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The Noradrenaline Receptor Coupled Adenylate Cyclase System in Brain

Lack of Modification by Changes in the Availability of Serotonin

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Summary. The present studies were undertaken to ascertain whether or not an alteration in the availability of serotonin (5HT) can modify central noradrenergic function at the level of the noradrenaline (NA) receptor coupled adenylate cyclase system in brain. The chronic but not acute administration of the 5HT uptake inhibitors amitriptyline and chlorimipramine reduced the sensitivity of the cyclic AMP generating system to NA in the limbic forebrain. This subsensitivity was linked to a decrease in the B_{max} value of β-adrenergic binding sites without appreciable changes in the K_d values, as assessed by specific ^{3}H-dihydroalprenolol binding. The specific 5HT uptake inhibitor fluoxetine did not change either the responsiveness of the cyclic AMP generating system to NA or the density of β-adrenergic receptor sites. Raphé lesions which selectively reduced the level of 5HT also did not cause any changes in the neurohormonal responsiveness or the density of β-adrenergic receptor sites. In contrast, medial forebrain bundle lesions which reduced the levels of both 5HT and catecholamines (NA and dopamine) in the forebrain, increased the responsiveness of the cyclic AMP generating system to NA. It can thus be concluded that a selective change in the availability of 5HT per se does not modify noradrenergic receptor function at the level of the NA receptor coupled adenylate cyclase system. The subsensitivity of the noradrenergic receptor system developed following amitriptyline and chlorimipramine may in all likelihood be due to the in vivo conversion to the secondary amines, nortriptyline and desmethylchlorimipramine respectively. These secondary amine metabolites are potent inhibitors of the NA reuptake and consequently could be responsible for the demonstrated in vivo down-regulation of central adrenergic receptor function (homospecific down-regulation).

Key words: Limbic cyclic AMP generating system – Noradrenergic down-regulation – β-Adrenergic receptors – Amitriptyline – Chlorimipramine – Fluoxetine – Raphé lesions – Medial forebrain bundle lesions

Introduction

Various prototypes of antidepressant drugs including tricyclic antidepressants, MAO inhibitors, iprindole and mianserin, and also electroconvulsive treatment (ECT) decrease, upon chronic administration, the responsiveness of the noradrenaline (NA) receptor coupled adenylate cyclase system in brain (Vetulani and Sulser 1975; Vetulani et al 1976a, b; Schultz 1976; Schmidt and Thornberry 1977; Frazer et al. 1978; Stone 1979; Gillespie et al. 1979). This subsensitivity is generally but not invariably linked to a reduction in the density of β-adrenergic receptors (Banerjee et al. 1977; Sarai et al. 1978; Bergstrom and Kellar 1979a, b; Wolfe et al. 1978; Clements-Jewery 1978; Gillespie et al. 1979; Mishra et al. 1979, 1980). Since most, if not all, clinically effective antidepressant drugs and ECT elicit subsensitivity of central adrenergic receptor function upon repeated but not acute administration, the hypothesis has been advanced that the well known delayed therapeutic action of antidepressant treatments may be related to this delayed alteration in central noradrenergic receptor function rather than to the acute pharmacological effects elicited by these drugs and by ECT (Sulser 1978).

Serotonergic function in brain has been implicated in the pathogenesis of affective disorders (Carlsson 1976) and a reciprocal functional relationship between serotonin (5HT) and NA has been suggested (Brodie and Shore 1957; Stein and Wise 1974; Samanin and Garattini 1975). It was thus of interest to ascertain whether or not a change in the availability of 5HT could modify noradrenergic receptor function at the level of the NA receptor coupled adenylate cyclase system. The present studies were designed to test this possibility. Thus, we determined the sensitivity of the noradrenergic cyclic AMP generating system to NA and the density of β-adrenergic receptor binding sites following the administration of the 5HT reuptake inhibitors amitriptyline, chlorimipramine and fluoxetine (increase in the availability of 5HT) and following lesions of the dorsal and medial raphé (decrease in the availability of 5HT).

Methods and Materials

Animals and Surgical Procedures. Male Sprague-Dawley rats weighing 200–220 g were used for all experiments involving studies with drugs. The animals had free access to water and standard laboratory diet (Purina Food Company, St. Louis, MO, USA) and were maintained under standard laboratory conditions with a controlled 12h light-dark cycle. To determine neurohormonal responses to NA and specific β-adrenergic receptor binding, the animals were decapitated at specific intervals after the last injection of the drugs or 7 and 14 days following the surgical procedures (lesions of the medial forebrain bundle and dorsal and medial raphé respectively). The brains were rapidly removed, cortex and limbic forebrain area dissected as previously described (Blumberg et al. 1976) and prepared for studies on the noradrenergic cyclic AMP generating

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system, the content of biogenic amines and on specific β-adrenergic receptor binding.

For the lesion experiments, male Sprague-Dawley rats weighing 300–350 g at the time of surgery, were anesthetized with 45 mg/kg sodium pentobarbital and given 0.06 mg/kg atropine sulfate to reduce respiratory difficulties. The animals were then mounted in a stereotaxic instrument for lesioning using a stainless steel, monopolar electrode (0.25 mm in diameter, insulated except for 0.5 mm at the tip). For all animals, the head was positioned in the flat skull orientation in which bregma and lambda are in the same horizontal plane; coordinates are given to correspond to the König and Klippel (1963) atlas.

To lesion the medial forebrain bundle (MFB), the electrode was lowered on the right side of the brain according to the following coordinates: 4.0 mm anterior to the interaural line, 1.4 mm lateral to the saggital suture and 7.5 mm down from the top of the brain. A 3 mA dc current was passed for 10 s between the cranial anode and an interaural cathode. Sham lesioned animals had the electrode lowered but no current was passed. The raphé lesions were carried out in 2 steps. The electrode was lowered according to the following coordinates: 0.35 mm anterior, 0.2 mm lateral and 6.7 mm deep. A 3 mA dc current was delivered for 5 s to lesion the median raphé. The electrode was then raised out of the brain, the tip was cleaned and the electrode was again lowered, this time to a depth of 4.7 mm to lesion the dorsal raphé nucleus (3 mA for 5 s). Sham lesioned animals had the electrode lowered to the level of the dorsal raphé, but no current was passed. All animals received a prophylactic injection of Bicillin (150,000 units) immediately following surgery.

In animals with MFB lesions, the limbic forebrain area was removed 7 days following surgery as described above. The remainder of the brain was placed in formalin for several days and then sliced on a freezing microtome in 60 μ sections. Photomicrographs, made directly from these slices, were used to verify the accuracy of the lesions.

Raphé lesioned animals were killed 2 weeks following surgery. In our first study, we compared the effects of the lesions on the levels of 5HT and catecholamines in the limbic forebrain vs. the rest of the brain to determine whether biochemical changes in the brain minus the limbic forebrain could be used as an index of changes in the limbic forebrain. Since the biochemical changes in both parts of the brain were nearly identical, in all subsequent studies, the brain was removed, the limbic forebrain excised, and a coronal cut made approximately at the level of the inferior colliculus. The portion of the brain rostral to the cut was used to determine the levels of 5HT, NA and DA while the caudal portion was placed in formalin for histological identification of the lesion site.

All drugs were administered intraperitoneally, amitriptyline and chlorimipramine in a dose of 20 mg/kg/day for a period of 2 weeks. Since fluoxetine, in contrast to chlorimipramine, exerts a very long duration of action (Fuller et al. 1978), this specific 5HT uptake inhibitor was administered in a dose of 10 mg/kg/day.

Biochemical Procedures. Studies on NA sensitive adenylate cyclase were performed according to procedures previously described (Blumberg et al. 1976; Robinson et al. 1978). Briefly, frontal cortex and limbic forebrain area were sliced by using a McIlwain tissue chopper set at 0.3 mm. The slices were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4; 95% O_2 – 5% CO_2). The cyclic AMP response was established by exposing the slices for 10 min to various concentrations of NA. Following aspiration of the buffer solution, the slices were frozen with liquid nitrogen and homogenized in 0.3 N perchloric acid. Cyclic AMP was isolated by ion exchange chromatography (Blumberg et al. 1976) and assayed by the protein binding method of Gilman (1970).

The status of β-adrenergic receptors was determined according to Bylund and Snyder (1976) using ^{3}H-dihydroalprenolol (DHA) as a ligand. Cortical brain tissue was frozen on dry ice and stored below –20° C until assayed. The tubes containing 150 μl of the tissue suspension and a ligand concentration of 0.5 to 10 nM ^{3}H-DHA in a final volume of 1 ml of Tris buffer were incubated for 10 min at 37° C, at which time 2 ml of ice cold Tris buffer were added to the tissue homogenate and immediately filtered through a Whatman GF/C glass filter. Non-specific binding was determined in the presence of 50 μM *d,l*-propranolol. The filters were rinsed 3 times with 4 ml ice cold Tris buffer and placed in 10 ml of Triton cocktail (5.0 g PPO, 0.1 g POPOP, 500 ml Triton, 1 l toluene). The radioactivity was determined in a liquid scintillation spectrometer. The binding data were analyzed by the method of Scatchard (1949). Protein was assayed according to Lowry et al. (1951).

For the assays of biogenic amines in animals having received raphé lesions, the tissue was homogenized in 8 ml of 0.1 N HCl. For the determination of 5HT, a 2 ml aliquot of the homogenate was transferred to a centrifuge tube containing 2.5 ml of 0.5 M borate buffer, pH 10, which was saturated with NaCl and n-butanol, 4 g of sodium chloride, 5 ml of n-butanol which was saturated with water, and 40 μl of 5 N NaOH. After vigorous shaking for 10 min and centrifugation, 5 HT in the butanol phase was determined by the method of Curzon and Green (1970). The remaining homogenate was homogenized again after the addition of 0.2 ml of concentrated perchloric acid. After the addition of 0.5 ml of 10 N potassium acetate, samples were centrifuged at 30,000 × g for 10 min. The supernatant was titrated to pH 8.4 with 3 M Tris, poured into polycarbonate tubes containing 200 mg of alumina (aluminum oxide active, neutral, Brockman grade 1, BDH Chemicals Ltd., Poole, GB) and the contents were mixed on a precipitate washer (Multi-Purpose Rotator Scientific Products, McGaw Park, IL, USA). The alumina-catecholamine complex was washed twice with water, and then eluted by the addition of 1.1 ml of 0.2 N HCl containing 0.05% sodium metabisulfite. NA and DA in the eluate were determined by the fluorometric method of Laverty and Taylor (1968).

In the experiment involving medial forebrain bundle lesions, the amines were assayed in one-half of the limbic forebrain sample using high pressure liquid chromatography with electrochemical detection (LCEC) according to the method of Perry (1980). Tissue samples were homogenized in 4.5 ml of acidified butanol (0.85 ml concentrated HCl/l) containing 50 ng of alpha-methyldopamine as an internal standard for the catecholamines. The homogenate was centrifuged at 3,000 × g for 20 min and the supernatant was transferred to 13 ml centrifuge tubes containing 4 ml of heptane and 1.4 ml of 0.1 N HCl. Following extraction (vortexing for 1 min), centrifugation, and aspiration of the organic phase, 5HT in the 0.1 N HCl was assayed by LCEC.

The chromatograph was a model LC-304 (Bioanalytical Systems, West Lafayette, IN, USA) liquid chromatograph with a glassy carbon amperometric detector equipped with a 25 cm × 4 mm i.d. reverse phase C_8 (DuPont Zorbax βQ) column. A 50 μl aliquot of the sample was injected into a six port valve equipped with a 200 μl sample loop. The mobile phase consisted of 0.1 M Na_2HPO_4 – 0.05 M citric acid – 10% methanol at pH 5. The mobile phase was pumped at a rate of 1.0 ml/min and the 5HT eluted from the column with a retention time of approximately 6 min (a ghost peak likely to be butanol dissolved in the acetate solution eluted with a retention time of about 12 min). The detector potential was set at +0.75 V versus the Ag/AgCl reference electrode.

A 1.0 ml aliquot of the 0.1 N HCl phase containing the catecholamines was transferred to a 1.5 ml centrifuge tube containing 50 mg of acid washed alumina. The pH was raised to 8.4 by the addition of 80 μl of 3 M TRIS and the catecholamines were adsorbed onto the alumina by vortexing for 1 min. The alumina was washed with 1 ml of 0.1 M TRIS (pH 7) and the catecholamines were eluted by the addition of 400 μl of 0.2 N formic acid. Particulate matter was removed by centrifugation at 12,000 × g for 5 min. The NA, DA, and alpha-methyldopamine were assayed by injecting 50 μl onto the LCEC equipped with a Vydac TP cation exchange column (25 cm × 4 mm i.d.) using the mobile phase described above with the addition of 0.075 M KCl at a flow rate of 1.4 ml/min. All other chromatographic conditions were identical to those listed above for the assay of 5HT. NA, DA, and alpha-methyldopamine eluted from the column with retention times of approximately 4, 9.5, and 13 min, respectively.

In order to calibrate the procedure, standard curves (3 points plus a blank) for each of the compounds were run through the assay. The recovery of 5HT was essentially 100% while that of the catecholamines was approximately 60%.

Drugs and Chemicals. Drugs and chemicals were obtained from the following sources: *l*-norepinephrine HCl, protein kinase from beef heart and adenosine 3′,5′-cyclic monophosphate from Sigma Chemical Co., St. Louis, MO, USA; tritiated adenosine 3′,5′-monophosphate (specific activity 41.1 Ci/mmole) and tritiated ^{3}H-dihydroalprenolol (specific activity 45 Ci/mmole) from New England Nuclear Corporation, Boston,

Table 1. Effect of 5HT uptake inhibitors on the cyclic AMP response to noradrenaline in the rat limbic forebrain. Amitriptyline (20 mg/kg), chlorimipramine (20 mg/kg) or fluoxetine (10 mg/kg) were injected i.p. daily and the animals sacrificed 24 h after the last injection. The response equals the NA stimulated level of cyclic AMP minus the basal level of the nucleotide. The numbers in parentheses designate the number of animals; each animal sample was analyzed in duplicate

	Duration of treatment (days)	Cyclic AMP pmoles/mg protein ± SEM		Control %
		Basal level	Response to 100 μM NA	
Control	1	19.45 ± 2.37 (6)	156.14 ± 8.03 (4)	100
Amitriptyline		16.47 ± 2.54 (6)	143.43 ± 16.62 (8)	92
Control		19.45 ± 2.37 (6)	156.14 ± 8.03 (4)	100
Chlorimipramine		14.70 ± 2.50 (6)	125.07 ± 8.48 (8)	80
Control		15.34 ± 3.46 (5)	117.96 ± 15.42 (6)	100
Fluoxetine		17.60 ± 1.51 (7)	112.77 ± 7.06 (9)	96
Control	7	15.60 ± 1.68 (6)	152.37 ± 16.77 (4)	100
Amitriptyline		15.79 ± 2.34 (6)	101.51 ± 10.94 (8)*	66
Control		15.60 ± 1.68 (6)	152.37 ± 16.77 (4)	100
Chlorimipramine		15.07 ± 1.30 (6)	103.71 ± 7.54 (8)*	68
Control		17.01 ± 0.39 (5)	161.15 ± 8.28 (6)	100
Fluoxetine		19.36 ± 2.76 (7)	131.36 ± 12.02 (9)	81
Control	14	21.99 ± 2.80 (4)	113.38 ± 10.43 (4)	100
Amitriptyline		23.86 ± 4.46 (8)	78.65 ± 7.97 (8)*	69
Control		21.66 ± 3.20 (5)	132.46 ± 8.81 (6)	100
Chlorimipramine		23.96 ± 2.55 (7)	82.12 ± 6.09 (9)**	62
Control		19.38 ± 2.25 (5)	127.71 ± 19.76 (6)	100
Fluoxetine		18.36 ± 2.04 (7)	127.70 ± 10.62 (9)	100

* $P < 0.01$, ** $P < 0.001$

MA, USA); *d,l*-propranolol was purchased from Ayerst Laboratories, New York, NY, USA.

The uptake inhibitors were generously donated as follows: amitriptyline (Merck, Sharp and Dohme Laboratories, West Point, PA, USA); chlorimipramine HCl (Ciba-Geigy Corp., Summit, NJ, USA) and fluoxetine HCl (Eli Lilly Laboratories, Indianapolis, IN, USA).

Statistics. The statistical evaluation of the data was carried out by the two tailed Student *t*-test (drug studies) and analyses of variance (lesion studies).

Results

Effect of 5HT Uptake Inhibitors on Basal Levels of Cyclic AMP and on Responsiveness of the Cyclic AMP Generating System to Noradrenaline. The acute or chronic administration of the 5HT uptake inhibitors amitriptyline, chlorimipramine and fluoxetine for 14 days did not change the basal level of the nucleotide in the limbic forebrain thus confirming earlier data (Mishra and Sulser 1978). Moreover, a single dose of the drugs did not significantly alter the neurohormonal response to 100 μM NA (concentration of NA that causes maximum cyclic AMP response). However, treatment with amitriptyline and chlorimipramine for 1 or 2 weeks reduced significantly the responsiveness of the system to NA (Table 1). The specific 5HT uptake inhibitor fluoxetine did not significantly alter at any time either the basal level of the nucleotide or the neurohormonal response to 100 μM NA thus excluding the possibility that a change in the maximal responsiveness could have been missed in preliminary studies in which a concentration of 10 μM NA (approximate EC_{50} value) was used (Mishra and Sulser 1978).

Table 2. Effect of repeated administration of amitriptyline and chlorimipramine on specific β-adrenergic receptor binding in rat cortex. The animals received amitriptyline (20 mg/kg) or chlorimipramine (20 mg/kg) for a period of 7 days and were sacrificed 24 h after the last injection. B_{max} and K_d values for specific ^{3}H-DHA binding were determined by Scatchard analysis of the binding data using no less than 5 concentrations of ligand ranging from 0.5 to 4 nM ^{3}H-DHA. The values are expressed as mean values ± SEM for determinations from 6 animals, each animal sample being analyzed in triplicate

Treatment	B_{max} fmoles ^{3}H-DHA/mg protein ± SEM	K_d nM
Control	171 ± 6	1.55 ± 0.25
Amitriptyline	148 ± 5*	1.24 ± 0.10
Chlorimipramine	148 ± 4*	1.21 ± 0.08

* $P < 0.02$

Effect of 5HT Uptake Inhibitors on Specific β-Adrenergic Receptor Binding in the Rat Cortex. A single dose of amitriptyline (20 mg/kg), chlorimipramine (20 mg/kg) or fluoxetine (10 mg/kg) did not change the specific ^{3}H-DHA binding in cortex 1 day following the intraperitoneal administration. However, repeated administration of chlorimipramine and amitriptyline for 7 days reduced significantly the number of β-adrenergic binding sites. Scatchard analysis of the data revealed that this change was due to a decrease in the density of β-adrenergic binding sites without an appreciable change in the K_d values (Table 2). In contrast, chronic administration of fluoxetine did not alter specific β-

Table 3. Effect of raphé lesions on the concentration of biogenic amines in brain

(*a*) Comparative effects of raphé lesions on the level of serotonin (5HT), noradrenaline (NA) and dopamine (DA) in the limbic forebrain and the rest of the brain[a]

	5HT		NA		DA
	Limbic forebrain	Rest of brain	Limbic forebrain	Rest of brain	Rest of brain
	μg/g ± SEM		μg/g ± SEM		μg/g ± SEM
Sham-operated ($n = 6$)	0.60 ± 0.06	0.39 ± 0.02	0.73 ± 0.04	0.33 ± 0.02	1.13 ± 0.07
Raphé lesion ($n = 8$)	0.28 ± 0.05*	0.22 ± 0.02*	0.89 ± 0.07	0.38 ± 0.02	1.23 ± 0.04

(*b*) Levels of biogenic amines in the rest of the brain[a] whose limbic forebrain area was used for the determination of the cyclic AMP response to NE

	5HT	NA	DA
Sham-operated ($n = 20$)	0.59 ± 0.0	0.48 ± 0.01	1.01 ± 0.02
Raphé lesion ($n = 20$)	0.20 ± 0.02*	0.47 ± 0.01	0.99 ± 0.03

[a] After removal of the limbic forebrain, a second cut was made at the level of the inferior colliculus. The portion caudal to the cut was used for histology and the remainder comprised the part designated as rest of the brain

** $P < 0.001$

Table 4. Cyclic AMP responses to NA in rat limbic forebrain following raphé lesions. The animals were sacrificed 14 days following raphé lesions and the cyclic AMP responses to NA determined as described in Methods and Materials. The response designates the NA stimulated level of cyclic AMP minus the basal level of the nucleotide. Numbers in parentheses indicate the number of individual animal samples, that is, the limbic forebrain from each brain was divided in half and each half served as an individual sample which was analyzed in duplicate. Samples were assigned to groups such that no group contained two samples from the same animal

	Cyclic AMP in pmoles/mg protein ± SEM			
	Basal level	Response		
		3 μM NA	30 μM NA	300 μM NA
Controls (sham-operated)	16.75 ± 3.01 (6)	27.40 ± 5.42 (12)	73.83 ± 5.04 (12)	91.42 ± 7.79 (10)
Raphé lesions	15.10 ± 3.88 (5)	30.12 ± 7.29 (12)	74.63 ± 6.23 (12)	73.14 ± 6.73 (10)

adrenergic ^{3}H-DHA binding [fluoxetine vs. control at a ligand concentration of 10 nM ^{3}H-DHA: 199 ± 19 (19) and 160 ± 19 (7) fmoles DHA/mg protein ± SEM].

Effect of Raphé Lesions on the Level of Biogenic Amines in Brain and on the Hormonal Responsiveness of the NA Receptor Coupled Adenylate Cyclase System. Preliminary data with 6 animals per group indicated that there were no differences in the levels of biogenic amines in the brains of non-manipulated control animals and sham lesioned animals (5HT, μg/g ± SEM: 0.635 ± 0.035 and 0.652 ± 0.041; NA, μg/g ± SEM: 0.384 ± 0.015 and 0.346 ± 0.006; DA, μg/g ± SEM: 0.699 ± 0.028 and 0.687 ± 0.020 respectively). Therefore, for all subsequent experiments, sham-lesioned animals were used as the control group. In our first lesion experiment, we compared the effects of raphé lesions on the levels of 5HT, NA and DA in the limbic forebrain and in the remainder of the brain 14 days after lesioning. As can be seen in Table 3a, the lesions produced a marked and selective reduction in 5HT. The degree of reduction of 5HT was similar in the limbic forebrain and in the rest of the brain thus indicating that levels of 5HT in the rest of the brain could be used as an index of changes in 5HT in the limbic forebrain.

This selective manipulation of the 5HT system did not change either the basal level of cyclic AMP in the limbic forebrain or alter the responsiveness of the cyclic AMP generating system to various concentrations of NA (Table 4). Table 3b presents the levels of 5HT, NA and DA from brain of animals used in these experiments confirming the selective depletion of 5HT. The localization of the lesions was verified histologically as described in Methods and Materials.

Effect of Medial Forebrain Bundle Lesions on the Level of Biogenic Amines and on the Responsiveness of the Cyclic AMP Generating System to NA in the Limbic Forebrain. In contrast to raphé lesions which selectively reduced the level of 5HT, lesions of the medial forebrain bundle also significantly decreased the concentration of the catecholamines noradrenaline and dopamine (Table 5). There were no differences in the content of biogenic amines in the limbic forebrain between control side and sham lesioned side. While the basal levels of the cyclic nucleotide were not altered by MFB lesions, the cyclic AMP response to NA was significantly increased on the lesioned side (Table 6) whereas the sham operation had no effect. The specific β-adrenergic ^{3}H-DHA binding was not significantly changed under these experimen-

Table 5. Effect of unilateral lesions of the medial forebrain bundle (*MFB*) on the concentration of biogenic amines in the limbic forebrain. The animals were killed by decapitation 1 week following lesioning of the MFB and the brain tissue analyzed for the content of biogenic amines as described under methods.

	Noradrenaline	Dopamine	Serotonin
	μg/g ± SEM		
Left side (control)	0.71 ± 0.02 (5)	2.81 ± 0.11 (5)	0.99 ± 0.10 (5)
Right side (sham lesioned)	0.69 ± 0.03 (5)	2.41 ± 0.23 (5)	0.99 ± 0.07 (5)
Left side (control)	0.64 ± 0.02 (8)	2.91 ± 0.09 (8)	1.09 ± 0.02 (8)
Right side (MFB lesion)	0.30 ± 0.03 (8)*	0.33 ± 0.03 (8)*	0.29 ± 0.01 (8)*

* $P < 0.001$

Table 6. Altered cyclic AMP response to noradrenaline (NA) in rat limbic forebrain following lesions of the medial forebrain bundle. Lesions of the medial forebrain bundle were performed as described under Methods and Materials. The animals were sacrificed 7 days following the lesions and the cyclic AMP response to NA determined. The response equals the NA stimulated level of cyclic AMP minus the basal level of the nucleotide. The number in parentheses designates the number of animals, each animal sample being analyzed in duplicate. The basal levels were in pmoles/mg protein ± SEM: 15.70 ± 0.65 (18)

	Cyclic AMP response to 10 μM NA pmoles/mg protein ± SEM
Left side (control)	36.15 ± 4.06 (13)
Right side (sham-operated)	43.24 ± 5.63 (13)
Left side (control)	41.87 ± 3.30 (13)
Right side (lesioned)	60.33 ± 8.28 (13)*

* $P < 0.05$

tal conditions [Lesioned vs. unlesioned side at a concentration of 6 nM ^{3}H-DHA: 136 ± 7 (8) and 133 ± 5 (8) fmoles/mg protein ± SEM]. Because of the lack of sufficient limbic forebrain tissue, a Scatchard analysis could not be performed in the same halves of the limbic forebrain. However, there was no difference in B_{max} and K_d values for ^{3}H-DHA binding between samples of the cortex from the lesioned and the unlesioned side (results not shown).

Discussion

The results of the present investigation on the effect of various 5HT uptake inhibitors on the sensitivity of the NA receptor coupled adenylate cyclase system confirm and extend previous data published from our laboratory (Mishra and Sulser 1978). Since down-regulation of the noradrenergic receptor system in brain has been shown to be associated with a reduction in the maximal response (R_{max}) to NA without a change in the EC_{50} value (concentration of agonist that causes half-maximal response), it is pertinent that the R_{max} value was significantly reduced following chronic administration of amitriptyline and chlorimipramine whereas the responsiveness to a maximal concentration of NA was not altered following chronic administration of the specific 5HT uptake inhibitor fluoxetine. Similar negative data with fluoxetine have been previously reported by Schmidt and Thornberry (1977). Since fluoxetine is a more selective inhibitor of 5HT uptake than amitriptyline and chlorimipramine and its in vivo N-demethylation to norfluoxetine does not alter either the potency or the selectivity for inhibiting 5HT reuptake (Wong et al. 1975), it can be concluded that a selective increase in the availability of 5HT does not modify the responsiveness of the cyclic AMP generating system to NA. Also, raphé lesions, which caused a selective reduction in the concentration of 5HT in the forebrain did not modify the neurohormonal responsiveness to NA. In contrast, lesions of the medial forebrain bundle, a procedure which markedly reduced the levels of both 5HT and catecholamines caused a significant increase in the sensitivity of the cyclic AMP generating system to NA in slices of the ipsilateral limbic forebrain area as compared to that in slices from the contralateral non-lesioned limbic forebrain area. Both the decrease in the concentration of NA and the magnitude of enhancement of the cyclic AMP response to NA in slices from the limbic forebrain area are comparable to the changes reported by Dismukes et al. (1975) in the rat cortex and hippocampus following lesions of the medial forebrain bundle. Though it has been suggested on the basis of turnover studies that amitriptyline lacks an effect on brain NA systems (Tang et al. 1978), the present results indicate that this drug shares its action on noradrenergic receptor function with that of other antidepressant drugs (Sulser 1979).

Though multiple mechanisms may be responsible for drug induced changes in the sensitivity of the NA receptor coupled adenylate cyclase system in brain, the subsensitivity following treatment with clinically effective antidepressants and with ECT is generally linked to a significant and rather specific decrease in the density of β-adrenergic receptors (Banerjee et al. 1977; Bergstrom and Kellar 1979a, b; Sarai et al. 1978; Wolfe et al. 1978; Gillespie et al. 1979). The recent findings that chronic treatment with a number of antidepressant drugs decreases (Peroutka and Snyder 1980) while ECT increases (Kellar et al. 1981) the density of $5HT_2$ receptors labelled by spiroperidol are interesting and deserve further study. The down-regulation of noradrenergic sensitivity caused by amitriptyline and chlorimipramine, linked to a decrease in the B_{max} value of β-adrenergic binding sites, is compatible with current views on homospecific down-regulation of noradrenergic receptor function. Thus, the tertiary amines amitriptyline and chlorimipramine are in vivo converted to their corresponding secondary amines, nortriptyline and desmethylchlorimipramine respectively, which are potent inhibitors of the NA reuptake (Koe 1976; Thomas and Jones 1977) and thus increase agonist (NA) receptor occupancy.

While most if not all clinically effective antidepressant treatments cause a down-regulation of central noradrenergic receptor function (it remains to be seen whether the specific 5HT uptake inhibitor fluoxetine will prove to be an effective antidepressant treatment and thereby represent an exception to the rule), the molecular mechanisms involved in the development of subsensitivity may be quite different. Thus, central noradrenergic subsensitivity is not invariably linked

to a reduction in the B_{max} value of β-adrenergic receptors (Mishra et al. 1979, 1980). It is also of interest in this regard that the increased sensitivity of NA observed 1 week following lesions of the medial forebrain bundle was not accompanied by an increase in the density of β-adrenergic receptors. Other factors such as changes in membrane properties affecting the coupling of receptors and adenylate cyclase may contribute to changes in the overall sensitivity of the system and may precede changes in receptor numbers during the process of development of neuronal sub- or supersensitivity to NA (Sulser and Mobley 1981).

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