ELSEVIER

European Journal of Pharmacology 294 (1995) 349–352

ejp

Short communication

3-Amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide, a new class of $GABA_B$ receptor antagonist in central and peripheral preparations

David I.B. Kerr [a,*], Jennifer Ong [a], Robert Hughes [b], Rolf H. Prager [a]

[a] *Department of Anaesthesia and Intensive Care, The University of Adelaide, Adelaide, South Australia 5005, Australia*
[b] *Department of Chemistry, Flinders University, Bedford Park, South Australia 5042, Australia*

Received 12 October 1995; accepted 17 October 1995

Abstract

Racemic 3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide (AHPNS), a sulphonamide analog of GABA, reversibly and competitively antagonised the concentration-dependent depression of cholinergic twitch contractions by baclofen, in the electrically stimulated guinea-pig isolated ileum, with a pA_2 of 4.0 ± 0.2. In the rat neocortex, maintained in Mg^{2+}-free medium, AHPNS (100–500 μM) also reversibly antagonised the baclofen (10 μM)-induced suppression of spontaneous discharges. AHPNS is a new class of $GABA_B$ receptor antagonist that has central and peripheral blocking actions.

Keywords: $GABA_B$ receptor; Baclofen; AHPNs ((*R*,*S*)-3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide); $GABA_B$ receptor antagonist; Ileum, guinea-pig; Neocortex, rat

1. Introduction

Originally, antagonists for bicuculline-insensitive $GABA_B$ receptors were developed from baclofen (4-amino-3-(4-chlorophenyl)-butanoic acid), the prototypic agonist (Bowery et al., 1981), by isosteric replacement of the carboxylate moiety with a phosphonic group (phaclofen) (Kerr et al., 1987) or a sulphonic group (saclofen) (Carruthers et al., 1995; Kerr et al., 1989). An additional 2-hydroxy substituent on the latter provided 2-hydroxysaclofen (Kerr et al., 1988), although only as a racemate, which has been much used in functional studies (Curtis et al., 1988; Harrison et al., 1990; Lambert et al., 1989). Furthermore, 3-aminopropylsulphonic acid (3-APS), the corresponding sulphonic analog of GABA (4-aminobutanoic acid), was earlier shown to be a $GABA_B$ receptor antagonist (Giotti et al., 1983), although its use is complicated by potent $GABA_A$ receptor agonist properties. In preliminary studies, we confirmed the antagonism with 3-APS and found the congener 3-amino-2-hydroxypropylsulphonic acid (2-OH-3-APS) to be a $GABA_B$ receptor antagonist with much reduced agonist activity at $GABA_A$ receptors (Kerr et al., 1990). As an extension of these studies on sulphonic analogs of GABA, we have now examined sulphonamide derivatives of 2-OH-3-APS, representative of a new class of $GABA_B$ receptor antagonists. We here show that the (*R*,*S*)-3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide analog (AHPNS; see chemical structure in Fig. 1) is a $GABA_B$ receptor antagonist, with no $GABA_A$ agonist activity, in the guinea-pig isolated ileum and rat neocortical slice.

2. Materials and methods

2.1. Guinea-pig ileal preparations

Male guinea-pigs, weighing between 200–400 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 oxygenated Krebs-bicarbonate solution as previously described (Ong et al., 1994). After 60 min equilibration in Krebs

* Corresponding author. Tel.: 61-8-303-5163; fax: 61-8-232-3283.

SSDI 0014-2999(95)00688-5

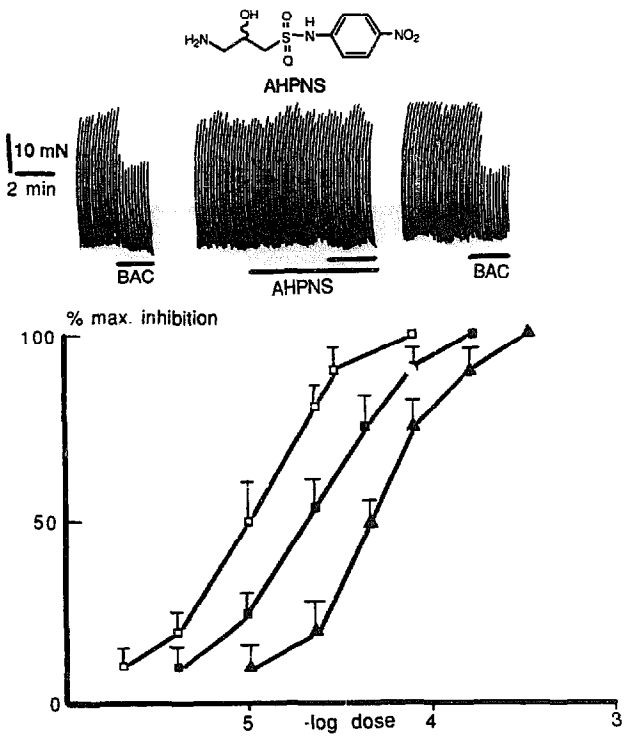

Fig. 1. Antagonism of the baclofen (BAC; 10 μM)-induced depression of cholinergic twitch contractions in the guinea-pig isolated ileum by the $GABA_B$ receptor antagonist (*R,S*)-3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide (AHPNS; 500 μM), with the chemical structure shown. There was a recovery of control response to baclofen with tissue wash-outs between drug applications ($n = 6$). Dose-response curves for baclofen-induced depression of cholinergic ileal twitch contractions, in the presence and absence of AHPNS. The dose-response curve for baclofen (□) was shifted to the right in a parallel manner by AHPNS (■ 200 μM; ▲ 500 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 6 determinations.

solution, pulses (duration 0.5–1 ms, frequency 0.15 Hz, just submaximal voltage) 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 percentage of the 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. Three concentrations of the antagonist were tested on 6 different preparations, and the pA_2 value was derived 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. Rat neocortical slice preparations

Outbred male adult Sprague-Dawley rats (250–350 g) were decapitated, their brains rapidly removed and immersed for 15 min in ice-cold Krebs solution oxygenated with 95% O_2 and 5% CO_2. 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 1.5–2 mm wide. Using a superfusion method based on a grease-gap system as previously reported (Ong et al., 1990), the neocortex was initially superfused with Mg^{2+}-containing Krebs medium at 28°C delivered by a peristaltic pump at 1 ml/min, and allowed to equilibrate for 30 min, followed by Mg^{2+}-free medium. For the Mg^{2+}-free medium, $MgSO_4$ was omitted. DC potentials between the cingulate cortex and corpus callosum were monitored by Ag/AgCl electrodes via agar/saline bridges with a high-input impedance DC amplifier, and responses were displayed on a chart recorder.

After a period of equilibration for 60 min under Mg^{2+}-free conditions, the neocortical slices developed spontaneous paroxysmal discharges. Drugs added to the superfusing medium were subsequently applied to the cortical side of the tissues for 5–10 min, usually at 30 min intervals depending on the recovery of the responses to control level. In this arrangement, recording cortex against corpus callosum, the spontaneous paroxysmal depolarisations caused an upward deflection in Mg^{2+}-free medium. Each experiment was repeated on 6 slices from 3 different animals.

2.3. Drugs

(*R,S*)-Baclofen was a gift from Ciba-Geigy (Basel, Switzerland), and the sulphonamide analogs (*R,S*)-3-

amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide analog (AHPNS), and the corresponding *N*-(4-chlorophenyl)- and *N*-(4-methoxyphenyl)-sulphonamides were synthesised by R. Hughes and R.H. Prager (Flinders University, South Australia).

3. Results

In the guinea-pig isolated ileum, racemic baclofen elicited a concentration-dependent depression of cholinergic twitch contractions, antagonised by the sulphonamide analog of GABA, AHPNS (200 and 500 μM respectively) (Fig. 1; $n = 6$). This antagonism was reversible and competitive, since AHPNS (200 and 500 μM) produced a clear rightward shift in the concentration-response curve for baclofen in a surmountable manner, with the mean calculated pA_2 value for AHPNS being 4.0 ± 0.2. The estimated EC_{50} value for baclofen-induced depression of twitch responses was 10 μM. As illustrated in Fig. 1, the depressant effect of baclofen (BAC, 10 μM) was completely antagonised by AHPNS (500 μM), with a recovery of the baclofen response to control level after tissue wash-out within 30 min. AHPNS did not show any $GABA_A$ agonist or $GABA_B$ partial agonist activity, as it did not affect the amplitude of the twitch contractions, or induce a contractile response.

Rat neocortical slices maintained in Mg^{2+}-free medium for 60 min showed repetitive paroxysmal discharges, which were consistently modified by baclofen in a concentration-dependent manner (1–50 μM; Ong et al., 1990). Using the present method of recording the discharges, it is difficult to construct quantitative concentration-response curves for baclofen, but nevertheless, its relative potency could be derived from the minimal dose causing total arrest of the discharges. In this study, the baclofen (BAC) concentration which effectively suppressed the discharges was 10 μM, this being antagonised by AHPNS over a concentration range of 100–500 μM. AHPNS (500 μM) itself did not affect the discharge rate or amplitude, showing an absence of $GABA_B$ agonist activity, but did antagonise the baclofen (10 μM)-induced suppression of spontaneous discharges ($n = 6$). Using 500 μM AHPNS, there was a complete recovery of the spontaneous activity, and the depressant response to baclofen after drug wash-out (Fig. 2; $n = 6$). AHPNS did not exhibit any $GABA_A$ agonist or antagonist activity, as it did not elicit any $GABA_A$ agonist effects, or block $GABA_A$ receptor-mediated suppression of spontaneous discharges induced by the $GABA_A$ receptor agonist 3-aminopropylsulphonic acid (50 μM).

Further *N*-substituents with less electron withdrawing effect than the 4-nitrophenyl substituent were also tested in the ileum and neocortex. Of these, the *N*-(4-chlorophenyl)-sulphonamide analog of AHPNS showed a weaker antagonist activity (approximate $pA_2 < 3.0$), whilst the *N*-(4-methoxyphenyl)-sulphonamide analog was inactive in the ileal preparations (data not shown). Both the *N*-(4-chlorophenyl)- and *N*-(4-methoxyphenyl)-sulphonamides did not affect $GABA_B$ receptor-mediated function in the neocortex, at concentrations of up to 1 mM. Furthermore, alkyl substituents (*i*- or *n*-butyl) at the ω-amino group, did not improve the low antagonist potency of the *N*-(4-chlorophenyl) analog of AHPNS in the neocortex or ileum.

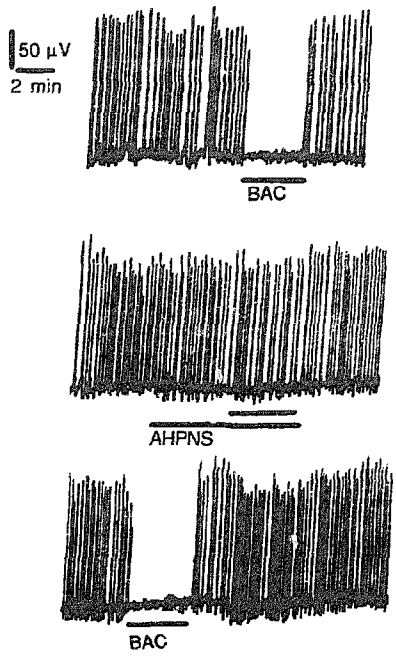

Fig. 2. In the rat isolated neocortical slice, maintained in Mg^{2+}-free Krebs medium, the depressant action of baclofen (BAC, 10 μM) on spontaneous discharges was completely and reversibly antagonised by 3-amino-2-hydroxy-*N*-(4-nitrophenyl)-propanesulphonamide (AHPNS; 500 μM). The control response to BAC was subsequently re-established after tissue wash-out ($n = 6$).

4. Discussion

In the present study, the sulphonamide analog of GABA, AHPNS, proved to be a competitive $GABA_B$ receptor antagonist in the guinea-pig ileum, yielding a pA_2 value of 4.0 at peripheral $GABA_B$ receptor sites, comparable to that of phaclofen (Kerr et al., 1990). In the spontaneously discharging rat neocortical slice, maintained in Mg^{2+}-free Krebs medium, AHPNS again exhibited antagonism of baclofen-induced suppression of spontaneous discharges, indicative of antagonist actions at central $GABA_B$ receptors. Furthermore, AHPNS is selective for $GABA_B$ receptors, as it shows neither $GABA_A$ agonist nor antagonist properties in the neocortical slice.

Antagonist potency of AHPNS at presynaptic $GABA_B$ receptors is relatively weaker than that of the related $GABA_B$ receptor antagonist saclofen or 2-hydroxysaclofen (Kerr et al., 1988, 1989), but may be improved by appropriate alteration of the sulphonamide moiety. In the sulphonamides AHPNS and its *N*-(4-chlorophenyl) analog, the pK_a of the substituted sulphonamide moiety evidently plays a part in determining their $GABA_B$ receptor antagonist potency, the pK_a in turn being dependent on the electronegativity of the *N*-substituent. Here, the *N*-(4-nitrophenyl) substituent on AHPNS, which has a pK_a of 6.1, is substantially more electronegative than the *N*-(4-chlorophenyl) of this less potent analog, with a pK_a near 9, whilst in the inactive *N*-(4-methoxyphenyl) analog, the 4-methoxy substituent is frankly electron donating. Thus, the more powerful the electron withdrawal properties of the *N*-substituent at the sulphonamide moiety, the more potent was the $GABA_B$ receptor antagonist action in these compounds. These results suggest that analogs of AHPNS with even stronger electron withdrawing substituents should yield more potent $GABA_B$ receptor antagonists. This provides an attractive, versatile structure-action profile which we are currently pursuing, particularly since similar compounds are known to penetrate the central nervous system. In conclusion, AHPNS represents a new class of $GABA_B$ receptor antagonist that is anticipated to provide members able to penetrate the central nervous system upon parenteral administration.

Acknowledgements

We thank the Australian Research Council for the award of a Research Fellowship to J.O. and for financial support.

References

Bowery, N.G., A. Doble, D.R. Hill, A.L. Hudson, J.S. Shaw, M.J. Turnbull and R. Warrington, 1981, Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals, Eur. J. Pharmacol. 71, 53.

Carruthers, N.I., J.M. Spitler, S.C. Wong, D.J. Blythin, X. Chen, H.J. Shue and S. Mittelman, 1995, Synthesis and resolution of β-(aminomethyl)-4-chlorobenzeneethanesulfinic acid a potent $GABA_B$ receptor ligand, Biorg. Med. Chem. Lett. 5, 237.

Curtis, D.R., B.D. Gynther, D.T. Beattie, D.I.B. Kerr and R.H. Prager, 1988, Baclofen antagonism by 2-hydroxy-saclofen in the cat spinal cord, Neurosci. Lett. 92, 97.

Giotti, A., S. Luzzi, S. Spagnesi and L. Zilletti, 1983, Homotaurine: a $GABA_B$ antagonist in guinea-pig ileum, Br. J. Pharmacol. 79, 855.

Harrison, N.L., D.M. Lovinger, N.A. Lambert, T.J. Teyler, R.H. Prager, J. Ong and D.I.B. Kerr, 1990, The actions of 2-hydroxy-saclofen at presynaptic $GABA_B$ receptors in the rat hippocampus, Neurosci. Lett. 119, 272.

Kerr, D.I.B., J. Ong, R.H. Prager, B.D. Gynther and D.R. Curtis, 1987, Phaclofen: a peripheral and central baclofen antagonist, Brain Res. 405, 150.

Kerr, D.I.B., J. Ong, G.A.R. Johnston, J. Abbenante and R.H. Prager, 1988, 2-Hydroxy-saclofen: an improved antagonist at central and peripheral $GABA_B$ receptors, Neurosci. Lett. 92, 92.

Kerr, D.I.B., J. Ong, G.A.R. Johnston, J. Abbenante and R.H. Prager, 1989, Antagonism at $GABA_B$ receptors by saclofen and related sulphonic analogues of baclofen and GABA, Neurosci. Lett. 107, 239.

Kerr, D.I.B., J. Ong and R.H. Prager, 1990, $GABA_B$ receptor agonists and antagonists, in: GABA-B Receptors in Mammalian Function, eds. N.G. Bowery, H. Bittiger and H.-R. Olpe (Wiley, Chichester) p. 29.

Lambert, N.A., N.L. Harrison, D.I.B. Kerr, J. Ong, R.H. Prager and T.J. Teyler, 1989, Blockade of the late IPSP in rat CA_1 hippocampal neurons by 2-hydroxy-saclofen, Neurosci. Lett. 107, 125.

Ong, J., D.I.B. Kerr and G.A.R. Johnston, 1990, Effects of potassium channel blockers on baclofen-induced suppression of paroxysmal discharges in rat neocortical slices, Neurosci. Lett. 112, 216.

Ong, J., D.I.B. Kerr, G. Lacey, D.R. Curtis, R. Hughes and R.H. Prager, 1994, Differing actions of nitropropane analogs of GABA and baclofen in central and peripheral preparations, Eur. J. Pharmacol. 264, 49.