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Short communication

The (S)-enantiomer of 2-hydroxysaclofen is the active $GABA_B$ receptor antagonist in central and peripheral preparations

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Abstract

In the guinea-pig isolated ileum, (*RS*)-(±)-baclofen induced a depression of cholinergic twitch contractions, reversibly and competitively antagonised by (*S*)-2-hydroxysaclofen ($pA_2 = 5.2 \pm 0.2$), but not by (*R*)-2-hydroxysaclofen. The depression of excitatory field potentials by baclofen (5 μM) in rat CA1 hippocampal slices was antagonised by (*S*)-2-hydroxysaclofen (100 μM) ($pA_2 = 4.3$), whilst in rat neocortex, (*S*)-2-hydroxysaclofen (50–500 μM) antagonised the baclofen (10 μM)-induced suppression of spontaneous discharges, the (*R*)-enantiomer being inactive. These results show that (*S*)-2-hydroxysaclofen is the active antagonist at central and peripheral $GABA_B$ receptors.

Keywords: $GABA_B$ receptor; (*RS*)-(±)-Baclofen; (*S*)-2-Hydroxysaclofen; (*R*)-2-Hydroxysaclofen; Ileum, guinea pig; Hippocampus, rat; Neocortex, rat

1. Introduction

Baclofen (4-amino-3-(4-chlorophenyl)butyric acid) is a specific agonist for G-protein coupled $GABA_B$ receptors which are bicuculline insensitive receptors for the inhibitory neurotransmitter GABA (4-aminobutyric acid). The absolute configuration of the active enantiomer (*R*)-(−)-baclofen is known (Chang et al., 1982), the (*S*)-(+)-enantiomer being virtually inactive (Hill and Bowery, 1981), the same being found for the respective (*R*)- and (*S*)-enantiomers of the congener β-phenyl-GABA (Ong et al., 1993). Antagonists for $GABA_B$ receptors were originally developed from baclofen by isosteric replacement of the carboxylate moiety with a phosphonic group (phaclofen) (Kerr et al., 1987) or a sulphonic group (saclofen), an additional 2-hydroxy substituent on the latter yielding 2-hydroxysaclofen (Curtis et al., 1988; Kerr et al., 1988; Lambert et al., 1989). The pharmacological and physiological actions of these antagonists in blocking $GABA_B$ receptor-mediated responses in a variety of central and peripheral preparations have been well characterised (for review, see Kerr and Ong, 1992).

Recently, phaclofen has been resolved using chiral chromatography, and the absolute stereochemistry of the active enantiomer (*R*)-(−)-phaclofen established, the (*S*)-enantiomer again being inactive (Frydenvang et al., 1994). Chromatographic separation of the enantiomers of saclofen and 2-hydroxysaclofen has also been achieved (Vaccher et al., 1993), but insufficient material was obtained to allow the configuration of the active enantiomer to be assigned. More recently, however, chiral synthesis has provided the two enantiomers of saclofen, with the (*R*)-(−)-enantiomer again being the active antagonist whilst (*S*)-(+)-saclofen is ineffective at $GABA_B$ receptors. From their circular dichroism curves, (*R*)-(−)-saclofen very likely has the same configuration as that of (*R*)-(−)-baclofen (Carruthers et al., 1995). We have now prepared the (*R*)- and (*S*)-enantiomers of 2-hydroxysaclofen, utilising Sharpless epoxidation of 2-(4-chlorophenyl)-3-hydroxypropene (Katsuki and Sharpless, 1980), and here report that the active antagonist at central and peripheral $GABA_B$ receptors is (*S*)-2-hydroxysaclofen, which has

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the same stereochemistry as that in (*R*)-baclofen (see Discussion).

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 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 a Grass FT03 force transducer, 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. 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. Drug volumes never exceeded 1% of the total bath volume, and all drugs were dissolved in distilled water.

2.2. Rat hippocampal slice preparations

Transverse hippocampal slices were prepared from male Sprague-Dawley rats, 250–350 g, as previously described (Ong et al., 1993). Slices were superfused with ACSF (32 ± 0.2°C) equilibrated with 95% O_2/5% CO_2. Glass microelectrodes for extracellular recording were filled with ACSF, the tips broken back to give a resistance of 1–3 MΩ, and placed in the stratum radiatum of the CA1 subfield of the hippocampal slice. A monopolar tungsten electrode was used to stimulate the Schaffer collateral/commissural fibre pathway in the stratum radiatum. Field potentials were evoked in response to constant current stimuli of 0.1 ms duration at 0.05 Hz. Evoked field potentials were digitised, recorded to disk for off-line analysis, and displayed on a microcomputer based data acquisition and analysis system. Three waveforms recorded over 1 min were averaged, and such minute values used for all data analysis. Experiments were repeated in 4 slices from 2 animals. The rate of rise of the field excitatory postsynaptic potential (fEPSP) was measured as the slope of the initial segment of the fEPSP, calculated as the first order derivative, and the data was presented as mean S.E.M. ($n = 4$).

2.3. 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.

2.4. Drugs

(*RS*)-(±)-Baclofen was a gift from Ciba-Geigy (Basel, Switzerland), and (*R*)-(−)- and (*S*)-(+)-2-hydroxysaclofen hydrochloride were synthesised by K.

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3. Results

In the guinea-pig isolated ileum, (*RS*)-(±)-baclofen elicited a depression of cholinergic twitch contractions, in a concentration-dependent manner (Fig. 1a), sensitive to the $GABA_B$ receptor antagonist (*S*)-2-hydroxysaclofen. (*S*)-2-Hydroxysaclofen (50 μM) produced a clear rightward shift in the concentration-response curve for baclofen in a surmountable manner. The estimated EC_{50} value for baclofen-induced depression of twitch responses was 10 μM. As illustrated in Fig. 1b, the depressant effect of baclofen (BAC, 8 μM) was reversibly antagonised by (*S*)-2-hydroxysaclofen ((*S*)-2-OH-SAC, 100 μM) but not by (*R*)-2-hydroxysaclofen ((*R*)-2-OH-SAC, 100 μM). The responses to baclofen returned to control levels on repeated washing of the tissue with Krebs solution within 30 min intervals. (*S*)-2-Hydroxysaclofen antagonised the effects of baclofen at concentrations of 50, 100 and 200 μM, whilst (*R*)-2-hydroxysaclofen did not affect the responses to

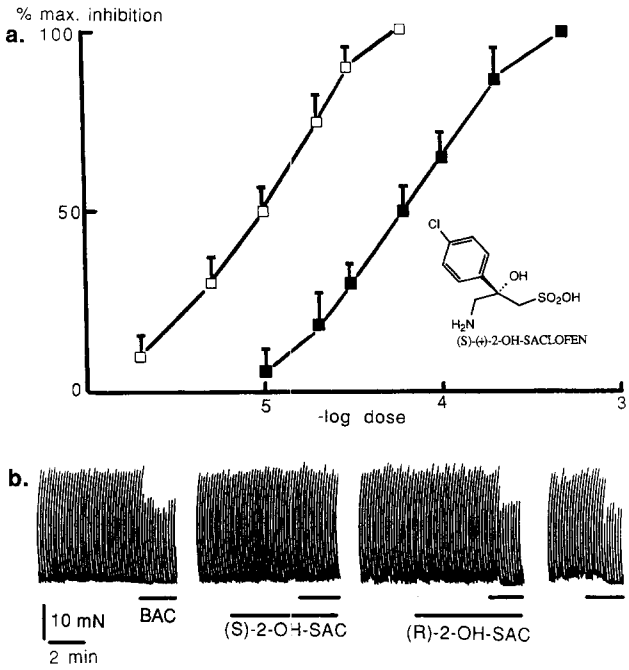

Fig. 1. (a) Dose-response curves for (*RS*)-(±)-baclofen (BAC)-induced depression of cholinergic ileal twitch contractions, in the presence and absence of (*S*)-2-hydroxysaclofen hydrochloride ((*S*)-(+)-2-OH-SACLOFEN), with the chemical structure shown. The dose-response curve for baclofen (□) was shifted to the right in a parallel manner by (*S*)-2-hydroxysaclofen (■ 50 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. (b) Antagonism of the baclofen (BAC; 8 μM)-induced depression of cholinergic twitch contractions in the guinea-pig isolated ileum by the $GABA_B$ receptor antagonist (*S*)-2-hydroxysaclofen ((*S*)-2-OH-SAC; 100 μM), but not by the enantiomer (*R*)-2-hydroxysaclofen ((*R*)-2-OH-SAC; 100 μM). (*S*)-2-Hydroxysaclofen reversibly antagonised the baclofen-induced depression of ileal twitch contractions, with tissue wash-outs between drug applications, and recovery of control responses to baclofen ($n = 6$).

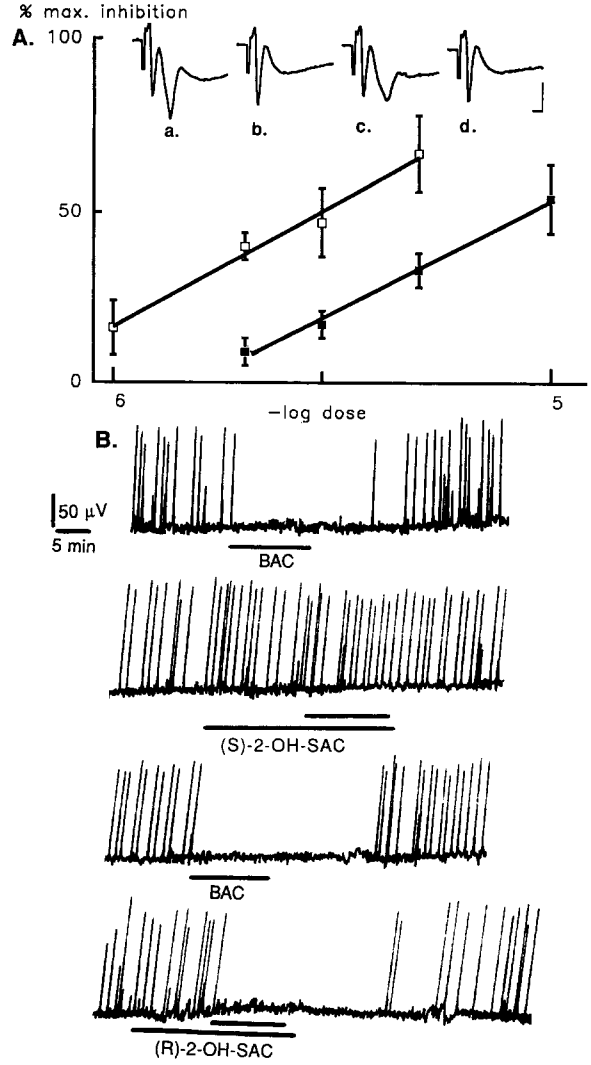

Fig. 2. (A) Concentration-response curves for the depression of field excitatory postsynaptic potentials by racemic baclofen alone, and in the presence of (*S*)-2-hydroxysaclofen (100 μM). Data are presented as mean S.E.M. ($n = 4$), the solid lines are the result of least squares linear regression. Averaged field potentials evoked in the hippocampal CA1 by stimulation in the adjacent stratum radiatum showing (a) control, (b) in the presence of (*RS*)-baclofen (5 μM), depression of field EPSP and population spike, (c) antagonism of this depression by (*S*)-2-hydroxysaclofen (100 μM), with restoration of field EPSP and population spike, and (d) (*R*)-2-hydroxysaclofen (100 μM) did not antagonise the baclofen-induced depression of field potentials, and without any influence on the prevolley. (B) Complete and reversible antagonism by (*S*)-2-hydroxysaclofen ((*S*)-2-OH-SAC; 50 μM) of the reduction of the amplitude and frequency of spontaneous discharges in rat isolated neocortical slice preparations by baclofen (BAC, 10 μM). The control response to BAC was subsequently re-established after washing out BAC and (*S*)-2-OH-SAC ($n = 6$). The enantiomer (*R*)-2-hydroxysaclofen ((*R*)-2-OH-SAC; 100 μM) did not affect the baclofen-induced suppression of discharges ($n = 4$).

baclofen, even at a maximal concentration of 500 μM. The mean calculated pA_2 value for (*S*)-2-hydroxysaclofen was 5.2 ± 0.2 ($n = 6$). (*S*)-2-Hydroxysaclofen alone did not affect the amplitude of the twitch contractions, nor did it have any $GABA_A$ or $GABA_B$ partial agonist activity.

In the CA1 area of the hippocampal slice, racemic baclofen depressed the fEPSP rate of rise in a concentration-dependent manner without affecting the prevolley (Fig. 2A), such depression of the fEPSP resulting from baclofen activation of presynaptic $GABA_B$ receptors on the Schaffer collaterals (Harrison et al., 1990). The EC_{50} for baclofen depression of the fEPSP was 3.0 μM. (*S*)-2-Hydroxysaclofen antagonised the action of baclofen at this presynaptic $GABA_B$ receptor site. Fig. 2A shows that (*S*)-2-hydroxysaclofen (100 μM) caused a rightward shift of the concentration-response curve of baclofen, with an EC_{50} for baclofen of 8.5 μM. Assuming competitive inhibition, this gave a pA_2 value of 4.3 for the (*S*)-enantiomer at this particular receptor. Antagonism of the $GABA_B$ receptor by 2-hydroxysaclofen was specific for the (*S*)-enantiomer, as can be seen from the averaged field potentials in Fig. 2A, where (*S*)-2-hydroxysaclofen (100 μM), but not (*R*)-2-hydroxysaclofen antagonised the depression of the fEPSP, and generation of the population spike, due to baclofen (5 μM).

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 (*RS*)-(±)-baclofen (BAC) concentration which effectively halted all discharges was 10 μM; the discharges were completely suppressed by baclofen, which was readily washed-out within 30 min with a complete recovery of the spontaneous activity (Fig. 2B; $n = 6$). (*S*)-2-Hydroxysaclofen, over a concentration range of 100–500 μM, did not affect the discharge rate or amplitude, but did antagonise the baclofen (10 μM)-induced suppression of spontaneous discharges ($n = 6$). On the other hand, (*R*)-2-hydroxysaclofen (100 μM) did not affect the baclofen-induced responses (Fig. 2B; $n = 6$), even at concentrations up to 1 mM.

4. Discussion

Of the enantiomers of 2-hydroxysaclofen, only (*S*)-2-hydroxysaclofen exhibited antagonist activity at $GABA_B$ receptor-mediated depression of excitatory transmission in the guinea-pig ileum and rat hippocampal slice. This antagonist activity was more potent ($pA_2 = 5.2$) in the ileum than in the CA1 area of the hippocampus ($pA_2 = 4.3$), suggesting that the $GABA_B$ receptors in these two regions are not identical. Such lower potency of 2-hydroxysaclofen in the hippocampus has been seen previously (Harrison et al., 1990), and the potency of (*RS*)-2-hydroxysaclofen calculated from the data in the latter study gives an approximate pA_2 of 4, i.e. half that of (*S*)-2-hydroxysaclofen itself. However, in the latter, the concentration-response curves showed a marked departure from parallel, which suggests partial agonist/antagonist properties of the active (*S*)-enantiomer at this particular receptor.

Superposition of (*R*)-(−)-baclofen and (*R*)-(−)-phaclofen (Frydenvang et al., 1994) indicates that these two analogues adopt similar configurations in the crystalline state, these low energy conformations presumably reflecting those adopted during their binding at the $GABA_B$ receptor recognition site. From their circular dichroism curves, the stereochemistry of the (*R*)-(−)-enantiomer of saclofen, lacking a 2-hydroxy substituent, is also very likely similar to that of (*R*)-(−)-baclofen (Carruthers et al., 1995). As can be seen from Fig. 1a, the hydroxy substituent in (*S*)-2-hydroxysaclofen has the same configuration as does the hydrogen in the (*R*)-(−)-enantiomers of baclofen, phaclofen and saclofen. However, since the 2-hydroxy substituent assumes highest priority under the Cahn, Ingold, Prelog sequence rule, this enantiomer is properly designated as (*S*)-2-hydroxysaclofen, thus the stereochemistry of (*S*)-2-hydroxysaclofen is in fact the same as that in the (*R*)-enantiomers of baclofen, β-phenyl-GABA, phaclofen and saclofen. The relevance of a hydroxyl group in hydroxy GABA analogs has already been discussed by Falch et al. (1986), as well as by Kristiansen and Fjalland (1991) and Kristiansen et al. (1992). Although (*S*)-2-hydroxysaclofen combines the configurations of the β-*p*-chlorophenyl-GABA analogs, and of 3-hydroxy-GABA, that are active at $GABA_B$ receptors, nevertheless, in 2-hydroxysaclofen, the precise contribution of the 2-hydroxy substituent to the binding and antagonist properties remains to be determined. In conclusion, these results show that (*S*)-2-hydroxysaclofen, having the same absolute configuration as (*R*)-(−)-baclofen, is the active antagonist at central and peripheral $GABA_B$ receptors.

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References

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.

Chang, C.H., D.S.C. Yang, C.S. Yoo, B.C. Wang, J. Pletcher, M. Sax and C.F. Terrence, 1982, Structure and absolute configuration of (*R*)-baclofen monochloride, Acta Chryst. B38, 2065.

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.

Falch, E., A. Hedegaard, L. Nielsen, B.R. Jensen, J. Hjeds and P. Krogsgaard-Larsen, 1986, Comparative stereostructure-activity studies on $GABA_A$ and $GABA_B$ receptor sites and GABA uptake using rat brain membrane preparations, J. Neurochem. 47, 898.

Frydenvang, K., P. Krogsgaard-Larsen, J.J. Hansen, A. Mitrovic, H. Tran, C.A. Drew and G.A.R. Johnston, 1994, $GABA_B$ antagonists: resolution, absolute stereochemistry and pharmacology of (*R*)- and (*S*)-phaclofen, Chirality 6, 583.

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

Hill, D.R. and N.G. Bowery, 1981, ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain, Nature 290, 149.

Katsuki, T. and K.B. Sharpless, 1980, The first practical method for asymmetric epoxidation, J. Am. Chem. Soc. 102, 5974.

Kerr, D.I.B. and J. Ong, 1992, GABA agonists and antagonists, Med. Res. Rev. 12, 593.

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.

Kristiansen, U. and B. Fjalland, 1991, Ligand structural specificity of $GABA_A$ receptors in guinea pig ileum, Pharmacol. Toxicol. 68, 332.

Kristiansen, U., A. Hedegaard, C. Herdeis, T.M. Lund, B. Nielsen, J.J. Hansen, E. Falch, H. Hjeds and P. Krogsgaard-Larsen, 1992, Hydroxylated analogues of 5-aminovaleric acid as 4-aminobutyric $acid_B$ receptor antagonists: stereostructure-activity relationships, J. Neurochem. 58, 1150.

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, D.J. Doolette, R.K. Duke, K.N. Mewett, R.D. Allan and G.A.R. Johnston, 1993, *R*-(−)-β-Phenyl-GABA is a full agonist at $GABA_B$ receptors in brain slices but a partial agonist in the ileum, Eur. J. Pharmacol. 233, 169.

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.

Vaccher, C., P. Berthelot and M. Debaert, 1993, Direct chromatographic separation of the enantiomers of phaclofen, saclofen and hydroxysaclofen – influence of the anionic moiety, J. Chromatogr. A657, 213.