Br. J. Pharmacol. (1991), **102**, 5–6 **SPECIAL REPORT**

Phosphinic acid analogues of GABA are antagonists at the $GABA_B$ receptor in the rat anococcygeus

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CGP 35348 (3-aminopropyl(diethoxymethyl)phosphinic acid) and 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA) were tested in the rat anococcygeus muscle against CGP 27492 (3-aminopropylphosphinic acid), a selective $GABA_B$ agonist, for their antagonist activity. Their antagonist potency was compared with that of 2-hydroxysaclofen. The pA_2 values for CGP 35348, 3-APHPA and 2-hydroxysaclofen were 5.38, 4.86, 4.45 respectively in the rat anococcygeus muscle. These results confirm the previous reports of $GABA_B$ antagonist activity for these compounds and show a marginal improvement in potency over 2-hydroxysaclofen.

Introduction A physiological role for $GABA_B$ receptors in the mammalian central nervous system has recently been suggested (Dutar & Nicoll, 1988) as a result of work with the $GABA_B$ antagonist, phaclofen (Kerr *et al.*, 1987). However phaclofen, and analogues derived from it such as saclofen and 2-hydroxysaclofen, bind relatively weakly to the $GABA_B$ receptor (Bowery, 1989) and are effective only at high concentrations in pharmacological assay systems (Kerr *et al.*, 1987; Curtis *et al.*, 1988; Hills *et al.*, 1989). A series of phosphinic acids have recently been reported to show $GABA_B$ receptor antagonist activity (Bittiger *et al.*, 1990). They are similar in structure to the potent $GABA_B$ receptor agonist CGP 27492 (see Figure 1) (Hills *et al.*, 1989), which is the phosphinic acid derivative of GABA. Here we have examined two of these compounds for $GABA_B$ antagonist activity in the field-stimulated rat anococcygeus muscle.

Methods Rat anococcygeus muscles were prepared as previously described (Hills *et al.*, 1989). Three cumulative agonist concentration-response curves were constructed on each preparation; control curve to agonist alone, curve in the presence of antagonist and wash out curve with a 30 min period between each. At least four concentrations of antagonists were tested on at least four preparations. pA_2 values were derived from Schild plots following statistical analysis carried out using a statistical package (fitline in RSE) and 95% confidence intervals calculated.

The following compounds were used; 3-amino-propylphosphinic acid (CGP 27492, for reference see Hills *et al.*, 1989; prepared by W. Howson at SmithKline Beecham); 2-hydroxysaclofen (Tocris Neuramin); 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348); 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA, Ciba Geigy). The Ciba-Geigy compounds were prepared as described in their recent patent (Bayliss *et al.*, 1989). All compounds were dissolved in distilled water, dilutions being made in distilled water, and compounds were added to the organ bath in volumes no greater than 1% total volume.

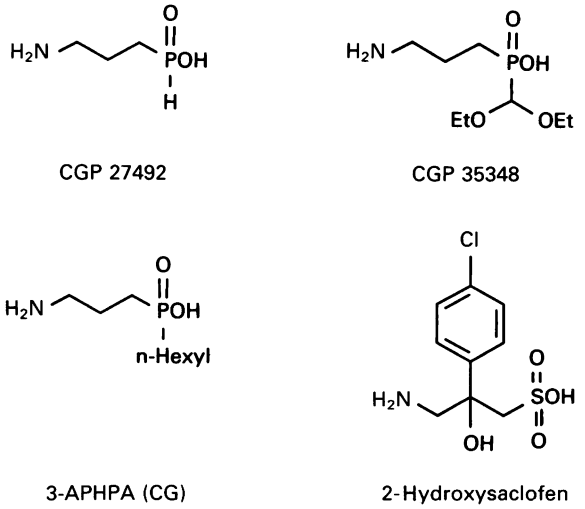

Figure 1 Chemical structures of the $GABA_B$ agonist 3-aminopropylphosphinic acid (CGP 27492) and the $GABA_B$ antagonists, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348), 3-aminopropyl(n-hexyl)phosphinic acid (3-APHPA) and 2-hydroxysaclofen.

Results 3-Aminopropylphosphinic acid caused a concentration-dependent inhibition of the electrical stimulation induced contractions in the rat anococcygeus muscle as previously reported (Hills *et al.*, 1989). The IC_{50} value obtained was $0.09 \pm 0.01\,\mu M$ ($n = 12$) and 2-hydroxysaclofen caused a concentration-dependent reversible antagonism. The pA_2 value obtained from the Schild plot was 4.45 (4.23, 4.79 95% CL) with a slope of 1.03 (0.72, 1.31 95% CL) indicating competitive antagonism. Similarly, CGP 35348 and 3-APHPA, caused a concentration-dependent, reversible antagonism of concentration-response curves to the $GABA_B$ agonist. The pA_2 value obtained from straight line Schild plots for CGP 35348 was 5.38 (4.4, infinity 95% CL) with a calculated slope of 0.67 (−0.10, 1.44 95% CL), and for 3-APHPA, 4.86 (4.68, 5.10 95% CL) with a slope of 0.95 (0.76, 1.13 95% CL). Although CGP 35348 appears to be the most potent antagonist, despite repeated experimentation, the confidence limits obtained are wide. All three compounds tested caused a parallel shift in the concentration-response curve to 3-aminopropylphosphinic acid.

Discussion These results provide the first demonstration of functional $GABA_B$ receptor antagonism by phosphinic acids in the peripheral tissue studied here. CGP 35348 has previously been shown to antagonize baclofen-mediated hyperpolarizations in hippocampal cells at $100\,\mu M$ and to be active *in vivo* against baclofen at $30\,mg\,kg^{-1}$ in the rotarod test (Bittiger *et al.*, 1990). No published data are available which specifically relate to 3-APHPA (see Bayliss *et al.*, 1989) and we are therefore able to confirm reported data with CGP 35348 and extend the information available by demonstrating functional $GABA_B$ antagonism in the rat anococcygeus with CGP 35348 and 3-APHPA.

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Previous work with 2-hydroxysaclofen, the sulphonic acid analogue of baclofen, has reported antagonism of $GABA_B$-mediated inhibition in the guinea-pig ileum (Kerr *et al.*, 1988) and other tissues (Kerr *et al.*, 1988; Curtis *et al.*, 1988) and in our experiments, clear antagonism was obtained in the rat anococcygeus.

Although these results confirm that CGP 35348 and 3-APHPA are functional $GABA_B$ antagonists *in vitro*, they fail to show any significant improvement in terms of potency over antagonists such as 2-hydroxysaclofen either studied here or elsewhere. They are however active *in vivo* (Bayliss *et al.*, 1989; Bittiger *et al.*, 1990) and this together with their CNS penetrating properties (Olpe *et al.*, 1989), may well turn out to be the feature of this class of compounds which makes them superior to those already available.

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