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Short communication

Evaluation of 3-aminopropanesulphonamide analogues of GABA as antagonists at $GABA_B$ receptors in peripheral and central preparations

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Abstract

Cholinergic twitch responses in the guinea-pig isolated ileum, and spontaneous discharges in rat neocortical slices, were depressed by the $GABA_B$ receptor agonist baclofen. These actions were reversibly antagonised by the sulphonamide derivatives (*R,S*)-2-hydroxy-3-phthalimidopropanesulphonamide (HPIPS), 3-amino-*N*-benzoylpropanesulphonamide (ABPS) and 3-phthalimidopropanesulphonamide (PIPS) which produced rightwards shifts of the baclofen concentration–response curves, with pA_2 values ranging between 4.1 and 4.3 in both preparations. From these results, HPIPS, ABPS and PIPS constitute a novel class of antagonists at $GABA_B$ hetero-receptors.

Keywords: Baclofen; $GABA_B$ receptor antagonist; Brain rat slice; Sulphonamide analogue; Ileum, guinea pig

1. Introduction

To date, there are two broad classes of antagonists for the G-protein-coupled $GABA_B$ receptors; the *N*-benzyl-aminopropylphosphinic acids with a variety of P-alkyl substituents on the phosphinic moiety (Froestl and Mickel, 1997), and a series of 5-substituted-1,4-morpholineacetic acids (Blythin et al., 1996), none of which have so far shown any discrimination between $GABA_B$ auto- and hetero-receptors. The new lead compound 3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide (AHPNS) was originally shown to be an antagonist at central and peripheral $GABA_B$ hetero-receptors (Kerr et al., 1995), but lacking activity at $GABA_B$ autoreceptors (Kerr and Ong, 1996). To further extend our studies on sulphonamide derivatives of GABA as a potential, additional class of antagonists for these receptors, we have now examined a series of analogues related to AHPNS. Since their actions at peripheral and central neurones have not been reported previously, three of these sulphonamides have been chosen for evaluation, namely 3-phthalimidopropanesulphonamide (PIPS), its hydroxy-analogue (*R,S*)-2-hydroxy-3-phthalimidopropanesulphonamide (HPIPS), and 3-amino-*N*-benzoylpropanesulphonamide (ABPS), the *N*-benzoyl analogue of AHPNS (Fig. 1). Using guinea-pig isolated ileal preparations, and rat neocortical slices, we have now characterised their pharmacological properties, and evaluated their potencies as antagonists at $GABA_B$ receptors.

2. Materials and methods

2.1. Guinea-pig ileal preparations

All experiments described here were performed in strict accordance with the National Health and Medical Research Council Code of practice for the care and use of animals for experimental purposes in Australia. Male guinea-pigs, weighing between 200 and 400 g, were killed by cervical dislocation. Segments of the terminal ileum, 2–3 cm in length, were quickly removed and mounted in 5 ml organ baths containing modified Krebs–bicarbonate solution of the following composition [mM]: NaCl 125, KCl 3.0, NaH_2PO_4 1.4, $NaHCO_3$ 25, $CaCl_2$ 2.0, $MgSO_4$ 1.0 and glucose 10 (pH 7.4 at 37°C). The Krebs solution was continuously aerated with a gas mixture of 95% O_2 and 5% CO_2 as previously described (Ong et al., 1994). After 60 min equilibration in Krebs solution, pulses (duration 0.5–1 ms, frequency 0.15 Hz, just submaximal voltage)

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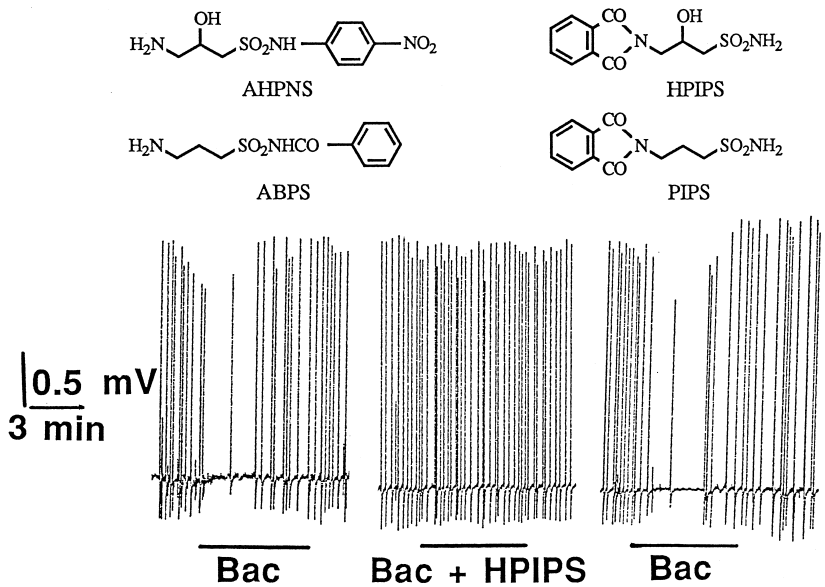

Fig. 1. Chemical structures of a series of sulphonamide related analogues of GABA showing the following: (*R,S*)-2-hydroxy-3-phthalimidopropanesulphonamide (HPIPS), 3-amino-*N*-benzoylpropanesulphonamide (ABPS) and 3-phthalimidopropanesulphonamide (PIPS). For comparison, the original lead (*R,S*)-3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide analog (AHPNS) is shown. In a typical experiment, baclofen (Bac; 1 μM) induced a suppression of spontaneous discharges which was reversibly antagonized by HPIPS (300 μM), with a recovery of the control response to baclofen (Bac; 1 μM) upon wash-out of the test compounds.

were delivered from a Grass S48 stimulator to give transmural stimulation of cholinergic intrinsic neurones. Effects of drug treatments were examined on repetitive twitch contractions evoked by field stimulation, elicited through ring electrodes positioned around the segments of the ileum. Mechanical activity of the longitudinal muscle was recorded isometrically using Grass FT03 force transducers, and changes in tension were displayed on a Grass Model 79 polygraph.

The $GABA_B$ receptor agonist baclofen was applied at 20 min intervals, and the antagonist added 3–5 min before the agonist was tested. Control responses to the agonist were routinely re-established after washing out the antagonist. Concentration–response curves to baclofen, in the presence and absence of different doses of the antagonist were constructed and the inhibitory response to baclofen was calculated as percent maximum response to baclofen. By interpolation from the concentration–response curve, the half maximally-effective agonist concentration (EC_{50}) was derived for the agonist baclofen alone, and in the presence of the antagonists. Three concentrations of each antagonist were tested on six different preparations, and the pA_2 value was derived as an average from the relationship $pA_2 = \log(CR - 1) - \log[B]$, where $(CR - 1)$ is the concentration ratio $- 1$, and $[B]$ the antagonist concentration. All numerical data on the concentration–response curves were expressed as means ± S.E.M. Student's *t*-test for paired and unpaired samples was used to assess the significance ($P < 0.05$) of differences between mean values of the concentration–response effects; *n* represents the number of preparations used for each drug treatment. Drug volumes never exceeded 1% of the total bath volume, and all drugs were dissolved in distilled water.

2.2. Preparation of rat neocortical slices

Rat neocortical slices were prepared from halothane anaesthetized outbred male adult Sprague–Dawley rats (250–350 g) which were decapitated. The brains were rapidly dissected out and immersed for 30 min in ice-cold oxygenated Krebs solution gassed with 95% O_2:5% CO_2 (pH 7.4) of the following composition (in mM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11, $MgSO_4$ 1.3. Cerebral cortical slices (400 μM thick) were prepared by cutting coronal sections using a vibraslice microtome (Campden Instruments, UK) and a radial wedge was cut from each side of the dorsal mid-line to yield slices of cingulate cortex and corpus callosum 2–3 mm wide. The slices were subsequently equilibrated in gassed Krebs solution at room temperature (20–23°C) for 60 min prior to experimentation.

Using a superfusion method based on a grease-gap system as described previously (Ong et al., 1990), the slices from the neocortex were superfused with gassed Mg^{2+}-free Krebs medium at 25°C delivered by a peristaltic pump at 1 ml/min. $MgSO_4$ was omitted in the Mg^{2+}-free medium. DC potentials between the cingulate cortex and corpus callosum were monitored on a chart recorder using Ag/AgCl electrodes, agar/saline bridges and a high input-impedance DC amplifier. The neocortical slices developed spontaneous paroxysmal discharges after a period of equilibration in Mg^{2+}-free Krebs medium for

10–15 min. The $GABA_B$ receptor agonist baclofen, added to the superfusing medium, was applied to the cortical side of the tissue for 2 min and the preparation was allowed 30 min recovery between drug applications. The antagonist was first superfused for 2 min and then added together with the agonist for a further 2 min. Results were quantified by counting the number of spontaneous discharges in 5 min epochs, in the absence and presence of test compounds, and the values expressed as a percentage depression of the average control discharge rate before the addition of drugs; depending on the concentration of the agonist, its effects outlasted the period of application. Concentration–response curves for the agonist were constructed, in the absence and presence of the antagonist. The EC_{50} value, that is the concentration which produced 50% inhibition of the discharge rate, was calculated from the concentration–response curve, and estimates of apparent pA_2 values were made. Each experiment was repeated on 8 slices obtained from at least 3 different animals and data expressed as mean ± S.E.M.

2.3. Drugs

Racemic baclofen was a gift from Novartis Pharmac (Basel, Switzerland), and the sulphonamide analogues (*R*,*S*)-2-hydroxy-3-phthalimidopropanesulphonamide (HPIPS), 3-amino-*N*-benzoylpropanesulphonamide (ABPS) and 3-phthalimidopropanesulphonamide (PIPS) were synthesised by R.H. Prager and J. Bowden (Flinders University, South Australia). 3-Aminopropanesulphonic acid was purchased from Sigma.

3. Results

The sulphonamide analogues, HPIPS, ABPS and PIPS were investigated for antagonist actions at $GABA_B$ receptors using baclofen-induced depression of electrically-evoked cholinergic twitch contractions in the guinea-pig isolated ileal preparations. On its own, racemic baclofen elicited a concentration-dependent inhibition of twitch responses (estimated EC_{50} value = 3 ± 1.0 μM) which was reversibly antagonised by HPIPS, ABPS and PIPS (100, 300 and 600 μM; $n = 6$ for each concentration). Within 30 min, the baclofen response recovered to control level after tissue wash-out of the drugs. All three compounds antagonised surmountably the inhibitory responses to baclofen, producing clear rightward shifts in the baclofen concentration–response curve, with apparent pA_2 values of 4.2 ± 0.05 for HPIPS, 4.2 ± 0.1 for ABPS, and 4.1 ± 0.15 for PIPS, respectively. Fig. 2a shows representative data for HPIPS (100, 300 and 600 μM) that produced a concentration-dependent rightward shift of the baclofen concentration–response curve with no significant alteration in the maximal response to baclofen ($n = 6$). None of the sulphonamide analogues in this series had any effect on the amplitude of the twitch contractions, nor did they affect contractile responses to the $GABA_A$ receptor agonist 3-aminopropanesulphonic acid (10 μM).

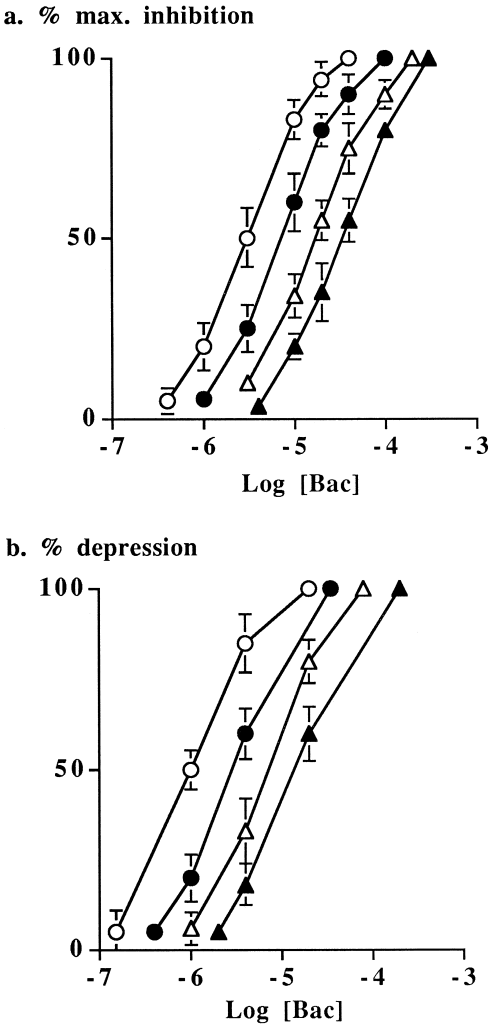

Fig. 2. Concentration–response curves for (*R*,*S*)-baclofen-induced (a) inhibition of cholinergic twitch contractions in the guinea-pig isolated ileum, and (b) suppression of the frequency of spontaneous discharges in the rat isolated neocortical slices, in the absence and presence of (*R*,*S*)-2-hydroxy-3-phthalimidopropanesulphonamide (HPIPS). (a) In the ileum, the concentration-response curve for baclofen (**O**) was shifted to the right, in a parallel fashion by HPIPS (● 100, △ 300, and ▲ 600 μM). Responses are represented as a percentage of the maximal depression induced by baclofen, expressed as a 100%. Each point represents the mean and standard error of the mean of six determinations. (b) In the neocortex maintained in Mg^{2+}-free Krebs medium, the concentration–response curve for baclofen (**O**) was displaced to the right, in a parallel fashion by HPIPS (● 100, △ 300, and ▲ 600 μM). Values are expressed as a percentage depression of the control discharge rate. Each point represents the mean and standard error of the mean of eight determinations.

Rat neocortical slices superfused with Mg^{2+}-free Krebs medium exhibited spontaneous depolarisations within 30 min. Once these were established at a stable rate, baclofen, superfused for 2 min, reduced the frequency of discharges in a concentration-dependent manner. The depressant ef-

fects to baclofen generally lasted some 5–15 min and returned to baseline levels within 20 min following the initial wash-out of the drug. Fig. 1 shows a representative experiment in which application of baclofen (Bac; 1 μM) for 2 min suppressed the firing rate by 50% over a 6 min period, as indicated by the horizontal bar, before returning to baseline levels. Pre-treatment with HPIPS (300 μM) alone for 2 min did not affect the discharge rate or amplitude, but in combination with baclofen (1 μM) for 2 min, reversibly antagonised the baclofen-induced suppression of spontaneous discharges (Fig. 1). Following wash-out of the baclofen and HPIPS, there was a complete recovery of the spontaneous activity and the depressant response to baclofen (1 μM) within 30 min. As illustrated in Fig. 2b, the threshold concentration for baclofen was 0.15 μM and the estimated half-maximally effective concentration was 1 μM, with a maximal depression at 20 μM. In order to quantify the antagonist potencies of HPIPS, ABPS AND PIPS, the effects of three standard concentrations of these compounds (100, 300 and 600 μM) on the baclofen concentration–response curve were measured (Fig. 2b; $n = 8$). Increasing concentrations of these sulphonamide analogues caused a progressive shift of the baclofen concentration–response curve to the right, without depression of the maximum response. Upon wash-out, there was a complete recovery of the spontaneous activity, and the depressant response to baclofen. Using the ratio method and averaging, this yielded apparent pA_2 values of 4.3 ± 0.2, 4.1 ± 0.15 and 4.2 ± 0.1, respectively for HPIPS, ABPS and PIPS. When applied alone, none of these compounds affected the discharge rate or amplitude, nor did they affect depressive responses to the $GABA_A$ receptor agonist 3-aminopropanesulphonic acid (10 μM).

4. Discussion

In the present study, HPIPS, ABPS and PIPS, sulphonamide derivatives of GABA, exhibited reversible and competitive antagonism of baclofen-induced responses in the guinea-pig isolated ileum and rat neocortical slices. In both preparations, all three analogues produced parallel rightward shifts in the concentration–response curves to baclofen, yielding comparable pA_2 values ranging between 4.3 and 4.1. Furthermore, they were selective for $GABA_B$ receptors, as they showed no $GABA_A$ receptor antagonist properties, being inactive against responses mediated by the $GABA_A$ receptor-agonist 3-aminopropanesulphonic acid. They also did not have any agonist actions at $GABA_A$ or $GABA_B$ receptors since they did not affect electrically-elicited responses to ileal twitch contractions, or spontaneous activity in the rat neocortex under Mg^{2+}-free conditions. These results indicate that HPIPS, ABPS and PIPS display similar potencies at $GABA_B$ hetero-receptors in central and peripheral tissues, as does the 2-hydroxy-nitrophenyl sulphonamide analogue AHPNS (Fig. 1) which was the first sulphonamide analogue reported to block $GABA_B$ receptors (Kerr et al., 1995). When compared to the baclofen phosphono-analogue phaclofen, the antagonist potency of the latter at $GABA_B$ hetero-receptors is slightly weaker than that of HPIPS, ABPS and PIPS ($pA_2 = 4.0$ for phaclofen; Kerr et al., 1987) which are in turn about ten times weaker than the sulphonic analogue 2-hydroxysaclofen (Kerr et al., 1988).

In the original sulphonamides AHPNS and its *N*-(4-chlorophenyl)-analogue which lack a substituent on the ammonium functionality, increasing the electron-withdrawing property of the *N*-substituent on the sulphonamide brought about an improvement in the $GABA_B$ receptor antagonist activity (Kerr et al., 1995). Here, this is again born out with the appearance of improved antagonist potency at $GABA_B$ hetero-receptors due to the stronger electronegativity of the *N*-benzoyl substituent on the sulphonamide moiety of ABPS. More unexpected was the similar improvement of potency in PIPS and HPIPS, where the sulphonamide moiety itself has no *N*-substituent, the improvement instead being brought out by the conversion of the 3-amino functionality to a protected phthalimido group. Although these analogues display a much weaker affinity for $GABA_B$ receptors than *N*-benzyl-substituted 3-aminopropanephosphinic acid antagonists developed by Novartis Pharmac (Froestl and Mickel, 1997), nevertheless, they are novel from a structure–action viewpoint since there have not been any examples of phthalimido substituents at the amino-functionality exhibiting antagonist properties at $GABA_B$ receptors. As with AHPNS (Kerr and Ong, 1996), preliminary data indicates that HPIPS, ABPS and PIPS have no activity at $GABA_B$ autoreceptors modulating [^{3}H]-GABA release evoked by electrical stimulation of rat brain slices, raising the possibility that these sulphonamides may provide a potential tool for distinguishing $GABA_B$ receptor subtypes pharmacologically; however, further work is required to substantiate this. In any case, the phthalimidopropanesulphonamide analogues proved to be an interesting and novel class of $GABA_B$ receptor antagonists, particularly if their potencies could be further improved.

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