Biochemical Pharmacology, Vol. 34, No. 5, pp. 679–683, 1985.
Printed in Great Britain.

0006–2952/85 $3.00 + 0.00

EFFECT OF CHRONIC DESMETHYLIMIPRAMINE OR ELECTROCONVULSIVE SHOCK ON SELECTED BRAIN AND PLATELET NEUROTRANSMITTER RECOGNITION SITES

MARC S. ABEL,* DONALD E. CLODY, LAWRENCE P. WENNOGLE† and LAURENCE R. MEYERSON

Department of CNS Research, Medical Research Division of American Cyanamid Co., Lederle Laboratories, Pearl River, NY 10965, U.S.A.

(*Received* 6 *February* 1984; *accepted* 27 *June* 1984)

Abstract—Rats were treated with electroconvulsive shock (ECS), desmethylimipramine (DMI), ECS plus DMI, or diazepam. *In vitro* analyses showed that chronic ECS produced an elevated density of recognition sites for [^{3}H]imipramine (IMI) in platelet membranes, but had no effect on membrane preparations derived from cortical tissue. A similar elevation in receptor binding was seen exclusively in platelets after chronic ECS plus DMI, whereas no effect was observed with DMI alone. Equilibrium dissociation constant (K_D) values for [^{3}H]IMI were also increased in platelet membranes from rats given chronic ECS or ECS plus DMI treatment. Chronic ECS or DMI administration produced a decreased density of β-adrenergic recognition sites in frontal cortex and cerebellum as assessed by [^{3}H]dihydroalprenolol (DHA) binding. The combination of ECS plus DMI produced a similar decrease. In addition, chronic diazepam administration produced a down-regulation of the β-adrenergic receptor only in the cerebellum. These data provide evidence for the differential regulation of brain and peripheral neurotransmitter recognition sites.

Depressive illness is a heterogeneous psychopathology with a multitude of proposed etiologies and therapies. Certain therapeutic regimens have in common the ability to modulate selected neurotransmitter recognition sites. For example, when administered chronically, electroconvulsive shock treatment (ECS) [1] and tricyclic antidepressive drugs such as desipramine (DMI) [2] produce a down-regulation of the rodent cortical β-adrenergic receptor. This may be due to increased availability of neurotransmitter in the synaptic junction. The time course for the development of receptor subsensitivity is comparable to that for the onset of clinical improvement (2–3 weeks) [3]. This alteration in receptor sensitivity may be a necessary factor for the successful treatment of only a sub-population of depressed patients. Not all patients show clinical improvement after treatments which down-regulate the β-adrenergic system, and not all clinically efficacious antidepressive therapies produce alterations in the β-adrenergic system. For example, iprindole [4], mianserin [5], and alprazolam [6] are antidepressive agents that do not down-regulate the cortical β-adrenergic system. Also, rapid-eye-movement sleep deprivation, a proposed therapeutic regimen for depression [7], does not produce cortical β-adrenergic subsensitivity [8, 9] (see, however, [10]).

Whereas an alteration in the β-adrenergic system may be a common molecular endpoint for effective therapy, other neurochemical systems have been recognized as possible indices for the diagnosis of depression. Reductions in the binding capacity to human platelets of [^{3}H]imipramine (IMI), an agent which labels the serotonin (5-HT) uptake/allosteric regulator complex, is correlated with the diagnosis of clinical depression [11, 12].

The present study has investigated the relationship between receptor regulation in brain and platelet. It was designed to assess the effect of chronic administration of DMI, ECS, or both on binding parameters of the β-adrenergic receptor in rat cortex and cerebellum as well as [^{3}H]IMI binding parameters in cortex and platelets from the same experimental animals. The plasticity of the cerebellum was examined because of the well established noradrenergic system innervating the cerebellum from the locus ceruleus. Diazepam was included as a centrally acting non-antidepressive agent.

MATERIALS AND METHODS

Chronic animal treatment. Male Wistar rats (Royalhart Farms, New Hampton, NY), weighing 150–180 g, were housed in group cages (five per cage) with food and water *ad lib.* under a 12–12 hr light–dark cycle (lights on at 7:00 a.m.).

Animals were treated for 14 days with either distilled water, desipramine (DMI, 10 mg/kg, i.p., b.i.d.), electroconvulsive shock (ECS, 75 mA, 0.3-sec duration, via corneal electrodes, once per day), DMI plus ECS, or diazepam (5 mg/kg, i.p., once a

* Author to whom all correspondence should be addressed.
† Present address: Department of Neuroscience, Ciba-Geigy Corp., Summit, NJ 07901.

day). The last dose of DMI was administered in the morning of day 14. After a 24-hr washout period, the animals were killed by decapitation. The cerebral cortex and cerebellum were dissected from each animal, weighed, and stored at −20°.

Platelet isolation and [^{3}H]*imipramine binding.* [^{3}H]IMI binding to platelets was performed using a modification of previously described techniques [13]. Trunk blood from decapitated rats was collected into 2.5 ml of an antiprotease/anticoagulant buffer containing 0.05 M Tris (pH 7.5), 0.12 M NaCl, 5 mM KCl, 5 mM EDTA, 1 mM ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA), 0.1 mM phenylmethylsulfonylfluoride (PMSF), 5 units/ml aprotinin and 0.5 mg/ml pepstatin (all from Sigma). All procedures were conducted at 0–4° using polyethylene labware. Blood from five animals was pooled to provide adequate numbers of platelets for biochemical measurements. After centrifugation of the blood at 250 *g* for 20 min, the supernatant fraction (platelet-rich plasma) was further centrifuged at 2500 *g* for 10 min and washed twice with buffer. The pellets were dispersed in buffer (1/2 original blood vol.) and sonicated four times with 10-sec pulses using a Branson Sonifier (microprobe). The suspension was centrifuged for 20 min at 20,000 *g* and the membranes were resuspended in buffer to a protein concentration of 3 mg/ml.

Saturation isotherms of [^{3}H]imipramine binding to platelets were performed in triplicate at radioligand concentrations of 2.6, 4.6, 8.3, 10.9 and 19.9 nM. Corrections were made for non-specific binding by assaying parallel incubation tubes containing 10 μM desmethylimipramine. Individual incubations had a total assay volume of 250 μl and contained 0.3 mg protein. The samples were equilibrated for 90 min at 0°. Assay tube contents were then filtered under vacuum through Whatman GF/B filters, and washed with 2 × 5 ml cold buffer (0.05 M Tris, 0.12 M NaCl, 0.005 M KCl, pH 7.5). Filters were placed in scintillation vials containing 10 ml Beckman Ready Solv-HP counting solution, and radioactivity was determined with a Beckman LS-350 liquid scintillation spectrometer.

Cortical membrane isolation and [^{3}H]*imipramine binding.* Frozen cortices from the same animals used for platelet [^{3}H]IMI binding were thawed and homogenized in 50 vol. of buffer (0.05 M Tris pH 7.5, 0.12 NaCl, 0.005 M KCl). Homogenization was performed using a Tekmar Ultra Turrax with a 30-sec pulse at 60% power. Membranes were centrifuged at 20,000 *g* for 10 min and then washed twice and resuspended to 2 mg protein/ml. Duplicate tubes containing 0.4 mg protein were equilibrated, filtered, washed, and analyzed essentially as described above for platelet studies. Non-specific binding was assessed by including desmethylimipramine (100 μM) in parallel incubations. Saturation isotherms were generated using [^{3}H]imipramine concentrations of 0.8, 1.2, 1.9, 3.0, 4.8, 7.6, 12.5 and 21.5 nM.

[^{3}H]*DHA binding.* Specific [^{3}H]dihydroalprenolol (DHA) binding was determined using modifications of methods previously described [14–16]. Briefly, frozen cortical tissue was thawed and prepared for the assay by homogenization (20:1, v/w) in 50 mM Tris–HCl buffer, pH 8.0, using a Tekmar Ultra Turrax set to 60% power for 30 sec. The homogenate was centrifuged at 49,000 *g* for 15 min and the resultant pellet was resuspended in buffer (6 mg wet weight/ml). Saturation isotherms were conducted using eight concentrations of [^{3}H]DHA. Triplicate assay tubes contained in a final volume of 1 ml: 0.1 to 1.5 nM [^{3}H]DHA (101 Ci/mmole, New England Nuclear), tissue (3 mg wet weight) and Tris–HCl buffer (50 mM, pH 8.0). Non-specific binding was determined using 10 μM propranolol in parallel incubations. After incubating at room temperature (23°) for 15 min, the samples were filtered under vacuum through Whatman GF/B filters on a Millipore manifold. Each tube was washed twice with 5 ml of cold buffer. Filters were placed in scintillation vials containing 10 ml of Beckman Ready Solv-HP counting solution, and radioactivity was determined in a Beckman LS350 liquid scintillation spectrometer. Cerebellar tissue samples were assayed in exactly the same manner with the exception that the total volume per sample tube was 250 μl.

Protein determination. Protein concentrations were determined by the protein-dye binding method [17] utilizing the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA). Bovine gamma globulin was used as the standard.

Data analysis and statistical evaluation. Data from saturation isotherms were subjected to Scatchard transformations [18]. The line of best fit for these data was generated using a computer program for least squares linear regression analysis. Intercepts and slopes from this line yielded receptor density (B_{max}) and equilibrium dissociation constant (K_D) values respectively.

Intergroup B_{max} and K_D values were statistically evaluated using an analysis of variance (ANOVA). When the ANOVA indicated significance, individual groups were compared to the control group using a two-sample *t*-test. P values less than or equal to 0.05 were taken to indicate significant differences.

RESULTS

The [^{3}H]DHA binding parameters for cortical membranes after each treatment are listed in Table 1. Specific binding represented 60% of total [^{3}H]-DHA binding at the K_D concentration in both cortex and cerebellum. The mean linear correlation coefficients (r) for [^{3}H]DHA Scatchard analyses in the cortex and cerebellum were 0.95 and 0.92 respectively. Chronic administration of DMI, ECS or the combination of ECS + DMI produced a decrease in the B_{max} for [^{3}H]DHA binding to membranes from the cerebral cortex. Diazepam did not affect the receptor density in cortex. No significant alterations in ligand equilibrium dissociation constants (K_D) were observed in the cortex after any treatment (ANOVA, $P > 0.1$).

The profile of [^{3}H]DHA binding to cerebellar membranes after each treatment is similar to that seen in the cortex (Table 1). Rats treated with DMI, ECS or the combination of ECS + DMI had a reduced B_{max}. In addition, and in contrast to the cortex, chronic treatment with diazepam decreased

Table 1. Effect of chronic administration of psychotropic treatments on cortical and cerebellar [^{3}H]DHA binding*

	Cortex		Cerebellum	
	B_{max} (fmoles/mg protein)	K_D (nM)	B_{max} (fmoles/mg protein)	K_D (nM)
Control	85.8 ± 1.8	0.42 ± 0.03	62.8 ± 1.1	0.40 ± 0.06
ECS	69.5 ± 1.1†	0.41 ± 0.04	29.2 ± 1.9‡	0.27 ± 0.02§
DMI	47.8 ± 7.7§	0.35 ± 0.08	27.6 ± 4.7‖	0.24 ± 0.03§
ECS + DMI	54.5 ± 3.7§	0.50 ± 0.02	35.3 ± 8.4§	0.23 ± 0.03§
Diazepam	82.2 ± 6.1	0.40 ± 0.03	40.1 ± 2.8§	0.32 ± 0.05

* Values represent means ± S.E.M. of data determined by linear regression analysis of Scatchard analyses (see Materials and Methods); N = 3–4 for each group.
† $P < 0.002$ as compared to control.
‡ $P < 0.001$ as compared to control.
§ $P < 0.02$ as compared to control.
‖ $P < 0.005$ as compared to control.

the B_{max} in the cerebellum. Also, significant decreases in the K_D were observed in the ECS, DMI, and ECS + DMI groups.

[^{3}H]*IMI binding*. Specific binding represented 60% of total [^{3}H]IMI binding at the K_D concentration in both platelet and cortex. The mean linear correlation coefficients (r) for [^{3}H]IMI Scatchard analyses in the platelet and cortex were 0.91 and 0.93 respectively.

No differences in either B_{max} or K_D for [^{3}H]IMI binding to cortical membranes were found between pretreatment groups (Table 2, ANOVA: B_{max}, $P > 0.1$; K_D, $P > 0.1$). However, a trend was apparent such that the combined treatment of ECS + DMI produced an elevation in the B_{max} that was significant when analyzed by the t-test. Surprisingly, a markedly elevated B_{max} was observed for [^{3}H]IMI binding to platelet membranes prepared from rats treated with ECS or ECS + DMI (Table 2). No change was seen in B_{max} after DMI treatment alone. The K_D values for the ECS and ECS + DMI groups were elevated significantly compared to control values.

DISCUSSION

This study supports the notion that important differences exist for the regulation of [^{3}H]IMI binding to peripheral sites (platelet) and central sites (brain).

Down-regulation of the rodent cortical β-adrenergic recognition site is a well-established phenomenon after chronic ECS [1] or DMI [16, 19–22] treatments. The present study supports and extends those findings. In addition, a novel observation was that chronic ECS + DMI produced a decrease in β-adrenergic receptor density that was not either an additive or potentiative effect of the individual treatments. This suggests that the down-regulation produced by both treatments is mediated by a common mechanism(s).

β-Adrenergic B_{max} values in the cerebellum were modulated by all the treatments employed. In each condition, there was a significant reduction in receptor density as assessed by [^{3}H]DHA saturation experiments. Kinnier *et al.* [23] reported a similar reduction in the specific binding of [^{3}H]DHA to cerebellar membranes from rats treated chronically with imipramine. In contrast, however, Keller *et al.* [24] reported no effect of chronic ECS on cerebellar [^{3}H]DHA binding. These other reports of cerebellar [^{3}H]DHA binding after antidepressive treatment were conducted using single concentration determinations of specific binding and no K_D values were presented. If the 40% decrease in K_D (increased

Table 2. Effect of chronic administration of psychotropic treatments on cortical and platelet [^{3}H]IMI binding*

	Cortex		Platelet	
	B_{max} (fmoles/mg wet wt)	K_D (nM)	B_{max} (fmoles/mg protein)	K_D (nM)
Control	21.9 ± 1.1	8.8 ± 0.93	775 ± 74	7.4 ± 0.5
ECS	20.0 ± 2.2	7.9 ± 1.1	1572 ± 172†	15.3 ± 3.8†
DMI	21.8 ± 2.8	10.5 ± 1.7	949 ± 126	10.6 ± 1.9
ECS + DMI	29.0 ± 4.2	12.8 ± 3.0	1651 ± 154†	13.2 ± 1.8‡
Diazepam	21.8 ± 1.2	8.2 ± 0.7	866 ± 61	7.9 ± 1.4

* Values represent means ± S.E.M. of data determined by linear regression analysis of Scatchard analyses (see Materials and Methods). Platelets from five rats were pooled to produce each (N). N = 5–6 for cortex, N = 3 for platelet.
† $P < 0.05$ as compared to controls.
‡ $P < 0.01$ as compared to controls.

ligand affinity) reported in the present paper has any functional significance, it may be a compensatory response to the concomitant decrease in B_{max} observed in those groups. The differential response of the cortex and the cerebellum suggests that the beta receptors in the two regions are subject to different types of regulation. Our observation that diazepam decreased cerebellar B_{max} values suggests an interaction either at a molecular or an interneuronal level between benzodiazepine (BDZ/GABA) and β-adrenergic systems in that region.

The literature regarding the plasticity of imipramine recognition sites after treatment with various antidepressive agents is inconsistent and varies with the region studied. For example, chronic administration of imipramine is reported to produce IMI recognition site down-regulation in the hypothalamus [25] and the hippocampus [23, 26]. However, in the cortex a decrease [27] or no change in B_{max} was found [20, 23, 28]. Similarly, chronic administration of DMI has been reported to either decrease the density of cortical sites labeled by [^{3}H]IMI [21, 22, 29, 30], or have no effect [20]. Our data support the latter finding in that no change was observed in cortical [^{3}H]IMI binding after 2 weeks of DMI treatment. This is also consistent with the observation that 3 weeks of chronic DMI administration are required to down-regulate cortical [^{3}H]-IMI recognition sites, whereas no change in density is detected after 2 weeks of chronic treatment (M. L. Barbaccia, personal communication). Because the administration of chronic ECS alone also did not alter IMI recognition site levels in the cortex, it may be stated that, in our study, traditional therapeutic regimens for depression do not regulate cortical binding sites labeled by [^{3}H]IMI. These treatments, however, may affect sites that have been altered due to depression, and normal laboratory animals may not be representative of this human psychopathology.

In contrast to the data for the cortex, IMI binding to platelet receptor sites was up-regulated by chronic ECS treatment. The combination of ECS + DMI also increased the density of platelet IMI receptors, although not to a greater extent than ECS alone. This finding is interesting in light of the clinical reports that depressed individuals have fewer platelet [^{3}H]IMI binding sites than normal control subjects [11, 12, 31]. The density of sites labeled by [^{3}H]IMI in platelets from depressed patients has been shown to increase to "normal" levels after tricyclic antidepressive therapy [31] or to remain the same [12]. Therefore, the etiology of the elevation in platelet IMI binding seen in the present study may not solely reflect an effect on neurotransmitter reuptake *per se* (since DMI did not produce a modulation), but rather a more generalized response to ECS treatment. The significance of increased K_D values for [^{3}H]IMI binding to platelet membranes after chronic ECS or DMI is yet to be determined. In contrast to the present finding of differential regulation of [^{3}H]IMI binding in cortical and platelet membranes, Briley *et al.* [25] report a concomitant decrease in [^{3}H]IMI binding to hypothalamic and platelet membranes after chronic administration of tricyclic drugs. There are several important differences between the studies including species (cat vs rat), treatment regimen (IMI vs DMI) and tissue sampled (hypothalamus vs cortex). These experimental variations may explain the different findings.

Chronic treatment with ECS + DMI was the only regimen capable of altering both [^{3}H]DHA and [^{3}H]-IMI binding in brain and platelet. Apparently, a robust treatment such as this is necessary to produce measurable changes in the systems studied.

Finally, [^{3}H]IMI binding in the platelet has been proposed and utilized as a marker for certain psychopathological states, but it is clear from the present study that the platelet and brain receptors are not regulated in identical ways by clinically efficacious treatments for depression. Therefore, because biochemical markers in the brain and platelet do not always regulate in concert, it is prudent that both tissues be evaluated to completely assess the molecular effect of various pharmacologic agents.

Acknowledgements—The authors would like to express their appreciation to Carmela Nardella for her excellent clerical assistance during the preparation of this manuscript and to Dr. Bernard Beer for his continued support of this research.

REFERENCES

1. D. A. Bergstrom and K. J. Kellar, *Nature, Lond.* **278**, 464 (1979).
2. S. P. Banerjee, L. S. Kung, S. J. Riggi and S. K. Chanda, *Nature, Lond.* **268**, 455 (1977).
3. I. Oswald, V. Brezinova and D. L. F. Dunlevy, *Br. J. Psychiat.* **120**, 673 (1972).
4. T. D. Reisine, D. C. U'Prichard, N. L. Wiech, R. C. Ursillo and H. I. Yamamura, *Brain Res.* **188**, 587 (1980).
5. S. Clements-Jewery, *Neuropharmacology* **17**, 779 (1978).
6. V. H. Sethy and D. H. Hodges, Jr., *Res. Commun. Chem. Path. Phamac.* **36**, 329 (1982).
7. G. W. Vogel, A. Thurmond, P. Gibbons, K. Sloan, M. Boyd and M. Walker, *Archs gen. Psychiat.* **32**, 765 (1975).
8. M. S. Abel, F. Villegas, J. Abreu, F. Gimino, S. Steiner, B. Beer and L. R. Meyerson, *Brain Res. Bull.* **11**, 729 (1983).
9. M. Radulovacki and N. Micovic, *Brain Res.* **235**, 393 (1982).
10. E. Mogilnicka, S. Arbilla, H. Depoortere and S. Z. Langer, *Eur. J. Pharmac.* **65**, 289 (1980).
11. K. B. Asarch, J. C. Shih and A. Kulcsar, *Commun. Psychopharmac.* **4**, 425 (1980).
12. S. Z. Langer and R. Raisman, *Neuropharmacology*, **22**, 407 (1983).
13. L. P. Wennogle, B. Beer and L. R. Meyerson, *Pharmac. Biochem. Behav.* **15**, 975 (1981).
14. R. W. Alexander, J. N. Davis and R. J. Lefkowitz, *Nature, Lond.* **258**, 437 (1975).
15. D. B. Bylund and S. H. Snyder, *Molec. Pharmac.* **12**, 568 (1976).
16. L. R. Meyerson, H. H. Ong, L. L. Martin and D. B. Ellis, *Pharmac. Biochem. Behav.* **12**, 943 (1980).
17. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
18. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
19. K. J. Kellar and D. A. Bergstrom, *Neuropharmacology* **22**, 401 (1983).
20. C. M. Lee, J. A. Javitch and S. H. Snyder, *Science* **220**, 626 (1983).

21. G. Racagni, I. Mocchetti, G. Calderini, A. Battistella and N. Brunello, *Neuropharmacology* **22**, 415 (1983).
22. R. Raisman, M. S. Briley and S. Z. Langer, *Eur. J. Pharmac.* **61**, 373 (1980).
23. W. J. Kinnier, D. M. Chuang and E. Costa, *Eur. J. Pharmac.* **67**, 289 (1980).
24. K. J. Kellar, C. S. Cascio, D. A. Bergstrom, J. A. Butler and P. Iadarola, *J. Neurochem.* **37**, 830 (1981).
25. M. Briley, R. Raisman, S. Arbilla, M. Casadamont and S. Z. Langer, *Eur. J. Pharmac.* **81**, 309 (1982).
26. M. L. Barbaccia, O. Gandolfi, D. M. Chuang and E. Costa, *Proc. natn. Acad. Sci. U.S.A.* **80**, 5134 (1983).
27. M. L. Barbaccia, N. Brunello, D. M. Chuang and E. Costa, *Neuropharmacology* **22**, 373 (1983).
28. P. Plenge and E. T. Mellerup, *Psychopharmacology* **77**, 94 (1982).
29. N. Brunello, D. M. Chuang and E. Costa, in *Typical and Atypical Antidepressants: Molecular Mechanisms* (Eds. E. Costa and G. Racagni), p. 179. Raven Press, New York (1982).
30. G. Racagni, I. Mocchetti, L. DeAngelis, M. Baraldi and E. Cuomo, in *Typical and Atypical Antidepressants: Molecular Mechanisms* (Eds. E. Costa and G. Racagni), p. 185. Raven Press, New York (1982).
31. B. E. Suranyi-Cadotte, P. L. Wood, N. P. Vasavan Nair and G. Schwartz, *Eur. J. Pharmac.* **85**, 357 (1982).