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$GABA_B$ receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area

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1 2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) is a recently reported positive allosteric modulator of γ-aminobutyric acid $(GABA)_B$ receptors. In this study, we assessed the ability of CGP7930 to modulate the baclofen-induced depression of dopamine (DA) neuron activity *via* the activation of $GABA_B$ receptors in the ventral tegmental area in rat midbrain slices.

2 The selective $GABA_B$ receptor agonist, baclofen, depressed the spontaneous firing rate of DA neurons in a concentration-dependent manner ($EC_{50} = 0.27\,\mu M$, $n = 11$). CGP7930 ($30\,\mu M$) significantly ($P < 0.05$) shifted the baclofen concentration–response curve to the left ($EC_{50} = 0.15\,\mu M$, $n = 5$). The effects of baclofen alone or baclofen coapplied with CGP7930 were fully blocked by $1\,\mu M$ (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl] (phenylmethyl) phosphinic acid (CGP55845), a potent and selective $GABA_B$ receptor antagonist.

3 In similar experiments, *N*-[3,3-diphenylpropyl]-α-methylbenzylamine (fendiline) (30 or $50\,\mu M$), a compound shown to potentiate $GABA_B$ receptor-mediated cortical hyperpolarisation, also significantly enhanced the inhibitory effect of baclofen.

4 It is therefore concluded that the recently reported $GABA_B$ receptor modulators, CGP7930 and fendiline, can enhance $GABA_B$ receptor-mediated depression of DA neuronal activity. This finding suggests a therapeutic potential for $GABA_B$ potentiators for the treatment of diseases associated with a hyperfunctional mesocorticolimbic system.
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Abbreviations: CGP55845, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid; CGP7930, 2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol; DA, dopamine; fendiline, *N*-[3,3-diphenylpropyl]-α-methylbenzylamine; GABA, γ-aminobutyric acid; VTA, ventral tegmental area

Introduction

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system, and GABAergic neurotransmission provides the inhibitory tone that controls the excitability of the neuronal network. Two types of receptors are known to mediate GABAergic inhibition: the $GABA_{A/C}$ receptors, which are ligand-gated chloride channels, and the $GABA_B$ receptors, which are G-protein-coupled receptors (GPCRs) (Bowery *et al*., 2002).

$GABA_B$ receptors are a member of the 'family 3' GPCRs, which also includes the metabotropic glutamate receptors (mGluR1–8). The $GABA_B$ receptor exists as a heterodimer, composed of subunits designated $GABA_{B(1)}$ (with 1a and 1b isoforms) and $GABA_{B(2)}$ (Kaupmann *et al*., 1998; White *et al*., 1998). The agonist binding site in the $GABA_B$ receptor resides on the $GABA_{B(1)}$ subunit (Kniazeff *et al*., 2002), while the $GABA_{B(2)}$ subunit links to G proteins (Galvez *et al*., 2001) of the pertussis toxin-sensitive family, that is, Gi/Go (Odagaki & Koyama, 2001).

The activation of $GABA_B$ receptors triggers a number of inhibitory responses at both pre- and postsynaptic locations (Misgeld *et al*., 1995). Presynaptic $GABA_B$ receptors act to inhibit neurotransmitter release, mainly through the inhibition of voltage-gated calcium channels (Doze *et al*., 1995; Isaacson, 1998). Direct inhibition of neurotransmitter exocytosis by the activation of $GABA_B$ receptors has also been reported (Capogna *et al*., 1996). Postsynaptic $GABA_B$ receptor activation has been shown to activate multiple K^+ channel subtypes, leading to slow inhibitory postsynaptic potentials (Luscher *et al*., 1997). A number of physiological functions involving $GABA_B$ receptors have been elucidated by the generation of knockout mice for the $GABA_{B(1)}$ subunit. These animals, while appearing normal at birth, develop spontaneous seizures, hyperalgesia, hyperlocomotor activity, memory impairment and anxiety (Prosser *et al*., 2001; Schuler *et al*., 2001; Mombereau *et al*., 2004). In addition, baclofen, a selective $GABA_B$ agonist, is used in the treatment of spasticity associated with cerebral palsy, tetanus, multiple sclerosis, stiff-man syndrome and dystonia (Hill & Bowery, 1981). Malan *et al*. (2002) demonstrated that intrathecal administration of a $GABA_B$ antagonist led to an increase in tactile

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allodynia and thermal hyperalgesia in rats, suggesting that the inhibition of $GABA_B$ receptor activity may lower pain thresholds. Several studies showed that baclofen is capable of alleviating pain in surgically induced neuropathic pain models (Hwang & Yaksh, 1997; Patel *et al*., 2001; Malan *et al*., 2002). Human trials have also shown that intrathecal injection of baclofen can have analgesic effects in patients with chronic pain, specifically in cases where patients had experienced failure with other treatments (Zuniga *et al*., 2000).

Baclofen has also been shown to limit self-administration of a variety of addictive substances in animal models including alcohol, cocaine, heroine and nicotine (Colombo *et al*., 2000; Xi & Stein, 2000; Fattore *et al*., 2002), and early clinical results in human trials suggest efficacy of baclofen in reducing cocaine craving (Ling *et al*., 1998). Some evidence also exists for baclofen anxiolytic effects in different animal models (Mombereau *et al*., 2004). However, the use of baclofen is limited, both in preclinical studies and for clinical use, by its side effects, which include sedation (at higher doses), nausea, nightmares, headaches and dizziness (Ling *et al*., 1998), and by the developments of tolerance in patients, as a result of constant receptor activation and subsequent desensitisation (Abel & Smith, 1994).

Recently, a class of positive allosteric modulator of $GABA_B$ receptors, exemplified by 2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) (Urwyler *et al*., 2001), has been reported to potentiate responses to GABA and baclofen in a number of experimental systems. CGP7930 increased the potency and maximal efficacy of GABA in GTPγ[^{35}S] binding assays on native rat brain membranes, and recombinant $GABA_{B(1b)/(2)}$ heterodimeric receptors stably expressed in Chinese hamster ovary (CHO) cells. In *Xenopus* oocytes expressing either $GABA_{B(1a)/(2)}$ or $GABA_{B(1b)/(2)}$ heterodimers, the potency of GABA to activate inwardly rectifying K^+ channels was increased by CGP7930. GABA induced Ca^{2+} signalling in human embryonic kidney cells (HEK 293) expressing $GABA_{B(1)/(2)}$ heterodimers, coupled to the promiscuous G-protein $G_{\alpha qo5}$, was also potentiated by CGP7930. In addition, the potency of baclofen to inhibit spontaneous oscillatory activity in rat cortical neurones was increased by CGP7930. Another series of positive allosteric modulators of $GABA_B$ receptors has recently been described, exemplified by GS39783 (Urwyler *et al*., 2003). GS39783 has similar *in vitro* effects on $GABA_B$ receptors as CGP7930, but it has also been shown to possess *in vivo* anxiolytic-like activity, without the side effects of baclofen (Cryan *et al*., 2004). Apart from these compounds, arylalkylamines (fendiline (*N*-[3,3-diphenylpropyl]-α-methylbenzylamine) for example), L-amino acids and dipeptides have also been shown to potentiate $GABA_B$ receptor activity (Kerr *et al*., 2002; Kerr & Ong, 2003). In those experiments using rat neocortical slices, these compounds potentiated the hyperpolarisation induced by the $GABA_B$ receptor-selective agonist, baclofen, in terms of both the potency and the efficacy.

In this study, we assessed the ability of the well-characterised $GABA_B$ receptor allosteric modulator, CGP7930, to modulate $GABA_B$ receptor-mediated depression of spontaneous firing, an intrinsic pacemaker-like activity of dopamine (DA) neurons, in the ventral tegmental area (VTA) of rat midbrain slices. DA neurons in the VTA project to various brain regions, such as the nucleus accumbens, the prefrontal cortex and the hippocampus. DA release is associated with various physiological (reward, attention and memory) and pathological (substance abuse, Parkinson's disease and schizophrenia) functions. Preclinical and clinical evidence demonstrated that baclofen given systemically or locally into the VTA reduced cocaine craving behaviours (Brebner *et al*., 2000). Electrophysiological recordings *in vitro* showed that baclofen, by activating $GABA_B$ receptors, can hyperpolarise DA cells (Madden & Johnson, 1998), resulting in the reduction of the DA cell firing rate (Erhardt *et al*., 2002). By measuring baclofen-induced inhibition of the spontaneous firing activity of VTA DA neurons in midbrain slices, we can assess the ability of $GABA_B$ potentiators, CGP7930 and fendiline, to modulate $GABA_B$ receptor-mediated depression of DA neurons.

Methods

Midbrain slices were prepared from male Sprague–Dawley rats (25–30 days old). Rats were killed by the dislocation of the neck, according to the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (U.K.). The brain was quickly removed and immediately immersed in ice-cold artificial cerebrospinal fluid (ACSF), constantly oxygenated with 95% O_2/5% CO_2. The composition of the ACSF was (in mM): NaCl 123, NaH_2CO_3 22, NaH_2PO_4 1.25, KCl 3.75, D-glucose 10, $CaCl_2$ 2.5 and $MgSO_4$ 1.2. A block of brain tissue containing the midbrain was cut out from the whole brain and affixed to a stainless-steel stage with cyanoacrylic glue, and then immersed in a tissue chamber constantly cooled to 4°C on a Campden vibroslicer. Coronal slices (350 μm) were cut and three to four slices containing the substantia nigra and the VTA were harvested and placed in an incubation chamber containing ACSF, at 27–28°C and bubbled with 95% O_2/5% CO_2, for at least 1 h before recording. More details on slice preparation and electrophysiological recordings can be found in Chen *et al*. (2003).

For electrophysiological recordings, one slice was placed in the recording chamber and was constantly superfused with oxygenated ACSF at a flow rate of 2.5–3 ml min^{-1} at a temperature of 33–34°C. The VTA was visually identified as a grey area medial to the substantia nigra and the medial lemniscus, a white fibre tract.

Single-cell extracellular recordings were made using glass microelectrodes (tip diameter $\sim$1 μm) filled with ACSF, giving an impedance of 3–6 MΩ. Extracellular action potentials (APs) from a single cell usually displayed constant amplitude and shape. The signal was recorded in the AC mode and amplified 1000 times. Signals were digitised using a CED1401 plus (CED, Cambridge, U.K.) and captured and stored on a PC using Spike 2 software (CED, Cambridge, U.K.).

DA and non-DA neurons in the VTA were distinguished by well-defined electrophysiological characteristics (Grace & Onn, 1989; Johnson & North, 1992; Chen *et al*., 2003), and their responses to DA. Extracellular APs recorded from DA neurons have positive–negative waveform usually with a large negative phase. The duration of the APs is around 3 ms and firing rate is between 0.5 and 4 Hz. DA neurons usually exhibit regular pacemaker-like firing pattern and inhibition of firing frequency upon bath perfusion of 50 μM DA. In contrast, non-DA neurons exhibit short duration APs ($<$2 ms), high firing rates ($>$4 Hz) and no inhibition of firing frequency upon

application of DA (Johnson & North, 1992). The majority (~80%) of the spontaneous firing cells recorded in the VTA were DA neurons rather than the non-DA neurons. Recordings were normally stable for up to 3–4 h.

A control baseline firing frequency of a DA cell was firstly established for more than 5 min. Then, test compounds were delivered to the slice *via* switching from the control ACSF to a drug-containing ACSF, which was continued for 10 min to allow for the plateau effect to be reached. The percentage change in frequency during drug application over the baseline frequency was then calculated. In experiments utilising escalating concentrations of drugs without a washing period in between, the plateau frequency was used for the calculation of percentage changes from the initial baseline. Data were only collected from experiments where, after the cumulative applications, the firing frequency was either washed back or reversed by an antagonist, to the initial baseline level. An average of six 10 s bins containing five to 40 spikes was normally used for the analysis of frequency. Concentration–response curves (CRCs) were constructed using data obtained in separate experiments and mean ± s.e. were calculated for n slices. Agonist (baclofen) CRCs were fitted according to the equation:

$$i/i_{max} = 1/\{1 + (EC_{50}/[agonist])^{n_H}\} \quad (1)$$

Curve fitting was performed using Graphpad prism 3.02 software (Graphpad software, Inc., San Diego, U.S.A.).

DA hydrochloride and fendiline were from Sigma-RBI (Poole, U.K.). (*RS*)-Baclofen and CGP 55845 were from Tocris (Avonmonth, U.K.). CGP7930 was synthesised at the Lilly chemistry laboratorie, U.K. Numerical data in the text and error bars in figures are expressed as the mean ± s.e.m. Statistical comparisons were made with two-tailed Student's t-test, unpaired, unless stated, paired.

Results

Baclofen-induced depression of DA cell firing rate

In this study, the spontaneous firing of VTA DA cells was recorded extracellularly from 55 cells. DA cells exhibit regular firing with frequencies ranging between 0.5 and 3.0 Hz, with an average of 1.8 ± 0.1 Hz. In all the DA cells tested, the $GABA_B$ receptor agonist baclofen depressed the firing rate in a concentration-dependent manner (Figure 1). In this set of experiments ($n = 11$), baclofen at 0.01, 0.1, 0.3 and 1 μM was applied in the perfusate for 10 min in a cumulative manner. With increasing concentrations of baclofen, stepwise decreases in firing frequency were observed. Full block of firing was achieved by 1 μM baclofen in 7/11 experiments, and by 10 μM in the remaining four experiments. The per cent inhibition from the baseline frequency by each concentrations of baclofen was calculated. Fitting the pooled data with a sigmoidal CRC yield an EC_{50} of 0.27 μM, with a 95% confidence interval between 0.22 and 0.34 μM.

At the end of each bacofen concentration–response experiment, the baseline frequency was recovered either by washing in the control ACSF or by the application of the $GABA_B$ receptor-selective antagonist, CGP 55845, at 1 μM (Figure 1a). In each experiment, the maximum blocking effects of baclofen (1 or 10 μM) was fully reversed (5/5 experiments). (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid (CGP55845) alone at 1 μM had no significant effect on the spontaneous firing rate of DA cells ($n = 3$).

CGP7930 enhanced the inhibitory effect of baclofen

After having established the potent and dose-dependent depression of VTA DA cell spontaneous activity by baclofen,

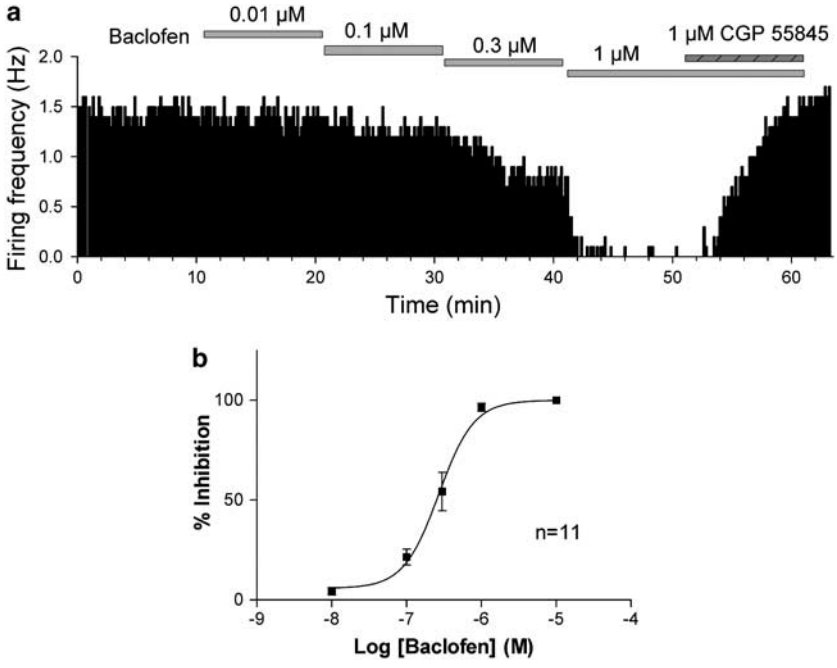

Figure 1 Baclofen bath applied showed concentration-dependent inhibitory actions on VTA DA cell spontaneous firing frequencies in rat midbrain slices (a). The inhibition was reversed by 1 μM CGP55845. (b) Baclofen CRC (from 11 experiments) yielded an EC_{50} of 0.27 μM (95% confidence interval between 0.22 and 0.34 μM).

we evaluated the effects of the recently reported $GABA_B$ potentiator CGP7930 on baclofen-induced inhibition. CGP7930 was applied 10 min before the first addition of baclofen and kept continuously in the perfusate throughout the experiments (Figure 2a). CGP7930 alone (10 or 30 μM) did not have significant effects on baseline activity ($n=8$). CGP7930 (10 μM) did not affect the responses of baclofen, resulting in a CRC ($n=3$, filled diamonds in Figure 2b) overlapping with the baclofen control CRC (filled squares in Figure 2b). However, in the presence of 30 μM CGP7930, the CRC ($n=5$, filled triangles in Figure 2b) shifted to the left compared with the baclofen control (filled squares in Figure 2b). An EC_{50} of 0.15 μM was calculated from the baclofen plus 30 μM CGP7930 CRC, with a 95% confidence interval between 0.11 and 0.21 μM, which is significantly different from the control baclofen CRC (see above). Individual $\log EC_{50}$'s from each experiments were also calculated and compared (Student's *t*-test) between the baclofen control (mean $\log EC_{50} = -6.57 \pm 0.05$ M) and the baclofen plus 30 μM CGP7930 (mean $\log EC_{50} = -6.83 \pm 0.07$ M) sets, and a significant difference ($P<0.05$) was found. Significant potentiation by 30 μM CGP7930 was seen mainly at lower concentrations of baclofen (0.01, 0.1 and 0.3 μM) (Figure 2b). In addition, the maximum block of firing obtained with 1 μM baclofen plus 30 μM CGP7930 was fully reversed by 1 μM CGP 55845 (Figure 1a), confirming a specific $GABA_B$-mediated effect.

Fendiline enhanced the effect of baclofen

The experiments above clearly showed that 30 μM CGP7930 potentiated baclofen-induced inhibition of VTA DA cell activity. A class of arylalkylamines (fendiline, prenylamine, F 551 and NPS 467) were also recently reported to act as positive modulators at $GABA_B$ receptors, when tested in rat neocortex (Kerr *et al*., 2002). We have therefore evaluated the effects of fendiline, on the baclofen CRC, utilising the same experimental paradigm as described above for CGP7930. Again, fendiline alone at 30 μM had no effect on baseline activity ($102.3 \pm 7.7\%$ of control, $n=3$), and the inhibition caused by baclofen plus fendiline was fully reversed by 1 μM CGP55845 ($n=3$, Figure 3b). The EC_{50} obtained from sigmoidal fitting of the baclofen plus fendiline CRC (filled triangles in Figure 3a) was found to be 0.13 μM (95% confidence interval between 0.11 and 0.15 μM), which is significantly shifted to the left compared with the control baclofen CRCs (filled squares in Figure 3a). Significant difference was found when comparing each individual $\log EC_{50}$'s in the two sets of experiments ($P<0.05$, mean $\log EC_{50}$ for fendiline experiments is -6.90 ± 0.03 M).

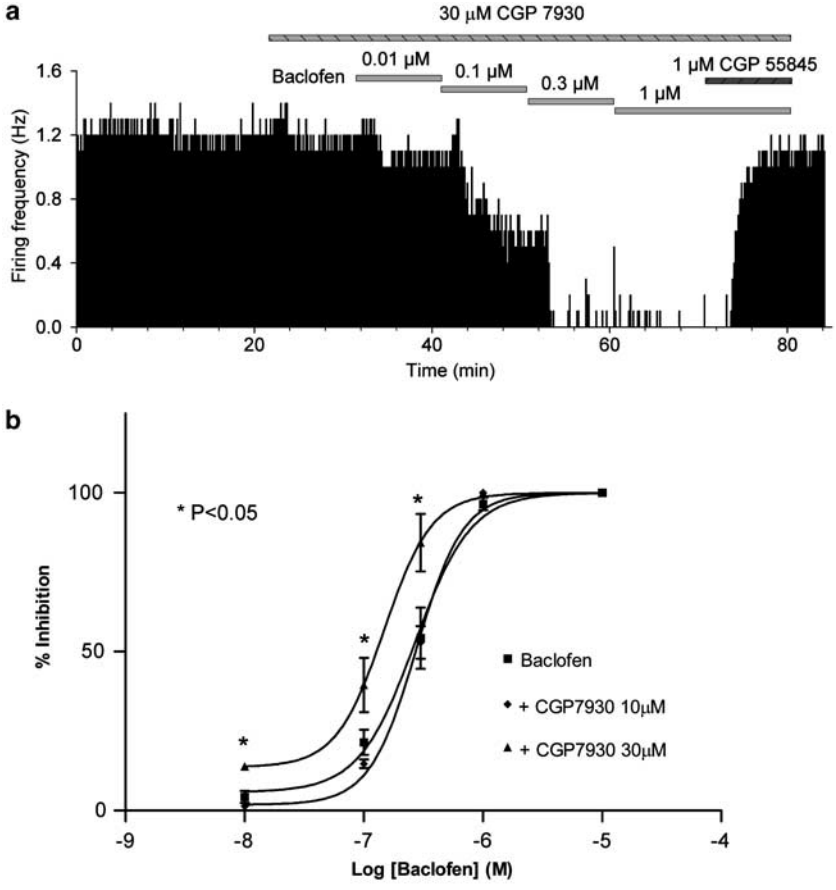

Figure 2 CGP7930 at 30 μM potentiated the actions of baclofen. (a) CGP7930 was applied for 10 min prior to the escalating concentrations of baclofen. CGP7930 had no effect on basal spontaneous activity of VTA DA cells, and the maximum effect of baclofen plus CGP7930 was reversed by 1 μM CGP 55845. (b) Baclofen CRCs in the presence of 10 or 30 μM ($n=3$ and 5, respectively) CGP7930 were plotted against the control baclofen CRC ($n=11$). Baclofen plus 30 μM CGP7930 CRC was significantly ($P<0.05$) shifted to the left compared with the baclofen control CRC.

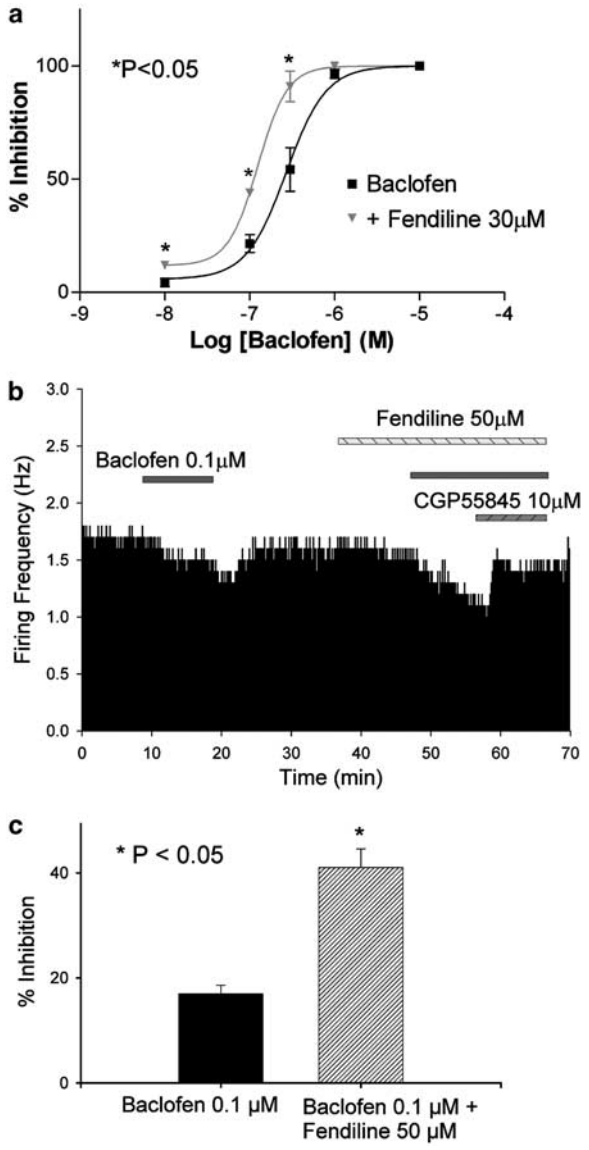

Figure 3 (a) Fendiline 30 μM also shifted the baclofen CRC significantly ($P<0.05$, $n=3$) to the left. (b) Direct comparison of baclofen and baclofen plus fendiline in the same recording was performed. Fendiline at 50 μM enhanced the inhibitory effect of 0.1 μM baclofen applied for 10 min. When fendiline was applied alone (10 min), no significant effect on basal activity was observed, and the enhanced effect of fendiline plus 0.1 μM baclofen was largely reversed by 1 μM CGP 55845. (c) An average of five experiments showed that the inhibitory effect of 0.1 μM baclofen was significantly (*$P<0.05$, paired t-test) enhanced by 50 μM fendiline.

The potentiating effect of 50 μM fendiline was also tested with baclofen repeats (Figure 3b). In these experiments, a control response to baclofen at a low concentration (0.1 μM) was obtained. After the response was washed out for 20 min, 50 μM fendiline was applied alone for 10 min (during which time no significant inhibition was observed), and then coapplied with 0.1 μM baclofen. Baclofen coapplied with fendiline produced a greater effect compared with baclofen alone. An average of $128\pm19.1\%$ potentiation by 50 μM fendiline is shown in Figure 3c ($n=5$). CGP55845 (10 μM) was able to reverse the effects of baclofen plus fendiline to $75\pm21\%$ of the control ($n=3$, Figure 3b).

At concentrations of 100 μM, fendiline alone depressed DA cell activity, but the effect was not reversed by CGP55845 ($n=4$), indicating a non-$GABA_B$-mediated effect.

Discussion

In this study, we have shown that the novel $GABA_B$-positive modulator, CGP7930, potentiated the inhibitory action of a $GABA_B$ receptor-selective agonist, baclofen, on VTA DA cell activity in acutely prepared rat brain slices, without causing any significant agonist effects by itself. This finding demonstrates, for the first time, that native $GABA_B$ receptors in the DA system can undergo positive allosteric modulation by CGP7930, which suggests a therapeutic potential for $GABA_B$-positive modulators in the regulation of mesocorticolimbic system dysfunctions.

Baclofen concentration dependently suppressed the spontaneous activity of VTA DA cells ($EC_{50}=0.27$ μM), with a maximum block at 1 μM, confirming that the spontaneous activity of VTA DA cells is highly sensitive to baclofen (Kerr *et al*., 2002; Onali *et al*., 2003). The mechanism underlying baclofen-induced depression of spontaneous activity in DA cells has been shown to be *via* the activation of $GABA_B$ receptors, and probably the subsequent increase in the membrane K^+ conductance (Madden & Johnson, 1998). The basal spontaneous activity was not affected by the application of the selective $GABA_B$ receptor antagonist CGP55845, showing a low level of endogenous $GABA_B$ receptor activity in this slice preparation.

During the experimental time course of the present study, no apparent desensitisation of $GABA_B$ receptors was observed. Cumulative applications of baclofen with escalating concentrations, applied over a period of 40 min, caused step-wise decreases in the firing frequency, without loss of effects. Rapid desensitisation to baclofen was reported in a hippocampal culture preparation, but mainly at high concentrations (>30 μM) of baclofen (Couve *et al*., 2002; Tosetti *et al*., 2004). Thus, the lack of apparent desensitisation in our experiments could be attributed to the low concentrations of baclofen used in our experimental time course, or even a different subunit expression or an alternative splice variant that may occur in the VTA (Bowery *et al*., 2002).

CGP7930 is the first well-described $GABA_B$ receptor-positive allosteric modulator, and was shown to be active in a number of membrane and cell-based assays (Urwyler *et al*., 2001). CGP7930, like several of its structural analogues, has characteristics similar to allosteric modulators of other family 3 GPCR (Pin *et al*., 2001), which do not possess intrinsic agonist activity, yet enhance the affinity and efficacy of ligand binding. Functionally, CGP7930 increased the potency and efficacy of GABA stimulation of GTPγ[^{35}S] binding in both recombinant and native membrane preparations. In our experiments, in the presence of either 30 μM CGP7930 or fendiline, the potency of baclofen in inhibiting DA cell activity was enhanced, as shown by the left-shift of baclofen CRCs and a significant reduction of EC_{50}'s. The complete block of firing activity by baclofen, however, precluded our ability to test for potentiation of baclofen E_{max}. In our experiments, full block of firing activity may not need the occupancy of all the $GABA_B$ receptors present. This kind of ceiling effect may also be present in other functional assays, like calcium fluctuations in

cortical neuronal cultures, as reported (Urwyler *et al*., 2001). In addition, neither CGP7930 nor fendiline exerted any significant effects on its own. This could be explained by the lack of endogenous $GABA_B$ tonic effects on DA cells in the VTA. Although we have recorded spontaneously firing GABAergic neurons in the VTA (see Methods, also see Grace & Onn, 1989), the amount of GABA release onto VTA DA cells may be too low, under our experimental conditions, to activate $GABA_B$ receptors. Altogether, both CGP7930 and fendiline demonstrated their properties as receptor modulators, by potentiating the agonist-induced responses, without activating the $GABA_B$ receptors directly by themselves.

We do not have full CRCs for potentiators that would allow for the determination of their potencies in this functional assay using a native preparation. However, some comparisons of the potentiators' potencies in other functional assays can be made. In GABA-stimulated GTPγ[^{35}S] binding assays, the EC_{50} value of CGP7930 was 1–5 μM (Urwyler *et al*., 2001). In a later study to examine the modulation of adenylyl cyclase activity by $GABA_B$ receptor activation in membrane preparations from various brain regions, the EC_{50} values of CGP7930 was between 10 and 30 μM (Onali *et al*., 2003). In our experiments, using a brain slice preparation from mature rats, 10 μM CGP7930 failed to show a significant effect, while 30 μM CGP7930 was effective. Higher concentrations could not be tested due to poor solubility of the compound. Tissue penetration may represent the cause for the slightly lower potency of CGP7930 in our slice preparation, but other factors, such as different subunit expression and different developmental stages, may also be considered.

Fendiline, as a $GABA_B$ potentiator, was only tested once before in rat neocortical slices using a grease-gap technique to record baclofen-induced hyperpolarisation, with fendiline (10–50 μM) potentiating baclofen (3–100 μM) induced hyperpolarisation (Kerr *et al*., 2002). The potency and efficacy of baclofen were both enhanced in this assay. It is interesting to note that baclofen was less potent in the neocortical slice than in our VTA slice preparation, but the per cent potentiation by 30 μM fendiline was greater in the neocortical slice. However, fendiline at concentrations higher than 50 μM was found to inhibit the spontaneous activity of DA cells *via* a non-$GABA_B$-mediated action, since the inhibition was not reversed by the $GABA_B$ antagonist CGP55845. These inhibitory effects was also noted by Kerr *et al*. (2002), who stated that at concentrations greater than 50 μM, fendiline could stimulate the Ca^{2+} sensing receptor, another family 3 GPCRs, interfering with $GABA_B$-mediated actions. It is also possible that the effects were due to fendiline's ability to block L-type calcium channels (the mechanism for which the drug is used therapeutically). Studies in the rat hippocampus have found that calcium channel block occurs in the 100–200 μM concentration range (Jones & Heinemann, 1987). The mechanisms underlying the direct inhibition by fendiline remains unclear, and is beyond the scope of this investigation. It is also interesting to note that fendiline, along with a few other arylalkylamines, amino acids and dipeptides, was recently shown not to act as allosteric modulators at recombinant or native $GABA_B$ receptors in a number of assays, whereas CGP7930 was shown to be a positive allosteric modulator (Urwyler *et al*., 2004). Further work is required to elucidate the exact mechanism underlying the potentiating effect by fendiline on $GABA_B$ receptor-mediated responses in brain slices.

Subsequent to the reports on CGP7930 and its analogues, GS39783 and its analogues (Urwyler *et al*., 2003) were also reported as positive allosteric modulators of the $GABA_B$ receptors. In a recent report, both CGP7930 and GS39783 have shown efficacy in reducing cocaine self-administration in rats (Smith *et al*., 2004). It is not surprising to see that in these whole animal experiments with cocaine reinforcement schedules there is a considerable level of $GABA_B$ receptor activity that allow for the potentiators to show their synergistic effects with endogenously released GABA, possibly within the highly sensitised mesolimbic DA system. DA neurons projecting from the VTA to the nucleus accumbens have been implicated in reward processes. Our results showing that CGP7930 can potentiate a $GABA_B$ receptor-mediated inhibition on DA neurons may help to explain these behavioural effects, and reinforce the hypothesis that $GABA_B$-positive allosteric modulation could be beneficial for the treatment of substance abuse and dependence.

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