Effects of the putative antagonists phaclofen and δ -aminovaleric acid on GABA_B receptor biochemistry

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1 Phaclofen and δ -aminovaleric acid (δ -AVA) have been reported to be antagonists at γ -aminobutyric acid_B (GABA_B) receptors. Phaclofen, δ -AVA and related compounds were examined for potency and specificity at GABA_B and GABA_A receptors in rat cortical membranes labelled with [³H]-(-)-baclofen and [³H]-muscimol, respectively. Additionally phaclofen and δ -AVA were examined in two functional tests of central GABA_B activity in rat cortical slices, namely the inhibition of forskolin-stimulated cyclic AMP accumulation, and the potentiation of isoprenaline-stimulated cyclic AMP accumulation.

2 δ -AVA (IC₅₀ = 11.7 μ M) was 20 fold more potent than phaclofen (IC₅₀ = 229 μ M) on GABA_B receptor binding. All compounds possessing a phosphonic acid group, including phaclofen, which were active at GABA_B receptors were inactive at GABA_A receptors, while δ -AVA was equally potent at both receptors. Several compounds exhibited Hill coefficients of less than unity in displacing [³H]-(-)-baclofen binding.

3 (-)-Baclofen inhibited forskolin-stimulated cyclic AMP accumulation (IC₅₀ = 7.9 μ M) but this effect was not stereospecific. Phaclofen (1 mM) was inactive against this inhibition but produced a potentiation of the forskolin effect. δ -AVA (1 mM) failed to antagonize the effect of baclofen; rather it mimicked baclofen.

4 (-)-Baclofen (10 μ M) potentiated isoprenaline-stimulated cyclic AMP accumulation, an effect antagonized by phaclofen (1 mM). δ -AVA (1 mM) may be a weak antagonist but also potentiated basal cyclic AMP accumulation.

5 We conclude that neither δ -AVA nor phaclofen are potent specific GABA_B receptor antagonists.

Introduction

Receptors for the inhibitory neurotransmitter γ -aminobutyric acid (GABA) are divided into two subtypes, GABA_A and GABA_B, on the basis of their pharmacological specificity (Hill & Bowery, 1981). The GABA_A receptor is a well characterized complex of binding sites for GABA, the benzodiazepines, and barbiturates together with an associated chloride ion channel, (see for example Olsen, 1981). Potent GABA_A agonists include GABA, muscimol and isoguvacine while bicuculline and its methohalide salts are potent antagonists (Curtis *et al.*, 1970). Baclofen is a potent GABA_B agonist with no activity at GABA_A receptors. However there is a lack of potent and specific GABA_B receptor antagonists. δ -Aminovaleric acid (δ -AVA) has been shown to antagonize the action of (-)-baclofen in the periphery (Muhyaddin *et al.*, 1982) and more recently in the CNS (Watling & Bristow, 1986), although δ -AVA is equally potent at GABA_A and GABA_B

receptors (Bowery *et al.*, 1983). The phosphonic acid derivative of baclofen, phaclofen, has also been reported to antagonize the actions of baclofen in the periphery (Kerr *et al.*, 1987) and this compound has recently been reported to antagonize central GABA_B receptors (Dutar & Nicoll, 1988). We describe here the use of filtration binding assays with [³H]-(-)-baclofen and [³H]-muscimol to look at the potency of an extensive range of compounds, including δ -AVA, phaclofen and related compounds at GABA_B receptors and their specificity for GABA_B compared with GABA_A receptors. However, such ligand binding assays do not provide any information on whether compounds active at GABA_B receptors are agonists or antagonists; such a differentiation can only be made by use of functional assays of GABA_B receptor activity. We have therefore also examined the actions of δ -AVA and phaclofen on two reported actions of (-)-baclofen. These are the inhibition by (-)-baclofen of forskolin-stimulated cyclic AMP accumulation (Hill &

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Dolphin, 1984; Wojcik & Neff, 1984; Hill, 1985; Karbon & Enna, 1985; Xu & Wojcik, 1986) and the potentiation by (–)-baclofen of the stimulation of cyclic AMP production by agonists such as nor-adrenaline, isoprenaline and vasoactive intestinal peptide (VIP) (Hill & Dolphin, 1984; Karbon & Enna, 1985; Watling & Bristow, 1986).

A preliminary account of some of this work has been presented to the British Pharmacological Society (Robinson *et al.*, 1988).

Methods

Animals

In all experiments male Lister Hooded rats (200–250 g) were used, obtained from Harlan Olac, Bicester.

Radioligand binding assays

A frozen osmotically-shocked membrane preparation from rat cortex was used for both [³H]–(–)-baclofen and [³H]–muscimol binding assays.

Frozen cortex was homogenized in 6 volumes ice-cold buffer (10 mM Tris-HCl, pH 7.4, containing 0.32 M sucrose), using a Potter Elvehjem homogenizer fitted with a teflon pestle. The homogenate was centrifuged at 500 *g* for 10 min. The resulting supernatant was stored on ice and the pellet rehomogenized and centrifuged as above. This was repeated so that three pooled supernatants gave a total suspension of 18 volumes *g*^{–1} tissue wet weight. The suspension was centrifuged at 30,000 *g* for 30 min at 4°C and the resulting crude synaptosomal membrane pellet resuspended in 60 volumes ice cold distilled water using an Ultra-Turrax homogenizer. The membrane suspension was given a second osmotic shock by use of centrifugation and resuspension in distilled water as before. Membrane pellets were harvested by centrifugation at 30,000 *g* for 12 min at 4°C and stored at –20°C for [³H]–(–)-baclofen binding. For [³H]–muscimol binding, following freezing at –20°C overnight, membrane pellets were thawed at room temperature, resuspended in 90 volumes distilled H₂O using an Ultra-Turrax homogenizer and harvested by centrifugation at 30,000 *g* for 12 min at 4°C. All membrane pellets were stored at –20°C for up to 2 months prior to their use in binding assays.

The activity of ligands at the GABA_B receptor was assayed by a [³H]–baclofen equilibrium binding assay as described by Hill & Bowery (1981). The binding procedure was novel, however, in that binding was terminated by filtration rather than centrifugation. Frozen membrane pellets were thawed, resuspended in assay buffer (50 mM Tris-HCl, pH 7.4

containing CaCl₂, 2.5 mM) using an Ultra-Turrax homogenizer and incubated at room temperature for 45 min. The membranes were harvested by centrifugation at 30,000 *g* for 12 min at 20°C, and then washed in assay buffer a further three times, with 15 min incubations between each centrifugation. Final equilibrated membrane pellets were resuspended in assay buffer (1–2 mg protein ml^{–1}) and assayed immediately. The binding assay was performed in 96 well microtitre plates in a total incubation volume of 200 µl, and routinely utilized 30 nM [³H]–baclofen. Specific binding was defined with an excess of GABA (100 µM). Binding was initiated by the addition of tissue (approximately 0.25 mg protein) to the microtitre well. Following 10 min incubation at 25°C the binding was terminated by filtration through glass fibre filters using a Skatron semi-automatic cell harvester. Filters were rapidly washed with approximately 1 ml ice cold assay buffer in a 2 s wash time. Following drying, the radioactivity on the filters was measured by liquid scintillation counting.

The activity of ligands at the GABA_A receptor was assayed by a [³H]–muscimol equilibrium filtration binding assay (Williams & Risley, 1979). Frozen membrane pellets were thawed, resuspended in ice cold assay buffer (50 mM Tris-HCl, pH 7.4) by use of an Ultra-Turrax homogenizer and centrifuged at 30,000 *g* for 10 min at 4°C. The resulting pellet was resuspended in assay buffer (1–1.5 mg protein ml^{–1}) and used immediately in the binding assay. The assay was essentially as described above for [³H]–(–)-baclofen binding, but 10 nM [³H]–muscimol and approximately 0.2 mg protein were used and the assay was incubated on ice for 10 min. Following filtration, the filters were rapidly washed with approximately 4.5 ml ice cold assay buffer in a 7 s wash time.

Forskolin- and isoprenaline-stimulated cyclic AMP accumulation

Modulation by baclofen of forskolin- and isoprenaline-stimulated cyclic AMP accumulation was measured essentially as described by Hill (1985). Slices (300 × 300 µm, 45°) of cerebral cortex were cut with a McIlwain tissue chopper and were washed for 90 min at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4, containing (mM): NaCl 118.5, KH₂PO₄ 1.18, MgSO₄ 1.18, NaHCO₃ 25, CaCl₂ 2.54 and glucose 11) and continually gassed with O₂/CO₂ (95/5%). The buffer was changed every 30 min. Aliquots (50 µl) of packed slices were preincubated with (–)-baclofen (and test drugs where appropriate) for 15 min at 37°C before the addition of buffer, forskolin or isoprenaline to give a final incubation volume of 1 ml. Slices were routinely incubated with 0.5 µM forskolin

Figure 1 Synthesis of phaclofen. Reagents were (a) NaOEt, diethyl oxalate; (b) Na₂CO₃, HCHO; (c) PBr₃; (d) P(OEt)₃; (e) PtO₂, H₂(g); (f) 6N HCl.

for 20 min or with 1 μ M isoprenaline for 10 min. In the experiments in which forskolin was used, the buffers contained the phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX) during the 15 min pre-incubation and 20 min incubation with forskolin. Slices were rapidly sedimented by centrifugation, the supernatants removed, and 20 μ l concentrated HCl (10M) added. After at least 30 min at room temperature, the slices were neutralised with 236 μ l 1M NaOH and sedimented by centrifugation. The supernatant was removed and the cyclic AMP content measured with a cyclic AMP assay kit, based on the protein binding assay of Brown *et al.* (1971). The residual slices were solubilized in 1M NaOH containing SDS (0.7% w/v) and Na₂CO₃ (2% w/v) prior to analysis of the protein. Data are expressed as

pmol cyclic AMP accumulated mg⁻¹ protein and were analysed by a one-way analysis of variance followed by a protected one-tailed *t* test.

Protein estimation

Protein concentration of membrane suspensions and solubilized tissue slices was determined by a phenol reagent technique (Dawson & Heatlie, 1984) with bovine serum albumin used as standard.

Synthesis of phaclofen

Phaclofen (3-amino-2-(4-chlorophenyl) propylphosphonic acid) was conveniently synthesized by the route shown in Figure 1. No attempt was made to

Table 1 Potencies (pIC_{50}) and Hill coefficients (n_H) of compounds in $GABA_B$ and $GABA_A$ binding assays

Compound	$GABA_B$		$GABA_A$
	pIC_{50}	n_H	pIC_{50}
GABA	7.59 ± 0.09	0.67 ± 0.05	7.00
(-)-Baclofen	7.17 ± 0.15	0.77 ± 0.11	i.a
SL 75102	7.12 ± 0.26	0.61 ± 0.08	6.42
3-Hydroxy-GABA	5.95	1.0	6.16
(+)-Baclofen	5.17 ± 0.08	0.94 ± 0.04	i.a
Muscimol	5.68 ± 0.18	0.54 ± 0.05	8.05
3-Aminopropane sulphonic acid	5.60 ± 0.11	0.60 ± 0.04	7.31
4-Aminobutyl phosphonic acid	5.14 ± 0.13	0.67 ± 0.03	i.a
3-Phenyl-GABA	5.11 ± 0.10	0.75 ± 0.12	i.a
3-Aminopropyl phosphonic acid	5.00 ± 0.12	0.89 ± 0.09	i.a
δ -Aminovaleric acid	4.93 ± 0.15	0.62 ± 0.17	5.14
2(3-Chlorophenyl)GABA	4.75 ± 0.11	0.81 ± 0.02	i.a
δ -Aminolevulinic acid	4.50 ± 0.07	1.32 ± 0.17	3.46
4-Guanidinobutyric acid	4.33	0.77	4.31
6-Aminohexanoic acid	4.17 ± 0.02	0.83 ± 0.08	i.a
Kojic acid	3.87	1.19	i.a
Phaclofen	3.64 ± 0.17	0.77 ± 0.17	*i.a
Isoguvacine	i.a	—	7.27
4-Imidazole acetic acid	i.a	—	6.10
Bicuculline methiodide	i.a	—	4.70

Data are means $\pm$ s.e.mean, where applicable from 2–16 experiments using triplicate assays. i.a, less than 10% displacement at $100 \mu M$; *i.a, less than 10% displacement at 1 mM. 4-Aminopyridine, 7-aminoheptanoic acid, 2-(4-chlorophenyl)ethylamine, 3-guanidinopropionic acid, kynurenic acid, L-kynurenine, L-kynurenine sulphate, D,L-pipecolic acid and progabide all displaced less than 10% [3H]-(-)-baclofen binding at $100 \mu M$.

optimise the reported yields. This route has the advantage of avoiding the photochemical step that is an integral part of the published method of synthesis of phaclofen (Chiefari *et al.*, 1987).

Drugs

Cyclic AMP 3H radioassay kits were supplied by Amersham International plc (Aylesbury). [3H]-(-)-baclofen ($39 Ci mmol^{-1}$) and [3H]-muscimol ($17.5 Ci mmol^{-1}$) were obtained from New England Nuclear, Du Pont (UK) Ltd. 4-Imidazole acetic acid and 3-hydroxy-GABA were supplied by Aldrich Chemical Co, and isoguvacine by Research Biochemicals Inc., U.S.A. SL75102 (4-[(4-chlorophenyl)(5-fluoro-2-hydroxy phenyl) methylene] amino butyric acid) and progabide were gifts from Synthelabo, Paris, France and (+)- and (-)-isomers of baclofen were gifts from Ciba Geigy Ltd., Switzerland. All other fine chemicals were obtained from Sigma Chemical Co., Poole.

Results

GABA receptor binding assays

The binding of [3H]-(-)-baclofen to rat cortical membranes was measured by a filtration binding

method. In initial experiments it was observed that single point binding data were comparable using the centrifugation and filtration assay. However, when filtration was used the specific binding represented a much greater percentage of the total binding than when centrifugation was used ($67 \pm 8\%$, $n = 11$ instead of $21 \pm 6\%$, $n = 5$, both given as mean $\pm$ s.e.mean). The binding of [3H]-(-)-baclofen (10 – 150 nM) was saturable and Scatchard analysis revealed a dissociation constant (K_D) of 73 nM and B_{max} of 0.34 pmol mg^{-1} protein. The K_D was 99 nM when derived by kinetic analysis (data not shown).

An extensive range of compounds was tested for activity in displacing 30 nM [3H]-(-)-baclofen binding. The potencies and Hill coefficients of active compounds were derived by Hill analysis of the displacement curve (Table 1). Compounds which exhibited activity at $GABA_B$ receptors were further analysed in [3H]-muscimol binding. The potencies of compounds in displacing [3H]-muscimol (10 nM) are also presented in Table 1.

Forskolin-stimulated cyclic AMP accumulation

Forskolin stimulated the production of cyclic AMP in slices of rat cortex in a concentration-dependent manner, saturating at $1.5 \mu M$ (data not shown); $0.5 \mu M$ forskolin was used in all subsequent experiments.

Figure 2 Effects of baclofen isomers on basal and forskolin-stimulated ($0.5 \mu\text{M}$) cyclic AMP accumulation in slices of rat cerebral cortex. (–)-Baclofen (hatched columns) and (+)-baclofen (stippled columns) were present at $10 \mu\text{M}$; control (open columns). Data are mean from three experiments using triplicate assays; s.e.mean shown by vertical bars.

Neither (–)- or (+)-baclofen, at a concentration of $10 \mu\text{M}$, had any effect on basal cyclic AMP accumulation (in the absence of forskolin) but at $10 \mu\text{M}$ both isomers produced 40% inhibition of forskolin-stimulated cyclic AMP accumulation (Figure 2). The inhibition by baclofen of forskolin-stimulated cyclic AMP accumulation was not stereoselective as (–)-baclofen and (+)-baclofen were equipotent (IC_{50} values (μM): (–)-baclofen, 7.9 ± 4.0 , (+)-baclofen, 8.0 ± 4.0 , $n = 3$ in triplicate assays). At high concentration (1 mM), GABA mimicked (–)-baclofen in inhibiting forskolin-stimulated cyclic AMP accumulation. The effect of the GABA_A agonist, isoguvacine was inconsistent (data not shown).

At a concentration of 1 mM , phaclofen and δ -AVA were without effect on basal cyclic AMP accumulation (in the presence of IBMX, Figure 3). δ -AVA mimicked (–)-baclofen in inhibiting forskolin-stimulated cyclic AMP accumulation and did not show any significant reversal of the inhibition produced by (–)-baclofen (Figure 3).

Phaclofen appeared to reverse the inhibition by (–)-baclofen of forskolin-stimulated cyclic AMP

Figure 3 Effects of phaclofen and δ -aminovaleric acid (δ -AVA) on the inhibition by (–)-baclofen of forskolin-stimulated cyclic AMP production in slices of rat cerebral cortex. Phaclofen and δ -AVA (both at 1 mM) were tested as indicated on basal (open columns), forskolin-stimulated ($0.5 \mu\text{M}$, stippled columns) and forskolin-stimulated cyclic AMP accumulation in the presence of $10 \mu\text{M}$ (–)-baclofen (hatched columns). Data are mean from 4–8 experiments using triplicate assays; s.e.mean shown by vertical bars. Basal cyclic AMP accumulation in the presence of phaclofen or δ -AVA was not significantly different from the control value; $P > 0.05$. Forskolin-stimulated cyclic AMP accumulation in the presence of phaclofen, δ -AVA and/or (–)-baclofen was tested for significant difference from the control forskolin-stimulated value: ** $P < 0.01$.

production but this was an indirect result of potentiation by phaclofen of the forskolin-stimulated cyclic AMP (Figure 3).

Isoprenaline-stimulated cyclic AMP accumulation

Isoprenaline ($1 \mu\text{M}$) doubled basal cyclic AMP accumulation in slices of rat cortex and this effect was potentiated by $10 \mu\text{M}$ (–)-baclofen (Figure 4). Phaclofen (1 mM) had no significant effect on cyclic AMP

Figure 4 Effects of phaclofen and δ -aminovaleric acid (δ -AVA) on the potentiation by $(-)$ -baclofen of isoprenaline-stimulated cyclic AMP production in slices of rat cerebral cortex. Phaclofen and δ -AVA (both at 1 mM) were tested, as indicated, on basal (open columns), isoprenaline-stimulated ($1 \mu\text{M}$, stippled columns) and isoprenaline-stimulated cyclic AMP accumulation in the presence of $10 \mu\text{M}$ $(-)$ -baclofen (hatched columns). Data are mean from 4–8 experiments using triplicate assays; s.e.mean shown by vertical bars. Basal cyclic AMP accumulation in the presence of phaclofen or δ -AVA was tested for significant difference from the control basal value. Isoprenaline-stimulated cyclic AMP accumulation in the presence of phaclofen, δ -AVA and/or $(-)$ -baclofen was tested for significant difference from the control isoprenaline-stimulated value; * $0.05 > P > 0.01$; ** $P < 0.01$.

accumulation in the absence or presence of isoprenaline but completely reversed the potentiation observed with $(-)$ -baclofen. At 1 mM, δ -AVA more than doubled basal cyclic AMP accumulation and therefore showed an apparent potentiation of isoprenaline-stimulated cyclic AMP accumulation. In the presence of δ -AVA, $(-)$ -baclofen showed a smaller potentiation of isoprenaline-stimulated cyclic AMP accumulation than control (Figure 4).

Discussion

The preparation of membranes and the assay used to measure GABA_B binding were essentially the same as originally reported by Hill & Bowery (1981) who used both [^3H]-GABA and [^3H]- $(-)$ -baclofen. However the methods described here differ in that membranes were prepared from rat cortex rather than whole brain, and the binding assay was terminated by filtration rather than centrifugation of the membranes. This change did not lead to a marked difference in single point binding of [^3H]-baclofen and B_{max} and K_D values were in the range reported by others (Drew *et al.*, 1984; Karbon *et al.*, 1983; Mennini *et al.*, 1986), although the B_{max} value was lower than that reported by Bowery *et al.* (1983). The use of filtration is quicker and more convenient than centrifugation and offers the major advantage of a greatly increased percentage specific binding signal.

With respect to the compounds presented in Table 1 which have previously been tested in GABA_B binding, the pharmacology described is in close agreement with Bowery *et al.* (1983), both in the order of potency and absolute potency in displacing [^3H]- $(-)$ -baclofen. The only discrepancy is that $(+)$ -baclofen is 10 fold more potent in our GABA_B assay than in that of Bowery *et al.* (1983).

The parallel study of compounds in both GABA_A and GABA_B binding assays (Table 1) enables some conclusions on their receptor subtype specificity to be made. SL 75012, 3-hydroxy-GABA, muscimol, 3-aminopropyl sulphonic acid and δ -AVA all lack GABA_B specificity as previously reported (Lloyd *et al.*, 1982; Bowery *et al.*, 1983; Falch *et al.*, 1986). All compounds active in GABA_B binding possessing a phenyl ring were inactive in GABA_A binding, and all the phosphonic acid derivatives active in GABA_B binding were inactive in GABA_A binding. This provides a basis for the GABA_B specificity of the putative antagonist phaclofen, and although phaclofen was 20 fold less potent than δ -AVA in displacing [^3H]- $(-)$ -baclofen binding (Table 1), it was specific.

When discussing potencies of compounds in the GABA_B binding assays, what must be borne in mind is that it is based on agonist binding and may represent binding to a high affinity agonist state. Consequently, the potency of antagonists in displacing [^3H]- $(-)$ -baclofen binding may not indicate absolute potency.

Forskolin stimulated the accumulation of cyclic AMP in slices of rat cortex and this was inhibited by $(-)$ -baclofen with a maximal inhibition of 40% (Figure 2), in agreement with previous workers (Hill & Dolphin, 1984; Hill, 1985; Karbon & Enna, 1985). Any effect of $(-)$ -baclofen which is via GABA_B receptors should be stereoselective with respect to the $(-)$ -isomer (Hill & Bowery, 1981). One group of

workers (Hill & Dolphin, 1984; Hill 1985) has shown that in the presence of IBMX, in slices of rat cortex, (–)-baclofen was at least 100 fold more potent than (+)-baclofen on forskolin stimulated cyclic AMP accumulation. We found that (–)-baclofen exhibited the same potency in inhibiting forskolin-stimulated cyclic AMP accumulation as reported by Hill (1985) but we were unable to demonstrate stereospecificity although this was demonstrated in both binding (Table 1) and in other studies on baclofen (Stirling *et al.*, 1989). Because of the lack of stereoselectivity and the fact that phaclofen does not antagonize we cannot therefore conclude that the action of baclofen on forskolin stimulated cyclic AMP accumulation is via GABA_B receptors.

To date, no pharmacology has been published for the baclofen/forskolin interaction. δ -AVA appeared to act as an agonist mimicking baclofen by inhibiting forskolin-stimulated cyclic AMP accumulation (Figure 3). It failed to antagonize the inhibition by (–)-baclofen. In contrast, phaclofen potentiated the effect of forskolin, and did not affect the (–)-baclofen inhibition of this activity.

Agonists, such as noradrenaline, isoprenaline and VIP, stimulate cyclic AMP production in brain slices, and (–)-baclofen augments this effect (Hill & Dolphin, 1984; Hill, 1985; Karbon & Enna, 1985; Watling & Bristow, 1986). Watling & Bristow (1986) reported that δ -AVA (1 mM) antagonized the potentiation by (–)-baclofen of VIP-stimulated cyclic AMP accumulation in slices of rat cortex. Our data clearly demonstrate that at 1 mM, phaclofen is an antagonist at the GABA_B receptors responsible for modulating isoprenaline-stimulated cyclic AMP accumulation (Figure 4). δ -AVA may also have acted as an antagonist in this system and partially attenuated the (–)-baclofen stimulation but this is difficult to conclude given the raised basal cyclic AMP levels produced by addition of δ -AVA alone. In conclusion, phaclofen, and possibly δ -AVA are antagonists at GABA_B receptors modulating agonist-stimulated cyclic AMP production.

The evidence that δ -AVA was a GABA_B antagonist came from rat anococcygeus muscle (Muhyaddin *et al.*, 1982) and guinea-pig ileum (Sawynok, 1986), although there has been some dispute as to whether the latter effect has a GABA_A component (Allan & Dickenson, 1986; Schwarz *et al.*, 1988). δ -AVA also antagonized the GABA_B receptor responsible for the (–)-baclofen-induced reduction of population spikes in hippocampal slices

(Nakahiro *et al.*, 1985) and reversed baclofen-induced hypothermia and analgesia (Hunter *et al.*, 1988). While δ -AVA may antagonize the GABA_B receptors augmenting agonist-stimulated cyclic AMP accumulation in brain slices (Watling & Bristow, 1986), we have previously shown that it is an agonist at the GABA_B receptor modulating K⁺-stimulated [Ca²⁺]_i in synaptosomes (Stirling *et al.*, 1989) and it also acted as an agonist in the forskolin system.

Phaclofen, first described as an antagonist at peripheral GABA_B receptors (Kerr *et al.*, 1987), has been shown to be an antagonist at postsynaptic GABA_B receptors modulating K⁺-conductances in hippocampal slices (Dutar & Nicoll, 1988) and at GABA_B receptors in the locus coeruleus (Olpe *et al.*, 1988). While we have shown that phaclofen antagonizes the potentiation by (–)-baclofen of isoprenaline-stimulated cyclic AMP accumulation, it appeared as an agonist at GABA_B receptors modulating synaptosomal K⁺-stimulated [Ca²⁺]_i (Stirling *et al.*, 1989) and has no effect on either the (–)-baclofen/forskolin interaction or on most of the behavioural tests we have used (Hunter *et al.*, 1988).

What is clear from the available data on putative GABA_B antagonists is that they do not always behave in the same manner in different systems, possibly because of receptor heterogeneity. It is striking that several of the compounds examined (Table 1) exhibit Hill coefficients markedly less than unity, supporting this concept. One tentative division could be pre- and postsynaptic GABA_B receptors. Phaclofen is an antagonist at postsynaptic but not presynaptic GABA_B receptors in the hippocampus (Dutar & Nicoll, 1988; Nicoll & Dutar, 1988). Furthermore phaclofen and δ -AVA are agonists at the GABA_B receptors modulating synaptosomal K⁺-stimulated [Ca²⁺]_i which are presumably presynaptic (Stirling *et al.*, 1989). It is not known whether the GABA_B receptors augmenting isoprenaline-stimulated cyclic AMP accumulation are pre- or postsynaptic, but as phaclofen, and probably δ -AVA, are antagonists, we suggest that they are postsynaptic even though phaclofen has been claimed to antagonize presynaptic rather than postsynaptic GABA_B receptors in the spinal cord (Kerr *et al.*, 1987), and the peripheral GABA_B receptors antagonized by δ -AVA are presynaptic (Muhyaddin *et al.*, 1982).

In conclusion, more potent and specific antagonists are necessary before we can fully characterize GABA_B receptors and define their roles in modulation of the nervous system.

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