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Increased $GABA_B$ receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks

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1 Addition of baclofen to a medium containing slices of mouse frontal cortex inhibited the potassium-evoked release of 5-hydroxytryptamine (5-HT) in a concentration-dependent manner. The degree of inhibition was increased in frontal cortex tissue taken from animals treated for 14 days with amitriptyline (10 mg kg^{-1}, twice daily) at all concentrations of baclofen tested (10^{-6} M – 10^{-4} M).

2 Administration of either desipramine, mianserin or zimeldine (10 mg kg^{-1} daily) for 14 days also approximately doubled the degree of inhibition evoked by addition of baclofen (10^{-5} M) to the medium.

3 One day of treatment with the antidepressant drugs did not alter the inhibitory effect of baclofen on K^+-evoked 5-HT release.

4 Addition of the antidepressant drugs to the medium had no effect on the K^+-evoked release of 5-HT.

5 Repeated administration of electroconvulsive shock (5 seizures spread out over 10 days), like amitriptyline, produced a significant enhancement of the baclofen-induced inhibition of 5-HT release over the range of baclofen concentrations studied. A single electroconvulsive shock had no effect.

6 These data suggest that repeated administration of the antidepressant drugs or electroconvulsive shock increases the function of the γ-aminobutyric acid $(GABA)_B$ receptor in the frontal cortex modulating 5-HT release and are consistent with the finding of increased $GABA_B$ receptor number in this region following various antidepressant treatments.

Introduction

Baclofen, the γ-aminobutyric acid $(GABA)_B$ receptor agonist, has been shown in several studies to inhibit the release of 5-hydroxytryptamine (5-HT) from preloaded slices of rat cortex (Bowery *et al.*, 1980; Schlicker *et al.*, 1984). Recently Gray & Green (1987) have shown that baclofen inhibits the potassium-evoked release of endogenous 5-HT from slices of mouse frontal cortex and partial characterization of this response again suggested that a $GABA_B$ receptor mechanism was involved.

Lloyd *et al.* (1985) have demonstrated that repeated administration to rats of a wide variety of antidepressant drugs or electroconvulsive shocks leads to a large increase in the number of $GABA_B$ receptors in the frontal cortex following these treatments. Recently a similar increase in $GABA_B$ receptor number was demonstrated in the frontal cortices of mice which had received repeated administration of imipramine (Suzdak & Gianutsos, 1986).

In the present study we have used the inhibition of potassium-evoked release of endogenous 5-HT from the mouse frontal cortex by baclofen as an index of $GABA_B$ receptor function. The aim of the study was to test the hypothesis that $GABA_B$ receptor function increases after various antidepressant treatments.

Part of this work has been presented in preliminary form to the British Pharmacological Society (Gray & Green, 1987).

Methods

Animals

Male mice C57B16 (Olac, Bicester) were housed in groups of 8 in conditions of constant temperature (21°C) and controlled lighting (light period, 07 h 00 min

–19 h 00 min) and fed an *ad libitum* diet of 41B pellets and tap water.

Measurement of 5-HT release

Mice were killed 24 h after the last treatment and the frontal cortices chopped in two directions at 45° at 300 μm intervals on a McIlwain chopper. The resulting prism-shaped slices were washed and then suspended in incubation tubes, containing 1 ml Krebs bicarbonate buffer (composition in mM: NaCl 126, KCl 1.8, KH_2PO_4 1.24, $MgSO_4$ 1.3 and $NaHCO_3$ 26) prewarmed to 37°C and gassed with 95% O_2, 5% CO_2, the resultant pH being 7.4. Pargyline (50 μM) and fluoxetine (5 μM) were present in the buffer throughout. The concentration of fluoxetine was lower than that used in our previous study (Gray & Green, 1987), since it was found that at concentrations of fluoxetine above 10 μM elevation of basal release occurred. The slices were incubated in 4 tubes for 15 min in the Ca^{2+}-free buffer, the medium being changed at 5 min intervals during this period. They were then centrifuged for 30 s at 1000 *g* and resuspended in 1 ml Krebs bicarbonate buffer containing calcium (2.4 mM). (±)-Baclofen was present in the medium added to two of the four tubes. After a further 5 min incubation, KCl (20 μl) was added to two of the tubes to raise the concentration to 35 mM; 20 μl of buffer was added to control tubes for measurement of basal 5-HT release. The tubes were incubated for a further 15 min, centrifuged for 30 s at 1000 *g* and 200 μl of the supernatant collected into Eppendorf centrifuge tubes containing 20 μl of perchloric acid (0.1 M, containing sodium metabisulphite, 400 mM). These tubes were stored on ice and 5-HT measured by high performance liquid chromatography with electrochemical detection, using the method of Molyneux & Clarke (1985). The slices were analysed for protein by the method of Lowry *et al.* (1951).

Dosage schedules

Groups of animals were treated for one or 14 days with intraperitoneal injections of the following antidepressant drugs: amitriptyline 10 mg kg^{-1} twice daily; zimeldine 10 mg kg^{-1} once daily; desipramine 10 mg kg^{-1} once daily; mianserin 10 mg kg^{-1} once daily. Control animals received injections of 0.9% saline. A further group of animals received electroconvulsive shocks (ECS) under halothane anaesthesia. Either a single shock or 5 shocks over 10 days were administered. Control animals received halothane anaesthesia plus application of ear-clip electrodes with no passage of current.

Drug sources

Drugs were obtained from the following sources (in parentheses): pargyline (Sigma, Poole, Dorset), fluoxetine (Eli Lilly Co., Indiana, U.S.A), amitriptyline (Merck, Sharp & Dohme, Hoddesdon), desipramine and (±)-baclofen (Ciba-Geigy, Horsham), mianserin (Organon, Oss, Holland), zimeldine (Astra Alab, Södertälje, Sweden).

Statistics

Data were analysed by use of Student's *t* test (unpaired). Data expressed as percentage changes were subjected to arc sin transformation before the use of the *t* test.

Results

Effect of repeated amitriptyline administration on the baclofen-induced inhibition of K^+-evoked 5-HT release

Mice were treated with saline or amitriptyline for 14 days as described in the 'Methods'. Twenty four hours after the final dose the mice were killed, slices of frontal cortex prepared and the release of 5-HT measured in the absence or presence of baclofen (10^{-5} M).

Chronic treatment with amitriptyline altered neither the basal release nor the K^+-evoked release of 5-HT (Table 1). However, the inhibition of K^+-evoked release of 5-HT produced by baclofen (10^{-5} M) was significantly greater in mice treated with amitriptyline (Table 1).

Next, a concentration-response curve for baclofen was produced. Baclofen induced a concentration-dependent inhibition of 5-HT release over the range 10^{-6}–10^{-4} M with a maximum inhibition at a baclofen concentration of 10^{-4} M (Figure 1). Repeated treatment with amitriptyline caused a shift to the left of this curve, a greater degree of inhibition of 5-HT release by baclofen being seen at every concentration examined (Figure 1). The maximum inhibitory response also appeared to increase (Figure 1). At the time the tissue was isolated (24 h after the last dose) no difference in cortical 5-HT content was observed between the saline- and drug-treated animals (data not shown).

Effect of other antidepressant drugs on baclofen-induced inhibition of 5-HT release

Mice were treated repeatedly with desipramine, mianserin and zimeldine (see Methods) and the degree of inhibition of 5-HT release induced by baclofen subsequently examined in slices of frontal cortex. A single concentration of baclofen (10^{-5} M) was used in all these studies.

Chronic treatment with all three drugs was found to enhance markedly the degree of inhibition of 5-HT

Table 1 Effect of addition of baclofen (10^{-5} M) on the K^+-evoked release of 5-HT from slices of frontal cortex of mice treated with amitriptyline

	Basal release		*K^+-evoked release*	
Treatment	*Saline*	*Amitriptyline*	*Saline*	*Amitriptyline*
No baclofen	1.4 ± 0.2 (4)	1.5 ± 0.1 (4)	4.3 ± 0.3 (4)	4.1 ± 0.4 (4)
Baclofen	1.4 ± 0.2 (4)	1.4 ± 0.2 (4)	2.6 ± 0.2 (4)†	0.8 ± 0.2 (4)*
% inhibition of K^+-evoked release by baclofen	—	—	43 ± 3	77 ± 3

Mice were injected twice daily with saline or amitriptyline (10 mg kg^{-1}) for 14 days. Results show the concentration of 5-hydroxytryptamine (5-HT) in the medium (expressed in ng mg^{-1} protein) both in the absence and presence of potassium (35 mM) and are presented as mean ± s.e.mean with number of observations in parentheses. †Significantly different from release in the absence of baclofen $P < 0.01$. *Significantly different from release in the presence of baclofen in saline-treated mice $P < 0.01$.

release induced by the addition of baclofen (10^{-5} M) to the incubate (Figure 2). However, as in the case of amitriptyline (see Table 1), none of the drugs altered either the basal or K^+-evoked release of 5-HT in the absence of baclofen (data not shown). Cortical 5-HT content was unaltered by repeated treatment with any of the antidepressant drugs studied.

Effect of a single day of treatment with antidepressant drugs on the influence of baclofen on 5-HT release

Administration of desipramine, mianserin, zimeldine or amitriptyline for one day failed to alter the degree of inhibition of the K^+-evoked 5-HT release, induced by addition of baclofen (10^{-5} M) to the incubate containing the cortical brain slices (Figure 3). Basal or K^+-evoked 5-HT release was not affected by one day of treatment with these drugs (data not shown).

Effect of addition of antidepressant drugs to the medium

When the antidepressant drugs under investigation were added to the medium at a concentration of 10^{-5} M, no effect on either basal release or the degree of inhibition of K^+-evoked release by baclofen (10^{-5} M) was observed (data not shown).

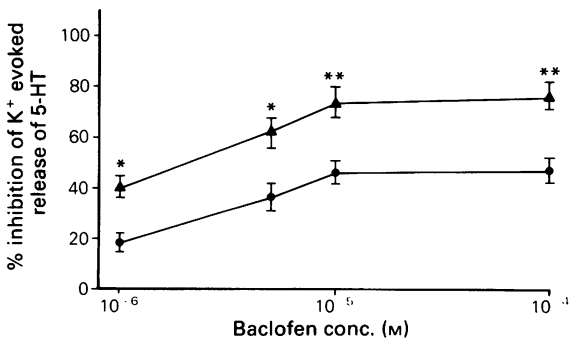

Figure 1 Effect of administration of amitriptyline or saline for 14 days on the inhibition of 5-hydroxytryptamine (5-HT) release by (±)-baclofen. The mean % inhibition of K^+-evoked release of 5-HT from frontal cortex taken from mice treated with saline (●) or amitriptyline (▲) in the presence of various concentrations of baclofen is shown. Vertical lines indicate s.e.mean. *$P < 0.05$, **$P < 0.01$; significantly different from control.

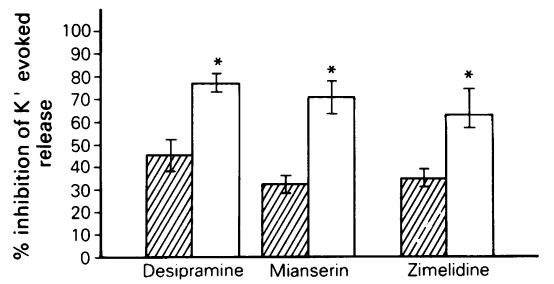

Figure 2 Effect of repeated administration of antidepressant drugs on the inhibition of 5-hydroxytryptamine (5-HT) release, from frontal cortex of mice by (±)-baclofen. Results show mean % inhibition of potassium-evoked release of 5-HT in the presence of (±)-baclofen (10^{-5} M) in saline-treated (hatched columns) and drug-treated (open columns) mice. Vertical lines indicate s.e.mean *$P < 0.05$; significantly different from control (saline-treated mice).

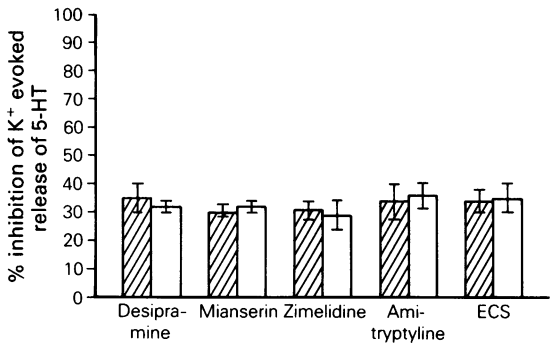

Figure 3 Effect of a single day of treatment with antidepressant drugs or electroconvulsive shock (ECS) on the inhibition of potassium evoked 5-hydroxytryptamine (5-HT) release by (±)-baclofen (10^{-5} M). Hatched columns represent control (saline/halothane-treated) mice and open columns represent drug or ECS-treated mice. Vertical lines indicate s.e.mean.

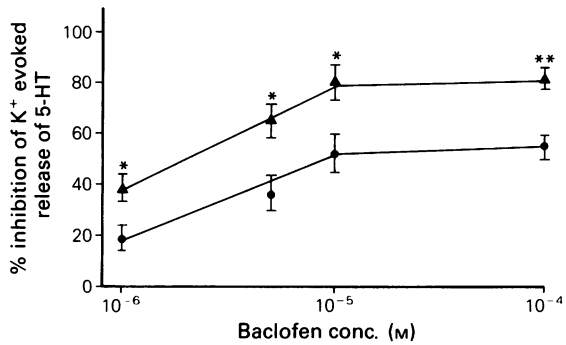

Figure 4 Effect of repeated administration (5 times over 10 days) of electroconvulsive shock (▲) or halothane (●), on the inhibition of potassium-evoked release of 5-hydroxytryptamine (5-HT) by various concentrations of (±)-baclofen. Vertical lines indicate s.e.mean. Significantly different from control tissue, $*P<0.05$, $**P<0.01$.

Effect of single and repeated administration of electroconvulsive shocks on the baclofen-induced inhibition of 5-HT release

Animals were anaesthetized with halothane and given either a single ECS or 5 ECSs spread over 10 days and killed 24 h after the single or repeated treatment. Control animals received halothane alone.

A single ECS had no effect on the degree of inhibition of K^+-evoked 5-HT release produced by addition of baclofen (10^{-5} M) to the medium (Figure 3). In contrast repeated ECS caused a significant increase in the inhibitory effect of baclofen at all concentrations of baclofen examined (Figure 4). Neither single nor repeated ECS altered either the basal or K^+-evoked release of 5-HT in the absence of baclofen (data not shown).

Effect of repeated flurazepam on baclofen-induced inhibition of 5-HT release

Animals were treated for 14 days with either saline or flurazepam (20 mg kg^{-1}). Repeated administration with the benzodiazepine did not alter either basal or K^+-evoked release of 5-HT in the absence of baclofen, or the degree of inhibition of K^+-evoked release in the presence of baclofen (10^{-5} M) (Table 2).

Discussion

The current study confirms the observation (Gray & Green, 1987) that baclofen inhibits K^+-evoked release of endogenous 5-HT from mouse frontal cortex slices in a concentration-dependent manner. In the present

Table 2 Effect of chronic treatment with flurazepam (20 mg kg^{-1}) on the K^+-evoked release of 5-HT from slices of mouse frontal cortex and the effect of baclofen on this release

	Basal release		*K^+-evoked release*	
Treatment	*Saline*	*Flurazepam*	*Saline*	*Flurazepam*
No baclofen	1.4 ± 0.2 (4)	1.6 ± 0.2 (4)	4.3 ± 0.4 (4)	3.9 ± 0.3 (4)
Baclofen	1.4 ± 0.1 (4)	1.4 ± 0.1 (4)	2.8 ± 0.3 (4)†	2.8 ± 0.2 (4)*
% inhibition of K^+-evoked release by baclofen	—	—	32 ± 4	28 ± 6

Mice were injected once daily with saline or flurazepam (20 mg kg^{-1} i.p.) for 14 days. Results show the concentration of 5-hydroxytryptamine (5-HT) in the medium (ng mg^{-1} protein) in the absence and presence of potassium (35 mM) and are presented as mean ± s.e.mean with number of obervations in parentheses. †Significantly different from release in the absence of baclofen $P<0.05$.

study this response has been used to investigate possible changes in $GABA_B$ receptor function following antidepressant drug administration.

Repeated (14 days) administration of various antidepressant drugs resulted in an increase in the inhibitory response of baclofen. These data are therefore completely consistent with the results of Lloyd *et al.* (1985) that $GABA_B$ receptor number increases after repeated antidepressant drug administration. Also consistent with the findings of Lloyd *et al.* (1985) were our observations that there was no change in the response to baclofen after 1 day of antidepressant drug administration, and that repeated treatment with ECS had the same effect as giving antidepressant drugs.

While much of our data has been presented in terms of percentage changes, the results shown in Table 1 demonstrate that the changed sensitivity to baclofen is not due to alterations in basal or K^+-evoked release (in the absence of baclofen) or a change in cerebral content of 5-HT.

It is interesting to note that the maximal response to baclofen is increased after chronic amitriptyline or ECS (Figures 1 and 4), a result which is difficult to explain solely in terms of an increase in receptor number. However, Lloyd *et al.* (1985) did detect the appearance of a second, low affinity, $GABA_B$ receptor, at least after desipramine treatment. The fact that several diverse antidepressant treatments and ECS, given in a manner rather analagous to the clinical administration of electroconvulsive therapy (ECT), all produced the same change suggests that an altered $GABA_B$ receptor function may be associated with the mechanism of action of antidepressants. This view is enhanced by the observation that another psychoactive compound namely flurazepam, an anxiolytic compound, did not induce a similar change. In agreement with our results, Suzdak & Gianutsos (1986) have recently produced evidence for increased $GABA_B$ function in the cerebral cortex of mice which had received repeated administration of imipramine. They showed that another index of $GABA_B$ receptor activity, namely the potentiation by baclofen of noradrenaline-induced adenylate cyclase stimulation, was increased after the antidepressant treatment.

The $GABA_B$ receptor in the current investigation is presumably situated as a heteroreceptor on a presynaptic 5-HT terminal in the frontal cortex. It is difficult to predict what the increased $GABA_B$ response would do in terms of overall transynaptic 5-HT function. Cortical postsynaptic $5\text{-}HT_2$ receptors in mice are decreased by antidepressant drugs but increased by ECS (Goodwin *et al.*, 1984). In rats postsynaptic $5\text{-}HT_{1A}$ receptor function has been shown to be decreased by both ECS and antidepressant drugs, although the behavioural response examined is unlikely to have been initiated in the frontal cortex (Goodwin *et al.*, 1987). Interestingly this response was also not altered by flurazepam treatment (Goodwin *et al.*, 1987).

The mechanism underlying the change in $GABA_B$ receptor number and function is not clear. Pilc & Lloyd (1984) demonstrated that $GABA_A$ receptor number was unchanged after several different antidepressant treatments. However, other changes in GABA function have been noted after antidepressant administration. For example, in rats both GABA synthesis and endogenous GABA release are decreased after ECS in several brain regions (Green, 1986). In contrast Sherman & Petty (1982) showed that the release of endogenous GABA was enhanced after administration of imipramine for four days. Furthermore, Korf & Venema (1983), using a push-pull cannula technique, have observed an increase in endogenous GABA release from the rat thalamus after acute administration of desmethylimipramine. In our present state of knowledge it is difficult to relate these changes in GABA function to the increased $GABA_B$ receptor number observed after antidepressant treatments.

In conclusion these data are consistent with the suggestion of Lloyd *et al.* (1985), that $GABA_B$ receptor number increases in the frontal cortex following antidepressant treatments and provide evidence that this change has a functional correlate. Furthermore, they demonstrate a consistent change in $GABA_B$ function following diverse antidepressant treatments to mice.

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References

BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDLEMISS, D.N., SHAW, J. & TURNBULL, M. (1980). (−)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*, 283, **92–94.**

GOODWIN, G.M., GREEN, A.R. & JOHNSON, P. (1984). $5\text{-}HT_2$ receptor characteristics in frontal cortex and $5\text{-}HT_2$ receptor-mediated head-twitch behaviour following antidepressant treatment to mice. *Br. J. Pharmac.*, **83,** 235–242.

GOODWIN, G.M. DE SOUZA, R.J. & GREEN, A.R. (1987). Attenuation by electroconvulsive shock and antidepressant drugs of the $5\text{-}HT_{1A}$ receptor-mediated hypothermia and serotonin syndrome produced by 8-OH-DPAT in the rat. *Psychopharmacology*, **91,** 500–505.

GREEN, A.R. (1986). Changes in γ-aminobutyric acid bio-

chemistry and seizure threshold. *A. New York Acad. Sci.*, volume no. 105–119.

GRAY, J.A. & GREEN, A.R. (1987). $GABA_B$-receptor mediated inhibition of potassium evoked release of endogenous 5-hydroxytryptamine from mouse frontal cortex. *Br. J. Pharmac.*, **91,** 517–522.

KORF, J. & VENEMA, K. (1983). Desethylimipramine enhances the release of endogenous GABA and other neurotransmitter amino acids from the rat thalamus. *J. Neurochem.*, **40,** 946–950.

LLOYD, K.G., THURET, F. & PILC, A. (1985). Upregulation of γ-aminobutyric acid ($GABA_B$) binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock. *J. Pharmac. exp. Ther.*, **235,** 191–199.

LOWRY, O.H., ROSEBOROUGH, N.J., FARR, A.L. & RANDALL, R.L. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193,** 265–276.

MOLYNEUX, S.G. & CLARKE, E.E. (1985). Precise determinations of 5-hydroxytryptamine in platelets and platelet-poor plasma. *Clin. Chem.*, **31,** 1573.

PILC, A. & LLOYD, K.G. (1984). Chronic antidepressants and GABA "B" receptors: a GABA hypothesis of antidepressant drug action. *Life Sci.*, **35,** 2149–2154.

SCHLICKER, E., CLASSEN, K. & GOTHERT, M. (1984). GABA receptor-mediated inhibition of serotonin release in the rat brain. *Naunyn-Schmiedebergs Arch. Pharmac.*, **326,** 99–105.

SHERMAN, A.D. & PETTY, F. (1982). Additivity of neurochemical changes in learned helplessness and imipramine. *Behav. Neur. Biol.*, **35,** 344–353.

SUZDAK, P.D. & GIANUTSOS, G. (1986). Effect of chronic imipramine or baclofen on GABA-B binding and cyclic AMP production in cerebral cortex. *Eur. J. Pharmac.*, **131,** 129–133.

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