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A comparison of the relative activities of a number of $GABA_B$ antagonists in the isolated vas deferens of the rat

[1]Judith M. Hills, Madelene M. Larkin & William Howson

SmithKline Beecham Pharmaceuticals Ltd, The Frythe, Welwyn, Herts. AL6 9AR

1 A series of $GABA_B$ receptor antagonists were tested against (±)-baclofen for activity on the presynaptic $GABA_B$ receptor in the rat vas deferens.

2 All the antagonists tested caused a rightward shift in the concentration-response curve to (±)-baclofen.

3 pA_2 values calculated from full Schild analysis were as follows: phaclofen, $pA_2 = 4.3$; δ-amino valeric acid, $pA_2 = 4.4$; 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348), $pA_2 = 5.0$; 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA), $pA_2 = 4.5$.

4 These results show that none of the above compounds possess potent antagonist activity at the $GABA_B$ receptor (i.e. $pA_2 > 6$) in this peripheral tissue. In addition, the more recently available phosphinic acid antagonists, appear to offer no great advance over the $GABA_B$ antagonists previously available.

Introduction

$GABA_B$ receptors are known to be located on peripheral autonomic and enteric nerve terminals (Bowery *et al.*, 1981; Ong & Kerr, 1983). Their pharmacological function appears to be the modulation of excitatory neurotransmitter release in adrenergic systems such as field stimulated guinea-pig atria, rat vas deferens and anococcygeus (Bowery *et al.*, 1981; Muhyaddin *et al.*, 1982) and cholinergic systems such as the guinea-pig ileum (Kaplita *et al.*, 1982; Ong & Kerr, 1983). There is evidence for the existence of intrinsic GABAergic neurones in the enteric nervous system (for review see Jessen *et al.*, 1987; Hills & Jessen, 1991) although their precise role in intestinal function is still uncertain. Recent reports suggest that GABAergic neurones may additionally be present in prevertebral sympathetic ganglia (Hills *et al.*, 1988), and in superior cervical ganglia (Kasa *et al.*, 1988; Wolff *et al.*, 1989). There is to date however, little evidence for a GABAergic innervation in the other autonomically innervated tissues such as the heart, and reproductive organs, studied here.

Limited success in investigations of the physiological significance of the $GABA_B$ receptor subtype has been obtained with the agonist, baclofen. However, few definitive studies have been undertaken because of the lack of an effective $GABA_B$ antagonist.

Recently, a number of investigators have used phaclofen to delineate the function of $GABA_B$ receptors in central systems (Dutar & Nicoll, 1988). This and other $GABA_B$ antagonists such as saclofen and 2-hydroxy saclofen have been considered to be more potent and more selective than previously used compounds such as δ-amino valeric acid (DAVA) and 4-aminobutylphosphonic acid (4-ABPA) (Kerr *et al.*, 1988; 1989). In addition to these compounds, Ciba-Geigy have published a patent (Baylis *et al.*, 1989) claiming $GABA_B$ antagonist activity for a series of phosphinic acids.

Here, using baclofen as agonist, we have examined the relative antagonist potencies of a range of $GABA_B$ antagonists (Figure 1), including two phosphinic acid analogues, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348, Bittiger *et al.*, 1989) and 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA) (Ciba-Geigy), on the isolated vas deferens of the rat as an *in vitro* functional pharmacological assay system.

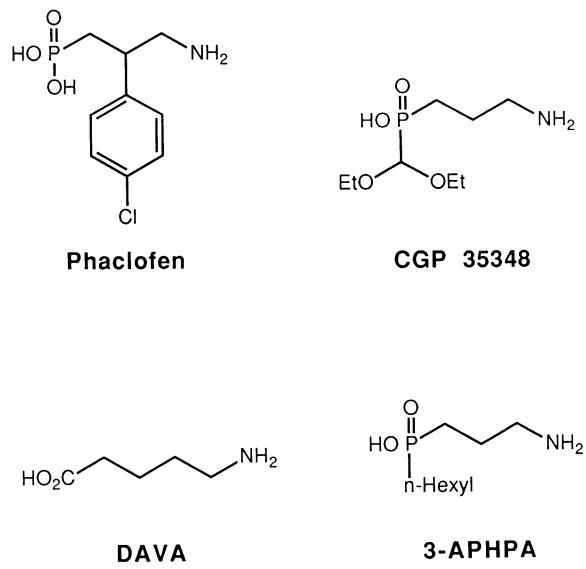

Figure 1 Chemical structures of the $GABA_B$ antagonists phaclofen, δ-amino valeric acid (DAVA), 3-aminopropyl(diethyloxymethyl)-phosphinic acid (CGP 35348) and 3-aminopropyl-(n-hexyl)phosphinic acid (3-APHPA) used in this study.

Methods

Male wistar rats (200–250 g) were killed by a blow to the head and their vasa deferentia removed. Vas deferens preparations were suspended between two platinum ring electrodes in organ baths containing modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.3, $NaHCO_3$ 16.3, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.7, which was continually gassed with $95\%O_2/5\%CO_2$. A resting tension of 1 g was applied and the preparations were field stimulated from Grass SD11 stimulators with the following stimulus parameters: 1 ms pulses of supramaximal voltage at 50 Hz for 100 ms every 10 s. Isometric muscle responses were measured with a strain gauge transducer (Biomed) and displayed on an Ormed multitrace pen recorder. Preparations were allowed to equilibrate for 1 h before the addition of a compound to the organ bath.

[1] Author for correspondence.

Baclofen concentration-response curves were constructed sequentially and the inhibitory response calculated as % maximum response to baclofen. Antagonists were tested by adding the appropriate concentration to the organ bath 10 min prior to baclofen. At least four concentrations of each antagonist was tested in the range 10–1000 μM, and each was tested on at least four vas deferens preparations from separate animals. pA_2 values were derived from full Schild analysis. Statistical evaluation has been carried out by linear regression analysis and 95% confidence intervals (CI) calculated for the pA_2 values and slopes obtained from the Schild analysis.

Drugs used

(±)-Baclofen (Research Biochemicals Inc.); δ-amino valeric acid (DAVA), (Sigma); phaclofen, (Tocris Neuramin); 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348) (W. Howson, SB) and 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA) (W. Howson, SB), were used. All compounds were dissolved in distilled water, subsequent dilutions being made in distilled water, and compounds were added to the organ bath in volumes no greater than 1% total volume.

Results

Baclofen inhibits the field stimulation-induced twitch response in the rat vas deferens (IC_{50} = 5.9 ± 1.4 μM, n = 8). Such an effect has been previously documented for the mouse vas deferens (Bowery *et al.*, 1981). Baclofen is thought to reduce the release of the excitatory neurotransmitter from the sympathetic nerve endings via $GABA_B$ receptors located presynaptically (Hill & Bowery, 1986).

Table 1 Antagonism of baclofen concentration-response curves in the isolated vas deferens by phaclofen, δ-amino valeric acid (DAVA), 3-aminopropyl(diethyloxymethyl)-phosphinic acid (CGP 35348) and 3-aminopropyl(n-hexyl)-phosphinic acid (3-APHPA)

Antagonist	pA_2	*Slope*
Phaclofen	4.25 (4.07, 4.55)	1.02 (0.66, 1.37)
DAVA	4.42 (4.16, 4.89)	0.86 (0.54, 1.18)
CGP 35348	5.03 (4.61, 5.98)	0.64 (0.35, 0.93)*
3-APHPA	4.55 (4.29, 4.96)	1.01 (0.66, 1.36)

The data are derived from Schild plots with at least 4 points obtained from at least 4 tissues from separate animals.
* Value is significantly different from one, * $P < 0.05$

All the antagonists tested produced a rightward shift in the concentration-response curve to (±)-baclofen. For each antagonist, increasing concentrations produced progressive near parallel shifts in the concentration-response curves and no reduction in the maximum response to (±)-baclofen was observed (Figure 2). pA_2 values for each antagonist and the slope of the plot derived from Schild analysis (Figure 3) are given in Table 1.

As may be seen from Table 1 and Figure 4a, the numerical differences in the pA_2 values obtained for the various antagonists tested were not great, the new Ciba-Geigy compounds (CGP 35348 and 3-APHPA) being only marginally more potent than for example, phaclofen or DAVA. Statistical analysis of the slopes of the plots (see Figure 4b), shows pha-

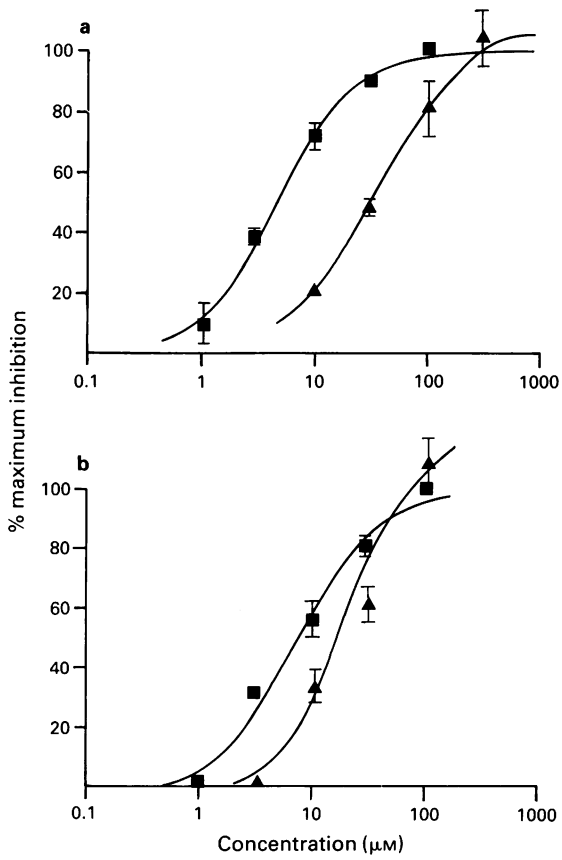

Figure 2 Concentration-response curves for the (±)-baclofen-mediated inhibition of the field stimulation induced twitch response in the rat vas deferens: (a) control curve (■) and curve in the presence of 3-aminopropyl(diethyloxymethyl)phosphinic acid (CGP 35348, 100 μM) (▲); (b) control curve (■) and curve in the presence of 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA, 30 μM) (▲) where $n > 4$.

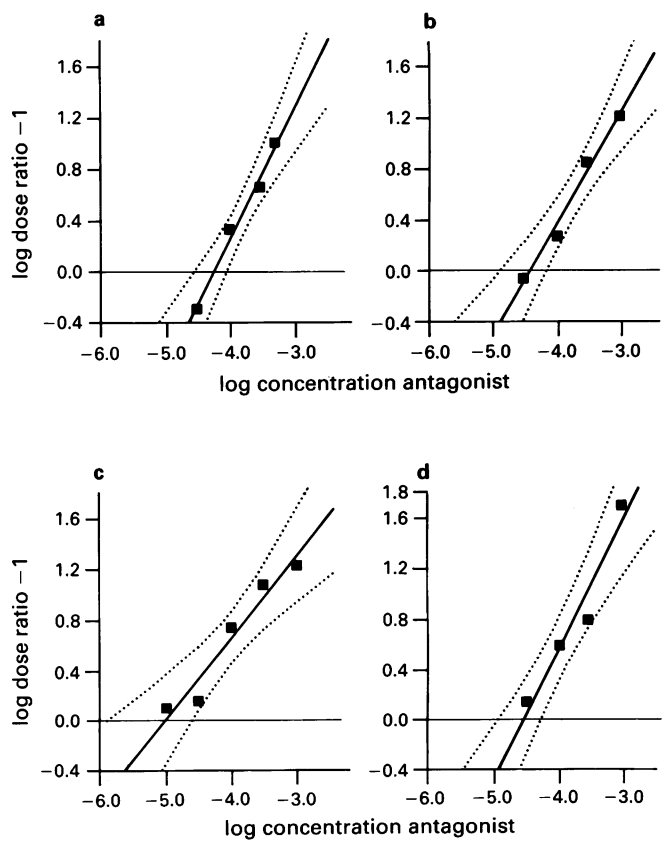

Figure 3 Schild plots (log dose ratio − 1 versus log antagonist concentration) for phaclofen (a), δ-amino valeric acid (DAVA) (b), 3-aminopropyl(diethyloxymethyl)phosphinic acid (CGP 35348) (c) and 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA) (d), tested against (±)-baclofen in the rat vas deferens. The graphs show the best line fit with 95% confidence intervals. The pA_2 may be obtained from the negative logarithm of the intercept on the x axis.

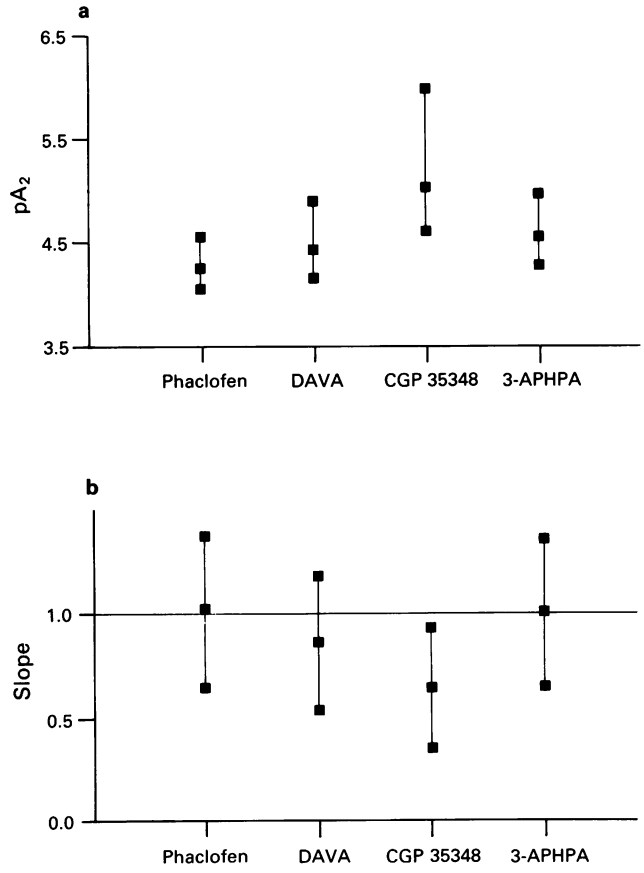

Figure 4 A graphical representation of calculated pA_2 values (a), and slopes (b) obtained from Schild analysis for phaclofen, δ-amino valeric acid (DAVA), 3-aminopropyl(diethyloxymethyl)phosphinic acid (CGP 35348) and 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA). The bars show the 95% CI for each value.

clofen, DAVA and 3-APHPA to have slopes close to one, and it can therefore be assumed that these compounds are true competitive GABA$_B$ antagonists. Although no reduction in the maximal agonist response was observed with CGP 35348, the Schild plot gave a slope significantly less than one, (see 95% CI for CGP 35348 on Table 1). A slope of less than one obtained from Schild analysis of antagonist data is normally taken to indicate non-competitive antagonism and thus to invalidate the pA_2 value so derived.

None of the antagonists tested showed agonist activity at high concentrations, indicating that they were not partial agonists. In the presence of the GABA$_B$ antagonists, high baclofen concentrations (100–300 μM) sometimes caused a greater inhibition of the twitch response than was observed with baclofen in the absence of antagonist (see Figure 2b). This phenomenon which caused a slight steepening of the concentration-response curves, occurred with all four of the antagonists tested in about 50% of experiments with each compound. There was a tendency towards the potentiation occurring at low antagonist concentrations for phaclofen and DAVA and at high antagonist concentrations for CGP 35348 and 3-APHPA, showing no clear association with compounds having a slope less than one in the Schild analysis.

Discussion

The results of studies using a variety of experimental systems indicate that compounds such as phaclofen and DAVA are antagonists of GABA$_B$ receptor-mediated pharmacological responses (Curtis *et al.*, 1988; Kerr *et al.*, 1988; 1989). Phaclofen has been claimed to be selective for GABA$_B$-mediated events (Dutar & Nicoll, 1988; Soltesz *et al.*, 1988; Karlsson & Olpe, 1989) although its binding shows only weak affinity for the GABA$_B$ receptor (phaclofen $IC_{50} = 118\,\mu M$) (Bowery, 1989). DAVA has been used as a GABA$_B$ antagonist since the early eighties (Muhyaddin *et al.*, 1982); however, results with this compound are often complicated by its GABA$_A$ agonist activity (Allan *et al.*, 1986; Bowery, 1989). The limited data available on CGP 35348 suggests that it is selective for the GABA$_B$ receptor, is active *in vitro* against baclofen at concentrations between 100–300 μM, and inhibits agonist binding in the rat cortex with an IC_{50} of 35 μM (Bittiger *et al.*, 1989). No data specifically relating to 3-APHPA have been published.

In agreement with previous reports, the pA_2 values obtained in our experiments are low and the antagonist phaclofen, which has a structure containing a chlorophenyl substituted ring, has similar activity in terms of antagonist potency, to the unsubstituted compounds, DAVA and the phosphinic acids (CGP 35348 and 3-APHPA). These relatively poor pA_2 values are borne out by the excessive concentrations often employed in pharmacological studies required to antagonize baclofen- or GABA-mediated effects (Kerr *et al.*, 1987; Dutar & Nicoll, 1988; Hunter *et al.*, 1989). The experiments with CGP 35348, suggest that this compound is not a true competitive antagonist. It is possible that there is allosteric modulation or non-receptor interaction which accounts for some of the observed antagonism seen with this compound. Recent reports have claimed that CGP 35348 is an effective antagonist against baclofen *in vivo* at doses of 30–100 mg kg^{-1} (Bittiger *et al.*, 1989).

The observation that potentiation of high baclofen mediated inhibition of twitch responses occurred in the presence of antagonist, is not readily explicable. One possible explanation for this effect may be an increase in tissue sensitivity to the agonist caused by the presence of the antagonist. This phenomenon was not further investigated in these experiments, although it would be of interest to see if the effect were peculiar to baclofen or occurred either with other GABA$_B$ agonists such as 3-aminopropylphosphinic acid or in other tissues where a postsynaptic rather than a presynaptic GABA$_B$ receptor was present.

In conclusion these results confirm that phaclofen, DAVA, CGP 35348 and 3-APHPA are weak but effective GABA$_B$ antagonists. Although the most potent antagonist, CGP 35348 (pA_2, 5.03), offers some improvement in antagonist activity, it would appear not to be a true competitive pharmacological antagonist at the GABA$_B$ receptor.

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