Br. J. Pharmac. (1987), **91**, 517–522

$GABA_B$-receptor mediated inhibition of potassium-evoked release of endogenous 5-hydroxytryptamine from mouse frontal cortex

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1 The effect of baclofen, the $GABA_B$-agent, on the potassium-evoked release of endogenous 5-hydroxytryptamine (5-HT) from slices of mouse frontal cortex has been investigated.

2 The release of endogenous 5-HT evoked by addition of K^+ (35 mM) was inhibited by (±)-baclofen in a dose-dependent manner with an IC_{50} of 0.1 μM.

3 Inhibition of K^+-evoked release of 5-HT was produced by (±)- and (−)-baclofen but not (+)-baclofen. This action of the (−)-enantiomer was not altered by the presence of the (+)-enantiomer.

4 Addition of GABA (0.1–10 μM) also induced a dose-dependent inhibition of 5-HT release. This effect was neither enhanced by flurazepam (1 μM) nor antagonized by bicuculline (10 μM).

5 The progabide metabolite, 4-([(4-chlorophenyl) (5-fluoro-2-hydroxyphenyl)methylene]amino)butyric acid (SL75.102) (1 μM) inhibited the K^+-evoked release of 5-HT by 61%.

6 These data suggest that baclofen is a potent inhibitor of the K^+-evoked release of endogenous 5-HT from the cortex and further indicate that the release of 5-HT may be controlled by a $GABA_B$-receptor located presynaptically.

Introduction

Baclofen, the γ-aminobutyric $acid_B$ ($GABA_B$)-receptor agonist (Bowery *et al.*, 1980), has been shown to inhibit the release of [^{3}H]-5-hydroxytryptamine evoked either by potassium (Bowery *et al.*, 1980), or by electrical stimulation (Schlicker *et al.*, 1984) from slices of rat frontal cortex.

Recently we have demonstrated that both baclofen and progabide, an agonist at both $GABA_A$- and $GABA_B$-receptors (Lloyd *et al.*, 1982) inhibited the head twitch behaviour elicited in the mouse by administration of 5-hydroxytryptophan (5-HTP), the 5-hydroxytryptamine (5-HT) precursor (Gray *et al.*, 1986a). However, the head twitch behaviour induced by the 5-HT agonist, 5-methoxy, *N*,*N*,-dimethyltryptamine (5-MeODMT) was unaffected by pretreatment with either progabide or baclofen. This suggested that $GABA_B$-receptor agonists *in vivo* were inhibiting 5-HT synthesis or release.

The present paper describes experiments *in vitro* designed to examine whether baclofen and GABA inhibit the release of 5-HT from mouse frontal cortex. We have measured the release of endogenous 5-HT rather than [^{3}H]-5-HT from preloaded slices since recent studies have shown that following various pretreatments differences in the release of both dopamine and GABA have been observed in results obtained using these two methods (Herdon *et al.*, 1985; Green, 1986; see also the Discussion). Some of these data have been presented in preliminary form elsewhere (Gray *et al.*, 1986b).

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Methods

Animals

Male C57B16 mice (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-hydroxytryptamine release

Mice were killed and the frontal cortex dissected on ice and chopped in 2 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 0.5 ml Krebs bicarbonate buffer (composition (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 (25 μM) were present in the buffer throughout. 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 0.5 ml Krebs bicarbonate buffer containing calcium (2.4 mM). Where appropriate, the test drug was present in the medium added to two of the four tubes. After a further 5 min incubation, KCl (10 μl) was added to two of the tubes to raise the concentration to 35 mM. The tubes were incubated for a further 20 min, centrifuged for 30 s at 1000 *g* and 200 μl of the supernatant collected into Eppendorf centrifuge tubes containing 20 μl perchloric acid (0.1 M containing sodium metabisulphite, 400 mM). Tubes were stored on ice and 5-HT measured by high performance liquid chromatography with electrochemical detection (Molyneux & Clarke, 1982), using a Gibson 302 pump with 802 manometric module coupled to a Rainin 10 cm 3 μm, C-18 reverse-phase analytical column fitted with a Brownlee 3 cm, 5 μm, C-18 pre-column. The mobile phase was sodium acetate solution (0.1 M) containing 15 v/v methanol adjusted to pH 4.2 with glacial acetic acid. This was pumped at a flow-rate of 0.1 ml min^{-1}. 5-Hydroxytryptamine was detected using a Bioanalytical Systems amperometric detector coupled to a TL-5 flow cell with glassy carbon electrode set at +0.75 V(Ag^+/AgCl). The slices were analysed for protein content by the method of Lowry *et al.* (1951).

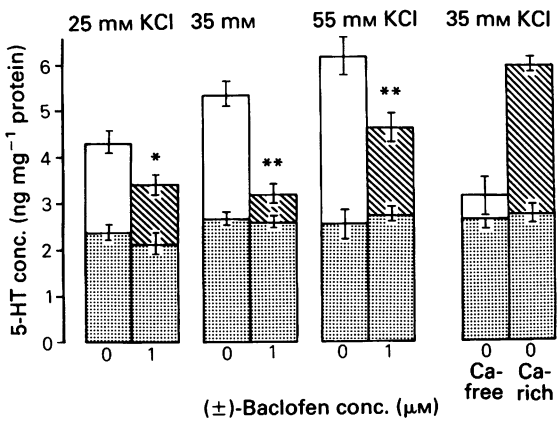

Figure 1 Effect of different concentrations of potassium on release of endogenous 5-hydroxytryptamine (5-HT) in the presence and absence of (±)-baclofen. Slices of mouse frontal cortex were incubated and the potassium concentration in half the tubes raised to 25, 35 or 55 mM. Half of the tubes contained (±)-baclofen (1 μM). Dotted sections indicate basal release, hatched areas release in the presence of (±)-baclofen (1 μM). The data on the right indicate the effect of removing external calcium from the medium on release evoked by 35 mM KCl. Results are expressed as mean of 4–6 experiments, with s.e.mean shown by vertical lines. Different from release in absence of (±)-baclofen: $*P<0.05$; $**P<0.01$.

Drug sources

Drugs were obtained from the following sources (in parentheses): (±)-Baclofen, (−)- and (+)-baclofen (Ciba-Geigy, Horsham); GABA, pargyline, bicuculline methiodide (Sigma, Poole, Dorset); fluoxetine (Eli Lilly Co., Indiana, U.S.A.); 4-([(4-chlorophenyl) (5-fluoro-2-hydroxyphenyl)methylene]amino) butyric acid (SL75.102; Synthélabo, Paris), flurazepam (Roche Products, Welwyn Garden City).

Statistics

Data were analysed by analysis of variance prior to using Student's *t* test (unpaired).

Results

Effect of different potassium concentrations on the release of 5-HT from slices of mouse frontal cortex

Slices were prepared and incubated as described in the Methods. Potassium chloride (10 μl) was added to half the tubes to raise the concentration in the medium to 25, 35 or 55 mM. A dose-dependent enhancement of 5-HT release over basal levels was seen (Figure 1). Addition of sodium chloride to elevate osmolarity to a similar degree to that after KCl addition did not affect basal release (data not shown). The concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the incubation medium was less than 5% of that of 5-HT, presumably due to the presence of the monoamine oxidase inhibitor.

Calcium-dependency of the release of 5-HT

Slices were incubated in groups of 4 tubes, with KCl (35 mM) being added to 2 of the tubes. In some parallel experiments calcium was omitted from the medium. Omission of Ca^{2+} from the medium did not alter basal release, but did completely abolish the potassium-evoked release of 5-HT (Figure 1).

Effect of (±)-baclofen on the release of 5-HT evoked by different concentrations of potassium

(±)-Baclofen (final concentration, 1 μM) was added to half of the tubes 5 min before addition of KCl (final concentration 25, 35 or 55 mM).

Addition of (±)-baclofen inhibited the K^+-evoked release of 5-HT without affecting basal release (Figure 1). This inhibition was more pronounced following addition of KCl at a concentration of 35 mM than 25 mM (Figure 1).

Effect of different concentrations of (±)-baclofen on the release of 5-HT evoked by 35 mM potassium chloride

Slices were prepared and incubated as before. To half of the tubes (±)-baclofen was added in the range 0.001–100 μM. Addition of (±)-baclofen produced a dose-dependent inhibition of the potassium evoked release of 5-HT (Figure 2). About 30% of the K^+-evoked release of 5-HT appears to be baclofen insensitive (Figure 3). The IC_{50} of baclofen-sensitive release was 0.1 μM (Figure 3). Basal release was not changed significantly at any of the concentrations examined (Figure 2).

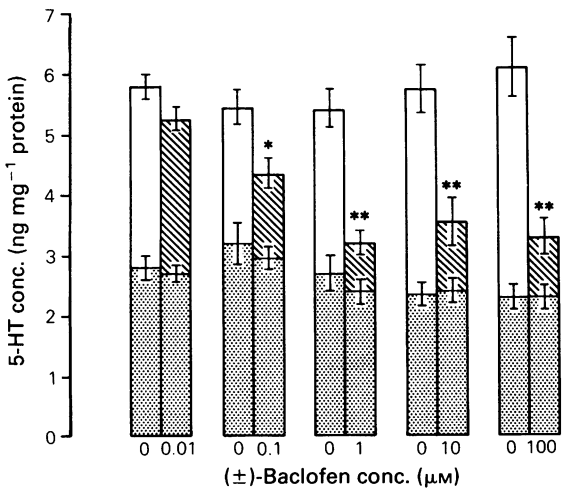

Figure 2 Effect of (±)-baclofen on the release of endogenous 5-hydroxytryptamine (5-HT) from mouse frontal cortex. Slices were incubated in the presence and absence of (±)-baclofen (0.01–100 μM). Basal release is indicated by the strippled columns; potassium-evoked release in the absence of (±)-baclofen is shown by the open columns, while release in the presence of (±)-baclofen is shown by the hatched columns. Results are shown as mean of 4–6 experiments with s.e.mean shown by vertical lines. Different from control: $*P<0.05$; $**P<0.01$.

Effect of the enantiomers of baclofen on the potassium evoked release of 5-HT

(−)-Baclofen (1 μM), (+)-baclofen (1 μM) or (−)-baclofen (1 μM) plus (+)-baclofen (1 μM) were included in the medium.

(−)-Baclofen inhibited the potassium-evoked release of 5-HT while (+)-baclofen was without effect (Table 1). The presence of (+)-baclofen did not alter the degree of inhibition of 5-HT release by the addition of (−)-baclofen (Table 1).

Effect of GABA and SL75.102 on the release of 5-HT

GABA (final concentration of 0.1 μM, 1 μM or 10 μM) or SL75.102 (final concentration 1 μM) was added to the medium.

Potassium-evoked release of 5-HT was inhibited in a dose-dependent manner in the presence of GABA, with no change in basal release (Figure 4).

The presence of SL75.102 (1 μM) also inhibited the potassium-evoked release of 5-HT without altering basal release (Figure 4).

Effect of bicuculline methiodide and flurazepam on the inhibition of potassium-evoked 5-HT release by GABA

When GABA (final concentration 1 μM) and bicuculline methiodide (final concentration 10 μM) were added to the incubation tubes, potassium-evoked

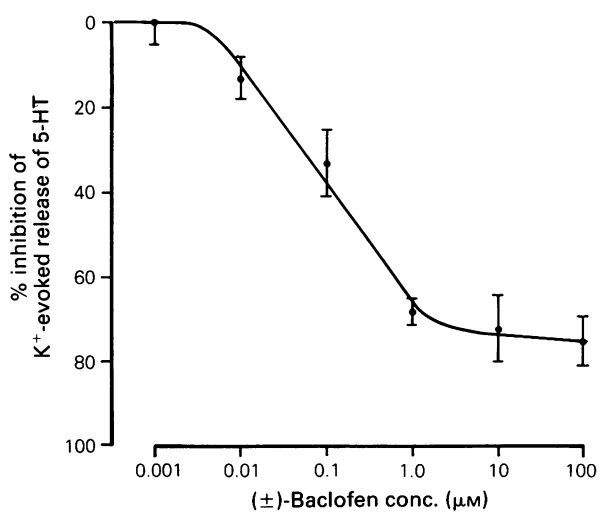

Figure 3 The percentage inhibition of the potassium-evoked release (total release minus basal release) in the presence of (±)-baclofen (0.001–100 μM). Each point represents mean of 4–6 experiments and is derived from data presented in Figure 2; s.e.mean shown by vertical lines.

Table 1 Effect of baclofen enantiomers on the potassium-evoked release of 5-hydroxytryptamine (5-HT) from mouse cortical slices

Drug added	*5-HT concentration* (ng mg^{-1} protein) *Basal release*	*Total release*	*K^+-evoked release*	*% inhibition of K^+-evoked release*
None	2.7 ± 0.2 (16)	5.9 ± 0.2 (16)	3.1 ± 0.2 (16)	—
(±)-Baclofen	2.5 ± 0.3 (4)	3.4 ± 0.2	0.9 ± 0.3 (4) **	66
(−)-Baclofen	2.6 ± 0.2 (4)	3.5 ± 0.2	0.9 ± 0.2 (4) **	73
(+)-Baclofen	2.7 ± 0.2 (4)	5.3 ± 0.2	2.7 ± 0.2 (4)	7
(−)-Baclofen plus (+)-baclofen	3.2 ± 0.2 (4)	4.2 ± 0.2	1.0 ± 0.2 (4) **	70

Baclofen enantiomers were added to the cortical slice preparations at a concentration of 1 μM. Results expressed as mean ± s.e.mean of 5-HT concentration in the medium before and after addition of K^+ (35 mM) with number of experiments in parentheses. Different from K^+-evoked release in control experiment: $^{**}P < 0.01$.

release of 5-HT was similar to that seen in the presence of GABA alone (Table 2). Similarly, the presence of flurazepam (1 μM) did not alter the inhibition of the potassium evoked release by GABA (Table 2).

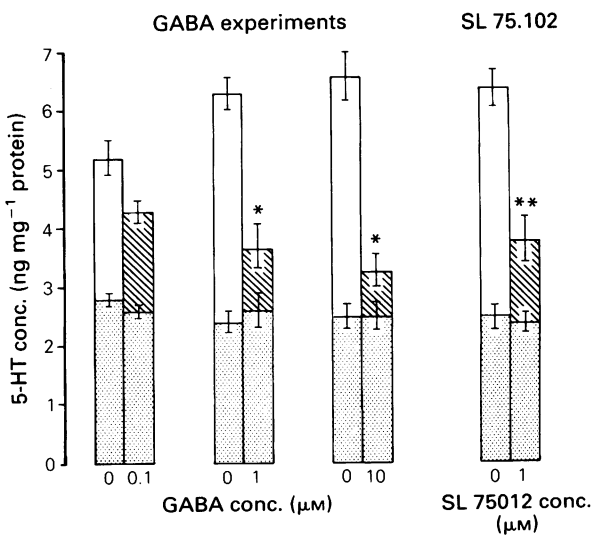

Figure 4 The effect of γ-aminobutyric acid (GABA) and 4-([4(chlorphenyl) (5-fluoro-2-hydroxyphenyl) methylene]-amino)butyricacid (SL75.102) on the release of 5-hydroxytryptamine (5-HT). Slices were incubated in the presence and absence of GABA (0.1–10 μM) or SL75.102 (1 μM). The stippled columns represent basal release; potassium-evoked release in the absence of drug is shown by the unshaded columns, while the hatched columns represent potassium-evoked release in the presence of GABA or SL75.102. Results are shown as mean of 4–6 experiments with s.e.mean shown by vertical lines. Different from control: $^{*}P < 0.05$; $^{**}P < 0.01$.

Discussion

The most striking finding from the study is the much lower doses of GABA and baclofen required to inhibit 5-HT release than those reported by either Bowery *et al.* (1980) or Schlicker *et al.* (1984). For example, the latter authors, who examined the effects of baclofen on electrically evoked release of [^{3}H]-5-HT from slices prepared from rat cortex, reported only a 30% inhibition in the presence of GABA (1 mM) while in the current study a 60% inhibition was observed at 1 μM. One possible reason for this apparent discrepancy is a species difference, mice rather than rats having been used in our study. Another reason could be that slices were incubated in our experiments rather than superfused. A more likely explanation, however, is that the release of endogenous 5-HT is modulated differently from that of [^{3}H]-5-HT. There is evidence that 5-HT exists in different storage pools within neurones (Auerbach & Lipton, 1985); these might be susceptible to heteroceptor-mediated effects to different degrees. Indeed, differences in the properties of the release of endogenous dopamine compared to [^{3}H]-dopamine have recently been described (Herdon *et al.*, 1985).

The potassium-dependency of the release of endogenous 5-HT is in agreement with the findings of Auerbach & Lipton (1985). The relationship of the inhibitory effect of baclofen with potassium concentration seems complex, a greater degree of inhibition being seen after stimulating with 35 mM potassium than at 25 mM. However, this finding is in agreement with the results of Bowery *et al.* (1980).

In the absence of a specific antagonist at the proposed $GABA_B$-receptor it is not possible to be completely certain that the inhibitory effects seen with GABA and baclofen are due to an action at this site. However, the dose-dependent nature of the inhibition with GABA and baclofen and the fact that the

Table 2 Effect of γ-aminobutyric acid (GABA) with and without bucuculline and flurazepam on the potassium-evoked release of 5-hydroxytryptamine (5-HT) from mouse cortical slices

Drug added	*5-HT concentration* (ng mg^{-1} protein) *Basal release*	*Total release*	K^+*-evoked release*	*% Inhibition of* K^+*-evoked release*
None	2.7 ± 0.2 (12)	6.1 ± 0.3 (12)	3.3 ± 0.2 (12)	—
GABA (1 μM)	2.6 ± 0.3 (4)	3.8 ± 0.3 (4)	1.2 ± 0.2 (4) **	67
GABA (1 μM) + bicuculline (10 μM)	2.6 ± 0.3 (4)	4.0 ± 0.3 (4)	1.3 ± 0.2 (4) *†	65
GABA (1 μM) + flurazepam (1 μM)	2.7 ± 0.3 (4)	3.8 ± 0.2 (4)	1.1 ± 0.1 (4) **†	59

Slices were incubated with GABA (1 μM) with and without bucuculline (10 μM) or flurazepam (1 μM). Results expressed as mean ± s.e.mean of 5-HT concentration in the medium before and after addition of K^+ (35 mM) with number of experiments in parentheses. Different from K^+-evoked release in control experiments: $*P<0.05$; $**P<0.01$; † not different from K^+-evoked release in presence of GABA alone.

baclofen-induced inhibition was stereospecific argue in favour of this possibility. Furthermore, as previously reported by Schlicker *et al.* (1984), the absence of antagonism of the inhibitory effect of GABA by bicuculline, an antagonist at the $GABA_A$ site, and the lack of potentiation by flurazepam, a drug which enhances the action of GABA at $GABA_A$-receptors (Haefeley, 1983) also argue against the involvement of $GABA_A$-receptors in the inhibitory response.

In behaviour studies on the 5-HT-mediated head twitch (Gray *et al.*, 1986a) progabide produced a similar inhibition of the response to that seen after baclofen. Since progabide is insoluble in water we were not able to examine its effects in the current *in vitro* studies. However, progabide has a major metabolite SL75.102 (Worms *et al.*, 1982) which has been shown to bind to both $GABA_A$- and $GABA_B$-receptors (Lloyd *et al.*, 1982). The effect of this compound was studied therefore in the current investigation. SL75.102 produced a similar inhibition of the potassium-evoked release of 5-HT to that seen in the presence of GABA or (±)-baclofen. This suggests that its inhibitory effects might be mediated through $GABA_B$-receptors and that progabide might have a similar mode of action.

The absence of antagonism by (+)-baclofen of the action of (−)-baclofen in inhibiting 5-HT release contrasts with the finding of Schlicker *et al.* (1984); however, it is consistent with our behavioural experiments in which no antagonism of the inhibition of head twitch seen after (−)-baclofen was seen when (+)-baclofen and (−)-baclofen were administered together (Gray *et al.*, 1986a).

Both presynaptic and postsynaptic inhibition have been noted electrophysiologically with baclofen (Newberry & Nicoll, 1985). Since the frontal cortex predominantly contains the terminals of 5-HT neurones, it seems probable that the inhibitory effects seen in our experiments are mediated presynaptically. This is in agreement with our previous behavioural experiments (Gray *et al.*, 1986a) which demonstrated that baclofen and progabide inhibited the head twitch evoked by the 5-HT precursor, 5-hydroxytryptophan but not that produced by the agonist 5-methoxy-*NN*-dimethyl-tryptamine.

Several other lines of evidence point to the importance of an inhibitory control of GABA on central 5-HT neurones in the brain. For example, Scatton *et al.* (1984) have pointed to the possibility of an inhibitory effect of GABA and GABA antagonists on 5-HT turnover in the raphé nucleus. This effect, presumably via an action on nerve cell bodies, was apparently mimicked by $GABA_A$ agonists.

Whether the *in vivo* effects of GABA-mimetic drugs on 5-HT function are more the result of their action at 5-HT cell bodies or terminals must await further investigation. Such studies would be simplified greatly by the existence of a selective $GABA_B$ antagonist drug.

J.A.G. is a MRC Clinical Training Fellow. We thank Professor D.G. Grahame-Smith for helpful discussions. We gratefully acknowledge the gifts of SL75.102 (Synthélabo, Paris), baclofen (Ciba-Geigy), flurazepam (Roche Products) and fluoxetine (Eli Lilly).

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(Received September 22, 1986.
Revised February 16, 1987.
Accepted March 9, 1987.)