHA binding. Values represent the means ± standard error of the mean of seven experiments.

Treatment	Specific DHA binding
	fmoles/mg protein
Sham-operated + 0.9% NaCl	1000 ± 50
Sham-operated + repeated DMI	650 ± 10[a]
Ganglionectomized + 0.9% NaCl	760 ± 30[a]
Ganglionectomized + repeated DMI	700 ± 50[a]

[a] $p < 0.01$ as compared with the sham-operated + 0.9% NaCl-treated group.

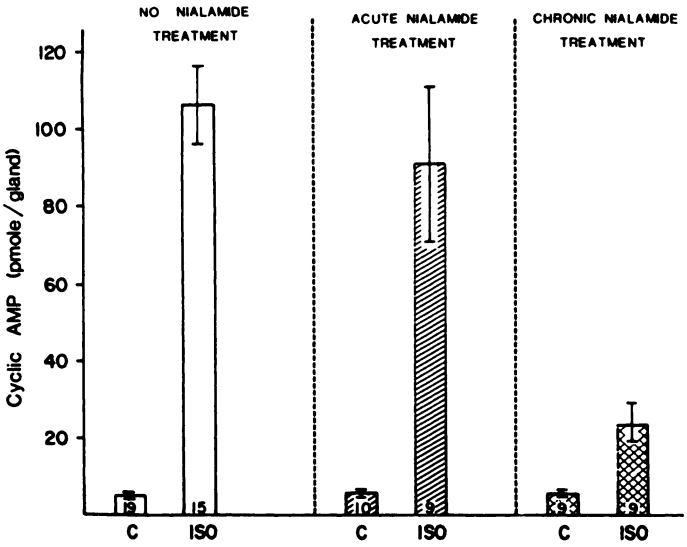

FIG. 2. *Effect of nialamide treatment on isoproterenol-stimulated cyclic AMP accumulation in the pineal gland in vivo*

Two groups of rats received either vehicle or nialamide (130 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). A third group of rats (acute nialamide) received eight injections of vehicle on days 1 to 4 followed by a single injection of nialamide (130 μmoles/kg, i.p.) on day 5. One hour after the final injection, the rats received intraperitoneal injections of either 0.1% ascorbate (controls; *C*) or isoproterenol (*ISO*, 2 μmoles/kg) and were decapitated 2.5 min later. Pineal glands were removed within 30 sec of decapitation, sonicated in 1 ml of 2.5% perchloric acid, and assayed for cyclic AMP as described under Methods. Values represent the means ± standard error of the mean of the number of rats indicated within each *bar*. In all cases, the concentration of cyclic AMP was significantly greater in pineal glands of rats treated with isoproterenol than in corresponding control rats (Student's *t*-test, two-tailed, $p < 0.005$). Repeated nialamide treatment significantly reduced the isoproterenol-induced increase in cyclic AMP as compared with that in vehicle-treated animals ($p < 0.001$) or animals treated acutely with nialamide ($p < 0.005$).

reduced in the glands of rats given repeated doses of the monoamine oxidase inhibitor.

A further analysis of the action of nialamide and DMI on *beta*-adrenergic receptors of rat pineal gland is presented in Table 5. Rats were treated repeatedly for 5 days with either DMI or nialamide, and the maximal number of binding sites for [^{3}H]DHA (B_{max}) and the apparent affinity of the ligand for the receptor (K_d) were estimated by Scatchard analysis. Table 5 shows that both antidepressant compounds significantly reduced the B_{max} but had no effect on the K_d, suggesting that the drugs reduced the number, but not the affinity, of *beta*-adrenergic receptor sites in this tissue.

TABLE 4

Effect of acute and repeated treatment with nialamide on binding of DHA in rat pineal gland

Two groups of rats received either 0.9% NaCl or nialamide (130 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). A third group of rats (acute nialamide) received injections of 0.9% NaCl on days 1–4 followed by a single injection of nialamide (130 μmoles/kg, i.p.) on day 5. All rats were decapitated 1 hr after the last injection. Pineal glands were removed, homogenized in 50 mM Tris-3 mM Mg^{2+} buffer (pH 8.0), and assayed for specific DHA binding. Values represent the means ± standard error of the mean of eight experiments.

Treatment	Specific DHA binding
	fmoles/mg protein
0.9% NaCl	650 ± 40
Acute nialamide	630 ± 50
Repeated nialamide	480 ± 30[a]

[a] $p < 0.005$ compared with 0.9% NaCl-treated animals as analyzed by Student's two-tailed *t*-test.

DISCUSSION

Previous studies showed that acute treatment of rats with the tricyclic antidepressant, DMI, enhances the ability of intraperitoneally administered norepinephrine to increase the concentration of cyclic AMP in the pineal gland (11). This effect is consistent with the widely held view that the clinical efficacy of antidepressant drugs is related to their ability to increase noradrenergic function in brain (4). However, the beneficial clinical effects of tricyclic antidepressants frequently are not observed for 2 to 3 weeks after therapy is initiated (23). Because of this, it is important to determine whether repeated administration of antidepressants produces the same effect on noradrenergic responsiveness as that noted with acute treatment. Studies in which DMI was administered repeatedly to rats indicated that the norepinephrine- or isoproterenol-stimulated accumulation of cyclic AMP in the pineal gland was abolished rather than enhanced (11).

The present results, which support and extend our previous findings, show that pineal glands taken from rats that had been given DMI for 5 consecutive days exhibited a reduced elevation of cyclic AMP in response to *beta*-adrenergic agonists added *in vitro*. The dose-response curves for norepinephrine- and isoproterenol-stimulated increases in the concentration of cyclic AMP suggest that the maximal response to the agonists was reduced by the repeated administration of DMI. These data are consistent with our finding that repeated injections of DMI or nialamide reduce the number of *beta*-adrenergic receptors in the pineal gland, but do not change the affinity of the receptors for DHA.

The decreased accumulation of cyclic AMP in response to *beta*-adrenergic agonists that was seen both *in vivo*

TABLE 5

Effect of repeated administration of DMI and nialamide on DHA binding in rat pineal gland

Three groups of rats received either 0.9% NaCl, DMI (40 μmoles/kg, i.p.), or nialamide (130 μmoles/kg, i.p.) twice daily for a total of nine injections (5 days). Rats were decapitated 1 hr after the final injection. The pineal glands from eight rats were pooled, homogenized in 50 mM Tris buffer (pH 8.0) containing 3 mM Mg^{2+}, and assayed for specific DHA binding as described under Methods, using concentrations of DHA ranging from 0.6 to 18 nM. The binding data were analyzed by the method of Scatchard (20). The values represent the means ± standard error of the mean of three experiments.

Treatment group	B_{max}	K_d
	fmoles/mg protein	*nM*
0.9% NaCl	970 ± 20	11 ± 1
DMI	620 ± 60[a]	8.8 ± 1.2
Nialamide	530 ± 50[a]	9.6 ± 1.6

[a] $p < 0.005$ comparing values from drug-treated rats with those of vehicle-treated control rats, as analyzed by Student's two-tailed *t*-test.

and *in vitro* following repeated doses of DMI may also have been due to decreases in the *beta*-adrenergic receptor-linked adenylate cyclase system or to an increase in phosphodiesterase activity. To distinguish between these two mechanisms, we measured these enzymes directly in pineal gland homogenates. The results showed that the catecholamine-induced activation of adenylate cyclase was markedly reduced in pineal glands of rats given DMI for 5 days. This treatment did not significantly alter the basal activity of the enzyme. These results suggest that the reduced activation of adenylate cyclase by norepinephrine in the DMI-treated rats reflects the loss of the receptor component of the *beta*-receptor-adenylate cyclase complex, and is consistent with previous findings of a decreased density of *beta*-adrenergic receptors following repeated treatment with DMI (11, 13). The fact that neither acute nor repeated doses of DMI significantly altered the phosphodiesterase activity of pineal glands at high or low substrate concentrations suggests further that an increase in the activity of phosphodiesterase did not contribute to the loss of noradrenergic responsiveness in the glands from rats given repeated doses of DMI.

Bilateral removal of the superior cervical ganglia causes the nerve endings in the pineal gland to degenerate and norepinephrine levels to be depleted within 24 hr (15). Presumably, once the nerve endings have degenerated, DMI should no longer be able to exert presynaptic effects. The finding that repeated doses of DMI failed to lower the density of *beta*-adrenergic receptors in rats subjected to superior cervical ganglionectomy suggests that DMI may be acting indirectly, perhaps by causing an increase in the concentration of norepinephrine at the receptor sites. DMI may produce this effect by at least two different mechanisms: (a) by blocking the reuptake of norepinephrine into the noradrenergic nerve endings or (b) by stimulating the release of norepinephrine from the nerve endings by producing presynaptic *alpha*-adrenergic receptor subsensitivity (24). Results similar to ours were obtained by Wolfe et al. (6), who showed that, in rats pretreated with 6-hydroxydopamine, DMI administration did not produce *beta*-adrenergic receptor subsensitivity in cerebral cortex. Thus, destruction of the noradrenergic innervation to tissues containing *beta*-adrenergic receptors prevents DMI from reducing the density of these receptors.

In an attempt to determine whether the subsensitivity observed following repeated treatment with DMI could be produced by another class of antidepressant drug that also increases the availability of norepinephrine at noradrenergic receptors, we examined the effects of the monoamine oxidase inhibitor, nialamide. Repeated administration of nialamide produced *beta*-adrenergic subsensitivity in the pineal gland similar to that produced by DMI. Moreover, as in the case of the tricyclic antidepressants, repeated but not acute doses of nialamide significantly reduced the accumulation of cyclic AMP following the *in vivo* administration of isoproterenol. Furthermore, this reduced *beta*-adrenergic responsiveness was accompanied by a decreased density of *beta*-adrenergic receptors in the glands after repeated nialamide administration.

Current hypotheses hold that, because it is a monoamine oxidase inhibitor, nialamide exerts its antidepressant action by increasing the amount of biogenic amine, such as norepinephrine, that is available to the receptors, thereby potentiating the actions of these amines. However, our data show that prolonged treatment with this drug causes a subsensitivity of adrenergic receptors.

Thus, the reduced elevation of cyclic AMP in response to norepinephrine stimulation that occurs in pineal gland and other brain areas after repeated antidepressant administration (5, 11) can be related to compensatory decreases in the density of *beta*-adrenergic receptors and the resulting decreased responsiveness of adenylate cyclase to *beta*-adrenergic agonists. A critical question that remains to be answered, however, is whether such compensatory changes actually inhibit noradrenergic transmission in brain *in vivo*. This question is complicated still further by other compensatory changes induced by the tricyclic drugs, such as the development of presynaptic *alpha*-receptor subsensitivity (24). Since stimulation of presynaptic *alpha*-receptors decreases the release of norepinephrine (25), *alpha*-receptor subsensitivity would be expected to cause an increase in the release of norepinephrine from noradrenergic terminals. However, noradrenergic responses induced by stimulating the locus coeruleus were reduced following repeated treatment of rats with antidepressant drugs (26). Furthermore, Siggins and Schultz (27) found that chronic treatment with antidepressants reduced the postsynaptic electrophysiological responsiveness to norepinephrine in rat cerebellar Purkinje cells.

The possibility that the decreased number of *beta*-adrenergic receptors caused by antidepressants may have clinical significance is supported by the evidence (a) that this phenomenon occurs in areas of the brain thought to be involved in antidepressant actions (6, 7, 12, 13, 28); (b) that both of the two major classes of antidepressants, namely the monoamine oxidase inhibitors and the tricyclics, reduce *beta*-receptors in brain, whereas a number of other types of centrally acting agents do not (29); and (c) that clinical improvement with antidepressant therapy and the development of *beta*-receptor subsensitivity occur only after repeated administration of the antidepressant (13, 23).

In conclusion, we have found that repeated but not acute administration of the antidepressant drugs DMI and nialamide decreases the responsiveness of the *beta*-adrenergic receptor-coupled adenylate cyclase system in the rat pineal gland *in vivo* and *in vitro*. Our findings showed further that this reduced noradrenergic responsivity is due, at least in part, to a reduction in the density of postsynaptic *beta*-adrenergic receptors. These results raise the possibility that decreased, rather than increased, noradrenergic transmission in brain may be an important component in the clinical effectiveness of antidepressant drugs.

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