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$GABA_B$ receptor antagonism by resolved (*R*)-saclofen in the guinea-pig ileum

David I.B. Kerr [a,*], Jennifer Ong [a], Claude Vaccher [b], Pascal Berthelot [b], Nathalie Flouquet [b], Marie-Pierre Vaccher [b], Michel Debaert [b]

[a] *Department of Anaesthesia and Intensive Care, University of Adelaide, Adelaide, South Australia 5005, Australia*

[b] *Laboratoire de Pharmacie Chimique et de Chimie Therapeutique, Faculté des Sciences, Pharmaceutiques et Biologiques, Université de Lille, BP 83, 59006 Lille Cedex, France*

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Abstract

The $GABA_B$ receptor antagonist saclofen (3-amino-2-(4-chlorophenyl)propylsulphonic acid) has been resolved by chiral high-performance liquid chromatography. The enantiomer (*R*)-saclofen, but not (*S*)-saclofen, reversibly antagonised the (*R,S*)-baclofen-induced depression of cholinergic twitch contractions in the guinea-pig ileum with an apparent pA_2 of 5.3. Also, 2-hydroxy-saclofen was resolved by the same method, its (*S*)-enantiomer yielding an apparent pA_2 of 5.0. This method provides a convenient resolution of these antagonists.

Keywords: $GABA_B$ receptor; (*R,S*)-Baclofen; (*R*)-Saclofen

$GABA_B$ receptors are bicuculline-insensitive receptors for the inhibitory neurotransmitter GABA (4-aminobutanoic acid) that are stereospecifically activated by baclofen (4-amino-3-(4-chlorophenyl)butanoic acid), agonist activity residing in the (*R*)-enantiomer of known absolute configuration (Chang et al., 1982). Saclofen (3-amino-2-(4-chlorophenyl)propylsulphonic acid) and its hydroxy analogue 2-hydroxy-saclofen (3-amino-2-hydroxy-2-(4-chlorophenyl)propylsulphonic acid) are antagonists at these receptors, derived by making a sulphonic replacement of the carboxyl group of baclofen (Kerr and Ong, 1992). Recently, both saclofen and 2-hydroxy-saclofen have been resolved by chiral analytical high-performance liquid chromatography (Vaccher et al., 1993). This analytical chiral separation has been scaled up to provide a convenient method for preparative isolation (> 99%) of their respective enantiomers, using an analytical crown ether column (CR +) under isocratic conditions (detailed in Vaccher et al., 1996). We here show that antagonist activity at $GABA_B$ receptors of the guinea-pig ileum resides in the (*R*)-enantiomer of saclofen.

$GABA_B$ receptor-mediated effects of baclofen, and the enantiomers of saclofen, were examined on repetitive cholinergic twitch contractions, evoked by field stimulation as previously described (Kerr et al., 1995). Concentration-response curves to baclofen, in the presence and absence of the antagonist were constructed, 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 have been expressed as means ± S.E.M. The number of preparations used for each experiment was $n = 4$.

Baclofen-induced depression of ileal twitch contractions was reversibly antagonised by (*R*)-saclofen, which alone did not affect the amplitude of the twitch contractions, nor did it have any $GABA_A$ or $GABA_B$ partial agonist activity. (*S*)-Saclofen was inactive. As shown in Fig. 1, using (*R*)-saclofen (25 μM), the concentration-response curve for the depression of the twitch by (*R,S*)-baclofen was shifted 6.3-fold to the right, giving an apparent pA_2 of 5.3 for (*R*)-saclofen. By contrast, (*S*)-saclofen up to 200 μM showed no such antagonism of baclofen in this preparation. Also shown is a concentration-response curve for baclofen in the presence of (*S*)-2-hydroxy-saclofen (50 μM), resolved by the same method; this enantiomer yielded

* Corresponding author. Tel.: +61 8 303-5163; fax: +61 8 303-3909 or +61 8 303-3788; e-mail: dkerr@medicine.adelaide.edu.au

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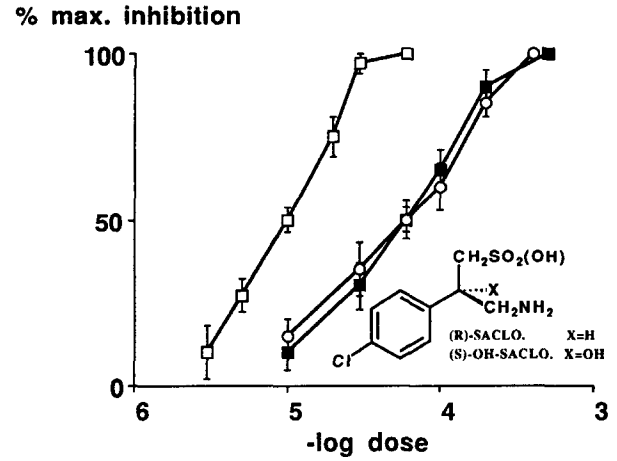

Fig. 1. Concentration-response curves for (*R,S*)-baclofen-induced depression of cholinergic ileal twitch contractions, in the presence and absence of (*R*)-saclofen ((*R*)-SACLO) and (*S*)-2-hydroxy-saclofen ((*S*)-OH-SACLO), with their chemical structures shown. The concentration-response curve for baclofen (□) was equally shifted to the right by (*R*)-saclofen (○, 25 μM) and by (*S*)-2-hydroxy-saclofen (■, 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 S.E.M. values of 4 determinations.

an apparent pA_2 of 5.0, whilst (*R*)-2-hydroxy-saclofen (400 μM) was inactive.

(*R*)-Saclofen was thus twice more potent than (*S*)-2-hydroxy-saclofen in antagonising ileal $GABA_B$ receptors, as has been shown for their racemates (Kerr and Ong, 1992). Chiral synthesis of (*R*)- and (*S*)-2-hydroxy-saclofen has already been described, with their absolute configurations assigned (Prager et al., 1995), and the antagonist activity at $GABA_B$ receptors found to reside in (*S*)-2-hydroxy-saclofen (Kerr et al., 1995). Because of the higher priority of the hydroxyl in (*S*)-2-hydroxy-saclofen, which replaces the hydrogen at the chiral center on C_2 of the propane backbone (Fig. 1), the Cahn, Ingold, Prelog rules dictate that the active enantiomer of 2-hydroxy-saclofen be designated as the (*S*)-enantiomer, despite its absolute configuration being the same as that of the antagonist (*R*)-phaclofen (Frydenvang et al., 1994), and the agonist (*R*)-baclofen itself (Chang et al., 1982). The conformations of phaclofen, saclofen and 2-hydroxy-saclofen in solution (D_2O) have recently been established using high-resolution (300 MHz) ^1H NMR, and shown to be the same as that of baclofen (Vaccher et al., 1995). Presumably, all these adopt the same conformation during binding at the $GABA_B$ receptor recognition site. The present results, using enantiomeric resolution of saclofen and 2-hydroxy-saclofen by preparative chiral high-performance liquid chromatography, confirm the correct assignment of their (*R*)- and (*S*)-enantiomers on the basis of their retention times, in the method of Vaccher et al. (1996).

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