Biochemical Pharmacology, Vol. 42, No. 8, pp. 1525–1528, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

DIFFERENTIAL EFFECTS OF ANTIDEPRESSANTS ON $GABA_B$ AND β-ADRENERGIC RECEPTORS IN RAT CEREBRAL CORTEX

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(*Received* 23 *February* 1991; *accepted* 16 *May* 1991)

Abstract—The effects of chronic administration of antidepressant drugs on β-adrenergic and γ-aminobutyric acid $(GABA)_B$ receptors have been assessed with radioligand binding. Tricyclics [imipramine (IMI), 30 mg/kg/day, and desmethylimipramine (DMI), 10 mg/kg day] or monoamine oxidase inhibitors [(±)-tranylcypromine (TCP), 1 mg/kg/day, and phenelzine (PLZ), 10 mg/kg/day] were administered to male Sprague–Dawley rats by constant infusion via Alzet 2ML4 osmotic minipumps for 28 days. Pumps were implanted s.c. in the interscapular region. On day 28 the animals were killed and their brains removed; [^{3}H]GABA binding to $GABA_B$ receptors was measured in frontal cortex and the remaining cortical tissue was used to measure [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to β-adrenoceptors. All drugs tested induced a significant decrease in density (B_{max}) of [^{3}H]DHA binding, although no significant changes in affinity (K_d) were observed. [^{3}H]GABA binding was not altered significantly by chronic antidepressant treatment. TCP-treated animals showed a tendency towards increased [^{3}H]-GABA binding, but the differences did not reach statistical significance. No effects on K_d were observed. These data do not support the proposal that an increase in the total population of cortical $GABA_B$ receptors is a common effect of chronic antidepressant treatment.

Antidepressant drugs have been used in the treatment of depression for over 30 years [1]. During this period the focus of research into their mechanism(s) of action has shifted from a study of acute neurochemical effects (e.g. inhibition of neurotransmitter degradation or reuptake [2]) to an analysis of delayed or adaptive neuronal changes associated with chronic drug treatments [3, 4]. As the time–course of these adaptive changes parallels the apparent onset of clinical improvement (usually 2–4 weeks after initiation of drug therapy [3]), emergent changes may be the key to the clinical efficacy of the drugs. A commonly observed adaptive change following chronic treatment with a wide variety of antidepressants is a modification of β-adrenoceptors. Vetulani and Sulser [5] demonstrated that chronic administration of desmethylimipramine (DMI) or iprindole, as well as repeated electroshocks, diminished the responsiveness of the noradrenaline-stimulated adenylate cyclase system linked to β-adrenoceptors in rat forebrain slices. Using radioligand binding techniques, decreased β-adrenoceptor number has been found in rat cortical tissue following chronic treatment with DMI, imipramine (IMI), amitryptiline, (±)-tranylcypromine (TCP) and phenelzine (PLZ) [6–8].

A current area of interest in the study of the effects of long-term antidepressant drug exposure is that of emergent changes seen with γ-aminobutyric acid (GABA) receptors. Lloyd *et al.* [9] observed that the repeated administration of antidepressants of every class—tricyclic (TCA), monoamine oxidase inhibitors (MAOIs), novel antidepressants, as well as repeated electroshocks—all produced a significant increase in the density (number) of $GABA_B$ receptors in rat frontal cortex. Due to the diversity of antidepressant drug classes that induced this effect, it was suggested that this increase may represent a common mechanism of antidepressant drug action [9]. Evidence for this effect of antidepressant drugs on GABA receptors is equivocal (e.g. see Refs. 10 and 11 vs 12 and 13). The present study was designed to investigate the generality of this purported antidepressant drug action and involved an analysis of the effects of chronic (28 days) administration of both MAOIs (PLZ, TCP) and TCAs (IMI, DMI) on $GABA_B$ binding in rat frontal cortex. These drugs represent frequently prescribed, clinically effective antidepressants, and, in the case of PLZ and TCP, antidepressants that have not previously been studied in the present context. β-Adrenoceptor changes were also investigated to allow a comparison to be made with published studies.

MATERIALS AND METHODS

Animals and treatments. Male Sprague–Dawley rats weighing 275–325 g were used. The animals were group housed (two per cage) under a 12-hr light/dark cycle at a temperature of 20 ± 1°. Food and water were freely available. Animals were randomly allocated to drug or vehicle treatment conditions. Each animal was deeply anesthetized with a mixture of ether and air, and an osmotic minipump (Alzet 2ML4, Alza Corp., Palo Alto, CA) was implanted s.c. in the interscapular region. Each pump was filled with a drug solution individually adjusted in concentration [14] or the distilled water vehicle according to the group allocation of each

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animal. Drug treatment groups received: PLZ sulfate, 10 mg/kg/day; TCP hydrochloride, 1 mg/kg/day; IMI hydrochloride, 30 mg/kg/day; or DMI hydrochloride, 10 mg/kg/day. The incision was sutured and, after recovery, the animals were replaced in normal housing conditions.

Immediately following 28 days of drug administration, the animals were killed by rapid decapitation and their brains removed and dissected over ice. Frontal cortex was used to measure [^{3}H]GABA binding to $GABA_B$ receptors. The remaining cortical tissue was used to measure [^{3}H]dihydroalprenolol (DHA) binding to β-adrenoceptors. Protein determinations were made according to the method of Lowry *et al.* [15], using bovine serum albumin as standard. Tissue from two to three animals was pooled for each [^{3}H]GABA binding estimation, while tissue from individual animals was used for [^{3}H]DHA estimates.

[^{3}H]*DHA binding.* The procedure for measuring cortical β-adrenoceptors was adapted from Bylund and Snyder [16]. Cerebral cortices were homogenized in 10 volumes of ice-cold 50 mM Tris·HCl buffer (pH 7.4). The homogenate was decanted into centrifuge tubes and buffer was added to yield a total dilution of 100 volumes. This homogenate was centrifuged at 40,000 *g* at 4° for 10 min. The supernatant was discarded and the pellet was resuspended in 10 volumes of buffer, subsequently made up to a 100-volume dilution. The resultant suspension was recentrifuged and washed once again as above. After the second centrifugation, the final pellet was resuspended in 10 volumes of buffer. Aliquots (100 μL) of this final suspension containing approximately 0.5 to 0.6 mg protein/mL were then incubated at 23° in the presence of 0 to 5.0 nM [^{3}H]-DHA.

Saturation analyses were performed in triplicate in a final volume of 1 mL. Binding was terminated by rapid filtration through Whatman GF/C filters with a Brandel Cell Harvester. Filters were washed three times with approximately 4 mL of ice-cold buffer. Nonspecific binding was estimated from parallel assay tubes containing 10 μM alprenolol. Following filtration, the filters were removed, dried, and placed into scintillation vials containing 5 mL of scintillation fluid (Ready–Safe, Beckman). After 12 hr the vials were vortexed and counted for 10 min on a Beckman LS-7000 counter. Specific binding, defined as the difference between total and nonspecific binding, was 85%.

[^{3}H]*GABA binding.* Membrane preparation was as described by Hill and Bowery [17]. Crude synaptic membranes (prepared within 72 hr of killing of the rats) were frozen at −20° for at least 24 hr. The frozen pellet was thawed and resuspended in 15 volumes of ice-cold 50 mM Tris·HCl buffer, pH 7.4, containing 2.5 mM $CaCl_2$. Additional buffer was added to yield a total of 100 volumes and the suspension was incubated at room temperature (23°) for 45 min. Following the incubation, the suspension was centrifuged at 16,000 *g* for 10 min. The resultant pellet was resuspended (15 volumes buffer), subsequently made up to 100 volumes, incubated at room temperature for 15 min, and centrifuged again at 16,000 *g* for 10 min. This final wash was repeated once. The final membrane suspension contained approximately 0.3 to 0.5 mg protein/mL.

For the binding assay, aliquots (500 μL) of the final membrane suspension were incubated for 10 min at room temperature in tubes containing isoguvacine (40 μM), [^{3}H]GABA (30 Ci/mmol) (1 mM) and one of seven concentrations of unlabeled GABA (5–160 nM) for a cold saturation curve. Buffer was added to a final volume of 1 mL. Nonspecific binding was defined using (±)-baclofen (100 μM) and represented approximately 60% of total binding. The incubation was terminated by centrifugation (48,000 *g*, 20 min). The resulting pellet was washed briefly and superficially with 4 mL of ice-cold distilled H_2O, followed by removal of the supernatant by aspiration. The pellet was dissolved in Protosol (300 μL) overnight before the addition of scintillation fluid (3 mL). After 12 hr, the samples were counted for 10 min (counting efficiency 43%).

Statistics. The maximal number of binding sites (B_{max}) and the dissociation constant (K_d) for binding were estimated using EBDA and LIGAND programs [18]. Changes in binding parameters were assessed with parametric ANOVA followed by Newman–Keuls multiple comparisons where appropriate. The critical two-tailed significance level was $\alpha \leq 0.05$.

RESULTS

The effects of chronic antidepressant treatment on [^{3}H]DHA binding characteristics are displayed in Table 1. From these data it is evident that each of the present drug treatments resulted in a significant decrease in B_{max}, although no significant changes in K_d were evident. The [^{3}H]DHA binding control values for B_{max} (92.6 ± 3.7 fmol/mg protein) and K_d (1.04 ± 0.10 nM) were consistent with previous reports [6, 16].

In contrast to the [^{3}H]DHA results, [^{3}H]GABA binding was not found to be significantly altered by chronic antidepressant treatment (Table 2). B_{max} estimates for each drug group were not significantly different from vehicle controls. TCP-treated animals showed a tendency towards increased [^{3}H]GABA binding, but the differences did not reach statistical significance. No effects on K_d were observed. B_{max} and K_d estimates for [^{3}H]GABA binding in control animals (708.6 ± 73 fmol/mg protein and 50.1 ± 5.8 nM, respectively) were in good agreement with previous studies [9, 11].

DISCUSSION

In the present experiment, chronic administration of PLZ, TCP, IMI and DMI induced a significant reduction in the number of cortical β-adrenoceptors. This result is in agreement with previous reports of reduced β-adrenoceptor density [6, 7] and function [5, 19–21] following repeated antidepressant treatment.

Literature reports detailing the effects of chronic administration of antidepressant drugs on $GABA_B$ receptors show considerable heterogeneity. With functional tests, $GABA_B$-mediated responses have been shown to be both enhanced and unaltered by repeated antidepressant treatment. Gray and Green

Table 1. Effect of chronic (28 days) antidepressant drug treatment on [^{3}H]DHA binding in rat cerebral cortex

Drug treatment	Dose (mg/kg/day)	K_d (nM)	B_{max} (fmol/mg protein)
Vehicle		1.04 ± 0.10	92.6 ± 3.7
PLZ	10	0.94 ± 0.15	69.1 ± 4.2*
TCP	1	0.84 ± 0.07	73.6 ± 3.8*
IMI	30	0.94 ± 0.16	57.6 ± 3.1*
DMI	10	1.46 ± 0.29	65.3 ± 2.6*

Values are means ± SEM (N = 6–8 saturation curves). Abbreviations: PLZ, phenelzine; TCP, (±)-tranylcypromine; IMI, imipramine; and DMI, desmethylimipramine.

* $P < 0.05$ compared to vehicle control animals.

Table 2. Effect of chronic (28 days) antidepressant drug treatment on [^{3}H]GABA binding in rat frontal cortex

Drug treatment	Dose (mg/kg/day)	K_d (nM)	B_{max} (fmol/mg protein)
Vehicle		50.1 ± 5.8	708.6 ± 73
PLZ	10	46.4 ± 10.6	837.7 ± 153
TCP	1	67.2 ± 5.5	1183.2 ± 146
IMI	30	38.8 ± 5.9	851.3 ± 107
DMI	10	52.5 ± 7.1	909.3 ± 131

Values are means ± SEM (N = 6 saturation curves).

[22] found that following chronic amitriptyline, mianserin, zimelidine or DMI, baclofen-induced inhibition of 5-hydroxytryptamine release in mouse frontal cortex was enhanced significantly. In addition, chronic IMI may enhance the baclofen-mediated increase in noradrenaline-stimulated cyclic AMP production in mouse cortex [10]. In contrast, McManus and Greenshaw [21] did not observe any increases in $GABA_B$ receptor function with an *in vivo* functional test. Repeated (21 days) administration of PLZ, TCP, IMI and DMI failed to alter the motor-suppressant effect of the GABA agonists progabide [23] and (±)-baclofen [24]. Szekely *et al.* [11], using an *ex vivo* functional test, also did not observe a change in $GABA_B$ receptor function. Chronic IMI and DMI failed to enhance the baclofen-mediated inhibition of forskolin-stimulated adenylate cyclase activity in frontal cortex membranes.

Radioligand binding studies of $GABA_B$ receptor changes have produced similarly inconsistent results. In parallel with the functional test reports, following chronic administration of antidepressant drugs, binding of [^{3}H]GABA to $GABA_B$ recognition sites has been reported to be both increased [9–11] and unaltered [12, 13] in cortical tissue. The study by Szekely *et al.* [11] included an additional measure of $GABA_B$ binding, using [^{3}H]baclofen as the radioligand. In contrast to the [^{3}H]GABA results, when [^{3}H]baclofen was used, no increase in the number of $GABA_B$ recognition sites was observed following chronic IMI or DMI administration. In the present study, we failed to observe any consistent changes in [^{3}H]GABA binding. Repeated (28 days) administration of PLZ, TCP, IMI and DMI did not alter significantly the binding of [^{3}H]GABA to $GABA_B$ recognition sites. Within the TCP-treated group, some animals showed clearly increased B_{max} values relative to the vehicle control group, although the between-groups difference was not statistically significant. This tendency was not evident with the other drug treatments.

The reasons for the great discrepancy in effects of antidepressants on $GABA_B$ binding and function are at present unknown. For the binding studies, methodological considerations are not likely to be of importance as virtually identical binding and tissue preparation protocols have been followed in the various studies. A recent report by Scherer *et al.* [25] detailing pharmacologically distinct subsets of $GABA_B$ receptors may provide an answer to the variable functional results, if these subtypes are indeed functionally distinct. Whether the existence of multiple $GABA_B$ receptors can also account for the mixed binding changes is unclear. The development of more selective $GABA_B$ agonists and antagonists will be of great value in assessing this question.

An important component of the present binding study was the inclusion of two clinically effective MAOIs, TCP and PLZ, that had not been analyzed previously for their effects on $GABA_B$ binding. The failure of PLZ to induce an increase in the number of $GABA_B$ receptors is consistent with its ability to alter GABA levels. Chronic administration of PLZ [26, 27] results in a significant increase in brain

GABA levels. Such an effect would not likely be associated with a concomitant increase in the number of receptors for that ligand. In fact, one would predict an associated decrease in receptor number.

In conclusion, chronic administration of four clinically effective antidepressants, representing both MAOI and TCA drug classes, reduced the number of β-adrenoceptors but did not alter significantly the number of $GABA_B$ receptors. This finding, together with other conflicting binding and functional studies, indicates that the influence of antidepressants on the total population of $GABA_B$ receptors is less consistent than previously thought. It may be necessary to examine the effects of antidepressants on subtypes of $GABA_B$ receptors to clarify the effects of these drugs on GABA function.

Acknowledgements—This research was supported by the Alberta Heritage Foundation for Medical Research (AHFMR) and the Provincial Mental Health Advisory Council (PMHAC). D. J. McManus is the recipient of an AHFMR studentship; Dr. A. J. Greenshaw is a Heritage Medical Scholar. The authors wish to thank Dr. G. B. Baker for his valuable discussion and Ms. S. Omura for her efficient typing of this manuscript. The assistance of Dr. D. D. Mousseau and Mr. P. R. Paetsch with DHA binding is gratefully acknowledged.

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