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Repeated administration of desipramine and a $GABA_B$ receptor antagonist, CGP 36742, discretely up-regulates $GABA_B$ receptor binding sites in rat frontal cortex

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1 $GABA_B$ receptor binding site densities within laminar regions of the rat frontal cortex were examined autoradiographically following repeated administration (21 days) of the antidepressants desipramine, paroxetine and amitriptyline in addition to the $GABA_B$ receptor antagonists, CGP 35348 and CGP 36742. β_1-Adrenoceptor autoradiography was studied in parallel with that for $GABA_B$ receptor sites.

2 The effects of these compounds were examined concomitantly on the $GABA_B$ receptor-mediated inhibition of forskolin- and enhancement of noradrenaline-stimulated cyclic AMP production.

3 $GABA_B$ receptor binding was increased by both desipramine (20 mg kg^{-1}, p.o. and 10 mg kg^{-1}, i.p.) and CGP 36742 (100 mg kg^{-1}, i.p.) in the outer laminar region of the frontal cortex by around 50% above control levels. Conversely, no significant changes were mediated by paroxetine, amitriptyline, CGP 35348 or the $GABA_B$ receptor agonist, baclofen.

4 With the exception of paroxetine, all compounds down-regulated the total β-adrenoceptor population throughout frontal cortical laminae which was attributable to the β_1-adrenoceptor subtype. In contrast, the reduction in β-adrenoceptors mediated by CGP 35348 and CGP 36742 did not occur as a consequence of reduced β_1-adrenoceptor numbers.

5 Protracted treatment with CGP 35348, failed to influence forskolin-stimulated cyclic AMP production; however, a significant increase in the accumulation of cyclic AMP produced in response to forskolin was seen after treatment with CGP 36742.

6 Such discretely localized changes in $GABA_B$ receptor densities induced by desipramine and CGP 36742 may provide an explanation for the discrepancies reported in membrane binding studies and possibly implicate a role for $GABA_B$ receptor antagonists in antidepressant therapy.

Keywords: $GABA_B$ receptors; antidepressants; autoradiography; β-adrenoceptors; adenylyl cyclase; $GABA_B$ receptor antagonists

Introduction

Since the introduction of the tricyclic antidepressant, imipramine in 1957, much research has focussed upon attempting to find a common biochemical denominator from which a neurochemical inbalance would trigger depression. The clinical observation that reserpine can produce depression suggested that the disease may be a consequence of a monoamine deficit. In support of this, synaptic levels of the monoamines, noradrenaline (NA) and 5-hydroxytryptamine (5-HT) are increased by both tricyclic antidepressants and monoamine oxidase inhibitors by the prevention of either their uptake or breakdown, respectively. Adaptations to the populations of central cortical monoamine receptors following chronic antidepressant administration are now well-documented. Down-regulation of the numbers of both β-adrenoceptors (Banerjee *et al.*, 1977) and 5-HT_2 receptors (Peroutka & Snyder, 1980) has been consistently found with a variety of antidepressant classes and is believed to be a prerequisite for their therapeutic actions.

Focus turned to the GABAergic system following the observations that the levels of γ-aminobutyric acid (GABA) are reduced in the cerebrospinal fluid (Gold *et al.*, 1980) and the plasma (Berrettini *et al.*, 1982) of depressed patients. In addition, since the GABA agonists, progabide and fengabine have antidepressant actions both in animal models as well as clinically (Lloyd *et al.*, 1983; Musch & Garreau, 1986), attention was directed to GABA receptors and in particular to the possible alterations in the $GABA_B$ receptor subtype following chronic antidepressant administration. This stemmed from observations of increased $GABA_B$ receptor binding to rat cerebral cortical membranes following subcutaneous infusions of a variety of antidepressants (Pilc & Lloyd, 1984; Lloyd *et al.*, 1985). These effects were substantiated using intraperitoneal administration (Szekely *et al.*, 1987) and also in mouse cortical membranes (Suzdak & Gianutsos, 1986). Moreover, $GABA_B$ receptors are reduced in the frontal cortex following olfactory bulbectomy (Lloyd & Pichat, 1986). Furthermore, increased $GABA_B$ receptor functionality with respect to baclofen-induced nociception is reduced by chronic desipramine (Borsini *et al.*, 1986) whereas baclofen-induced hypothermia and $GABA_B$ receptor-mediated inhibition of 5-HT release (Gray & Green, 1987) are enhanced by repeatedly administered antidepressants or electroconvulsive therapy (ECT) (Gray *et al.*, 1987). Additionally, chronic imipramine treatment enhances the $GABA_B$ receptor-mediated potentiation of NA-stimulated adenylyl cyclase activity (Suzdak & Gianutsos, 1986).

Not all researchers, however, have been able to demonstrate $GABA_B$ receptor up-regulation after such dosing regimes. Cross & Horton (1988) failed to observe any significant changes in $GABA_B$ binding sites following repeated administration of desipramine or zimeldine, despite the fact that 5-HT_2 binding site densities were significantly reduced in the same membrane preparations. Moreover, $GABA_B$ receptors are not altered in drug-free suicide victims (Cross *et al.*, 1988). Interestingly, although the binding of [^{3}H]-GABA to $GABA_B$ sites in rat frontal cortical membranes was increased after prolonged treatment with desipramine and imipramine, [^{3}H]-baclofen binding was unaffected by these drugs (Szekely *et al.*, 1987). Furthermore, although

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[^{3}H]-dihydroalprenolol binding sites were down-regulated by infusions of imipramine, desipramine, tranylcypromine and phenelzine, $GABA_B$ receptor densities were not influenced by these antidepressants (McManus & Greenshaw, 1991).

It was hypothesized that if indeed $GABA_B$ receptor densities are increased following repeated antidepressant administration, it could be that certain undetected changes may arise from a discrete localization within certain cerebral cortical laminae which would be considerably diluted in a membrane preparation. The aim of the present study, therefore, was firstly to employ receptor autoradiography in an attempt to resolve these discrepancies. Since this technique offers the advantage of retaining the morphology of the brain intact, such an approach should enable the detection of possible changes within individual laminal regions of the frontal cortex. Autoradiographical examination of $GABA_B$ receptor sites within laminar regions of the frontal cortex was investigated in parallel with β_1-adrenoceptors after treatment with a variety of antidepressants, an established $GABA_B$ receptor antagonist, CGP 35348 (Olpe *et al.*, 1990) and also a novel $GABA_B$ receptor antagonist, CGP 36742 (Bittiger *et al.*, 1992).

Secondly, since activation of $GABA_B$ receptors inhibits forskolin-induced stimulation of adenylyl cyclase (Wojcik & Neff, 1984; Karbon & Enna, 1985; Hill, 1985) and potentiates the accumulation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) produced in response to noradrenaline (Karbon & Enna, 1985; Hill, 1985), such receptor up-regulation might be detected through these $GABA_B$ receptor-linked transduction mechanisms. An increase in the number of $GABA_B$ binding sites might be expected to confer a greater inhibition of forskolin- and enhancement of noradrenaline-stimulated cyclic AMP production by (−)-baclofen. The effects of the compounds were therefore examined concomitantly on these $GABA_B$ receptor-linked second messenger responses.

Methods

Drug-treatment protocols

In one study, male Wistar rats (160–200 g; Interfauna) were anaesthetized with halothane, and subcutaneously implanted with Alzet 2002 minipumps (Alza Corporation, USA). The minipumps were filled with 200 µl of a concentration of imipramine sufficient to deliver a proposed daily infused dose of 10 mg kg^{-1} for 14 days. Following the removal of the minipumps under halothane anaesthesia, animals were separated into two groups to compare drug-free periods of 24 and 48 h. A second group of rats was injected intraperitoneally for 14 days with imipramine (5 mg kg^{-1}) in order to compare this route of administration with that of subcutaneous infusion. These animals were prepared for perfusion-fixation after a further 24 h and were subjected to halothane anaesthesia to control for its possible influence on the action of imipramine. For this study, the binding kinetic parameters B_{max} and K_D were assessed by non-linear regression analysis as described by DeBlasi *et al.* (1989), using a range of GABA concentrations at a fixed concentration of [^{3}H]-GABA (50 nM).

In a second study, male CFY rats (140–180 g; Interfauna) caged in groups of 5 animals were dosed orally for 21 days with amitriptyline (30 mg kg^{-1}), desipramine (20 mg kg^{-1}), paroxetine (10 mg kg^{-1}) or (±)-baclofen (10 mg kg^{-1}). Additionally, desipramine (10 mg kg^{-1}) and the $GABA_B$ receptor antagonists, CGP 35348 and CGP 36742 (both at a concentration of 100 mg kg^{-1}) were injected intraperitoneally. In this study, the effects of antidepressant treatment on $GABA_B$ receptor binding parameters were determined using saturation analysis by varying the concentration of [^{3}H]-GABA (37.5–300 nM; 4 concentrations). Binding to β-adrenoceptors, using (−)-[^{125}I]-iodopindolol (18.75–300 pM; 5 concentrations) and resolved β_1-adrenoceptors (in the presence of the β_2-adrenoceptor antagonist, ICI 118,551) was performed on adjacent sections. In addition, the effects of these compounds were examined on the $GABA_B$ receptor-mediated modulation of forskolin and noradrenaline-stimulated adenylyl cyclase activity by baclofen. Drug concentrations are expressed in terms of their salt:base ratios.

Autoradiographical procedures

Tissue preparation Male Wistar rats (180–250 g) were anaesthetized with sodium pentobarbitone (Nembutal, 40 mg kg^{-1}, i.p.) and perfused-fixed with 250 ml of 0.1% paraformaldehyde in 0.01 M phosphate-buffered saline (0.9%) (pH 7.4) via intra-cardiac administration through the left ventricle. Brains were mounted onto a cork slice with 'Tissue Tek' (Miles Scientific), frozen in iso-pentane cooled to approximately − 40°C in liquid nitrogen and stored at − 80°C until required. Parasagitally-orientated sections (10 µm) were cut at − 20°C with a cryostat and thaw-mounted onto glass microscope slides to be stored at − 20°C until use.

Localization of $GABA_B$ receptor binding sites Frozen brain sections were thawed for 45 min and pre-incubated at room temperature for 60 min in 200 ml Tris-HCl buffer (50 mM; pH 7.4) containing 2.5 mM $CaCl_2$. After thorough drying under ambient conditions, sections were incubated individually for 20 min with 150 µl buffer containing [^{3}H]-GABA. Selectivity for $GABA_B$ sites was achieved in the presence of 40 µM isoguvacine (to prevent binding to $GABA_A$ sites). Non-specific binding was defined by (−)-baclofen (100 µM). Following incubation, excess radiolabel was aspirated from the section which then received 2 × 3 s washes in room temperature buffer. Sections were allowed to dry under a stream of cold air prior to their apposition to tritium-sensitive 'Hyperfilm' (Amersham) for between 3 and 4 weeks to generate autoradiograms.

Localization of β-adrenoceptor binding sites Frozen brain sections were thawed for 45 min and then incubated for 60 min at room temperature in a Tris-HCl/saline buffer (20 mM Trizma base; 135 mM NaCl; pH 7.4) containing (−)-[^{125}I]-iodopindolol. β-Adrenoceptor binding was resolved into the β_1-adrenoceptor subtype in the presence of the β_2-adrenoceptor antagonist, ICI 118,551 (50 nM). Non-specific binding was defined by use of (−)-isoprenaline (200 µM). Following incubation, sections were washed for 2 × 5 min periods in buffer at 4°C, rinsed for 3 s in ice-cold distilled water to remove buffer salts and then quickly dried in a stream of cold air. Sections were apposed to 'Hyperfilm' for between 24 and 48 h to generate autoradiograms with calibrated brain paste standards made according to the following method of Clarke & Hall (1986).

Autoradiographical analysis After the required duration of exposure, 'Hyperfilm' was developed and fixed in D-19 developer and 'Unifix' (Kodak), respectively. Autoradiographical density measurements were restricted to the frontal cortex of rat brain which was taken to represent approximately the anterior one-third region of the cerebral cortex (as described by plate 82; Paxinos & Watson, 1986). Optical densities of radioligand binding were measured with a 'Quantimet 970' image analysis system (Cambridge Instruments) againt calibrated ^{3}H micro-scales (Amersham). Scatchard plots were constructed from optical density values, converted to fmol mg^{-1} tissue using the simple relationship:

$$1\text{nCi} = 1/\text{specific activity (Ci mmol}^{-1}) \times 1000 \text{ fmol}$$

Adenylyl cyclase studies

This protocol was based essentially on the methods of Hill (1985) and Watling & Bristow (1986). Freshly removed

brains from stunned and decapitated male Wistar rats (180–250 g) were dissected on ice and cross-chopped slices of cerebral cortex (250 μm × 250 μm) prepared with a McIlwain tissue chopper. Slices were dispersed in 10 ml Krebs-Ringer bicarbonate (KRB) buffer of the following composition (mM); NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 and ascorbic acid 0.005%. They were then transferred to a larger volume of KRB (100 ml/brain), continually gassed with O_2:CO_2 (95%:5%) and washed for 90 min (with one buffer change after 30 min). Following this, excess KRB buffer was aspirated and 50 μl aliquots of the resulting tissue suspension added to fresh KRB. Following pre-incubation for 10 min at 37°C, adenylyl cyclase activity was initiated by the addition of either forskolin (10 μM) or noradrenaline (100 μM) in 50 μl aliquots to give a final assay volume of 500μl. After 10 min the reaction was terminated in a boiling water-bath (3 min). Aliquots (50 μl) of the supernatant were then assayed for cyclic AMP content by a radioimmunoassay based on the method of Brown *et al.* (1972). Estimates of the protein concentrations of brain tissue were made by the method of Bradford (1976). Statistical evaluation of the effects of chronic drug treatment on both receptor binding parameters and adenylyl cyclase activity were made by oneway analysis of variance followed by Dunnett's multiple comparison test.

Radioligands and drugs

The following were used: [2,8-^{3}H]-adenosine 3',5'–cyclic phosphate ammonium salt (specific activity = 34.2 Ci mmol^{-1}); (−)-[^{125}I]-iodopindolol (specific activity = 2200 Ci-mmol^{-1}) (New England Nuclear); 4-amino-*n*-[2,3-^{3}H]-butyric acid (GABA) (specific activity = 92.5 Ci mmol^{-1}) (Amersham); isoguvacine (Cambridge Research Biochemicals); baclofen isomers, CGP 35348 (*p*-(3-aminopropyl)-diethoxymethyl-phosphinic acid) and CGP 36742 (*p*-(3-aminopropyl)-*p*-n-butylphosphinic acid) were kindly supplied by Dr H. Bittiger, Ciba-Geigy Ltd. Basel, Switzerland; ICI 118,551 (erythro-DL-1-(7-methylindan-4-y(oxy)-3-isopropyl-aminobutan-2-ol) was kindly supplied by ICI Pharmaceuticals, Macclesfield.

Drugs were dissolved in saline or distilled water.

Results

Chronic antidepressant administration on $GABA_B$ receptor binding

Administration for 14 days The effects of imipramine administered for 14 days either intraperitoneally (5 mg kg^{-1})

Table 1 The effect of imipramine, administered intraperitoneally and via subcutaneously implanted minipumps for 14 days, on [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) binding to $GABA_B$ sites in rat frontal cortex

Treatment	n	*Lamina I*	*Laminae II–III*	*Lamina V*	*Lamina VI*
			B_{max} (fmol mg^{-1} tissue)		
Control	5	211.9 ± 23.0	264.2 ± 26.8	197.4 ± 19.2	139.9 ± 18.6
Imipramine (1)	5	270.8 ± 29.8	305.4 ± 22.4	217.3 ± 23.5	134.4 ± 17.3
Imipramine (2)	5	231.4 ± 48.7	292.2 ± 55.0	215.9 ± 49.0	NC
Imipramine (3)	4	286.9 ± 42.0	319.2 ± 69.3	209.8 ± 69.3	151.3 ± 12.2
			K_D (nM)		
Control	5	77.5 ± 10.3	86.5 ± 15.9	90.6 ± 20.2	117.3 ± 24.1
Imipramine (1)	5	90.0 ± 19.1	95.2 ± 14.0	91.8 ± 15.6	110.2 ± 20.6
Imipramine (2)	5	81.8 ± 26.5	82.0 ± 36.6	118.0 ± 42.2	NC
Imipramine (3)	4	86.5 ± 15.9	95.8 ± 25.2	90.7 ± 46.6	114.8 ± 14.2

(1) 10 mg kg^{-1}, s.c., 24 h drug-free period
(2) 10 mg kg^{-1}, s.c., 48 h drug-free period
(3) 5 mg kg^{-1}, i.p., 24 h drug-free period
NC: not calculated

Table 2 The effects of repeatedly administered (21 days) antidepressants on the binding of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) to $GABA_B$ sites in rat frontal cortex

Treatment	*Dose* (mg kg^{-1})	*Lamina I*	*Laminae II–III*	*Lamina V*	*Lamina VI*
			B_{max} (fmol mg^{-1} tissue)		
Control		236.6 ± 25.0	254.6 ± 42.3	228.8 ± 38.5	209.4 ± 22.2
Desipramine	20 p.o.	316.0 ± 26.0*	299.2 ± 26.2	252.0 ± 23.9	237.7 ± 27.3
Amitriptyline	30 p.o.	290.5 ± 40.9	283.5 ± 34.4	239.8 ± 22.2	232.6 ± 27.8
Paroxetine	10 p.o.	268.5 ± 38.7	299.1 ± 44.3	NC	177.5 ± 29.2
Baclofen	10 p.o.	297.4 ± 24.9	266.4 ± 11.8	194.1 ± 10.1	250.4 ± 18.7
Desipramine	10 i.p.	352.0 ± 50.7*	275.1 ± 11.9	224.2 ± 4.8	190.8 ± 23.4
CGP 35348	100 i.p	241.4 ± 7.4	255.2 ± 16.9	190.4 ± 6.9	161.1 ± 7.3
CGP 36742	100 i.p.	367.1 ± 36.1*	307.4 ± 17.3	259.4 ± 11.6	221.1 ± 9.8
			K_D (nM)		
Control		70.1 ± 16.1	59.2 ± 19.3	74.6 ± 22.3	85.8 ± 17.4
Desipramine	20 p.o.	106.5 ± 26.8	72.2 ± 14.9	76.7 ± 13.5	85.6 ± 17.5
Amitriptyline	30 p.o.	76.9 ± 13.6	55.6 ± 10.2	68.9 ± 7.6	103.8 ± 18.1
Paroxetine	10 p.o.	84.1 ± 24.0	76.4 ± 21.2	NC	70.3 ± 31.2
Baclofen	10 p.o.	71.6 ± 1.5	48.5 ± 4.0	40.5 ± 6.6	111.3 ± 20.7
Desipramine	10 i.p.	81.0 ± 12.3	44.9 ± 11.2	55.9 ± 7.2	61.0 ± 24.4
CGP 35348	100 i.p.	59.5 ± 6.5	56.0 ± 8.2	41.7 ± 3.3	46.8 ± 6.4*
CGP 36742	100 i.p.	132.5 ± 20.3*	82.9 ± 10.1	90.8 ± 10.1	103.5 ± 10.9

*$P < 0.05$ Dunnett's multiple comparison test (n = between 3 and 5 rats/group)
NC: not calculated

or by continuous infusion (10 mg kg^{-1}) from subcutaneously implanted osmotic minipumps on [^{3}H]-GABA binding to GABA$_B$ receptors in rat frontal cortex are summarised in Table 1. Following a 24 h (but not 48 h) drug-free period, subcutaneous infusion of imipramine produced an apparent increase in GABA$_B$ binding sites in lamina I amounting to 28% above control; however, statistical significance was not achieved ($P = 0.074$). Nevertheless, this trend of increased GABA$_B$ binding in lamina I was also evidence following intraperitoneal injections of imipramine (5 mg kg^{-1}). Such changes were also reflected, but to a lesser extent, in laminae II and III. Since the osmotic pumps offered no apparent advantages over intraperitoneal injections, the latter route was used in the subsequent study for comparison with oral administration.

Administration for 21 days Since this second study involved drug administration by both oral and intraperitoneal routes, control values for [^{3}H]-GABA binding to GABA$_B$ sites were derived from the mean of six animals, three dosed orally and three dosed intraperitoneally, there being no significant difference in the results from these two groups.

Chronic treatment with desipramine for 21 days significantly increased the B_{max} of GABA$_B$ binding sites in lamina I by 34% and 49% (p.o. and i.p., respectively). By contrast, although amitriptyline and paroxetine (as well as the GABA$_B$ receptor agonist, (±)-baclofen), also appeared to increase the B_{max} of GABA$_B$ binding, the values did not differ significantly from control (Table 2).

The GABA$_B$ receptor antagonist, CGP 35348 (100 mg kg^{-1}, i.p.), failed to increase GABA$_B$ receptor numbers, however, CGP 36742, at the same dose produced a significant enhancement (+55%) of GABA$_B$ binding in lamina I, comparable to that mediated by desipramine. With the exception of CGP 36742, none of these treatments significantly altered the affinity of [^{3}H]-GABA for GABA$_B$ sites in any of the frontal cortical laminae studied. A significant reduction in receptor affinity was produced by CGP 36742, denoted by an increased K_D of 89% above control. Thus, it would appear that the ability of this compound to up-regulate markedly GABA$_B$ receptors possibly occurs at the expense of a reduction in the affinity of the endogenous ligand for its receptor. The effects of these chronic treatments are shown autoradiographically (Figure 1) and by Scatchard analysis (Figure 2).

Drug-induced modulation of β-adrenoceptor binding

Figure 3 shows the effects of the repeated administration of CGP 35348, CGP 36742 and the antidepressants, on binding to β-adrenoceptors in the frontal cortex, assessed autoradiographically using (−)-[^{125}I]-iodopindolol. Quantitative measurements of the radioligand binding were examined over the concentration range (18.75–300 pM) to produce Scatchard plots of total and β_1-adrenoceptor binding (Figure 4) from which B_{max} and $K_D$values were derived (Table 3).

Whilst no apparent reduction in binding was produced by paroxetine in the frontal cortex, chronic treatment with amitriptyline and desipramine produced a clear down-regulation of both total and β_1-adrenoceptor binding in all laminae of this region. Similarly, oral administration of the GABA$_B$ receptor agonist, baclofen, produced a β-adrenoceptor down-regulation of a similar magnitude to that mediated by amitriptyline. Resolution into the β_1-adrenoceptor subtype, revealed that the observed drug-induced modifications were attributable to this subsite in all frontal cortical laminae. Moreover, with the exception of desipramine (i.p.) in laminae II and III, no significant changes in receptor affinity were observed.

Both the GABA$_B$ receptor antagonists, CGP 35348, and CGP 36742, down-regulated the total β-adrenoceptor population to a similar degree as observed with amitriptyline. However, the resolved β_1-adrenoceptor subtype was not significantly affected by either treatment which may implicate the preferential involvement of β_2-adrenoceptors in this response. Furthermore, both GABA$_B$ receptor antagonists and desipramine significantly increased the affinity of the total β-adrenoceptor population (with the exception of CGP 35348 in laminae II and III) for (−)-[^{125}I]-iodopindolol, as exemplified by reduced K_D values of between 14% and 40%. In contrast, the affinities of resolved β_1-adrenoceptors were unaffected by such treatments (with the exception of desipramine in laminae II and III). By ranking the individual B_{max} values for GABA$_B$ receptors in desipramine- and CGP 36742-treated rats with the corresponding B_{max} values for β-adrenoceptors, no positive correlation was apparent (Spearman's rank correlation coefficient, $r_s = 0.13$; $P >> 0.05$).

Table 3 The effects of repeatedly administered (21 days) antidepressants on (−)-[^{125}I]-iodopindolol binding to β-adrenoceptors in rat frontal cortex

Treatment	*Dose* (mg kg^{-1})	*Total* *Lamina I*	*Total* *Laminae II–III*	*Total* *Laminae V–VI*	β_1 *Lamina I*	β_1 *Laminae II–III*	β_1 *Laminae V–VI*
		B_{max} (fmol mg^{-1} tissue)					
Control		1.88 ± 0.06	1.92 ± 0.05	1.51 ± 0.02	1.46 ± 0.06	1.55 ± 0.05	1.15 ± 0.04
Desipramine	20 p.o.	1.29 ± 0.08*	1.44 ± 0.04*	1.09 ± 0.04*	0.73 ± 0.06*	0.92 ± 0.06*	0.62 ± 0.05*
Amitriptyline	30 p.o.	1.37 ± 0.06*	1.57 ± 0.07*	1.22 ± 0.07*	0.97 ± 0.10*	1.27 ± 0.11*	0.92 ± 0.07*
Paroxetine	10 p.o.	1.78 ± 0.01	1.80 ± 0.01	1.36 ± 0.04	1.33 ± 0.03	1.38 ± 0.05	0.94 ± 0.04
Baclofen	10 p.o.	1.41 ± 0.08*	1.51 ± 0.01*	1.16 ± 0.02*	1.02 ± 0.09	1.14 ± 0.08*	0.79 ± 0.09
Desipramine	10 i.p.	0.98 ± 0.09*	1.21 ± 0.05*	0.92 ± 0.03*	0.69 ± 0.03*	0.85 ± 0.03*	0.57 ± 0.04*
CGP 35348	100 i.p.	1.40 ± 0.04*	1.56 ± 0.05*	1.20 ± 0.04*	1.26 ± 0.12	1.37 ± 0.14	1.03 ± 0.09
CGP 36742	100 i.p.	1.46 ± 0.05*	1.59 ± 0.07*	1.18 ± 0.06*	1.30 ± 0.08	1.40 ± 0.08	0.98 ± 0.07
		K_D (pM)					
Control		52.5 ± 5.3	46.5 ± 4.0	41.4 ± 2.4	83.7 ± 9.6	73.4 ± 8.7	81.6 ± 6.8
Desipramine	20 p.o.	48.2 ± 5.9	46.9 ± 1.3	40.3 ± 1.3	68.4 ± 9.5	62.7 ± 3.9	70.2 ± 7.1
Amitriptyline	30 p.o.	49.7 ± 1.7	49.6 ± 2.8	47.0 ± 4.3	64.7 ± 3.1	79.3 ± 4.5	84.5 ± 7.6
Paroxetine	10 p.o.	58.5 ± 2.5	53.4 ± 2.8	42.1 ± 2.1	90.2 ± 8.1	76.9 ± 5.6	68.9 ± 4.8
Baclofen	10 p.o.	48.0 ± 10.7	39.1 ± 2.4	36.0 ± 2.9	86.2 ± 11.7	73.4 ± 8.1	64.0 ± 3.7
Desipramine	10 i.p.	31.2 ± 5.2*	31.0 ± 2.8*	30.0 ± 3.2*	58.9 ± 11.1	48.2 ± 5.0*	55.8 ± 14.5
CGP 35348	100 i.p.	37.8 ± 2.2*	38.8 ± 2.7	35.4 ± 1.2*	79.3 ± 12.8	67.4 ± 10.8	68.4 ± 6.1
CGP 36742	100 i.p.	34.6 ± 1.5*	32.6 ± 3.4*	27.7 ± 3.2**	86.7 ± 7.1	73.9 ± 12.0	64.4 ± 8.0

*$P < 0.05$ Dunnett's multiple comparison test (n = between 3 and 5 rats/group)

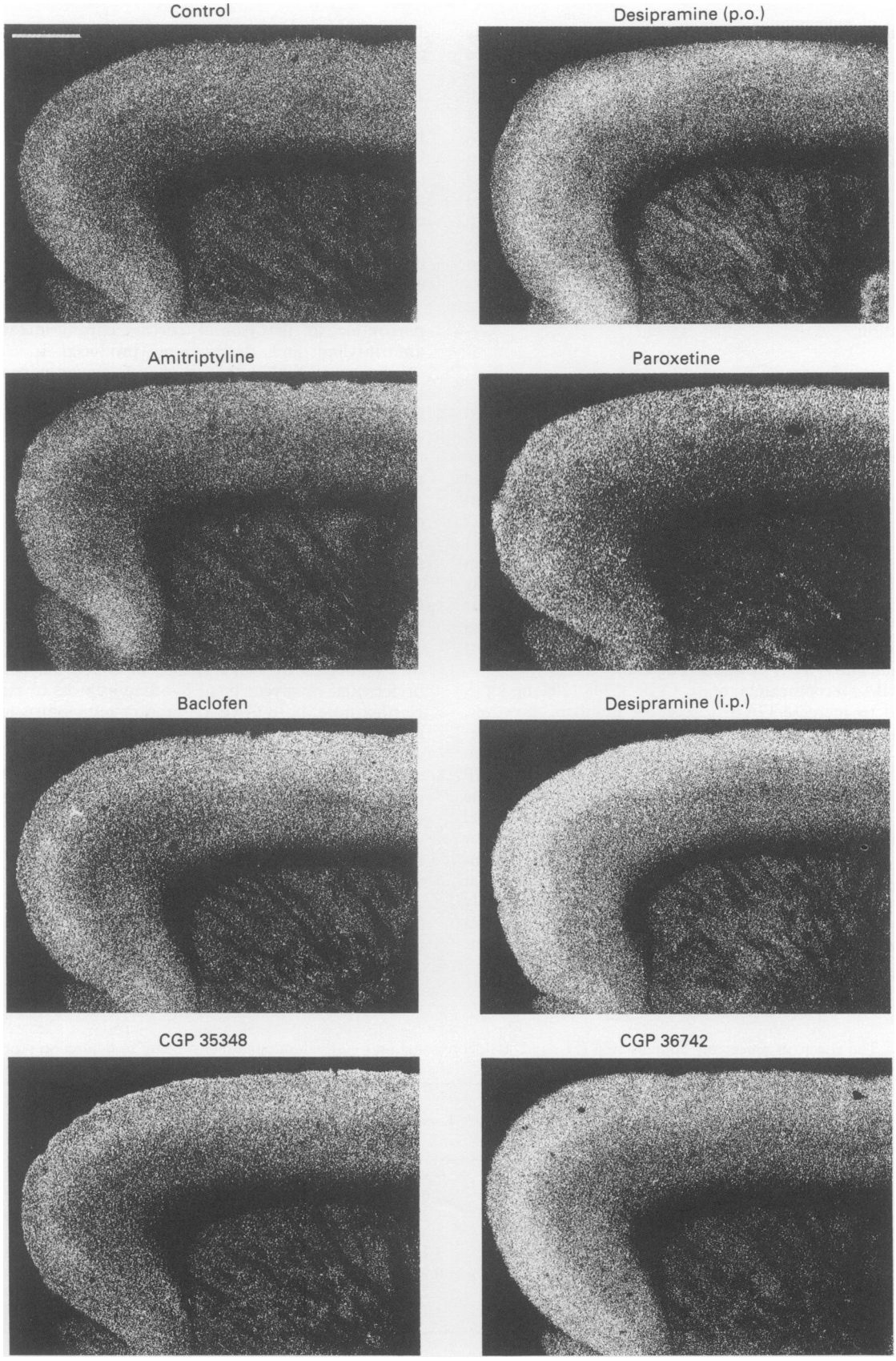


Figure 1 Autoradiograms showing the binding of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) (37.5 nM) to $GABA_B$ receptor sites in the frontal cortex of rats treated with antidepressants, CGP35348 and CGP 36742. Rats were treated orally with amitriptyline (30 mg kg^{-1}), desipramine (20 mg kg^{-1}), paroxetine (10 mg kg^{-1}) or baclofen (10 mg kg^{-1}) whilst intraperitoneal injections of desipramine (10 mg kg^{-1}), CGP 35348 (100 mg kg^{-1}) or CGP 36742 (100 mg kg^{-1}) were also administered for a period of 21 days. Following a 24 h drug-free period, animals were prepared for perfusion-fixation prior to $GABA_B$ receptor autoradiography. Sections were incubated for 20 min with [^{3}H]-GABA and the selective labelling of $GABA_B$ receptors was achieved in the presence of 40 μM isoguvacine. Non-specific binding was defined by 100 μM (−)-baclofen. Of particular interest is the increase in $GABA_B$ binding density in the outer laminae of the frontal cortex following protracted treatment with desipramine (p.o. and i.p.) and with CGP 36742. (Bar = 1 mm).

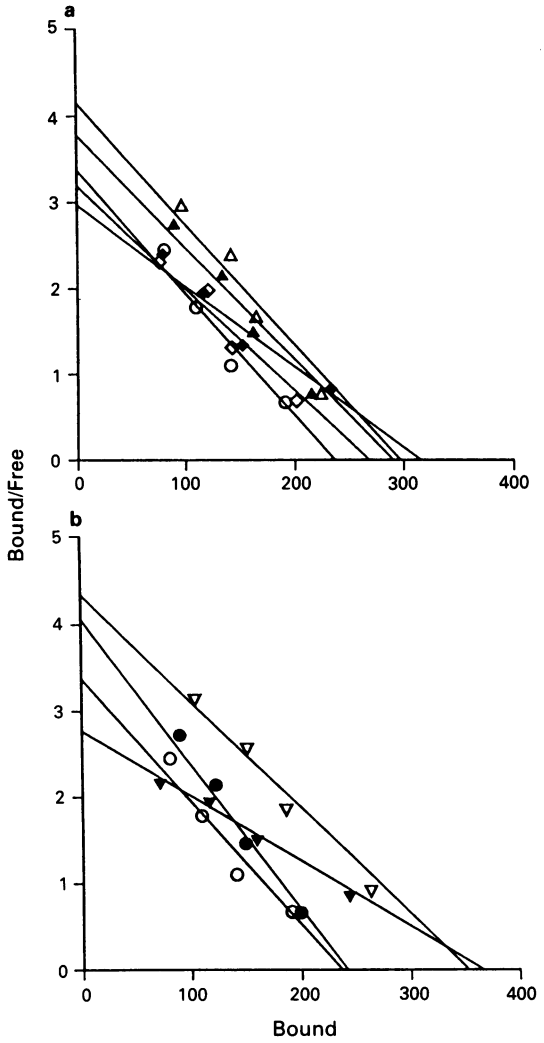


Figure 2 Scatchard plots of GABA$_B$ receptor binding in the frontal cortex (Lamina I) of control (○; $r = 0.97$) and (a) amitriptyline- (30 mg kg^{-1} ▲; $r = 0.91$), desipramine- (20 mg kg^{-1} ◆; $r = 0.84$), paroxetine- (10 mg kg^{-1} ◇; $r = 0.84$) or baclofen- (10 mg kg^{-1} △; $r = 0.94$) orally-treated rats. (b) Desipramine- (10 mg kg^{-1} ▽, $r = 0.96$), CGP 35348- (100 mg kg^{-1} ●; $r = 0.89$) or CGP 36742- (100 mg kg^{-1} ▼; $r = 0.97$) intraperitoneally-treated rats. K_D and B_{max} of the binding of [^{3}H]-GABA (at concentrations of 37.5, 75, 150 and 300 nM) were derived by linear regression analysis and data points represent the mean of 5 animals for which triplicate determinations were made. (Bound = fmol mg^{-1} tissue; Bound/Free = fmol mg^{-1} tissue/nM).

Effects of repeated antidepressant treatment on GABA$_B$ receptor transduction

A summary of the effects of desipramine, amitriptyline, baclofen, CGP 35348 and CGP 36742 on GABA$_B$ receptor-modulation of forskolin and noradrenaline-stimulated adenylyl cyclase is shown in Table 4. With the exception of tissue from rats treated intra-peritoneally with desipramine, forskolin-induced increases in cyclic AMP formation were significantly inhibited by (−)-baclofen (100 μM) in all cases. Similarly, the augmentation of noradrenaline-stimulated cyclic AMP production induced by (−)-baclofen amounted in most instances to around 100%, except in the case of desipramine (i.p.) where cyclic AMP levels were enhanced by only 66%. Moreover, the responsiveness of adenylyl cyclase to noradrenaline stimulation alone, was significantly reduced following repeated administration of amitriptyline (p.o.) and desipramine (i.p.). Although protracted treatment with the GABA$_B$ receptor antagonist, CGP 35348, failed to influence the forskolin-stimulated system, a significant increase in the accumulation of cyclic AMP produced in response to forskolin was seen after treatment with CGP 36742. Since the response to (−)-baclofen did not differ significantly from control tissue, this might suggest an increase in the GABA$_B$ receptor-mediated effect.

Discussion

The inconsistencies surrounding reports of the modulation of GABA$_B$ receptor populations by chronic treatment with antidepressants have remained largely unresolved. From a summary of the main findings, predominantly from five laboratories (Table 5), it is immediately apparent that many methodological differences abound which may account for the existing discrepancies. In an attempt to arbitrate these conflicting reports, the present study has examined the effects of a number of chronically administered antidepressants on the GABA$_B$ receptor population in the rat frontal cortex using receptor autoradiography. This approach has enabled a more detailed analysis of GABA$_B$ sites located within distinct laminal areas of this brain region and has therefore advanced the findings of existing studies in which only synaptic membranes of the frontal cortex had been employed.

The use of osmotic minipumps in drug administration confers the advantage of effecting a slow infusion over a required period which serves to maintain more consistent plasma drug levels than would perhaps be expected following daily oral or intraperitoneal injections. Although infusion and intraperitoneal injections of imipramine appeared to increase the B_{max} of GABA$_B$ receptor binding, most notably in

Table 4 The effects of chronic treatment with antidepressants and GABA$_B$ receptor antagonists GABA$_B$ receptor-modulated forskolin- and noradrenaline-stimulated adenylyl cyclase activity

Treatment	*Dose* (mg kg^{-1})	n	*Forskolin* *Basal*	*Alone*	+ (−)-*Baclofen*	*Noradrenaline* *Basal*	*Alone*	+ (−)-*Baclofen*
Control (p.o.)		5	0.31 ± 0.06	4.08 ± 0.56	2.49 ± 0.37^b	0.27 ± 0.04	1.36 ± 0.19	2.31 ± 0.42^b
Desipramine	20 p.o.	4	0.63 ± 0.06^c	5.28 ± 0.15	4.45 ± 0.29^{b,d}	0.87 ± 0.06^c	1.81 ± 0.18	2.77 ± 0.34^c
Amitriptyline	30 p.o.	4	0.26 ± 0.05	2.93 ± 0.39	1.96 ± 0.39^c	0.24 ± 0.07	0.69 ± 0.10^a	1.12 ± 0.07^c
Baclofen	10 p.o.	5	0.39 ± 0.05	5.06 ± 1.13	3.01 ± 0.46^b	0.24 ± 0.05	1.08 ± 0.14	1.74 ± 0.18^c
Control (i.p.)		5	0.36 ± 0.05	4.28 ± 0.50	2.99 ± 0.31^b	0.41 ± 0.06	1.59 ± 0.20^a	2.46 ± 0.35^c
Desipramine	10 i.p.	3	0.37 ± 0.08	1.87 ± 0.50^a	2.16 ± 0.64	0.33 ± 0.08	0.65 ± 0.06	0.93 ± 0.10^{b,d}
CGP 35348	100 i.p.	5	0.34 ± 0.08	4.03 ± 0.40	3.13 ± 0.17^b	0.32 ± 0.06	1.38 ± 0.06	2.08 ± 0.20^c
CGP 36742	100 i.p.	5	0.47 ± 0.06	6.19 ± 0.32^a	3.85 ± 0.40^c	0.53 ± 0.06	1.76 ± 0.12	2.91 ± 0.21^c

Values represent the mean (± s.e.mean) amount of cyclic AMP accumulated (pmol min^{-1} mg^{-1} protein).
$^aP < 0.05$ forskolin/noradrenaline stimulation alone vs control (Dunnett's multiple comparison test).
$^bP < 0.05$ forskolin/noradrenaline stimulation vs (−)-baclofen (100 μM) (Student's t test).
$^cP < 0.01$ forskolin/noradrenaline stimulation vs (−)-baclofen (100 μM) (Student's t test).
$^dP < 0.01$ (−)-baclofen response (100 μM), drug-treatment vs control (Dunnett's multiple comparison test).
$^eP < 0.05$ basal cyclic AMP levels vs control (Dunnett's multiple comparison test).

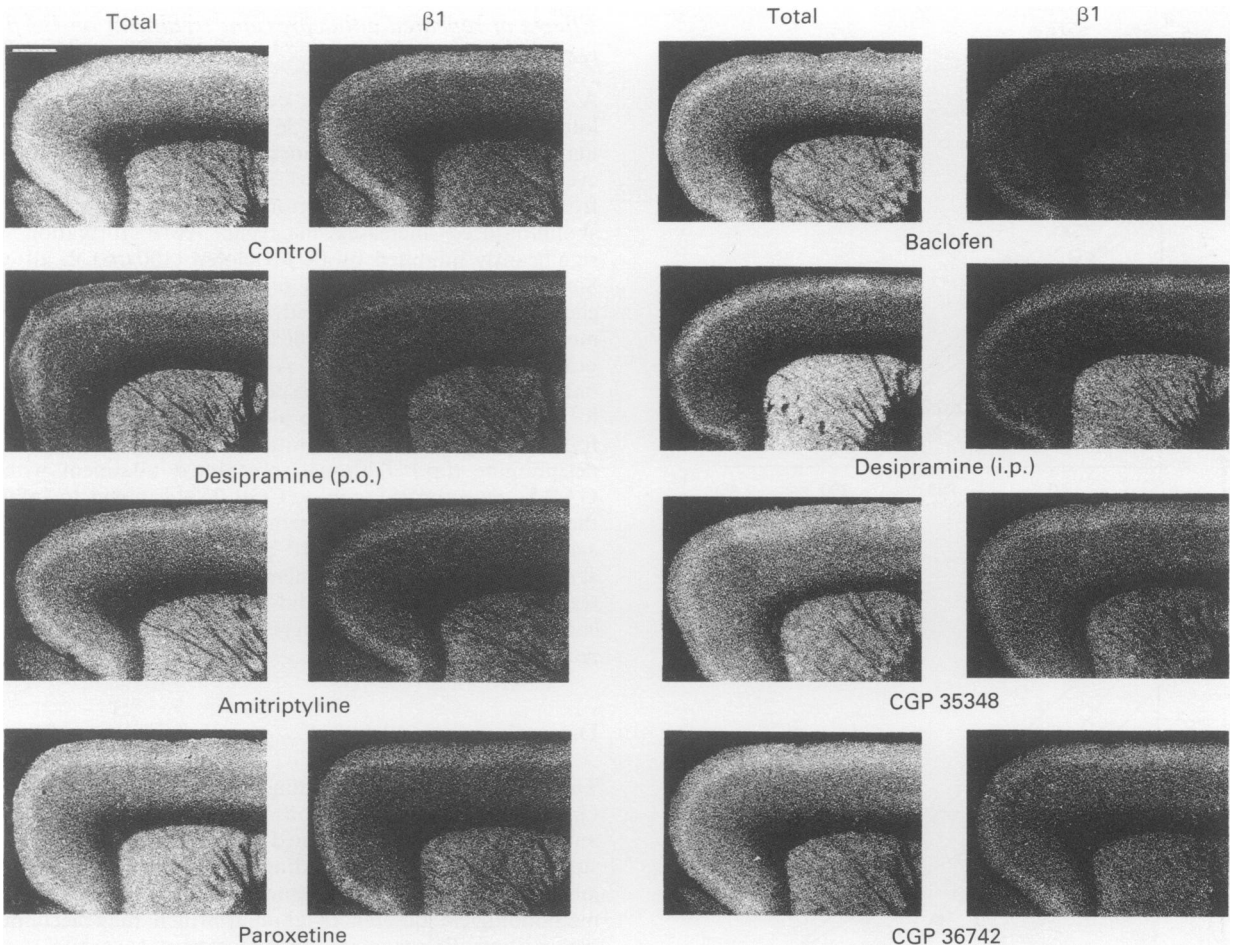


Figure 3 Autoradiograms of total (β_1 and β_2) and resolved β_1-adrenoceptor binding in parasagittal sections of the brains of rats treated with antidepressants, CGP35348 and CGP 36742. Rats were treated orally with amitriptyline (30 mg kg^{-1}), desipramine (20 mg kg^{-1}), paroxetine (10 mg kg^{-1}) or baclofen (10 mg kg^{-1}) whilst intraperitoneal injections of desipramine (10 mg kg^{-1}), CGP 35348 (100 mg kg^{-1}) or CGP 36742 (100 mg kg^{-1}) were also administered for a period of 21 days. Following a 24 h drug-free period, animals were prepared for perfused-fixation prior to β-adrenoceptor autoradiography. Sections were incubated for 60 min with (−)-[^{125}I]-iodopindolol (18.75–300 pM). Non-specific binding was defined by use of 200 μM (−)-isoprenaline whilst the selective labelling of β_1-adrenoceptors was achieved in the presence of the β_2-adrenoceptor antagonist, ICI 118,551 (50 nM). (Bar = 1 mm).

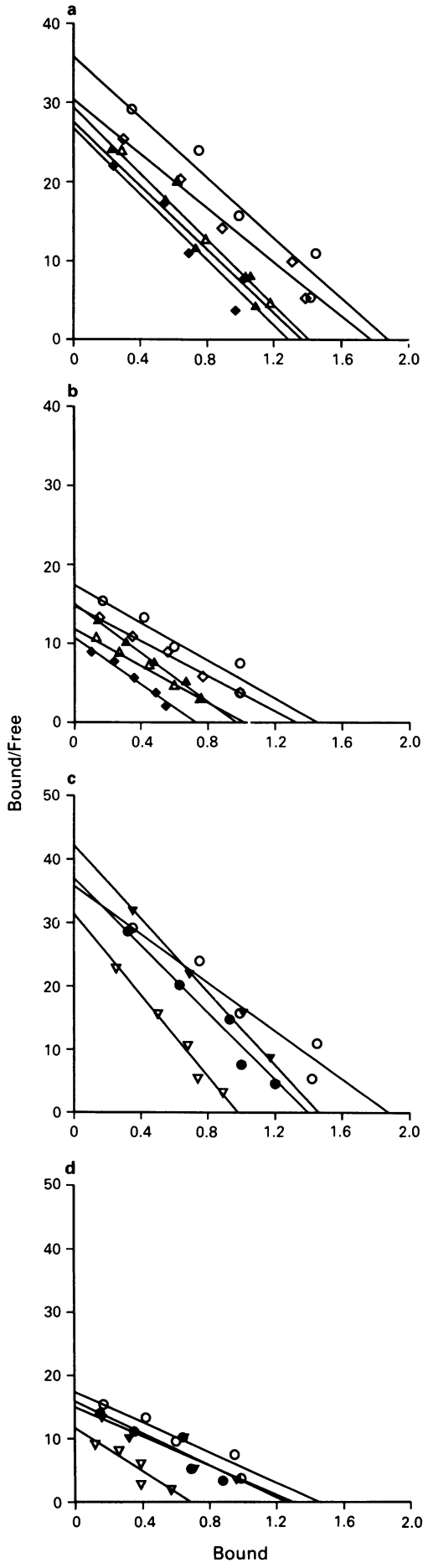
the outer lamina (I) of the frontal cortex, significance was not achieved. These findings agree with those of McManus & Greenshaw (1991) but are in contrast to the up-regulation of $GABA_B$ receptors mediated by both imipramine (Suzdak & Gianutsos, 1986; Szekely *et al.*, 1987) and its demethylated metabolite, desipramine (Pilc & Lloyd, 1984; Lloyd *et al.*, 1985; Szekely *et al.*, 1987).

The antidepressants employed in the second study (desipramine, paroxetine and amitriptyline) were selected on the basis of their ability to inhibit selectively the uptake of NA, 5-HT or both monoamines. From the localized increase in $GABA_B$ receptor binding induced by desipramine in lamina I of the frontal cortex, it could be interpreted that this modulation of $GABA_B$ receptors may have occurred as a consequence of the selective inhibition of noradrenaline uptake, since both amitriptyline and paroxetine were ineffective under the conditions employed in this study. However, this would contradict the findings of Lloyd *et al.* (1985) since $GABA_B$ receptor binding site densities in rat cortical membranes were up-regulated by chronic subcutaneous infusions of both amitriptyline and fluoxetine as well as desipramine.

In addition to the possible modulation of $GABA_B$ receptors, the effects of the antidepressants on β-adrenoceptor binding were also examined with the aim of correlating potentially up-regulated $GABA_B$ receptors with down-regulated β-adrenoceptors. In light of this, desipramine significantly reduced the β-adrenoceptor population in all laminae of the frontal cortex, attributable to a reduction in the β_1-adrenoceptor sub-type. These findings agree with the autoradiographical study of Ordway *et al.* (1988) who observed a reduction in β_1- but not β_2-adrenoceptors throughout the somatosensory cortex following administration of desipramine. Selective β_1-adrenoceptor modulation by desipramine has also been demonstrated in cortical membrane preparations (Minneman *et al.*, 1979; Beer *et al.*, 1987; Heal *et al.*, 1989) and the reduction in β_1-adrenoceptors mediated by amitriptyline, substantiates the findings of Heal *et al.* (1989) and Nelson *et al.* (1990).

The failure of paroxetine (10 mg kg^{-1}), to down-regulate β-adrenoceptors, at a concentration known to inhibit 5-HT uptake, confirms its lack of effect in membrane binding studies (Nelson *et al.*, 1990). Although receptor autoradiography has shown that the 5-HT uptake inhibitors fluoxetine and sertraline decrease β-adrenoceptors in rat frontoparietal cortex (Byerley *et al.*, 1987; 1988), the changes induced by fluoxetine (10 mg kg^{-1}) were marginal, with a larger, more widespread down-regulation induced by repeated doses of 30 mg kg^{-1}. The effects of sertraline were limited to deeper laminae of the cortex.

Repeated administration of the $GABA_B$ receptor agonist, baclofen, reduces the B_{max} of high affinity $GABA_B$ receptors in mouse cortical membranes as well as suppressing the



ability of baclofen to potentiate NA-stimulated cyclic AMP accumulation (Suzdak & Gianutsos, 1985a; 1986). In the present study, such a reduction of GABA$_B$ receptors was not apparent after chronic baclofen treatment which may reflect the differing routes of administration used, or, that after a period of 21 days had elapsed, tolerance to the effects of the agonist may have ensued. This treatment protocol did not, however, preclude a significant reduction in β-adrenoceptor populations.

The emergence of the centrally-active GABA$_B$ receptor antagonists, CGP 35348 (Olpe *et al.*, 1990) and CGP 36742 (Bittiger *et al.*, 1992), should facilitate our understanding of the possible physiological roles for central GABA$_B$ receptors. Although CGP 35348 enters the CNS, it does so rather rapidly and since its effects are short lasting (Olpe *et al.*, 1990), this could explain why after its repeated administrations, no alteration in GABA$_B$ receptor populations in the frontal cortex was observed. Conversely, CGP 36742 increased the B_{max} of GABA$_B$ receptors in lamina I, to a similar extent to that induced by desipramine. In view of the comparable affinities of the two antagonists (Olpe *et al.*, 1990; Bittiger *et al.*, 1992), this suggests that a possible longer half-life of CGP 36742 may be responsible for mediating the receptor regulation. It was therefore intriguing that whereas both antagonists reduced the total β-adrenoceptor population, the modulation was not attributable to the β_1-adrenoceptor sub-type (in contrast to the antidepressant effects already described). This indicates a possible involvement of β_2-adrenoceptors, although in cortical regions this sub-type constitutes only 20–25% of the total β-adrenoceptor population (Rainbow *et al.*, 1984; Beer *et al.*, 1987; Ordway *et al.*, 1988; De Paermentier *et al.*, 1989) and may be associated with non-nerve cell components such as glia and blood vessels (Minneman *et al.*, 1979). The discrepancy may point to different sensitivities between neuronal GABA$_B$ receptors and those functionally-linked to non-neuronal elements. Clearly, clarification of such a link between GABA$_B$ receptor antagonists and β_2-adrenoceptors requires further experimental investigation.

To provide a functional index of GABA$_B$ receptor modulation, the sensitivities of both forskolin- and noradrenaline-stimulated adenylyl cyclase systems were examined in brain slices following repeated administration of the aforementioned compounds. The sensitivity of adrenoceptors to noradrenaline stimulation was significantly reduced after chronic treatment with both desipramine (i.p.) and amitriptyline, which was likely to be a consequence of down-regulated β-adrenoceptors (Minneman *et al.*, 1979; Beer *et al.*, 1987; Heal *et al.*, 1989). Since the ability of (−)-baclofen to enhance the effect of noradrenaline was unaltered from control levels by these treatments, a drug-induced modulation of these particular GABA$_B$ receptors was not evident. The failure of desipramine (p.o.) to reduce the cyclic AMP response, despite down-regulated numbers of β-adrenoceptors

Figure 4 Scatchard plots of (a) total (β_1 and β_2) and (b) resolved β_1-adrenoceptor binding (in the presence of 50 nM ICI 118,551) in the frontal cortex (lamina I) of control (○; $r = 0.86$ and 0.89) and amitriptyline- (30 mg kg^{-1} ▲; $r = 0.93$ and 0.97), desipramine- (20 mg kg^{-1} ◆; $r = 0.87$ and 0.89), paroxetine- (10 mg kg^{-1} ◇; $r = 0.95$ and 0.99) or baclofen- (10 mg kg^{-1} △; $r = 0.95$ and 0.96) orally-treated rats. (c) Total (β_1 and β_2) and (d) resolved β_1-adrenoceptor binding (in the presence of 50 nM ICI 118,551) to the frontal cortex (lamina I) of control (○; $r = 0.86$ and 0.89) and desipramine- (10 mg kg^{-1} ▽ $r = 0.95$ and 0.81), CGP 35348- (100 mg kg^{-1} ●; $r = 0.93$ and 0.84) or CGP 36742- (100 mg kg^{-1} ▼; $r = 0.90$ and 0.83) intraperitoneally-treated rats. The kinetic parameters, K_D and B_{max} of the binding of (−)-[^{125}I]-iodopindolol (at concentrations of 18.75, 37.5, 75, 150 and 300 pM) were derived by linear regression analysis and data points represent the mean of 5 animals for which triplicate determinations were made. (Bound = fmol mg^{-1} tissue; Bound/Free = fmol mg^{-1} tissue/nM).

Table 5 Summary of published studies examining the effects of chronic antidepressant treatments on $GABA_B$ receptor binding in brain membranes

Reference	*Drugs*	*Dose* (mg kg^{-1})	*Duration*	*Drug-free period*	*Region/ species*	*Radioligand conditions*	*Results* re: $GABA_B$ B_{max}	*Additional findings*
Pilc & Lloyd (1984)	Amitriptyline Desipramine Citalopram Viloxazine Pargyline	10 s.c. 5 s.c. 10 s.c. 10 s.c. 20 s.c.	18 day mini-pumps	24 h	Frontal cortex Wistar Rat	[^{3}H]-GABA (8–160 nM) saturation	+ 63%** + 39%* + 52%** + 66%** + 57%*	only viloxazine increased $GABA_B$ binding acutely (10 nM). No chronic effects on $GABA_B$ binding, GABA levels or GAD activity.
Lloyd *et al.* (1985)	Nomifensine Desipramine Desipramine Zimeldine	5 s.c. 1.25 s.c. 5 s.c. 10 s.c.	18 days mini-pumps	72 h	Frontal cortex Wistar rat	[^{3}H]-GABA (10 nM) 1–750 μM GABA displacement	+ 16% + 41% − 3%[a] + 34%[a]	*In frontal cortex* DMI, Map, Vilox, Cital, Zimel, Nomi, Ami, Parg, Traz, Mian (s.c.), ECS; Fluox, Prog, Feng, Val (i.p.) produced increases in $GABA_B$ binding of between 27% and 88% at 10 nM [^{3}H]-GABA. *In Hippocampus* DMI, Vilox and Cital significantly increased $GABA_B$ binding.
Suzdak & Gianutsos (1986)	Baclofen Imipramine	10 i.p. 32 i.p.	14 days	24 hrs	Whole cortex Mouse (CD1)	[^{3}H]-GABA (1–1000 nM) saturation	− 16%*[b] + 17%*[c] + 24%*[b] + 15%*[c]	Chronic baclofen reduced the baclofen-induced potentiation of NA-stimulated adenylyl cyclase whereas imipramine augmented the potentiation.
Szekely *et al.* (1987)	Desipramine Imipramine Maprotiline	10 i.p. 7.5 i.p. 10 i.p.	21 days twice daily	48 h	Frontal cortex Sprague-Dawley rat	[^{3}H]-GABA (5–400 nM) [^{3}H]−(-)-Bac (5–150 nM) saturation	[^{3}H]-GABA [^{3}H]-(−)-Bac + 28%** No + 24%** change − 6%	Antidepressants failed to affect the potency of (−)-baclofen to inhibit forskolin-stimulated adenylyl cyclase in membrane studies.
Cross & Horton (1988)	Desipramine Zimeldine Desipramine Zimeldine	1.25 & 5 p.o. 1.25 & 5 p.o. 5 & 10 p.o. 5 & 10 p.o.	21 days twice daily	24 h	Whole cortex Frontal cortex Wistar rat	[^{3}H]-GABA (1 nM) 5–150 nM GABA displacement	No change	DMI (5 and 10 mg kg^{-1}, p.o.) and Zimel (10 mg kg^{-1}, p.o.) significantly reduced 5-HT_2 binding sites in frontal cortex but not hippocampus.
Cross *et al.* (1988)	Post-mortem depressed suicide victims	–	–	–	Frontal/Temporal cortex Human	[^{3}H]-GABA (1 nM) 5–300 nM GABA displacement	No change in K_D or B_{max} of drug-free or AD-treated suicide victims	Kinetic parameters were also unaffected in hippocampal membranes.

Table 5 Summary of published studies examining the effects of chronic antidepressant treatments on $GABA_B$ receptor binding in brain membranes

Reference	*Drugs*	*Dose* (mg kg^{-1})	*Duration*	*Drug-free period*	*Region/ species*	*Radioligand conditions*	*Results* re: $GABA_B$ B_{max}	*Additional findings*
Motohashi *et al.* (1989)	Lithium chloride	1.5 mEq kg^{-1}	14 days	24 h	Hippocampus	[^{3}H]-(−)-Bac(10–80 nM) saturation	+ 52%*	No changes in $GABA_B$ binding in frontal cortex or [^{3}H]-muscimol binding in either region.
	Carbamazepine	50 i.p.					+ 38%*	
					Wistar rat			
McManus & Greenshaw (1991)	Imipramine	30 s.c.	28 days	None	Frontal cortex	[^{3}H]-GABA (1 mM) 5–160 nM GABA displacement	No change	B_{max} of [^{3}H]-dihydroalprenolol binding reduced with all treatments.
	Desipramine	10 s.c.	mini-pumps					
	Tranylcypromine	1 s.c.			Sprague-Dawley rat			
	Phenelzine	10 s.c.						

[a]Changes refer to the high affinity components of a curvilinear Scatchard plot.
[b]High affinity binding site.
[c]Low affinity binding site.
*$P<0.05$; **$P<0.01$ Student's t test.

Abbreviations DMI (desipramine); Map (maprotiline); Vilox (viloxazine); Cital (citalopram); Zimel (zimeldine); Nomi (nomifensine); Ami (amitriptyline); Parg (pargyline); Traz (trazodone); Mian (mianserin); ECS (electroconvulsive shock); Fluox (fluoxetine); Prog (progabide); Feng (fengabine); Val (sodium valproate); NA (noradrenaline); GAD (glutamic acid decarboxylase); [^{3}H]-(−)-Bac ([^{3}H]-(−)-baclofen); AD (antidepressant).

is difficult to interpret but may be associated with the higher basal cyclic AMP content found in these animals which could have masked any drug-induced modulation of adenylyl cyclase activity. Protracted treatment with baclofen decreases the sensitivity of β-adrenoceptors to noradrenaline activation in addition to suppressing the ability of baclofen to augment this response (Suzdak & Gianutsos, 1986). Although there was some indication that such an effect may have occurred in the present study, significant differences from control values were not obtained. Furthermore, no additional drug-induced modulations of the $GABA_B$ receptor-mediated noradrenergic potentiation of β-adrenoceptor activation were detected.

Focussing on the forskolin-activated adenylyl cyclase system, with the exception of desipramine (i.p.), the (−)-baclofen-induced inhibition of activated cyclic AMP production was evident following all of the chronic treatments. Since protracted treatment with desipramine (p.o. and i.p.) as well as CGP 36742, significantly up-regulates $GABA_B$ receptor sites in the outer lamina (I) of the frontal cortex, it was anticipated that such an increase in the $GABA_B$ receptor population would be reflected by an enhancement of the ability of (−)-baclofen to inhibit the stimulatory action of forskolin. In the case of desipramine (p.o.), the levels of cyclic AMP produced in response to forskolin alone were not significantly increased after this treatment. However, although in the presence of (−)-baclofen, the amount of significantly increased after this treatment. However, in the presence of (−)-baclofen, the amount of accumulated cyclic AMP was significantly greater than the control value which may reflect $GABA_B$ receptor up-regulation.

Protracted treatment with CGP 36742 significantly increased the levels of cyclic AMP formed by forskolin alone. Moreover, although the percentage inhibition of this effect by baclofen was enhanced, unlike desipramine, the increased cyclic AMP levels observed in the presence of (−)-baclofen after treatment with CGP 36742 were not significantly different from the control response. This indicates an enhancement of the $GABA_B$ receptor-mediated effect and provides a functional correlate of the $GABA_B$ receptor up-regulation induced by CGP 36742. Moreover, since treatment with CGP 36742 failed to modulate those $GABA_B$ receptors associated functionally with the noradrenaline-stimulated adenylyl cyclase system, this further supports the contention that the $GABA_B$ receptors linked with these two transduction mechanisms may be heterogeneous.

In conclusion, of the compounds tested, only desipramine and CGP 36742 were able to mediate consistently an up-regulatory signal for $GABA_B$ receptors. Secondly, where drug-induced increases in the $GABA_B$ receptor population were evident in the frontal cortex, such changes were restricted to the outer lamina (I) of this region. Since desipramine specifically inhibits the uptake of noradrenaline, some speculation regarding the influence of this antidepressant (as well as CGP 36742) on $GABA_B$ receptors in this discrete anatomical location would seem most appropriately interpreted in relation to both the noradrenergic system and known markers of GABAergic activity.

Although desipramine enhances the release of GABA from the rat thalamus (Korf & Venema, 1983), the mechanism(s) through which $GABA_B$ receptors in the frontal cortex are up-regulated by chronic treatment with antidepressants still remain to be elucidated. Activation of $GABA_B$ receptors inhibits noradrenaline release in cerebral cortex (Bowery *et al.*, 1980; Suzdak & Gianutsos, 1985b), suggesting a presynaptic inhibitory action on noradrenergic terminals. Low (but not high) affinity $GABA_B$ sites are associated with noradrenergic cortical terminals since only the former are reduced following a unilateral lesion of the dorsal noradrenergic bundle (Karbon *et al.*, 1983). However, since the changes in $GABA_B$ receptor sites reported in the present study pertain to a high affinity component, this implies that such $GABA_B$ receptors may not necessarily be linked with the noradrenergic system in depression. Such a hypothesis,

however, contradicts the findings of Lloyd *et al.* (1985) and Suzdak & Gianutsos (1986) where both high and low affinity sites were increased by antidepressants.

One of the predicted effects of a $GABA_B$ receptor antagonist would be to inhibit the suppression of noradrenaline release induced by GABA acting at presynaptic $GABA_B$ receptors on noradrenergic terminals. Although there is no experimental evidence to support this at present, CGP 35348 inhibits the release of [^{3}H]-GABA from cortical slices (Waldmeier & Baumann, 1990). The $GABA_B$ receptors increased following chronic treatment with CGP 36742, again, are likely to represent presynaptic $GABA_B$ autoreceptors. Blockade of terminal $GABA_B$ receptors on noradrenergic neurones would lead to an increase in synaptic concentrations of noradrenaline, thus facilitating postsynaptic β-adrenoceptor down-regulation. Persistent occupation of a receptor by an antagonist would eventually lead to a compensatory 'supersensitization', thus those $GABA_B$ receptors up-regulated by chronic treatment with CGP 36742, could be attributed to these terminal receptors.

Such hypotheses are purely speculative at this stage and do not account for possible modulations of post-synaptic $GABA_B$ receptors or changes in other brain regions. Nonetheless, it is hoped that these findings will provide a significant step forward in our understanding of the role of $GABA_B$ receptors in depression.

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