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Arylalkylamines are a novel class of positive allosteric modulators at $GABA_B$ receptors in rat neocortex

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Abstract

Using grease-gap recording from rat neocortical slices, the γ-aminobutyric $acid_B$ ($GABA_B$) receptor agonists baclofen (3–100 μM) and SKF 97541 (3-aminopropyl-methylphosphinic acid) (1–30 μM) elicited reversible and concentration-dependent hyperpolarizing responses, with EC_{50} values of 10 and 3 μM, respectively. The hyperpolarizations were antagonised by the $GABA_B$ receptor antagonist Sch 50911 ((+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid) (1, 5 and 10 μM). Fendiline (*N*-[3,3-diphenylpropyl)-α-methylbenzylamine) (5–50 μM) and its congeners, prenylamine (*N*-[3,3-diphenylpropyl)-α-methylphenylethylamine) (10–100 μM) and F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine) (1–30 μM) reversibly enhanced hyperpolarizing responses to the agonists; such effects were reduced by Sch 50911. These arylalkylamines produced leftward shifts of the concentration–response curves, with a marked increase in the maximal hyperpolarization obtained, compared with the agonists alone, F551 being the most potent. These findings suggest that these arylalkylamines represent a new class of positive modulators of $GABA_B$ receptor-mediated function.

Keywords: Neocortex, rat; Baclofen; SKF 97541; Positive modulator; Fendiline; Prenylamine

1. Introduction

In general, allosteric modulators provide a novel means for the pharmacological manipulation of G-protein-coupled receptors (Holzgrabe and Mohr, 1998). Acting as they do at a site apart from the orthosteric binding region of the receptor protein, allosterically acting ligands represent an entirely different approach for affecting receptor function. Notably, they may delay dissociation by stabilizing the bound-agonist state, or act as subtype-selective enhancers of agonist binding, and promote receptor G-protein coupling. All these properties suggest that allosteric modulators may offer a number of potential pharmacological improvements over the use of conventional agonists, particularly in γ-aminobutyric $acid_B$ ($GABA_B$) receptors where subtype-selective enhancers, if found, would be a definite advantage.

Recently, a number of allosteric modulators have been described among members of Family 3 G-protein-coupled receptors, including extracellular Ca^{2+}-sensing receptors (Nemeth et al., 1998), metabotropic glutamate receptors (mglu1) (Knoflach et al., 2001) and $GABA_B$ receptors (Urwyler et al., 2001). In all these Family 3 receptors, the orthosteric binding site for the natural agonist lies within a specialized conserved Venus fly-trap region of the amino-terminal domain (Couve et al., 2000), whereas the allosteric modulators bind at a site apart from this specialized region of the amino-terminal domain. With the $GABA_B$ receptors, there is an additional complication, in that they exist in the central nervous system as heterodimers of the two similar related, but not identical receptor subtypes, $GABA_BR1$ (GBR1) and $GABA_BR2$ (GBR2). Curiously, only GBR1 shows agonist and antagonist binding within the Venus fly-trap region; the corresponding region of GBR2 shows no such binding, and its naturally occurring ligand(s) remains unknown. Importantly, however, GBR2 modulates GBR1 and is the main signalling receptor essential for G-protein coupling of the $GABA_B$ receptor heterodimer (Margeta-Mitrovic et al., 2001; Robbins et al., 2001).

It is already known that a variety of phenylalkylamines are potent allosteric modulators at extracellular Ca^{2+}-sensing receptors. These include fendiline (*N*-[3,3-diphenylpropyl)-α-methylbenzylamine) and prenylamine (*N*-[3,3-

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diphenylpropyl)-α-methylphenylethylamine) (see chemical structures in Fig. 1). Such alkylamines were originally introduced as coronary vasodilators, whilst fendiline emerged as the prototypical positive allosteric modulator for extracellular Ca^{2+}-sensing receptors (Nemeth et al., 1998). Elsewhere we have drawn attention to the similarity between the α-methylbenzyl moiety of fendiline and its congeners, and a corresponding region in $GABA_B$ receptor ligands (Kerr and Ong, 2001). It should be emphasized that elements of the fendiline structure are also immediately recognizable in two of the allosteric modulators, Ro 67-4853 and Ro 01-6128, for the related Family 3 mglu1 receptor, recently introduced by Knoflach et al. (2001). These similarities are sufficiently striking to suggest that phenylalkylamines akin to fendiline might also be allosteric modulators at $GABA_B$ receptors, which share sequence similarity with the other Family 3 G-protein-coupled receptors.

$GABA_B$ receptors can be subdivided into presynaptic heteroreceptors that inhibit synaptic transmission, together with presynaptic autoreceptors that similarly regulate the release of GABA itself, and postsynaptic receptors that reduce neuronal excitability (Kerr and Ong, 1995, 2001). Inhibition by these receptors is involved in a number of crucial physiological processes such as autonomic function, memory and cognition, as well as motor and sensory control including pain regulation and epilepsy. Indeed, $GABA_BR1$ subunit knockout mice lacking these receptors exhibit defects in all these processes, resulting in premature death (Prosser et al., 2001). Despite such gene targeting techniques and knockout technology, this approach does not provide evidence for pharmacologically or functionally distinct $GABA_B$ receptor subtypes.

Using baclofen as the agonist at $GABA_B$ receptor-mediated hyperpolarizations in rat neocortical slice preparations (Ong et al., 2001), our preliminary data revealed positive allosteric actions of fendiline at these receptors. This prompted us to explore further aspects of the structure–action requirements of this potentiating activity in fendiline and a variety of its derivatives, along with the related phenylalkylamine prenylamine and F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine) (Fig. 1). We now report that positive allosteric modulation of $GABA_B$ receptors is to be found in several such phenylalkylamines, which thus constitute a new class of potentiating modulators for these receptors that may provide the potential for discrimination between $GABA_B$ receptor subtypes.

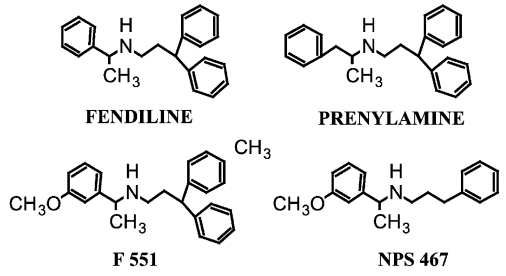

Fig. 1. Chemical structures of a novel class of arylalkylamine potentiators of $GABA_B$ receptors, including fendiline (*N*-[3,3-diphenylpropyl)-α-methylbenzylamine) and its structurally related congeners, prenylamine (*N*-[3,3-diphenylpropyl)-α-methylphenylethylamine) and F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine). F551 incorporates a 3-*O*-methyl substituent on the phenyl ring of the α-methylbenzyl moiety that is also found in the Ca^{2+} receptor potentiator NPS 467 (*N*-(3-phenylpropyl)-α-methyl-3-methoxybenzylamine), shown here for comparison.

2. Materials and methods

2.1. Rat neocortical slice preparations

All experiments 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 using established procedures described previously (Ong et al., 2001). 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 and $MgSO_4$ 1.3. Cerebral cortical slices (400 μm thick) were prepared by cutting coronal sections using a VIBROSLICE microtome (Campden Instruments, 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 1 h prior to experimentation.

Following the equilibration period, wedge-shaped slices from the neocortex were placed in a two-compartment Perspex perfusion chamber, where each wedge was placed across a septum, separating pools containing the cortex and white matter by a grease seal, using a superfusion method based on a grease-gap system as described previously (Horne et al., 1986). The grey matter was then continuously superfused with gassed Krebs medium at 25 °C delivered by a peristaltic pump at 1 ml/min. Potential changes induced by $GABA_B$ receptor agonists were recorded during 3-min applications of each agonist. Differential recordings (mV) between the cortex and white matter were measured with Ag/AgCl electrodes, and the DC potentials were monitored on a chart recorder using a high input-impedance DC amplifier. The white matter was immersed in a chamber containing Krebs solution, whilst the grey matter in the second chamber was superfused with gassed Krebs buffer at 25 °C delivered by a peristaltic pump at 1 ml/min. Here, Mg^{2+}-containing

Krebs medium was used throughout the experiments to eliminate the spontaneous discharges, since the latter tended to complicate the hyperpolarizing responses.

After 60-min equilibration, the $GABA_B$ receptor agonists were added to the superfusing medium and applied to the cortical side of the tissue for 3 min to achieve steady state concentrations within the recording chamber. Each preparation was allowed a minimum of 30 min recovery between drug applications. When examining the modulatory effects of a potentiator, the latter was first superfused for 5 min and then added together with the agonist for a further 3 min before tissue washout. In some experiments where the $GABA_B$ receptor antagonist was used to test the specificity of the potentiators, it was first superfused for 3 min and then added together with the test compounds and agonist. In each experiment, the responses to the agonist were reestablished after each drug application to control for the stability of the preparation. Results were quantified, and values expressed as a percentage of the maximum hyperpolarization obtained with the agonist alone were measured from the chart recordings. Concentration–response curves were constructed, in the absence and presence of the test agents. To test the positive modulatory activity of the compounds, they were applied with ascending concentrations and a fixed concentration of the agonist. Concentration–response profiles for all potentiators were constructed by measuring the peak amplitude during application of the compound and a fixed concentration of the agonist (EC_{50} of the agonist), calculating the percent increase relative to the agonist (alone) response and plotting the data as a function of potentiator concentration.

In other experiments, the concentration–response curves of the agonist were constructed, in the absence and presence of differing concentrations of the potentiator. The EC_{50} values were then calculated from the concentration–response curves, where the EC_{50} is the concentration giving a response equal to 50% of the maximally effective concentration. All numerical data on the concentration–response curves were expressed as means ± S.E.M. Each experiment was repeated on 8–24 slices obtained from 6 to 12 different animals. Comparison of the data was made using Student's *t*-test with $P < 0.05$ being significant.

2.2. Drugs

Racemic (±)-baclofen, SKF 97541 (3-aminopropyl-methylphosphinic acid) and Sch 50911 ((+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid) were purchased from Tocris Cookson (Bristol, UK). Fendiline (*N*-[3,3-diphenylpropyl)-α-methylbenzylamine) and prenylamine (*N*-[3,3-diphenylpropyl)-α-methylphenylethylamine) were from Sigma (MO, USA). F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine) (Fig. 1) was synthesized in house, by the reductive alkylation of commercially available 3,3-diphenylpropylamine and 2-methoxyacetophenone with sodium cyanoborohydride.

3. Results

3.1. Hyperpolarizations mediated by $GABA_B$ receptor agonists in neocortical slices

As described previously, when experiments were performed on neocortical wedges superfused with Mg^{2+}-containing Krebs solution, the $GABA_B$ receptor agonist baclofen induced concentration-dependent hyperpolarizing responses which were reversibly antagonised by Sch 50911, a selective $GABA_B$ receptor antagonist. These population hyperpolarizations were mediated through activation of inwardly rectifying K^+ channels, being sensitive to Ba^{2+} (0.1 mM) or Cs^+ (1 mM) (Ong et al., 2001). The onset of the baclofen-evoked hyperpolarization occurred 2 min after baclofen reached the slice and the maximal effect was reached within 3–5 min, after which restoration of the initial potential was completed within 30 min following reintroduction of drug-free Krebs solution with reestablishment of baseline controls. A typical example of this response is represented in Fig. 2.

Superfusion of neocortical slices with baclofen for 3 min, over a concentration range of 3–100 μM, consistently induced concentration-dependent hyperpolarizing responses (Fig. 3A,B; $n = 24$). Responses obtained by using different concentrations of this $GABA_B$ receptor agonist were normalized to those obtained by using a maximum concentration of the agonist. The concentration–response curve for baclofen was then plotted as a percentage of the normalized near-maximal hyperpolarizing response elicited by baclofen at 100 μM (100% response; $n = 24$). From this curve, an EC_{50} value of 10 μM for baclofen measured at half-maximal

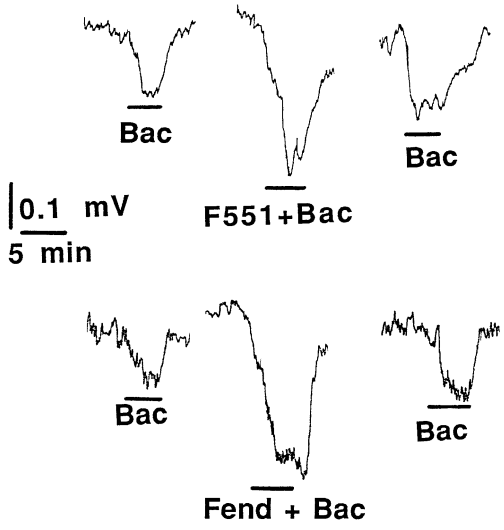

Fig. 2. Discontinuous records of the hyperpolarizing effects of baclofen (10 μM) in rat neocortical slices and the potentiating effects of F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine) (3 μM) and fendiline (Fend) (30 μM) on these responses in two separate preparations. The control responses to baclofen were subsequently reestablished upon tissue washout within 60 min. The interval between drug applications was 30–60 min.

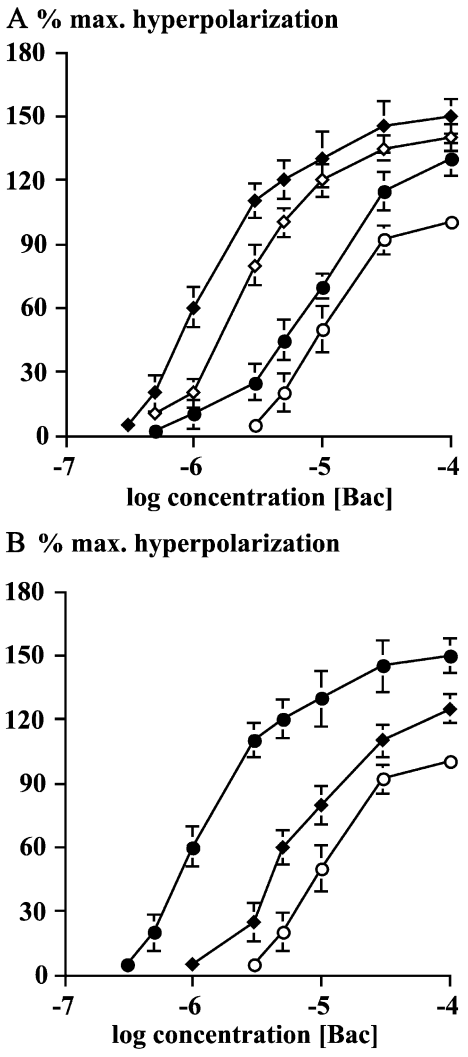

Fig. 3. Concentration–response curves for baclofen-induced hyperpolarizations recorded from the rat neocortical slices, in the absence and presence of the potentiator fendiline. (A) Complete concentration–response curves for baclofen alone (○), and the leftward shifts of the curves by various concentrations of fendiline (● 10 μM; ◇ 30 μM; ◆ 50 μM). (B) The baclofen (○) concentration–response curve was shifted to the left by fendiline (●) (50 μM), and the leftward shift of the concentration–response curve for baclofen in the presence of fendiline (50 μM) was partially reversed by the antagonist Sch 50911 ((+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid) (◆) (5 μM). Values are expressed as a percentage of the maximum hyperpolarization achieved by the agonist alone, and each point represents the mean and standard error of mean of 8–24 determinations.

effect was derived. The threshold for inducing a hyperpolarization response was around 3 μM, resulting in a 5% response, and the maximal effect was elicited by 100 μM baclofen (100% response). In general, full recovery to baclofen-induced responses was obtained only after 30 min of tissue washout.

Another $GABA_B$ receptor agonist, SKF 97541 (1–30 μM), also induced hyperpolarizing responses, similar to that of baclofen. In the present experiments, the half-maximal effective concentration of SKF 97541 (EC_{50}) in inducing hyperpolarizations of the neocortex was 3 μM (Fig. 4; $n=24$), some three times more potent than baclofen itself which has an EC_{50} value of 10 μM. Furthermore, as with baclofen, SKF 97541 elicited hyperpolarizations that were reversibly inhibited by Sch 50911 (1, 5 and 10 μM), with a rightward shift of the concentration–response curves (pA_2 value of 6.0; data not shown; $n=12$). This was readily washed out within 30 min. On its own, the antagonist Sch 50911 (1, 5 and 10 μM) did not evoke a potential shift in control slices. Like baclofen, the onset of the SKF 97541-evoked hyperpolarization came after 2 min of drug application. The maximal effect was achieved within 5 min, after which repolarization of the membrane potential occurred, with reestablishment of baseline conditions, within 30 min following superfusion of drug-free Krebs solution (figure not shown).

3.2. Effects of fendiline on $GABA_B$ receptor-mediated hyperpolarizations

Application of fendiline (5, 10, 30 and 50 μM) alone for 5 min had no detectable effect, but when cosuperfused with baclofen, it potentiated the baclofen-induced hyperpolarizing responses ($n=8$). Moreover, fendiline (30 μM) accelerated the onset of $GABA_B$ receptor-mediated hyperpolarizing activity induced by baclofen (10 μM), such that the response began within 30 s of the agonist reaching the slice, rather than the 2 min with baclofen alone (see Fig. 2). The reversibility of fendiline potentiation was examined by recording the hyperpolarizations to baclofen before, during

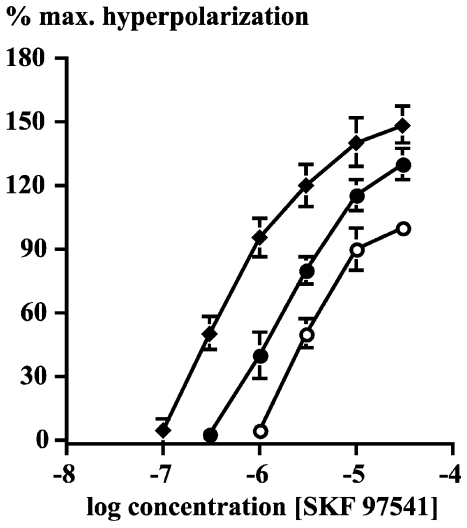

Fig. 4. Concentration–response curve for the effect of the enhancer fendiline on $GABA_B$ receptor agonist SKF 97541 (3-aminopropyl-methylphosphinic acid)-induced hyperpolarizations in rat neocortical slices. The SKF 97541 concentration–response curve (○) was shifted to the left by fendiline (◆) (50 μM), and the leftward shift of the concentration–response curve for SKF 97541 in the presence of fendiline (50 μM) was partially reversed by the antagonist Sch 50911 ((+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid) (●) (5 μM). Values are expressed as a percentage of the maximum hyperpolarization achieved by the agonist alone, and each point represents the mean and standard error of mean of 8–24 determinations.

and after co-application of the compound. Hyperpolarizing responses elicited by co-application of fendiline and baclofen were significantly more pronounced and lasted longer than the response recorded during the application of baclofen alone (Fig. 2). The control response to baclofen was reestablished by washing for 60 min.

Fendiline-induced enhancement of the hyperpolarizing response to baclofen was concentration-dependent, since the concentration–response curves for baclofen at different fixed concentrations of fendiline (10, 30 and 50 μM) revealed an increase of agonist potency as well as maximal efficacy (Fig. 3A; $n=8$ for each drug concentration). Fendiline significantly displaced the baclofen concentration–response curves to the left; in particular, at the highest concentration of 50 μM, fendiline also increased the maximal effect of baclofen at 100 μM to 150% of the control response (Fig. 3B; $n=8$). Concentrations higher than 50 μM were not used in the present experiments, since at higher concentrations, fendiline can stimulate the Ca^{2+}-sensing receptors and interferes with $GABA_B$ receptor-mediated actions. The concentration–response profile for baclofen alone had an EC_{50} value of 10 μM ($n=24$). In the presence of fendiline (50 μM), the potency of baclofen increased approximately sevenfold, such that the average EC_{50} value was 1.4 μM (Fig. 3B; $n=8$).

Application of baclofen alone induced responses that were reduced in the presence of Sch 50911. Following antagonist washout, subsequent application of fendiline augmented the response to baclofen in the same slices. Furthermore, this modulatory effect was mediated via $GABA_B$ receptors, since it was abolished by the competitive $GABA_B$ receptor antagonist Sch 50911 (5 μM; estimated pA_2 value of 6.0) (Fig. 3B; $n=8$). Pretreatment with Sch 50911 (5 μM) alone for 3 min did not affect the resting potential, but in combination with varying concentrations of baclofen and fendiline (50 μM) for 3 min, reversibly reduced the fendiline-induced enhancement of baclofen hyperpolarizations (Fig. 3B). Following washout of the compounds, there was a complete recovery of the response to baclofen within 60 min. The enhancing effects of fendiline on baclofen-induced responses were also diminished by two other concentrations of Sch 50911 (1 and 10 μM), which caused a progressive shift of the fendline–baclofen concentration–response curves to the right (data not shown; $n=8$ for each concentration of Sch 50911).

A further series of experiments were performed with increasing concentrations of fendiline on a fixed concentration of the agonist baclofen. Here, we generated concentration–response curves for the potentiation of baclofen at 10 μM by fendiline (5, 10, 30 and 50 μM) at the $GABA_B$ receptor (Fig. 5; $n=8–12$). From this, the EC_{50} value for fendiline potentiation was estimated at 20 μM. Similarly, SKF 97541-evoked hyperpolarizing responses were also reversibly potentiated by a maximal concentration of fendiline (50 μM), which shifted the SKF 97541 concentration–response curve to the left, with a maximal potentiation of

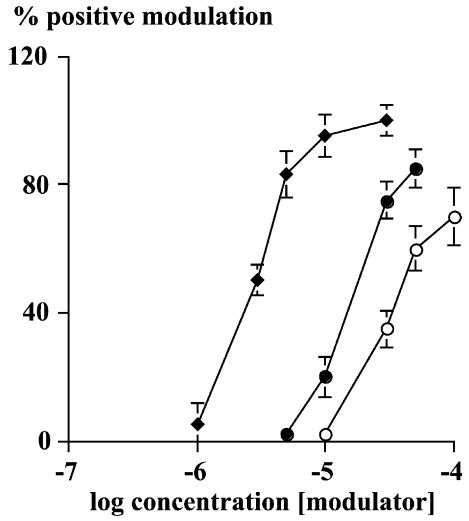

Fig. 5. Concentration–response profiles for the modulators prenylamine (○), fendiline (●) and F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine) (◆) in potentiating the hyperpolarizing responses induced by a fixed concentration of baclofen (10 μM), in rat neocortical slices, maintained in Mg^{2+}-containing Krebs solution. Values are expressed as a percentage of the hyperpolarization induced by baclofen alone, and each point represents the mean and standard error of mean of 8–12 determinations.

148% for the highest concentration of the agonist (30 μM) (Fig. 4; $n=8$). Here, the effects of fendiline in the presence of SKF 97541 could be prevented by the antagonist Sch 50911 (5 μM) in a reversible manner (Fig. 4; $n=8$). Lower concentrations of fendiline (5, 10 and 30 μM) also increased the hyperpolarizing effects of SKF 97541 in a concentration-dependent manner (data not shown; $n=8$), but to a lesser degree than that induced by 50 μM fendiline. The concentration–response profile for SKF 97541 alone had an EC_{50} value of 3 μM ($n=24$), but in the presence of fendiline (50 μM), the potency of SKF 97541 increased approximately fivefold, such that the average EC_{50} value was 0.67 μM ($n=8$).

3.3. Effects of F551 and prenylamine on $GABA_B$ receptor-mediated hyperpolarizations

The enhancing effects of F551 and prenylamine on hyperpolarizing potentials associated with the application of baclofen were examined. At all concentrations tested, each compound was devoid of any effect on the membrane potential when applied alone. In a typical experiment, Fig. 2 shows persistent potentiating action of F551 (3 μM) on baclofen (10 μM)-induced hyperpolarization, where the amplitude was markedly enlarged, followed by a slower recovery of the baclofen response. After 45-min washout in this slice, the response to baclofen was still enhanced, but returned to control level after further washing, requiring at least 60 min. Evidently, F551 was far more effective than fendiline itself in enhancing the amplitude of the hyperpolarizing potential. Similar to fendiline, the modulators

F551 and prenylamine potentiated the hyperpolarizing responses to baclofen and accelerated the onset of the hyperpolarizations within 30 s of the agonist being present. Both F551 and prenylamine substantially increased the effects of different concentrations of baclofen; the latter was less sensitive to prenylamine (50 μM) (Fig. 6; $n=8$) than to F551 (3 μM) (Fig. 7A; $n=8$). In the presence of F551 (3 μM), the potency of baclofen (EC_{50} value of 10 μM) increased approximately threefold, such that the average EC_{50} value was 3.2 μM (Fig. 7A). However, the weaker potentiator prenylamine (50 μM) gave only a twofold shift of the baclofen curve, with an EC_{50} value of 5 μM (Fig. 6).

Rather than to provide detailed concentration–response curves to baclofen in the presence of varying concentrations of F551 (1–30 μM) and prenylamine (10–100 μM), further experiments were performed with increasing concentrations of F551 and prenylamine on a fixed concentration of the agonist baclofen. The concentration–response profiles for F551 (1, 3, 5, 10 and 30 μM) and prenylamine (10, 30, 50 and 100 μM) in enhancing the hyperpolarizing effects elicited by the fixed concentration of baclofen (10 μM) show that the estimated EC_{50} value for F551 potentiation of baclofen responses was 3 μM, whilst the EC_{50} value for prenylamine was 30 μM (Fig. 5; $n=8–12$ for each modulator). Consequently, in comparison to the effects of fendiline, which has an estimated EC_{50} value of 20 μM, the analogue F551 was approximately 7 times more potent than fendiline itself, but 10 times more potent than prenylamine. From these curves, it is apparent that at the highest concentrations of each modulatory agent, F551 induced the biggest potentiation when compared to either fendiline or prenylamine, with the smallest potentiation (Fig. 5; $n=8–12$).

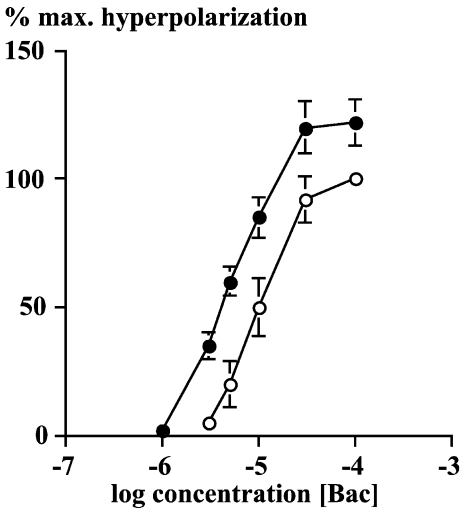

Fig. 6. Concentration–response curve for the effect of the enhancer prenylamine on baclofen-induced hyperpolarizations in rat neocortical slices. The baclofen concentration–response curve (○) was shifted to the left by prenylamine (●) (50 μM). Values are expressed as a percentage of the maximum hyperpolarization induced by baclofen alone, and each point represents the mean and standard error of mean of eight determinations.

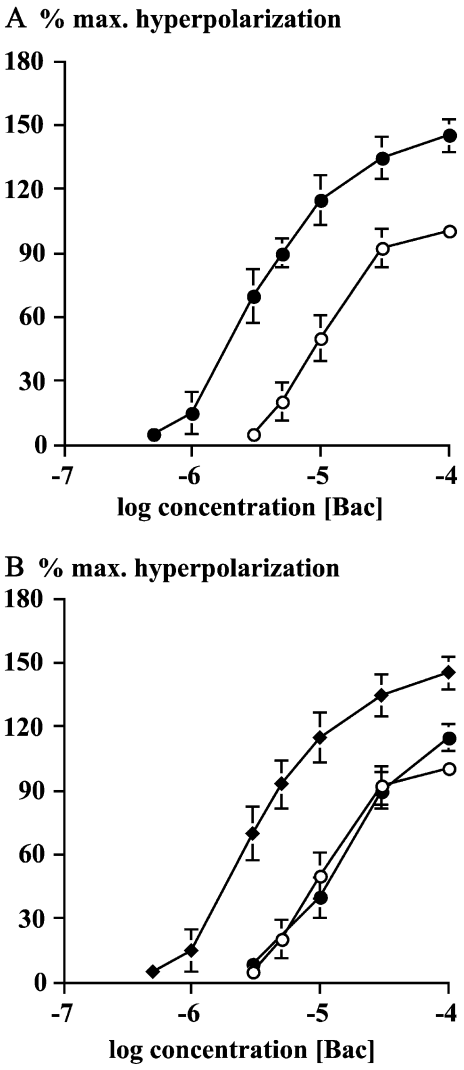

Fig. 7. Concentration–response curve for baclofen-induced hyperpolarizations in rat neocortical slices, in the absence and presence of the modulator F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine), and a subsequent reduction of the potentiation by the antagonist Sch 50911 ((+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid). (A) The baclofen concentration–response curve (○) was shifted to the left by F551 (●) (3 μM), and (B) the leftward shift of the concentration–response curve for baclofen in the presence of F551 (◆) was fully reversed by the antagonist Sch 50911 (●) (5 μM). Values are expressed as a percentage of the maximum hyperpolarization achieved by the agonist alone, and each point represents the mean and standard error of mean of 8–24 determinations.

However, when baclofen was applied at 100 μM, the maximal potentiation reached with 3 μM F551 was 145% (Fig. 7A), whilst the maximal effect for prenylamine was 122% at 50 μM (Fig. 6). Based on these results, F551 (3 μM) produced rather similar maximal potentiation as fendiline at 50 μM (150%). Although the extent of potentiation was close between the two compounds, nevertheless, F551 was far more potent than fendiline; indeed, 150% potentiation may be the maximum amplitude that can be reached. Sch 50911 (5 μM) reversibly reduced the positive modulatory actions of F551 (3 μM) on baclofen responses (Fig.

7B; $n=8$), as well as that of prenylamine (data not shown; $n=8$), similar to that observed with fendiline.

Similar results were found with the agonist SKF 97541. Responses to SKF 97541 were also markedly and reversibly potentiated in the presence of F551 (3 μM) (Fig. 8A; $n=8$) and prenylamine (50 μM) (Fig. 8B; $n=8$), with F551 being more potent than prenylamine. Over the concentration range of 1–30 μM F551 and 10–100 μM prenylamine, the amplitude of the hyperpolarizing potentials to a fixed concentration of SKF 97541 ($EC_{50}=3$ μM) was enhanced in a concentration-dependent manner (data not shown; $n=8-12$). These effects were also reversibly reduced by Sch 50911 (5 μM) (data not shown; $n=8-12$). The extent of potentiation for SKF 97541 by all three modulators maintained at the given concentration range was similar to that for baclofen, with the maximal potentiation obtainable at the highest concentration of SKF 97541 being the same as that of baclofen. The concentration–response profile for SKF 97541 alone had an EC_{50} value of 3 μM ($n=24$), but in the presence of F551 (3 μM), the potency of SKF 97541 increased approximately twofold, such that the average EC_{50} value was 1.3 μM ($n=8$). In the case of prenylamine, the potency of SKF 97541 increased approximately twofold, with an EC_{50} value of 1.6 μM ($n=8$). Notably, none of these modulators at their highest concentrations employed had any effects on depolarizing responses to GABA (100 μM) involving $GABA_A$ receptor-mediated actions, again confirming that these compounds were selective for $GABA_B$ receptor sites.

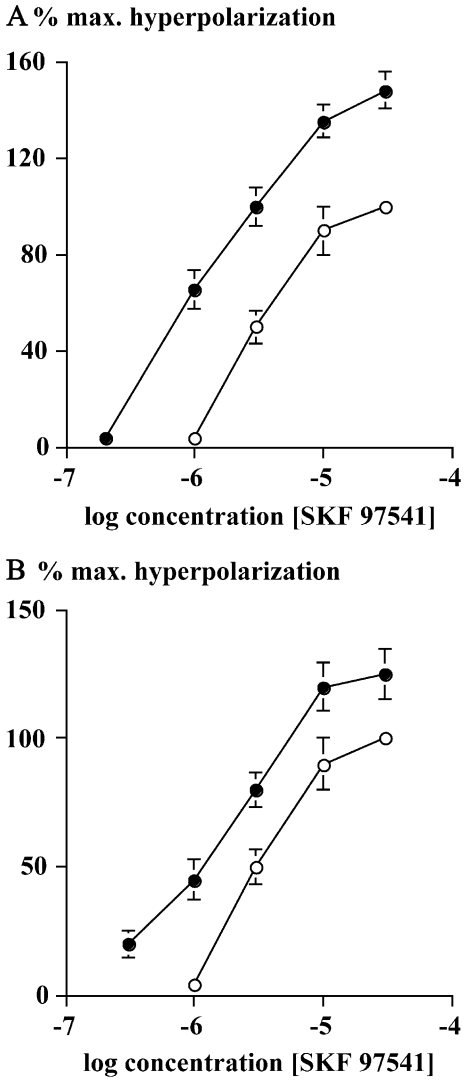

Fig. 8. Concentration–response curves for SKF 97541 (3-aminopropyl-methylphosphinic acid)-induced hyperpolarizations in rat neocortical slices, and the modulation by the potentiators F551 (*N*-[3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine) and prenylamine. (A) The curve for SKF 97541 (○) was shifted to the left by F551 (●) (3 μM), with an increase in the maximal response, as well as by (B) prenylamine (●) (50 μM). Values are expressed as a percentage of the hyperpolarization achieved by the agonist alone, and each point represents the mean and standard error of mean of 8–24 determinations.

4. Discussion

Our present results introduce a new class of positive allosteric modulators at $GABA_B$ receptors, based on the *N*-(3,3-diphenyl)propylamine backbone of fendiline. These arylalkylamines (fendiline, prenylamine and F551) showed little or no hyperpolarizing response in the absence of the $GABA_B$ receptor agonist baclofen, but potentiated responses to baclofen and produced a leftward shift of the baclofen concentration–response curve, with a marked increase in the maximal hyperpolarization obtained with baclofen alone, indicative of positive allosteric modulation at these receptors. Such responses were depressed by the $GABA_B$ receptor antagonist Sch 50911 (Ong et al., 1998) and, therefore, dependent on activation and modulation of $GABA_B$ receptors at neurons in the neocortex. The most potent modulator was F551, being 7 times more potent than fendiline itself, but 10 times more potent than prenylamine. Similar enhancing effects were also seen with the more potent $GABA_B$ receptor agonist SKF 97541 (Seabrook et al., 1990). The modulatory actions of fendiline, prenylamine and F551 were selective to $GABA_B$ receptors since they did not modulate $GABA_A$ receptor-mediated responses induced by GABA itself. Moreover, the lesser potentiating action of the phenylalkylamines on agonist-induced $GABA_B$ receptor-mediated responses in the presence of the antagonist is evidently due to antagonism of the agonist site of the receptor, producing a rightward shift of the agonist concentration–response curve, rather than antagonism of the allosteric site as such. Although so far, we have not found any compound that would antagonise the modulatory site of the $GABA_B$ receptor, nevertheless, compounds such as NPS 2143 (*N*-[(*R*)-2-hydroxy-3-(2-cyano-3-chlorophenoxy)-propyl]-1,1-dimethyl-2-(2-naphthyl)ethylamine) (Nemeth et al., 2001) bear a strong resemblance to ligands active at $GABA_B$ receptors, and might well be active at the $GABA_B$ receptor modulatory site even though NPS 2143 and other phenylalkylamines are inactive at the $GABA_B$ receptor agonist site (Nemeth, E.F., personal communications).

A further effect, related to this allosteric action of the arylalkylamines, also emerged; in the presence of the modulator, the hyperpolarization generated by a given baclofen concentration was not only increased in amplitude, but the duration also became prolonged. This suggests that the modulators in some way prevented desensitization, and that the dissociation of the agonist was delayed by the modulator, or the activation of the G-proteins prolonged. Furthermore, their modulatory action far outlasted the period of application, since potentiation of responses to baclofen could still be seen 30–50 min after tissue washout of the modulators. Comparing the positive modulatory effects of the three arylalkylamines used in the present study shows that modification of just the *N*-α-methylbenzyl moiety effectively alters their modulatory activity. Evidently, incorporation of an additional methylene to provide prenylamine, the α-methylphenylethylamine derivative of fendiline, leads to some twofold loss of modulatory potency, relative to the parent α-methylbenzylamine, and thus is to be avoided when searching for more potent modulators. However, F551 incorporates a 3-*O*-methyl substituent on the phenyl ring of the α-methylbenzyl moiety (Fig. 1), which markedly increases its potency, some sevenfold relative to fendiline. This substituent is also found in the corresponding phenylalkylamine-derived Ca^{2+} receptor potentiator NPS 467 (*N*-(3-phenylpropyl)-α-methyl-3-methoxybenzylamine) (Fig. 1) (Nemeth et al., 1998), and suggests that more potent $GABA_B$ receptor modulators may yet emerge from this series. There is a caveat in all this, however, since Ca^{2+} receptors are found on a variety of neurons, so that these $GABA_B$ receptor modulators may, by stimulating neuronal Ca^{2+} receptors, increase the neuronal Ca^{2+} content and modify their properties, which is to be avoided. Indeed, when using higher concentrations of the present $GABA_B$ receptor modulators, some development of spontaneous firing is at times seen in the quiescent slices, superfused with Mg^{2+} (1.3 mM)-containing Krebs solution to block *N*-methyl-D-aspartate receptors.

It is of interest that our preliminary studies with fendiline and F551, at levels not obviously affecting Ca^{2+} receptors, show no evidence of positive modulation at $GABA_B$ autoreceptors. Indeed, these compounds did not potentiate the action of baclofen in depressing electrically stimulated [^{3}H]GABA release in rat neocortical slices (Ong et al., 2001), at concentrations up to 20 μM for fendiline and 5 μM for F551, close to their EC_{50}s for potentiation of $GABA_B$ heteroreceptor-mediated hyperpolarization (unpublished observations, to be detailed elsewhere). If these results can be confirmed and extended, they offer the exciting possibility that positive allosteric modulators, based on the fendiline congeners, may provide some entirely novel means for selectively enhancing physiologically relevant responses mediated by $GABA_B$ receptor subtypes, where no subtype-selective agonists exist at present.

Where might these phenylalkylamines act at the $GABA_B$ receptor GBR1/GBR2 complex, when giving rise to the allosteric enhancement of agonist-dependent responses? Certainly we found no difference between the modulatory action on responses to baclofen or SKF 97541. Nonetheless, baclofen has a different mode of binding from that of other $GABA_B$ receptor agonists at the $GABA_BR1$ site (Galvez et al., 2000), suggesting that this site is not involved in the modulatory actions. In fact, the allosteric activation of Ca^{2+} receptors by the related phenylalkylamines NPS 467 and NPS 568 (*N*-(3-(2-chloro-phenyl)propyl)-α-methyl-3-methoxybenzylamine), in *Xenopus* oocytes, is due to their actions at the 7-transmembrane domain and not the extracellular amino-terminal domain of these receptors (Hammerland et al., 1998). There is a degree of sequence homology between $GABA_B$ receptors and Ca^{2+} receptors, both of which belong to Family 3 of G-protein-coupled receptors, and it seems entirely possible that the fendiline-derived phenylalkylamines used here might also act at a similar region in the 7-transmembrane domain of $GABA_B$ receptors. Apart from Ca^{2+} itself, the only other known allosteric modulator of $GABA_B$ receptor-mediated function is CGP 7930 (2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol) (Urwyler et al., 2001), which we have confirmed to be an effective potentiator of agonist-induced hyperpolarizations in our system (unpublished observations). Urwyler et al. (2001) have suggested that CGP 7930 might act at $GABA_BR2$ subunit to modulate the GBR1/GBR2 heterodimer, and whether these new phenylalkylamine potentiators might also act at this subunit remains to be seen. At any event, it is interesting that GBR2 receptors are modulatory on GBR1, which raises the question of naturally occurring ligands acting at the GBR2 modulatory site, an area we are currently pursuing.

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