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The morpholino-acetic acid analogue Sch 50911 is a selective $GABA_B$ receptor antagonist in rat neocortical slices

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Abstract

The pharmacological properties of (+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid (Sch 50911) were evaluated on $GABA_B$ receptors in rat neocortical slices. The $GABA_B$ receptor agonist, baclofen, produced a concentration-dependent depression of the frequency of spontaneous discharges in slices maintained in Mg^{2+}-free Krebs medium with an EC_{50} of 6 μM, reversibly antagonised by Sch 50911 (5, 10 and 25 μM) with an apparent pA_2 of 6.0 ± 0.1. The (−) enantiomer Sch 50910 (500 μM) and the racemic des-methyl analogue Sch 48588 (500 μM) were inactive. In slices preloaded with [^{3}H]GABA, Sch 50911 antagonised $GABA_B$ autoreceptors, increasing the electrically-stimulated ^{3}H overflow in a concentration-dependent manner, with an IC_{50} of 3 μM. The maximal effect (148 ± 10.5%) was found at 10 μM, but at 50 μM the response was reduced to 67 ± 19%. In contrast, evoked release was unaffected by Sch 50910 (100 μM) whilst Sch 48588 at 100 μM increased the overflow by 51.3 ± 11.6%. In summary, Sch 50911 is a relatively potent antagonist of considerable potential in studies of $GABA_B$ receptor function.

Keywords: Baclofen; $GABA_B$ receptor; $GABA_B$ heteroreceptor; $GABA_B$ autoreceptor; Sch 50911; Neocortical slice, rat

1. Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system that activates three distinct classes of receptors, $GABA_A$, $GABA_B$ and $GABA_C$ receptor subtypes (Bowery, 1993; Johnston, 1996). Baclofen (β-*p*-chlorophenyl GABA) is the prototypical agonist for the bicuculline-insensitive $GABA_B$ receptors that are coupled to a variety of effectors through pertussis toxin-sensitive G proteins (Bowery, 1993). Recently, two $GABA_B$ receptor splice variants have been cloned, showing sequence similarity to the metabotropic glutamate receptors (Kaupmann et al., 1997). In general, presynaptic $GABA_B$ receptors modulate synaptic transmission by depressing neurotransmitter release, including that of GABA itself, through autoreceptors (Pittaluga et al., 1987; Baumann et al., 1990; Waldmeier et al., 1994), whilst postsynaptic $GABA_B$ receptors contribute to the inhibitory control of overall neuronal excitability.

The original $GABA_B$ receptor antagonists, phaclofen and 2-hydroxy-saclofen, which were discovered in our laboratory (Kerr et al., 1987, 1988), are compounds based on bioisosteric replacement of the carboxylic moiety in baclofen. The first major improvements in the potency and bioavailability in $GABA_B$ receptor antagonists resulted from the synthesis of phosphinic bioisosteres of GABA (Froestl et al., 1995). Subsequently, the potency of these phosphinic analogues on $GABA_B$ receptors was increased greatly by the introduction of *N*-benzyl and 2-hydroxy substituents into the parent phosphinic analogues of GABA (Froestl et al., 1995; Froestl and Mickel, 1997), with the 2-hydroxy substituent being found in the original antagonist 2-hydroxy-saclofen (Kerr et al., 1988). A unique series of $GABA_B$ receptor antagonists has recently been described based on 2,5-disubstituted-1,4-morpholines (Kuo et al., 1994; Blythin et al., 1996). The most potent of these is (+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid (Sch 50911) (Fig. 1), first characterised as a selective, competitive, and orally-active antagonist at central and peripheral $GABA_B$ receptors (Bolser et al., 1995; Hosford et al., 1995). This compound not only displaces GABA binding

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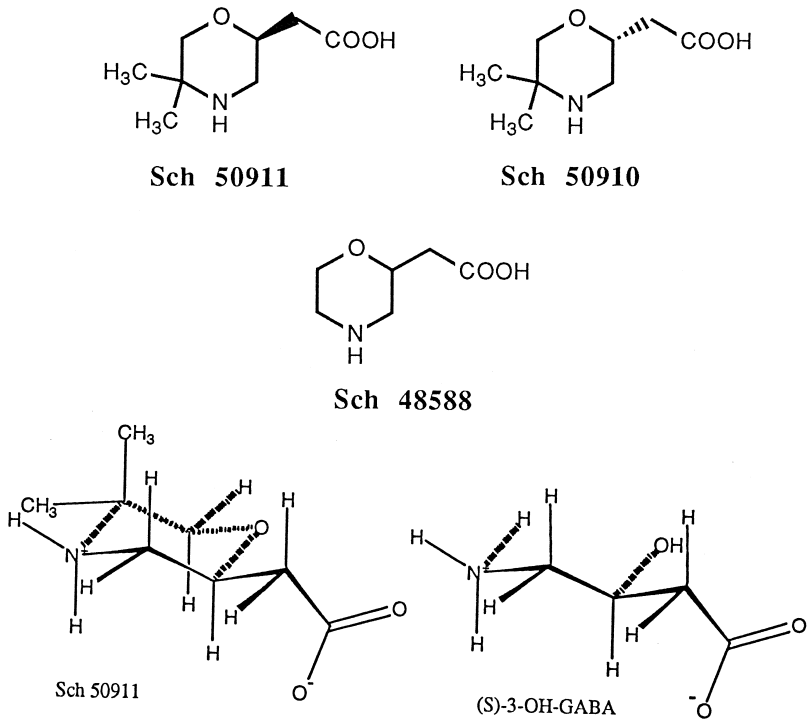

Fig. 1. Chemical structures of (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911), the enantiomer of Sch 50911 (−)-(R)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50910) and the racemic des-methyl analogue Sch 48588. Extended conformations of (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911) and (*S*)-3-hydroxy-GABA ((*S*)-3-OH-GABA), showing the identical positions of their oxygen atoms which can act as hydrogen bond acceptors.

to $GABA_B$ receptors in rat brain tissues, but also blocks the inhibitory responses to baclofen in the guinea-pig trachea and antagonises the central antitussive and respiratory depressant effects of baclofen in cats and guinea-pigs when administered systemically, indicating its ability to penetrate the blood brain barrier (Bolser et al., 1995). Sch 50911 was shown to be an effective, orally-active $GABA_B$ receptor antagonist in further in vivo studies in which it suppressed seizure activity through blocking $GABA_B$ receptors (Hosford et al., 1995). From a structure-activity viewpoint, such morpholinoacetic acids are of particular interest, since they represent GABA analogues partially frozen in an antagonist conformation which retains the N-substitution and β-hydroxy of the previous antagonists incorporated into the morpholine ring (Fig. 1).

To date, relatively little work has been done in characterising the pharmacological properties of these morpholino-acetic analogues, particularly using isolated tissues from the central nervous system. In the present study, we have evaluated the antagonist potencies of Sch 50911, its enantiomer (−)-(*R*)-5,5-dimethylmorpholinyl-2-acetic acid (Sch 50910), and the racemic des-methyl analogue (±)-Sch 48588 (Fig. 1), on baclofen-induced suppression of spontaneous discharges in rat neocortical slices, mediated through $GABA_B$ heteroreceptors. We have also examined the actions of these compounds on $GABA_B$ autoreceptors modulating GABA release, using electrically-evoked release of [^{3}H]GABA from brain slices preloaded with [^{3}H]GABA. In these preparations, Sch 50911 demonstrated moderately potent antagonist activity at $GABA_B$ receptors. Since its antagonist properties reside in the morpholine ring that partially restricts the molecular flexibility of this compound, Sch 50911 forms a novel class of antagonist at $GABA_B$ receptors.

2. Materials and methods

2.1. Preparation of rat neocortical slices

All studies described here were conducted in strict accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985), the Australian Code of Practice for the care and use of animals for scientific purposes of the National Health and Medical Research Council and The University of Adelaide Animal Ethics Committee. 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 (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 Instru-

ments, UK), and a radial wedge was cut from each side of the dorsal midline 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. Slices were then randomly assigned to the experiments described below.

2.2. Grease-gap recording of brain slices

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.

After a period of equilibration for 10–15 min under Mg^{2+}-free conditions, the neocortical slices developed spontaneous paroxysmal discharges. 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. Results were quantified by counting the number of spontaneous discharges in 10-min epochs, in the absence and presence of test compounds, and the values expressed as a percentage depression of the average control discharge rate, during the 10 min immediately before the addition of drugs. 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. Due to limited amounts of test compounds available, estimates of apparent pA_2 values were made using the relationship $pA_2 = \log (CR - 1) - \log [B]$, where the concentration ratio (CR), relative to corresponding controls, was produced by a single concentration of antagonist [B], assuming competitive antagonism and Schild regression close to unity. Each experiment was repeated on six to eight slices obtained from at least three different animals and data are expressed as mean ± S.E.M.

2.3. Neurochemical release studies

Pairs of neocortical slices were equilibrated in Krebs solution (37°C; 95% O_2:5% CO_2) for 40 min and then incubated in Krebs solution containing [^{3}H]GABA (0.1 μM) for 20 min. Each pair of slices was rinsed, placed in a small chamber and superfused at 1 ml/min with Krebs solution (37°C; gassed). Aliquots of superfusate were collected at 10 min intervals for the first five collections and for 5 min thereafter and their ^{3}H contents were assayed by liquid scintillation spectrometry. GABAergic neurons in the slices were stimulated through platinum field electrodes by square wave pulses (2 Hz, 2.0 ms duration, 25 mA) for 40 pulses at 10 min and for 300 pulses at 65 min (S_1), 100 min (S_2), and in some experiments at 130 min (S_3) after superfusion commenced. At the end of each experiment, the residual ^{3}H content in the neocortical slices was extracted in 0.4 M $HClO_4$ (containing EDTA, 3.0 mM and Na_2SO_3, 10 mM) at 4°C for at least 16 h and then assayed. From this data the fractional overflow of ^{3}H during each collection period was computed and the overflow per min was plotted (see Fig. 4). The GABA uptake inhibitor, NO-711 (10 μM), was added to the perfusion medium at the beginning of superfusion and remained throughout each experiment, while the three test compounds reported in this study were added 15 min prior to the second or third stimulation. Krebs solution used in these experiments was of the following composition (mM): NaCl (120), KCl (4.7), $NaHCO_3$ (25), KH_2PO_4 (1.0), $CaCl_2$ (2.5), $MgCl_2$ (1.0), glucose (5.5), and contained aminooxyacetic acid (AOAA; 0.05 μM). Incubation medium comprised [^{3}H]GABA (0.05 μM), GABA (0.05 μM) in Krebs solution containing AOAA (0.05 mM).

2.4. Resting and stimulation-induced overflows of [^{3}H]GABA

The resting overflow of ^{3}H is defined as the fractional overflow in the 10 min prior to stimulation. The stimulation-induced (SI) overflow for each stimulation, SIO_1, SIO_2 and SIO_3 was calculated by subtracting the resting overflow in the 5 min period prior to stimulation from the fractional overflow in the 5 min following the onset of stimulation at S_1, S_2 and S_3 respectively. The effects of the test compounds on the resting and SI-overflows of ^{3}H were determined by comparing the R_2/R_1 or R_3/R_1 and SIO_2/SIO_1 or SIO_3/SIO_1 ratios with the equivalent ratios in the absence of the compound. Hence, a putative antagonist of $GABA_B$ autoreceptors would increase the SI-overflow ratio.

2.5. Statistical analysis

The significance of the effect of a compound was assessed by paired or unpaired Student's *t*-test, with significance levels at $p < 0.05$.

2.6. Drugs

Baclofen was a gift from Novartis Pharma (Basel, Switzerland). Sch 50911, Sch 50910 and racemic (±)-Sch 48588 were synthesised in the Chemical Research Department at the Schering-Plough Research Institute (Kenilworth, NJ). 2, 3-[^{3}H][N]-GABA, specific activity 1.06 TBq/mmol was obtained from New England Nuclear (Boston, MA). Aminooxyacetic acid hemihydrochloride

(AOAA) was purchased from Sigma (MO, U.S.A.) and the GABA uptake inhibitor, NO-711 (1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid), was obtained from Research Biochemicals (Natick, MA).

3. Results

3.1. Effects of Sch 50911, Sch 50910 and Sch 48588 on baclofen-induced suppression of spontaneous discharges in rat neocortical slices

When superfused with Mg^{2+}-free Krebs medium, neocortical slices exhibited spontaneous depolarisations within 10–15 min. Once these were established at a stable rate, baclofen, superfused at concentrations ranging from 1–60 μM for 2 min, reduced the firing rate in a concentration-dependent manner. Fig. 2a shows a representative experiment in which baclofen (Bac; 30 μM) abolished the spontaneous activity for 8 min, with a subsequent slowing of the discharges for a further 5 min; these effects generally lasted some 10–15 min and returned to baseline levels within 20 min following the initial wash-out of the drug.

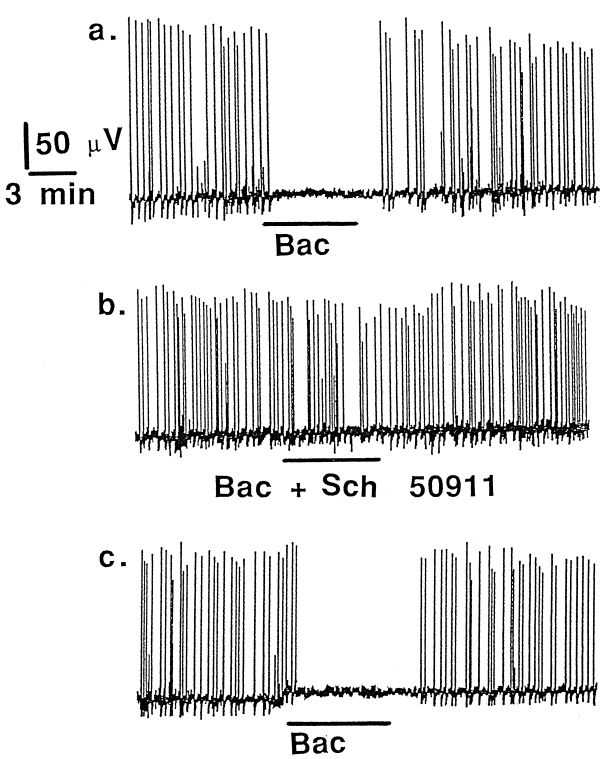

Fig. 2. Representative records from a typical experiment showing the effects of (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911) on the responses to baclofen (BAC) in the rat neocortical slice preparation, maintained in Mg^{2+}-free Krebs medium. (a) Baclofen (BAC; 30 μM) induced a suppression of spontaneous discharges (b) reversibly antagonised by Sch 50911 (10 μM) and (c) the control response to baclofen was subsequently reestablished upon wash-out of the test compounds.

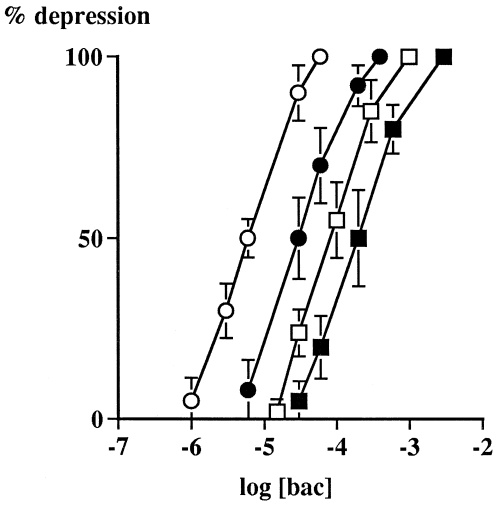

Fig. 3. Concentration–response curves for (R,S)-baclofen-induced suppression of the frequency of spontaneous discharges in the rat isolated neocortical slices, maintained in Mg^{2+}-free Krebs medium, in the absence and presence of (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911). The concentration–response curve for baclofen (○) was subsequently shifted to the right, in a parallel fashion, by Sch 50911 (● 5 μM; □ 10 μM; ■ 25 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.

As can be seen from the baclofen concentration–response curve in Fig. 3, the threshold concentration for the reduction in frequency of spontaneous activity by baclofen was 1 μM and the estimated half-maximally effective concentration (EC_{50} value) was 6 μM. Over the 10-min period, the maximal baclofen effect, with complete cessation of discharges, was obtained with 60 μM baclofen. No desensitization of the tissues to baclofen was detected, since continuous exposure to baclofen induced a consistent effect, with no resultant decrease in tissue responsiveness in all slices studied.

Pretreatment with Sch 50911 (10 μM) alone for 2 min did not affect the discharge rate or amplitude, but in combination with baclofen (30 μM) for 2 min, reversibly antagonised the baclofen-induced suppression of spontaneous discharges by 78% (Fig. 2b). Following wash-out of the compounds, there was a complete recovery of the spontaneous activity and the depressant response to baclofen (30 μM; Fig. 2c) within 30 min. To quantify the antagonist potency of Sch 50911, the effects of three concentrations of Sch 50911 (5, 10 and 25 μM) on the baclofen concentration–response curve were measured. Increasing concentrations of Sch 50911 caused a progressive shift of the baclofen concentration–response curve to the right, without depression of the maximum response. Using the ratio method and averaging, this yielded an apparent pA_2 value of 6.0 ± 0.1 (Fig. 3; $n = 8$). At a concentration of 50 μM, Sch 50911 itself did not elicit any partial agonist activity since it did not affect the spontaneous discharges, but antagonised the baclofen (30 μM) response

(data not shown, $n = 6$). By contrast, neither Sch 50910 (500 μM), the (−) enantiomer of Sch 50911, nor the des-methyl analogue Sch 48588 (500 μM) affected baclofen-induced responses or discharge frequency ($n = 6$).

3.2. Overflow of [^{3}H]GABA from rat neocortical slices

The resting overflow of ^{3}H into the Krebs solution superfusing neocortical slices, prelabelled with [^{3}H]GABA (0.1 μM), reached a near steady-state within 50 min of commencing superfusion (Fig. 4). In the presence of the GABA uptake inhibitor NO-711 (10 μM), electrical stimulation increased the overflow of ^{3}H approximately 2-fold in the 5-min collection following the onset of stimulation, an increase that returned to the resting level within a further 5 min. The magnitudes of both the resting and SI overflows of ^{3}H before and as a consequence of the second and third stimulations were almost identical with those during the collection periods at R_1 and S_1 (viz, $R_2/R_1 = 1.04 \pm 0.05$; $R_3/R_1 = 1.02 \pm 0.05$; $SIO_2/SIO_1 = 1.01 \pm 0.04$; $SIO_3/SIO_1 = 0.98 \pm 0.04$) (paired Student's *t*-test, R_2/R_1 cf. R_3/R_1, $p > 0.2$; SIO_2/SIO_1 cf. SIO_3/SIO_1, $p > 0.6$; $n = 8$). Hence, the significance of changes in the resting or SI overflow ratios produced by any of the compounds tested were calculated against the pooled R_2/R_1 plus R_3/R_1 or SIO_2/SIO_1 plus SIO_3/SIO_1 ratios.

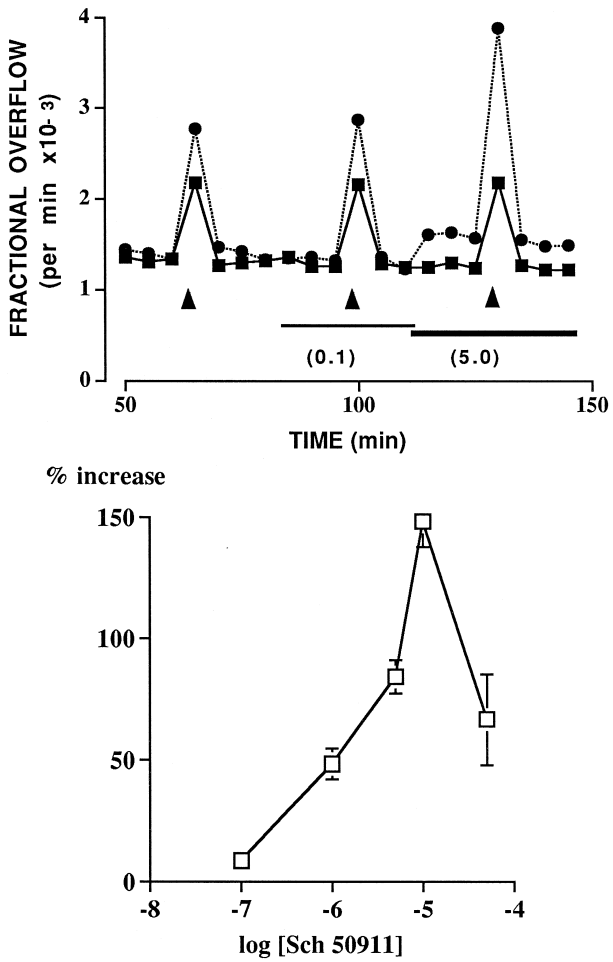

Fig. 4. The fractional overflow of ^{3}H per min from rat neocortical slices in a typical experiment in which two pairs of slices were stimulated at the arrows at 2 Hz for 300 pulses. Slices were pre-incubated with [^{3}H]GABA (0.1 μM) and superfused with Krebs solution containing NO-711 (10 μM). Prior to and during both the second and third periods of stimulation, Sch 50911 was added to the perfusion medium superfusing one pair of slices (●) at a concentration of 0.1 or 5.0 μM as shown by the bars. At the lower concentration, Sch 50911 had little effect on either the resting or stimulation-induced (SI) overflows of ^{3}H, whereas at 5.0 μM it increased both. Concentration–response curve to (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911) on the electrically-stimulated release of [^{3}H]GABA from rat neocortical slices. The data presented are means ± S.E.M. from four to six experiments calculated as a percentage of the increase above the same ratio in the absence of the antagonist.

3.3. Effects of Sch 50911, Sch 50910 and Sch 48588 on [^{3}H]GABA release

The effects of Sch 50911 on the release of [^{3}H]GABA from neocortical slices was examined at concentrations between 0.1 and 50 μM. As shown from the results of a typical experiment, and from the concentration–response curve (Fig. 4), the compound failed to affect the SI-overflow at a concentration of 0.1 μM, but facilitated the overflow of [^{3}H]GABA at 5 μM. Facilitation was maximal ($148.2 \pm 10.5\%$, $n = 4$) at a concentration of 10 μM, but was substantially lower ($66.5 \pm 18.7\%$, $n = 4$) at the highest concentration tested (50 μM). The EC_{50} for Sch 50911 was 3 μM. Although, in some experiments, Sch 50911 increased the resting overflow of ^{3}H (see Fig. 4), this effect was variable and at none of the concentrations tested did the compound enhance the mean resting overflow significantly. The (−) enantiomer Sch 50910 was without effect on the resting or SI-overflow of ^{3}H at the one concentration tested (100 μM). While Sch 48588 (10 μM) did not affect either the resting or SI-overflows, at a concentration of 100 μM, the SI-overflow was increased by $51.3 \pm 11.6\%$ (unpaired Student's *t*-test, $p < 0.005$, $n = 7$) and the resting overflow increased by $34.6 \pm 7.5\%$ (unpaired Student's *t*-test, $p < 0.001$, $n = 7$).

4. Discussion

Spontaneous paroxysmal discharges in rat neocortical slices superfused with Mg^{2+}-free Krebs medium is a well described phenomenon, involving excitatory amino acid neurotransmission, particularly *N*-methyl-D-aspartate receptors (Harrison and Simmonds, 1985; Horne et al., 1986; Aram and Lodge, 1988; Ong et al., 1990). Baclofen, a specific $GABA_B$ receptor agonist, effectively reduces the frequency of these discharges (Horne et al., 1986; Ong et al., 1990), an action that is attenuated by the selective $GABA_B$ receptor antagonists phaclofen and 2-hydroxysaclofen (Kerr et al., 1988, 1989). In the present study, the

suppression of spontaneous firing rate by baclofen was concentration-dependent and sensitive to Sch 50911, which reversibly antagonised the baclofen-induced depression of discharge rate in neocortical slices, with an apparent pA_2 value of 6.0 ± 0.1. The latter value is similar to the pA_2 value of 5.8 ± 0.004 for the antagonism of baclofen-induced inhibition of electrically-stimulated contractile responses in the guinea-pig trachea (Bolser et al., 1995). Moreover, in competitive binding studies, Sch 50911 has been shown to inhibit the binding of GABA to $GABA_B$ receptors in rat brain synaptosomes with an estimated IC_{50} of 1.1 μM, whilst in vivo, it blocks the antitussive and respiratory depressant effects of baclofen, indicating a central site of action (Bolser et al., 1995). By contrast, in the present study, neither Sch 50910, the (−) enantiomer of Sch 50911, nor the des-methyl analogue Sch 48588, had any effect on baclofen-induced depressant responses in the rat neocortex, even at a high concentration of 500 μM. The lack of effect of Sch 50910 confirms that the action of the active enantiomer Sch 50911 is stereoselective. This is in agreement with the binding data where the affinities of Sch 50910 and Sch 48588 for $GABA_B$ receptor binding sites were relatively weak (> 100 μM), when compared to the binding potency of Sch 50911 ($IC_{50} = 1.1$ μM; Bolser et al., 1995).

In rat brain cerebral cortical slices, Sch 50911 not only inhibits baclofen-induced augmentation of isoproterenol-stimulated cyclic AMP accumulation, but also antagonises baclofen-mediated inhibition of forskolin-stimulated cyclic AMP production, and may be useful in characterising the functional and pharmacological properties of $GABA_B$ receptors in biochemical assays (Cunningham and Enna, 1996). Furthermore, Hosford et al. (1995) showed that Sch 50911 was active in suppressing spontaneous absence seizures in the lethargic (*lh/lh*) mutant mouse model, as well as in γ-hydroxybutyrate or pentylenetetrazole-induced absence seizures. Notably, in each of these models, Sch 50911 was found to be more potent than any of the clinical agents used so far, such as ethosuximide, trimethadione or valproic acid, and $GABA_B$ receptors may well provide a rational therapeutic target for the treatment of absence seizures (Hosford et al., 1995). In this regard, it is noteworthy that Sch 50911 may also be useful in some of the clinical applications involving $GABA_B$ receptor activation which includes cognition, analgesia, gastric acid secretion and gut motility (Kerr and Ong, 1995).

In the present study, our findings that Sch 50911 increased the electrically-evoked release of [^{3}H]GABA from rat neocortical slices implies that this compound is an antagonist at $GABA_B$ autoreceptors. The antagonist action of Sch 50911 at $GABA_B$ autoreceptors appears to be stereospecific, given that the (−) enantiomer, Sch 50910, had no effect on the release of [^{3}H]GABA. However, upon increasing the concentration of Sch 50911 above that which produced maximal facilitation of [^{3}H]GABA release, this facilitation was reduced by more than 50%; we have no explanation for the latter, although a similar effect was also observed with the $GABA_B$ receptor antagonist CGP 55845A (see Fig. 3a in Waldmeier et al., 1994). On the other hand, Sch 50911 had no effect on spontaneous discharges at concentrations up to 50 μM. Despite the lack of activity of the racemate Sch 48588 on $GABA_B$ heteroreceptors in the spontaneously discharging slices, it appears to be a weak $GABA_B$ autoreceptor antagonist, given that it increased the stimulation-induced overflow of ^{3}H by 51.3% at 100 μM. Such weak antagonist actions have also been reported in functional and binding studies (Blythin et al., 1996). However, concentrations of Sch 48588 beyond 100 μM were not tested on evoked release, due to limited supply of the compound.

As a class the morpholino-acetic analogues examined here are of particular interest as $GABA_B$ receptor antagonists, since they represent GABA partially frozen in an extended conformation which evidently corresponds with the antagonist recognition site on the receptor. The absolute configurations of Sch 50910 and 50911 are known from X-ray crystallography, with the inactive (−)-enantiomer Sch 50910 in the (*R*)-form, having the 2-acetic moiety attached below the plane of the morpholine ring, a ring that adopts a chair conformation (Frydenvang et al., 1997). By contrast, the active antagonist (+)-enantiomer Sch 50911 exists in the (*S*)-form, with the 2-acetic moiety attached above the plane of the morpholine ring that again adopts a chair conformation (see the stereo-projection in Blythin et al., 1996). The latter projection makes clear the extended, zigzag, $GABA_B$ antagonist conformation of the GABA backbone, partially incorporated into the morpholine ring, although some flexibility is retained around the methylene group of the acetic acid moiety. This establishes the antagonist orientation of the substituted ammonium functionality, and of the morpholine oxygen which may be equivalent to that in the antagonist (*S*)-3-hydroxy-GABA (Falch et al., 1986). This interpretation is reinforced by our finding that (*S*)-3-hydroxy-GABA is a weak partial agonist/antagonist that blocks baclofen responses at $GABA_B$ receptors (Ong and Kerr, unpublished data). Such partial agonist properties of (*S*)-3-hydroxy-GABA in guinea-pig ileal preparations can also be seen in Fig. 6 of Kristiansen and Fjalland (1991). The two molecules, in their extended conformations, can place their oxygen atoms in essentially identical positions, even though Sch 50911 is an ether and (*S*)-3-hydroxy-GABA is an alcohol, and thus may interact similarly with the $GABA_B$ receptor since both ethers and alcohols can act as hydrogen bond acceptors (Fig. 1). One difference between the structures of the relatively weak des-methyl analogue Sch 48588 and of the more potent antagonist Sch 50911 resides in the two methyl substituents on the latter where antagonist activity may be related to the presence of a hydrophobic binding pocket in the $GABA_B$ receptor or a sterically unhindered region. Other substituents at the 5-position might well yield even more potent antagonists than Sch 50911, a compound

which is of great potential pharmacological and therapeutic interest.

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References

Aram, J.A., Lodge, D., 1988. Validation of a neocortical slice preparation for the study of epileptiform activity. J. Neurosci. Methods 23, 211–224.

Baumann, P.A., Wicki, P., Stierlin, C., Waldmeier, P.C., 1990. Investigations on $GABA_B$ receptor-mediated autoinhibition of GABA release. Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 88–93.

Blythin, D.J., Kuo, S.-C., Shue, H.-J., McPhail, A.T., Chapman, R.W., Kreutner, W., Rizzo, C., She, H.S., West, R., 1996. Substituted morpholine-2*S*-acetic acid derivatives: SCH 50911 and related compounds as novel $GABA_B$ antagonists. Bioorg. Med. Chem. Letts. 6, 1529–1534.

Bolser, D.C., Blythin, D.J., Chapman, R.W., Egan, R.W., Hey, J.A., Rizzo, C., Kuo, S.-C., Kretner, W., 1995. The pharmacology of SCH 50911: a novel, orally-active GABA-B receptor antagonist. J. Pharmacol. Exp. Ther. 274, 1393–1398.

Bowery, N.G., 1993. $GABA_B$ receptor pharmacology. Annu. Rev. Pharmacol. Toxicol. 33, 109–147.

Cunningham, M.D., Enna, S.J., 1996. Evidence for pharmacologically distinct $GABA_B$ receptors associated with cAMP production in rat brain. Brain Res. 720, 220–224.

Falch, E., Hedegaard, A., Nielsen, L., Jensen, B.R., Hjeds, J., Krogsgaard-Larsen, P., 1986. Comparative stereostructure-activity studies on $GABA_A$ and $GABA_B$ receptor sites and GABA uptake using rat brain membrane preparations. J. Neurochem. 47, 898–903.

Froestl, W., Mickel, S.J., 1997. Chemistry of $GABA_B$ modulators. In: Enna, S.J., Bowery, N.G. (Eds.), The GABA Receptors, Humana Press, Totowa, NJ, pp. 271–296.

Froestl, W., Mickel, S.J., Von Sprecher, G., Diel, P.J., Hall, R.G., Maier, L., Strub, D., Melillo, V., Baumann, P.A., Bernasconi, R., Gentsch, C., Hauser, K., Jaekel, J., Karlsson, G., Klebs, K., Maitre, L., Marescaux, C., Pozza, M.F., Schmutz, M., Steinmann, M.W., Van Riezen, H., Vassout, A., Mondadori, C., Olpe, H.R., Waldmeier, P.C., Bittiger, H., 1995. Phosphinic acid analogues of GABA: 2. Selective, orally active $GABA_B$ antagonists. J. Med. Chem. 38, 3313–3331.

Frydenvang, K., Enna, S.J., Krogsgaard-Larsen, P., 1997. (−)-(*R*)-5,5-Dimethylmorpholinyl-2-acetic acid ethyl ester hydrochloride, $C_{10}H_{20}NO_3^+ \cdot Cl^-$. Acta Crystallographica C: Crystal Structure Commun. 53, 1088–1091.

Harrison, N.L., Simmonds, M.A., 1985. Quantitative studies on some antagonists of ^{N}-methyl-D-aspartate in slices of rat cerebral cortex. Br. J. Pharmacol. 84, 381–391.

Horne, A.L., Harrison, N.L., Turner, J.P., Simmonds, M.A., 1986. Spontaneous paroxysmal activity induced by zero magnesium and bicuculline: suppression by NMDA antagonists and GABA mimetics. Eur. J. Pharmacol. 122, 231–238.

Hosford, D.A., Wang, Y., Liu, C.C., Carter Snead, O., 1995. Characterization of the antiabsence effects of SCH 50911, a $GABA_B$ receptor antagonist, in the lethargic mouse, g-hydroxybutyrate, and pentylenetetrazole models. J. Pharmacol. Exp. Ther. 274, 1399–1403.

Johnston, G.A.R., 1996. $GABA_C$ receptors: relatively simple transmitter-gated ion channels?. Trends Pharmacol. Sci. 17, 319–323.

Kaupmann, K., Huggel, K., Heid, J., Flor, P.J., Bischoff, S., Mickel, S.J., McMaster, G., Angst, C., Bittiger, H., Froestl, W., Bettler, B., 1997. Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. Nature 386, 239–246.

Kerr, D.I.B., Ong, J., 1995. GABAB receptors. Pharmacol. Ther. 67, 187–246.

Kerr, D.I.B., Ong, J., Prager, R.H., Gynther, B.D., Curtis, D.R., 1987. Phaclofen: a peripheral and central baclofen antagonist. Brain Res. 405, 150–154.

Kerr, D.I.B., Ong, J., Johnston, G.A.R., Abbenante, J., Prager, R.H., 1988. 2-Hydroxy-saclofen: an improved antagonist at central and peripheral $GABA_B$ receptors. Neurosci. Lett. 92, 92–96.

Kerr, D.I.B., Ong, J., Johnston, G.A.R., Prager, R.H., 1989. $GABA_B$-receptor-mediated actions of baclofen in rat isolated neocortical slice preparations: antagonism by phosphono-analogues of GABA. Brain Res. 480, 312–316.

Kristiansen, U., Fjalland, B., 1991. Ligand structural specificity of $GABA_A$ receptors in guinea pig ileum. Pharmacol. Toxicol. 68, 332–339.

Kuo, S.-C., Blythin, D.J., Kreutner, W., 1994. 2-Substituted morpholine and thiomorpholine derivatives as $GABA_B$ antagonists. WO 22843, prior, 26 March 1993.

Ong, J., Kerr, D.I.B., Johnston, G.A.R., Hall, R.G., 1990. Differing actions of baclofen and 3-aminopropylphosphinic acid in rat neocortical slices. Neurosci. Lett. 109, 169–173.

Pittaluga, A., Asaro, D., Pellegrinni, G., Raiteri, M., 1987. Studies on [^{3}H]GABA and endogeneous GABA release in rat cerebral cortex suggest the presence of autoreceptors of the $GABA_B$ type. Eur. J. Pharmacol. 144, 45–52.

Waldmeier, P.C., Wicki, P., Feldtrauer, J.-J., Mickel, S.J., Bittiger, H., Baumann, P.A., 1994. GABA and glutamate release affected by $GABA_B$ receptor antagonists with similar potency: no evidence for pharmacologically different presynaptic receptors. Br. J. Pharmacol. 113, 1515–1521.