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Effects of chronic oral administration of the antidepressants, desmethylimipramine and zimelidine on rat cortical $GABA_B$ binding sites: a comparison with 5-HT_2 binding site changes

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1 The effects of chronic oral administration of desmethylimipramine (DMI) or zimelidine (1.25 and 5 mg kg^{-1} twice daily for 21 days) were studied on rat whole cortical γ-aminobutyric $acid_B$ ($GABA_B$) binding sites. No changes in receptor affinity or number were found with either drug.

2 A subsequent study of $GABA_B$ binding sites using higher doses of these drugs (5 and 10 mg kg^{-1}) and rat frontal cortex was also without effect, when investigated 24 h after termination of drug administration or 72 h after DMI administration (5 mg kg^{-1}).

3 The number of frontal cortical 5-hydroxytryptamine$_2$ (5-HT_2) binding sites was significantly and dose-dependently decreased after both drugs, whereas the number of hippocampal 5-HT_2 binding sites was not significantly altered after either drug.

4 As the number of frontal cortical $GABA_B$ binding sites was unaltered whereas the number of 5-HT_2 binding sites was significantly decreased under identical study conditions, it may be concluded that the effects of antidepressant administration upon $GABA_B$ binding sites is a less consistent observation than their effects on 5-HT_2 binding sites.

Introduction

Adaptive changes in cortical monoamine receptor numbers after chronic antidepressant administration to rodents have been widely reported (reviews by Enna *et al.*, 1981; Sugrue, 1983). Decreases in the number of β-adrenoceptors and 5-hydroxytryptamine$_2$ (5-HT_2) receptors have been consistently found following chronic oral administration of a variety of antidepressant drugs (Goodwin *et al.*, 1984; Ask *et al.*, 1986).

With the increasing clinical evidence that CSF and plasma γ-aminobutyric acid (GABA) concentrations are reduced in depressed patients compared to controls (Gold *et al.*, 1980; Gerner & Hare, 1981; Petty & Schlesser, 1981), attention has recently been directed towards the GABA receptor and possible changes in the $GABA_A$ and $GABA_B$ subtypes following repeated antidepressant administration.

$GABA_A$ receptor numbers were decreased in the mouse cortex after chronic administration of imipramine and nomifensine (Suzdak & Gianutsos, 1985). An earlier study by Pilc & Lloyd (1984) in the rat did not obtain similar findings after repeated administration of five other antidepressants. However, these authors did observe a marked increase in the number of $GABA_B$ receptors in the rat frontal cortex with all antidepressants studied. This finding was later confirmed using a wider range of antidepressants, although the studies were predominantly performed at a single ligand concentration and the animals were killed 72 h after the termination of drug administration (Lloyd *et al.*, 1985). A subsequent demonstration of increased numbers of $GABA_B$ receptors in mouse frontal cortex following repeated imipramine administration has substantiated this finding (Suzdak & Gianutsos, 1986).

We now present the results of our investigations of the effects of chronic oral administration of desmethylimipramine (DMI) and zimelidine on rat whole cortical $GABA_B$ receptors using full saturation analysis. (These results were previously communicated to the British Pharmacological Society, Cross &

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Horton, 1986). In subsequent studies we have investigated the effects of higher doses of these two drugs on frontal cortical $GABA_B$ receptors and concomitantly studied the drug effects on frontal cortical and hippocampal 5-HT_2 receptors to provide a comparison with more widely reported findings. We have also compared $GABA_B$ receptor binding at two time intervals after termination of repeated DMI administration.

Methods

Whole cortical $GABA_B$ studies

DMI or zimelidine (1.25 and 5 mg kg^{-1}) were administered by gastric tube to male Wistar rats twice daily (between 08 h 00 min–10 h 00 min and 16 h 00 min–19 h 00 min) for 21 days in a dose volume of 5 ml kg^{-1}. Control animals received an equivalent volume of distilled water vehicle. Animals were weighed daily and the drug dosage, calculated as free base, adjusted accordingly. Further groups of rats received a single dose of drug at each dose level. Animals were killed 24 h after the final dose of drug.

Frontal cortical $GABA_B$ studies

Male Wistar rats were dosed twice daily with DMI and zimelidine as above except that the doses were increased to 5 and 10 mg kg^{-1}. A single dose of each drug was administered at the higher dose to further groups of rats. Animals were killed 24 h after the final dose of drug. A further group of rats was killed 24 or 72 h after single or repeated administration of DMI (5 mg kg^{-1}).

Membrane preparations

Animals were killed by cervical dislocation and the brain areas immediately dissected. For 5-HT_2 binding, frontal cortex and hippocampus were stored at − 20°C; for $GABA_B$ binding, whole or frontal cortex was placed in 0.32 M sucrose for membrane preparation according to the method of Bowery *et al.* (1983). The membranes were stored in 20 vol (v/w) 50 mM Tris HCl, pH 7.4 at − 20°C prior to assay. On the day of assay, membranes were thawed at room temperature and centrifuged at 50,000 *g* for 20 min. The pellet was resuspended in 50 vol (v/w) 50 mM Tris HCl, pH 7.4 containing 2 mM $CaCl_2$, incubated at room temperature for 15 min and centrifuged at 50,000 *g* for 10 min. This washing procedure was repeated two further times.

Tissue for 5-HT_2 binding was thawed in 0.25 M sucrose and membranes prepared as described by Leysen *et al.* (1982).

Binding assays

Binding assays were performed immediately after membrane preparation. For 5-HT_2 binding, membrane (equivalent to 10 and 20 mg wet weight for frontal cortex and hippocampus, respectively) was incubated for 10 min at 37°C in 50 mM Tris HCl, pH 7.7 containing [^{3}H]-ketanserin (77–92 Ci $mmol^{-1}$, New England Nuclear) at 8 concentrations (0.1–5 nM) in a volume of 2 ml. Non-specific binding was defined using 10^{-6} M methysergide (Sandoz Ltd). Membrane bound radioactivity was recovered by filtration under vacuum through Whatman GF/B filters (previously dipped in 0.1% (v/v) Triton X-100 to reduce filter binding). Filters were washed with 16 ml ice-cold buffer and radioactivity determined by scintillation counting at an efficiency of 40–44%.

For $GABA_B$ binding, membrane (equivalent to 35 mg original wet weight) was incubated for 15 min at room temperature in 50 mM Tris HCl pH 7.4, containing 2 mM $CaCl_2$, 40 μM isoguvacine (Cambridge Research Biochemicals), 1 nM [^{3}H]-GABA (57 Ci $mmol^{-1}$, Amersham International) alone and in the presence of unlabelled GABA (7 concentrations, 5–150 nM) in a volume of 1 ml. Non-specific binding was defined using 10^{-4} M (±)-baclofen. The reaction was terminated by rapid centrifugation at 16,000 *g* and the supernatants removed by aspiration. The pellets were dissolved in 0.3 ml Soluene-350 tissue solubilizer (Packard, UK) overnight before the addition of 10 ml acidified scintillation fluid and counting at an efficiency of 39–43%.

Aliquots of membrane were stored at − 20°C for subsequent protein determination (Lowry *et al.*, 1951), using bovine serum albumin as standard. The maximal number of binding sites (B_{max}) and equilibrium dissociation constant (K_D) were determined by non-linear regression analysis.

Statistics

Differences between group means were compared by use of Student's *t* test (2-tail, unpaired).

Results

Body weights and adverse drug effects

The rate of increase in body weight of rats receiving DMI or zimelidine was less than that of the controls at all dose levels studied. This effect was dose-related for both drugs and reached statistical significance at 5 mg kg^{-1}. The body weight data for the animals in the highest dose groups of both drugs is illustrated in Figure 1.

Some animals receiving 10 mg kg^{-1} p.o. DMI twice

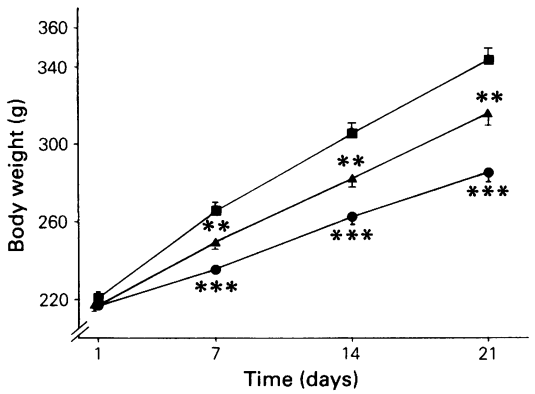

Figure 1 Mean body weights for control animals (■) and animals receiving desmethylimipramine (●) or zimelidine (▲) 10 mg kg^{-1} twice daily p.o. for 21 days ($n = 16$). Vertical lines indicate s.e.mean. $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle-treated controls.

daily developed swollen limbs during the last week of the study. In most cases (one animal was killed on day 16) the swelling did not seem to cause undue distress to the animals and some improved before the end of the dosing period with continued drug administration.

Whole cortical $GABA_B$ binding studies

Neither the number nor affinity of $GABA_B$ binding sites was altered in the rat whole cortex after a single dose of DMI or zimelidine at dose levels of 1.25 and 5 mg kg^{-1} p.o. or after repeated twice daily administration of these drugs for 21 days (Table 1).

Frontal cortical $GABA_B$ binding sites

In agreement with the findings in whole cortex, neither the number nor the affinity of frontal cortical $GABA_B$ binding sites was significantly altered following either a single dose (10 mg kg^{-1}) of drug (DMI, $K_D = 14 \pm 4$ nM, $B_{max} = 1.08 \pm 0.26$ pmol mg^{-1} protein; zimelidine, $K_D = 13 \pm 4$ nM, $B_{max} = 1.11 \pm 0.18$ pmol mg^{-1} protein, $n = 4$ for both groups) or after repeated twice daily administration at either dose level (Figure 2). Likewise $GABA_B$ binding sites did not differ in rats killed 72 h after repeated DMI administration (5 mg kg^{-1}) compared with controls or with those killed 24 h after repeated DMI (Table 2).

Frontal cortical 5-HT_2 binding sites

Following repeated oral administration of DMI at doses of 5 and 10 mg kg^{-1} twice daily there was a significant, dose-related decrease in the number of frontal cortical 5-HT_2 binding sites compared to controls (Figure 2). No changes in K_D or B_{max} values were found after a single dose of 10 mg kg^{-1} DMI ($K_D = 0.57 \pm 0.02$ nM, $B_{max} = 277 \pm 7$ fmol mg^{-1} protein, $n = 3$).

Following repeated zimelidine administration, the numbers of frontal cortical 5-HT_2 binding sites were also decreased compared to controls with a significant change occurring after 10 mg kg^{-1} (Figure 2). A single dose of zimelidine (10 mg kg^{-1}) decreased the number of 5-HT_2 binding sites with no change in K_D ($K_D = 0.54 \pm 0.05$ nM, $B_{max} = 239 \pm 10$ fmol mg^{-1} protein, $n = 3$, $P < 0.05$).

Hippocampal 5-HT_2 binding studies

Repeated administration of DMI or zimelidine at dose levels of 5 or 10 mg kg^{-1} twice daily did not significantly alter hippocampal 5-HT_2 binding sites, although there was a tendency towards a reduction in the number of sites in the DMI-treated animals. A single dose of DMI or zimelidine (10 mg kg^{-1}) had no effect on K_D or B_{max} values of 5-HT_2 binding sites in this brain region (Table 3).

Table 1 Effects of desmethylimipramine (DMI) and zimelidine on $GABA_B$ binding sites in the rat whole cortex

	DMI		*Zimelidine*	
Treatment groups	K_D	B_{max}	K_D	B_{max}
Controls	26 ± 3	1.29 ± 0.05	21 ± 2	1.30 ± 0.05
1.25 mg kg^{-1} single dose	30 ± 7	1.15 ± 0.05	34 ± 9	1.20 ± 0.13
1.25 mg kg^{-1} for 21 days	19 ± 1	1.25 ± 0.07	18 ± 1	1.25 ± 0.06
5 mg kg^{-1} single dose	30 ± 9	1.24 ± 0.07	22 ± 2	1.24 ± 0.11
5 mg kg^{-1} for 21 days	20 ± 1	1.27 ± 0.06	21 ± 2	1.25 ± 0.07

Values are means ± s.e.mean for control animals ($n = 8$) or animals given a single dose ($n = 4$) and those dosed twice daily for 21 days ($n = 8$) using tissue from individual animals. K_D = nM and B_{max} = pmol mg^{-1} protein.

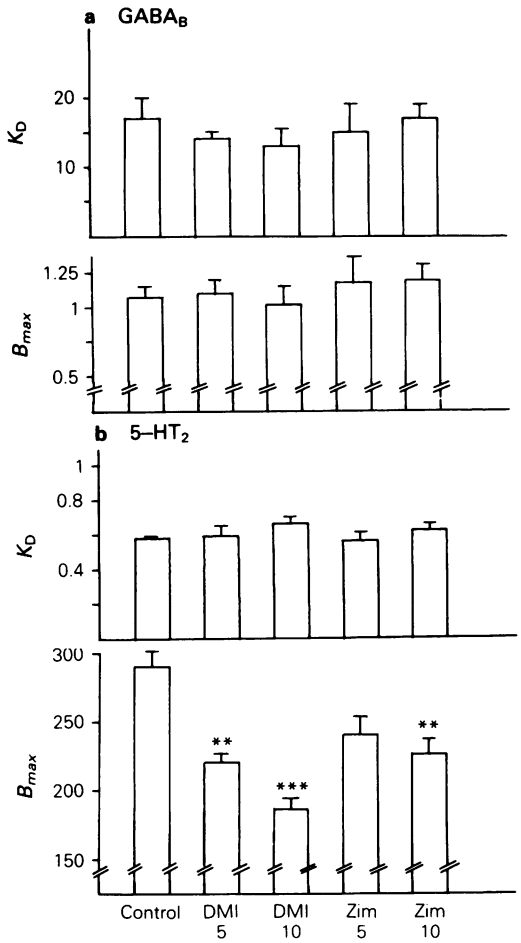

Figure 2 Frontal cortical $GABA_B$ (a) (K_D = nM, B_{max} = pmol mg^{-1} protein) and 5-hydroxytryptamine$_2$ (5-HT$_2$) (b) (K_D = nM, B_{max} = fmol mg^{-1} protein) binding sites in controls (n = 6–8) and animals given desmethylimipramine (DMI) or zimelidine (Zim) at 5 mg kg^{-1} (n = 3–4) and 10 mg kg^{-1} (n = 6–8) p.o. twice daily for 21 days using tissue from individual animals. Results are means and vertical lines indicate s.e.mean. **$P<0.01$, ***$P<0.001$ compared to controls.

Discussion

The observed decreases in rat body weight gain after chronic administration of DMI and zimelidine have been described in other studies (Hall *et al.*, 1984; Ask *et al.*, 1986). A single oral dose of tricyclic antidepressants including DMI in the rat has been found to inhibit markedly food intake (Blavet & DeFeudis, 1982). The selective 5-HT uptake inhibitor zimelidine may also evoke a decrease in growth rate by inhibiting food intake, as animal studies have illustrated that 5-HT may be involved in the suppression of feeding (Gottfries, 1981).

The number and affinity of $GABA_B$ binding sites in control animals compared well with published data (Pilc & Lloyd, 1984). However, our initial findings that neither DMI nor zimelidine altered the number of sites in the rat whole cortex, at doses where marked increases in frontal cortical receptor numbers had previously been observed (Lloyd *et al.*, 1985), prompted us to perform further investigations.

In the subsequent study the decreases in the number of frontal cortical 5-HT$_2$ binding sites following repeated oral administration of DMI and zimelidine were similar in magnitude to previous results (Fuxe *et al.*, 1982; Goodwin *et al.*, 1984). The lack of a significant reduction in the number of hippocampal 5-HT$_2$ binding sites suggests that the antidepressants used exerted some regional selectivity.

In agreement with our findings in whole cortex, $GABA_B$ binding sites in the frontal cortex were unaltered following chronic oral administration of DMI or zimelidine. We have also demonstrated that chronic intraperitoneal administration of these two drugs had no effect on $GABA_B$ binding sites in this brain area (Cross & Horton, 1987). These results do not replicate the observed increases in rat frontal cortical $GABA_B$ binding sites after a range of antidepressants (Lloyd *et al.*, 1985).

Methodological differences may account for the differing results. The route of drug administration may be important, as in the study by Lloyd *et al.* (1985), the majority of the drugs were administered subcutaneously using osmotic minipumps. This may

Table 2 Comparison of differing time intervals between termination of desmethylimipramine (DMI) administration and killing on $GABA_B$ binding sites in the rat frontal cortex

	24 h		*72 h*	
Treatment groups	K_D	B_{max}	K_D	B_{max}
Controls	28 ± 3	1.27 ± 0.10	27 ± 2	1.46 ± 0.06
5 mg kg^{-1} single dose	27 ± 2	1.24 ± 0.17	27 ± 6	1.35 ± 0.17
5 mg kg^{-1} for 21 days	23 ± 1	1.33 ± 0.09	23 ± 2	1.36 ± 0.06

Values are mean ± s.e.mean for control animals (n = 9–11) and animals given either a single dose (n = 4) and those dosed twice daily for 21 days (n = 8–11) using tissue from individual animals. K_D = nM and B_{max} = pmol mg^{-1} protein.

Table 3 Effects of desmethylimipramine (DMI) and zimelidine on [^{3}H]-ketanserin binding sites in the rat hippocampus

	DMI		*Zimelidine*	
Treatment groups	K_D	B_{max}	K_D	B_{max}
Controls	0.92 ± 0.12	42 ± 4	0.92 ± 0.12	42 ± 4
10 mg kg^{-1} single dose	1.11 ± 0.18	45 ± 7	0.96 ± 0.10	42 ± 3
5 mg kg^{-1} for 21 days	0.97 ± 0.15	37 ± 5	0.98 ± 0.13	39 ± 4
10 mg kg^{-1} for 21 days	1.21 ± 0.18	35 ± 4	0.93 ± 0.13	39 ± 4

Values are means ± s.e.mean for control animals (n = 7–8) or animals given a single dose (n = 4) and those dosed twice daily for 21 days (n = 7–8) using tissue pooled from two animals. K_D = nM and B_{max} = fmol mg^{-1} protein.

produce more stable concentrations of drugs within the brain than the twice daily oral administration we have used. However, this factor is unlikely to be of major importance, as in the same study an increase in $GABA_B$ binding was also observed after daily intraperitoneal administration of fluoxetine, progabide, fengabine and valproate.

Another methodological difference is that Lloyd *et al.* (1985) killed their animals 72 h after the last dose of drug and froze the tissue at − 80°C prior to membrane preparation. Since all the antidepressants we have tested (13 drugs including DMI, zimelidine, amitriptyline, imipramine, iprindole and mianserin) do not interact directly with $GABA_B$ receptor binding *in vitro* (at 10^{-4} M), it is not apparent why a period of 72 h after drug discontinuation was chosen. The present results, however, indicate that the time interval between the last dose of drug and death of the animals is not a contributing factor to the differing results obtained.

Recently, three functional studies of the effects of antidepressants on $GABA_B$-mediated responses have been published. Following chronic administration of amitriptyline, mianserin or zimelidine and also after repeated electroshocks, the ability of (±)-baclofen to inhibit 5-HT release in the mouse frontal cortex was significantly increased (Gray & Green, 1986). Chronic imipramine was also found to enhance the baclofen-mediated increase in noradrenaline-stimulated cyclic AMP accumulation in the mouse cortex (Suzdak & Gianutsos, 1986). These results are compatible with an increased functional activity of $GABA_B$ receptors after chronic antidepressant administration. However, Borsini *et al.* (1986) found that whereas a single dose of DMI significantly reduced the antinociceptive effect of (±)-baclofen in the rat, chronic administration had no effect on this $GABA_B$-mediated response, a result that would support our data after chronic drug administration.

In conclusion we have found no evidence to suggest that rat cortical $GABA_B$ binding sites are modified by chronic oral administration of DMI or zimelidine at doses where decreases in the number of frontal cortical 5-HT_2 receptors are apparent. This evidence together with conflicting functional studies suggests that the influence of antidepressants on the $GABA_B$ receptor may be less consistent than has been previously demonstrated.

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