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Electrophysiological actions of $GABA_B$ agonists and antagonists in rat dorso-lateral septal neurones *in vitro*

Christelle Bon & [1]Martin Galvan

Department of Pharmacology, Marion Merrell Research Institute, 16 rue d'Ankara, 67080 Strasbourg Cedex, France

1 The actions of $GABA_B$-receptor agonists and antagonists on rat dorso-lateral septal neurones *in vitro* were recorded with intracellular microelectrodes.

2 In the presence of 1 μM tetrodotoxin to prevent indirect neuronal effects caused by action potential-dependent neurotransmitter release, bath application of baclofen (0.1-30 μM) or SK&F 97541 (0.01-3 μM) evoked concentration-dependent hyperpolarizations which reversed close to the potassium equilibrium potential; the EC_{50}s were 0.55 and 0.05 μM, respectively. No significant desensitization was observed during prolonged agonist exposure ($\leqslant$ 10 min).

3 Hyperpolarizations induced by baclofen were antagonized in a competitive manner by the following $GABA_B$-receptors antagonists (calculated pA_2 values in parentheses): CGP 36742 (4.0), 2-OH saclofen (4.2), CGP 35348 (4.5), CGP 52432 (6.7) and CGP 55845A (8.3). Responses to SK&F 97541 were also antagonized by CGP 55845A ($pA_2 = 8.4$).

4 The amplitude of the late, $GABA_B$ receptor-mediated inhibitory postsynaptic potential (i.p.s.p.) was reduced by the $GABA_B$ antagonists as follows (means $\pm$ s.e.mean): CGP 55845A (1 μM) 91 $\pm$ 5%, CGP 52432 (1 μM) 64 $\pm$ 5%, CGP 35348 (100 μM) 82 $\pm$ 5%, CGP 36742 (100 μM) 76 $\pm$ 8%, and 2-OH saclofen (100 μM) 68 $\pm$ 3%.

5 It is concluded that neurones in the rat dorso-lateral septal nucleus express conventional $GABA_B$ receptors, which are involved in the generation of slow inhibitory postsynaptic potentials. CGP 55845A is the most potent $GABA_B$ receptor antagonist described in this brain area.

Keywords: $GABA_B$ agonists; $GABA_B$ antagonists; late inhibitory postsynaptic potentials (late i.p.s.p.); dorsolateral septal nucleus (DLSN); intracellular recording

Introduction

Through connections with the limbic system and the hypothalamus, the septal nuclei are involved in a variety of motivational, emotional and associative mechanisms (Raisman, 1966; DeFrance, 1976). A number of chemical neurotransmitters are involved in these intrinsic and extrinsic connections (Costa *et al.*, 1983) and electrophysiological investigations indicate that γ-aminobutyric acid (GABA) functions as an inhibitory synaptic transmitter on these neurones (McLennan & Miller, 1974; DeFrance *et al.*, 1975; Gallagher *et al.*, 1984). Immunohistochemical studies confirm the presence of GABA in the lateral septum (Panula *et al.*, 1984; Bowery *et al.*, 1987).

GABA interacts with two receptor subtypes designated $GABA_A$ and $GABA_B$, both of which are present in the peripheral and central nervous systems (Hill & Bowery, 1981). The bicuculline-sensitive $GABA_A$ receptors are associated with chloride-selective channels and mediate fast inhibitory postsynaptic potentials (Möhler, 1992) whilst $GABA_B$ receptors are insensitive to bicuculline and are coupled through a G-protein to neuronal potassium and calcium channels (Hill *et al.*, 1984; Dolphin & Scott, 1987). They can be activated by the antispastic drug, baclofen (Bowery *et al.*, 1980; 1983), and blocked by the baclofen analogues phaclofen and 2-OH saclofen (Kerr *et al.*, 1987; Harrison *et al.*, 1990). Presynaptic $GABA_B$ autoreceptors can regulate the release of GABA and presynaptic heteroreceptors have been shown to influence the release of glutamate via a reduction in Ca^{2+} influx (Potashner, 1979; Dunlap, 1981; Dolphin & Scott, 1987). Activation of postsynaptic $GABA_B$ receptors on central neurones elicits an increase in potassium conductance (Newberry & Nicoll, 1984; Gallagher *et al.*, 1984; Colmer & Williams, 1988) which hyperpolarizes the membrane potential, a mechanism underlying the late inhibitory postsynaptic potential (i.p.s.p.).

A large number of novel $GABA_B$ receptor antagonists have been described with different potencies measured in *in vitro* radioligand binding assays (Waldmeier *et al.*, 1994; Froestl *et al.*, 1995; Bolser *et al.*, 1995); however, there is little functional pharmacological data comparing the potencies of these compounds. We therefore investigated by intracellular recording whether 5 of these antagonists blocked postsynaptic $GABA_B$ responses induced by baclofen or SK&F 97541, two $GABA_B$ agonists in the dorso-lateral septal neurones. Then, we examined whether these same antagonists were able to reduce the amplitude of $GABA_B$-mediated late i.p.s.ps.

Methods

Slice preparation

Unanaesthetized male OFA rats (100-250 g; Iffa Credo, France) were killed by decapitation and the brains quickly removed and cooled for 1 min in ice cold Krebs solution. Frontal brain slices were cut at a thickness of 350 μm with a vibratome. A single slice containing the dorso-lateral septum was transferred to a perspex recording chamber where it was sandwiched between two nylon nets and completely submerged by continuously flowing Krebs solution (7.5-8 ml min^{-1}) at 32°C. The composition of the superfusate was as follows (mM): NaCl 119, KCl 3, NaH_2PO_4. H_2O 1.2, $MgCl_2$. $6H_2O$ 1.2, $NaHCO_3$ 25, $CaCl_2$. $2H_2O$ 2, D-glucose 10; gassed with 95% O_2 and 5% CO_2. The remaining slices were stored until required in a small holding chamber filled with oxygenated Krebs solution at 32-34°C. Drugs were added via the superfusion medium in fixed concentrations and there was a delay of about 30-40 s before the new solution arrived in the recording chamber.

[1] Author for correspondence.

Electrophysiological recordings

Intracellular recordings were obtained in the dorso-lateral septal area by standard intracellular recording techniques. Most recording electrodes were made from glass micropipettes filled with 3 M KCl (resistance, 70–100 MΩ). However, in order to record the late inhibitory postsynaptic potentials, electrodes were filled with 4 M potassium acetate (resistance, 75–150 MΩ). Electrical signals were amplified with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA, U.S.A.) and monitored on a digital oscilloscope and a chart recorder (RS 3400 Gould Instruments, France). Electrotonic potentials and evoked postsynaptic potentials were recorded and analysed with a personal computer running pCLAMP software (Axon Instruments). Neurones accepted for inclusion in the study met several criteria: stable resting membrane potential greater than −50 mV and membrane resistance superior to 50 MΩ. All experiments (except those involving evoked synaptic potentials) were performed in the presence of 1 μM tetrodotoxin (TTX) added to the superfusion medium to block action potential-dependent neurotransmitter release and thus prevent indirect effects of the compounds under study. Synaptic responses were evoked by electrical stimulation in the dorso-lateral septum with single stimulus pulses (10–32 V; 0.1 Hz) of 100 μs duration using a concentric stainless steel electrode pair; the stimulus strength was adjusted to be supramaximal for eliciting a late i.p.s.p.

Concentration-response curves

Ascending concentrations of $GABA_B$ agonists were applied to single dorso-lateral septal neurones for 2 or 3 min with wash periods between each application. In all cases, the maximal membrane potential change (mV) in the presence of the agonist was measured from the chart recordings. Antagonists were in contact with the slice for at least 30 min before applying ascending concentrations of agonists. The pA_2 was calculated using the following equation:

$$pA_2 = \log_{10}(DR - 1) - \log_{10}[B]$$

where [B] is the antagonist concentration and DR is the dose-ratio calculated from estimates of the EC_{50} value of the agonist in the presence and absence of antagonist.

Analysis of the results

Drug effects were defined as the maximal change in the membrane potential reached during agonist or antagonist superfusion or as the difference in the amplitude of synaptic potentials during control and treatment period with antagonist. Unless otherwise stated, data are expressed as the means ± standard error of the mean (mean ± s.e.mean). Statistical comparisons involving the two-tailed, paired and unpaired *t* test were performed as necessary using the programme Instat (GraphPad Software, San Diego, CA, U.S.A.).

Chemicals

Drug sources were the following: TTX and [±]-baclofen (Sigma, France); 2-OH-saclofen (Tocris Cookson Ltd, U.K). SK&F 97541 (3-aminopropyl methylphosphinic acid) and CPG 35348 (3-aminopropyl-diethoxy-methylphosphinic acid) were synthesized at Marion Merrell, Strasbourg, France. CGP

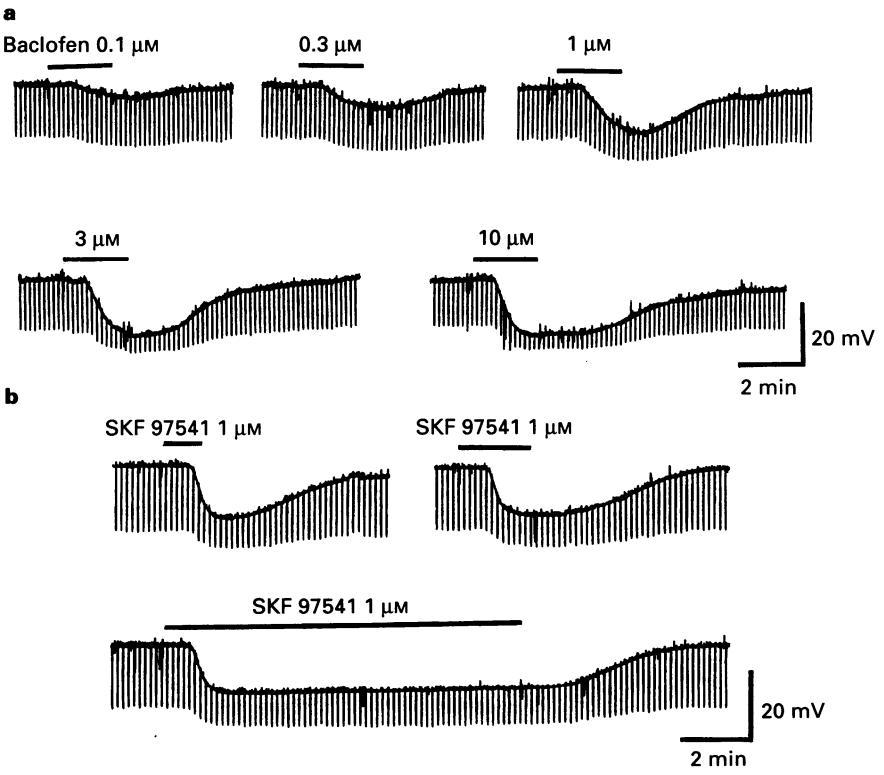

Figure 1 (a) Intracellular records showing that bath application of 0.1–10 μM baclofen (horizontal bars) induced a concentration-dependent hyperpolarization and a reduction in membrane resistance. Downward deflections represent hyperpolarizing electrotonic potentials in response to constant current pulses (−0.1 nA, 300 ms, 0.1 Hz). The membrane potential was held constant at −73 mV throughout the experiment. (b) SK&F 97541, 1 μM (horizontal bars) hyperpolarized the membrane potential and decreased membrane resistance. The hyperpolarization evoked by SK&F 97541 did not significantly desensitize during 1, 2 or 10 min bath applications. Electrotonic potentials were elicited by constant current pulses (−0.3 nA, 300 ms, 0.1 Hz); the membrane potential was held constant at −77 mV. The 30–40 s delay in the superfusion system (see Methods) has not been corrected for in this and subsequent figures.

52432 ([3-[[(3,4 dichlorophenyl)methyl]amino]propyl] (diethoxymethyl) phosphinic acid); CGP 55845A (3-[1-(S)-(3,4-dichloro-phenyl)-ethyl]amino-2(S)-hydroxy-propyl-benzyl-phosphinic acid (hydrochloride)) and CGP 36742 (3-amino-propyl-n-butyl-phosphinic acid) were supplied by Ciba-Geigy (Switzerland).

Results

Stable intracellular recordings included in this study were made from 59 rat dorso-lateral septal neurones with a resting membrane potential and membrane resistance of -73 ± 8 mV and 97 ± 35 MΩ respectively (means ± s.d.). No significant differences were noticed in the passive membrane properties of the groups of cells used to test the actions of the various agonists and antagonists (ANOVA; $P=0.54$ for membrane potential and $P=0.49$ for membrane resistance).

Effects of GABA$_B$ agonists on membrane potential

Application of baclofen (0.1–30 μM) produced a concentration-dependent hyperpolarization which was associated with a decrease of membrane input resistance (Figure 1a). The maximal effect was reached after about 2–3 min of drug application and repolarization of the membrane potential occurred within 1 to 7 min after reintroduction of drug-free Krebs solution. In 4 cells, 6 concentrations of baclofen were applied in a non-cumulative fashion and the mean calculated EC_{50} (the concentration required to produce a half-maximal response) was 0.6 ± 0.1 μM.

Bath application of SK&F 97541 (0.01–3 μM), a potent and selective GABA$_B$ agonist (Seabrook *et al.*, 1990) also induced a reversible, concentration-dependent hyperpolarization. The EC_{50} calculated from 4 cells was found to be 0.044 ± 0.003 μM; this was significantly different from that obtained with baclofen ($P<0.01$; unpaired two-tailed t test). No significant desensitization was observed during 1, 2 or 10 min applications of 1 μM SK&F 97541 (Figure 1b; $n=4$) or 10 μM baclofen ($n=5$). Current-voltage curves were constructed from steady state electrotonic potentials before and during application of 3 μM baclofen and 3 μM SK&F 97541 (Figure 2). The reversal potential for agonist induced hyper-polarization was -97.9 ± 3.6 mV ($n=5$) for baclofen and -102.6 ± 1.9 mV ($n=6$) for SK&F 97541; these values were not significantly different ($P=0.26$; unpaired two-tailed t test).

Actions of GABA$_B$ antagonists on baclofen- and SK&F 97541-induced hyperpolarization

In order to characterize fully the postsynaptic GABA$_B$ receptors in rat dorso-lateral septal neurones, the affinities of 5 selective GABA$_B$ antagonists were determined. Slices were preincubated in antagonist solution for at least 30 min and then concentration-response curves to baclofen (0.1–300 μM) were constructed; these were compared to the control data

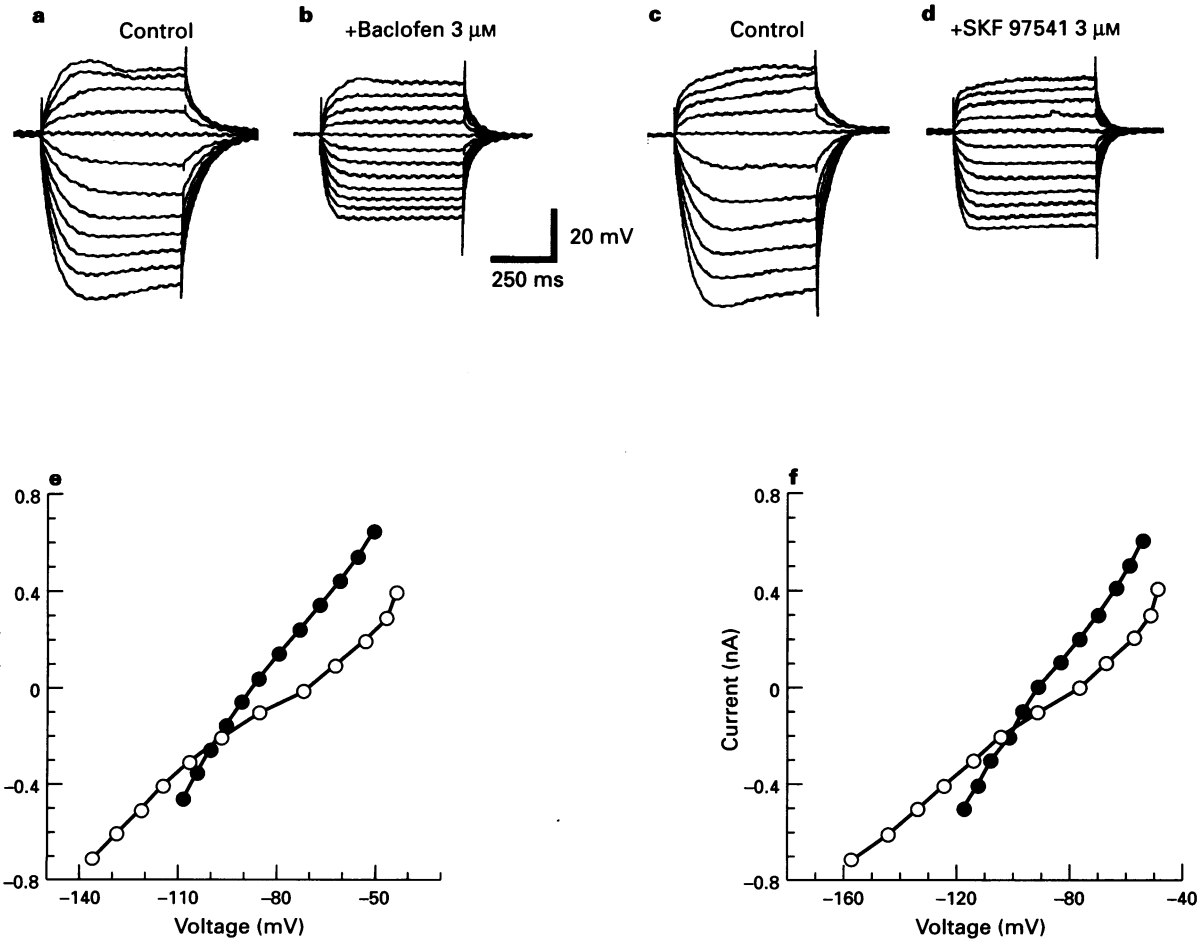

Figure 2 Electrotonic potentials recorded before and during application of 3 μM baclofen (a,b) or 3 μM SK&F 97541 (c,d). The 12 superimposed potentials resulted from 12 rectangular current pulses (in 0.1 nA steps). During agonist application, depolarizing direct current was injected to restore the membrane potential to the control level. (e) Complete current-voltage relationship recorded from the same cell as (a) in control solution (○) and in the presence of 3 μM of baclofen (●). The value of the reversal potential for the baclofen-induced hyperpolarization is about −99 mV. (f) Current-voltage curves from the same cell as (c) in control solution (○) and in the presence of 3 μM SK&F 97541 (●). The value of the reversal potential for the SK&F 97541-induced hyperpolarization is about −102 mV. Membrane potentials were held constant at (a,b) −73 mV and (c,d) −79 mV.

described above. All five antagonists tested induced a parallel shift in the baclofen concentration-response curve without a significant change in the maximal response (Figures 3 and 4a). At the concentrations employed in these experiments, none of the antagonists significantly changed the resting membrane potential or membrane resistance. Four of the compounds tested were rather weak antagonists and the pA_2 values calculated at the level of the EC_{50} from the mean concentration-response curves were as follows: CGP 36742, 4.0; 2-OH-saclofen, 4.2; CPG 35348, 4.5; CGP 52432, 6.7. However, CGP 55845A proved to be a very potent, competitive antagonist of both the action of baclofen and SK&F 97541, the calculated pA_2 values were 8.3 and 8.4, respectively (Figure 4). Figure 5 illustrates hyperpolarizations elicited in single cells before and during application of either 100 μM 2-OH saclofen, 1 μM CGP 52432 and 0.1 μM CGP 55845A. The recordings illustrate clearly the low potency of 2-OH saclofen and the high potency of CGP 55845A with CGP 52432 occupying an intermediate position.

Effect of $GABA_B$ antagonists on the late i.p.s.p.

Further experiments were performed to determine the potency of the 5 antagonists as blockers of the $GABA_B$-receptor mediated slow i.p.s.p. resulting from electrical stimulation of excitatory pathways in the septum. The drugs were applied until the maximal effect was reached and Figure 6a–c illustrates synaptic potentials obtained before, during and after addition of three different antagonists.

All 5 compounds significantly reduced the amplitude of the late $GABA_B$ receptor-mediated i.p.s.p. but not the early, $GABA_A$ receptor-mediated i.p.s.p. The % reduction in late i.p.s.p. amplitude compared to control was as follows: 1 μM CGP 55845A, 91±5% (n=5), 1 μM CGP 52432, 64±5% (n=5), 100 μM CGP 35348, 82±5% (n=4), 100 μM CGP 36742, 76±8% (n=4) and 100 μM 2-OH saclofen, 68±3% (n=4). It was interesting to note that 0.1 μM CGP 55845A, a concentration that significantly inhibited the action of bath applied baclofen and SK&F 97541, had little effect on evoked late i.p.s.ps (15±4% inhibition; n=5).

With the exception of CGP 55845A, the action of the antagonists was easily reversible by washing for 15 min in drug-free Krebs solution; only partial recovery was seen following addition of CGP 55845A and subsequent prolonged washing in drug-free Krebs solution (Figure 6).

Discussion

The purpose of this study was to characterize the postsynaptic $GABA_B$ receptors on single neurones in dorso-lateral septal nucleus by comparing the potencies of two $GABA_B$ agonists and five selective antagonists.

The membrane hyperpolarization obtained during bath application of baclofen was concentration-dependent and reversed close to the potassium equilibrium potential. These results are consistent with those previously described by Gallagher *et al.* (1984). A similar hyperpolarizing response has been described in numerous brain areas including the hippocampus, the cerebral cortex, the thalamus and the medulla (Inoue *et al.*, 1985; Howe *et al.*, 1987; Osmanovic & Shefner, 1987; Lacey *et al.*, 1988; Li & Guyenet, 1995). It has also been

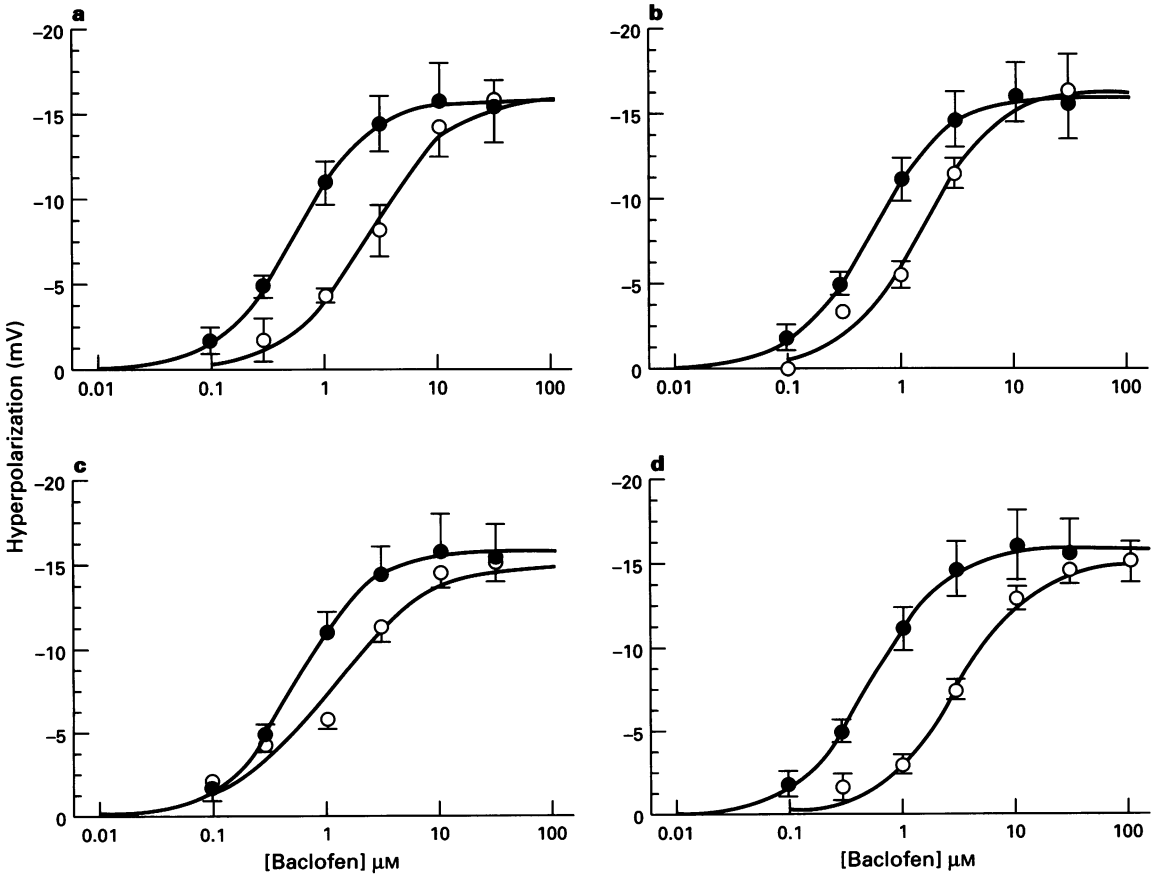

Figure 3 Complete concentration-effect curves for baclofen in the absence (●); n=5–7 cells) and presence (○) of (a) 100 μM CGP 35348 (n=2–4), (b) 100 μM 2-OH saclofen (n=2–4); (c) 100 μM CGP 36742 (n=4–5); (d) 1 μM CGP 52432 (n=3–4). The applied concentrations (μM) of baclofen are represented on the abscissa scale and the peak hyperpolarization in mV on the ordinate scale (mean±s.e.mean). The membrane potentials (mV) of the recorded cells were as follows (mean±s.e.mean): baclofen, −76±1.7 (n=9); CGP 35348, −74±4.6 (n=4); 2-OH saclofen, −69±2.5 (n=4); CGP 36742, −77±3.2 (n=5); CGP 52432, −76±3.6 (n=4).

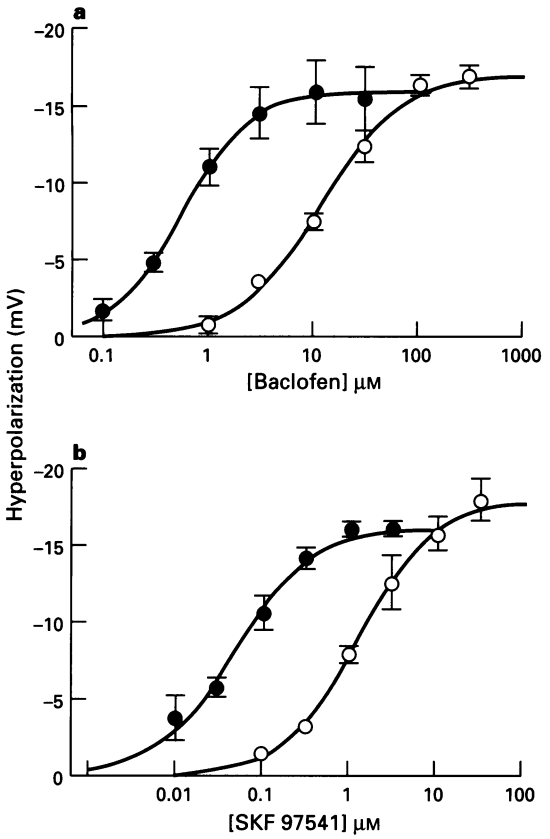

Figure 4 (a) Concentration-response curves for baclofen in the absence (●); $n=5-7$ cells) and presence (○) of 0.1 μM of CGP 55845A ($n=4$). (b) Concentration-response curves for SK&F 97541 in the absence (●; $n=3-7$) and presence (○) of 0.1 μM of CGP 55845A ($n=4$). Ordinates and abscissae are as in Figure 3. The membrane potentials (mV) of the recorded cells were as follows (mean±s.e.mean): baclofen, -76 ± 1.7 ($n=9$); SK&F 97541, -77 ± 1.6 ($n=7$); baclofen plus CGP 55845A, -74 ± 3.5 ($n=4$); SK&F 97541 plus CGP 55845A, -74 ± 3.0 ($n=4$).

reported that baclofen can act on presynaptic GABA$_B$ receptors in the cortex, the hippocampus and the cerebellum (Bonanno *et al.*, 1988; Davies *et al.*, 1990; Huston *et al.*, 1995) and interneuronal nerve terminals (Bonanno *et al.*, 1988; Misgeld *et al.*, 1989). In the hippocampus, baclofen seemed to be more potent on the pre- than on the postsynaptic receptors (Davies *et al.*, 1990).

SK&F 97541 also hyperpolarized the membrane potential, most probably via an increase in potassium conductance. The concentration-response curves for baclofen and SK&F 97541 were parallel, the maximum responses were similar as were the reversal potentials. Comparison of the EC_{50} values indicates that SK&F 97541 was about 10 fold more potent than baclofen. This is in agreement with functional studies on rat substantia nigra neurones (Seabrook *et al.*, 1990) and binding studies on rat brain membranes (Bowery, 1993). However, in an identified insect motor neurone, SK&F 97541 induced a membrane hyperpolarization whereas (-) L-baclofen failed to evoke any response (Bai & Sattelle, 1995). In the peripheral nervous system, it was reported that SK&F 97541 was about 10 times more potent than baclofen as an inhibitor of field stimulation-induced tracheal contraction (Chapman *et al.*, 1992) and about 5 times more potent than baclofen as an inhibitor of electrically stimulated contractions of intestinal smooth muscle preparations (Hills *et al.*, 1989).

The hyperpolarizing action of baclofen in the dorso-lateral septal neurones was antagonized by CGP 36742, 2-OH-saclofen, CPG 35348, CGP 52432 and CGP 55845A. Responses to SK&F 97541 were also blocked by CGP 55845A with a pA_2 value close to the one obtained with baclofen. Previous electrophysiological studies have also demonstrated that baclofen-induced hyperpolarizations could be antagonized by several of the GABA$_B$ antagonists that we employed: CGP 35348, (Newberry & Nicoll, 1984; Seabrook *et al.*, 1990; Bittiger *et al.*, 1990), 2-OH saclofen (Lambert *et al.*, 1989), CGP 52432 (Lanza *et al.*, 1993) and CGP 55845A (Davies *et al.*, 1993). In the present experiments, concentration-response curves were shifted in a parallel fashion to the right indicating competitive antagonism. This is consistent with ligand binding experiments where Hill coefficients of close to unity were measured for CGP

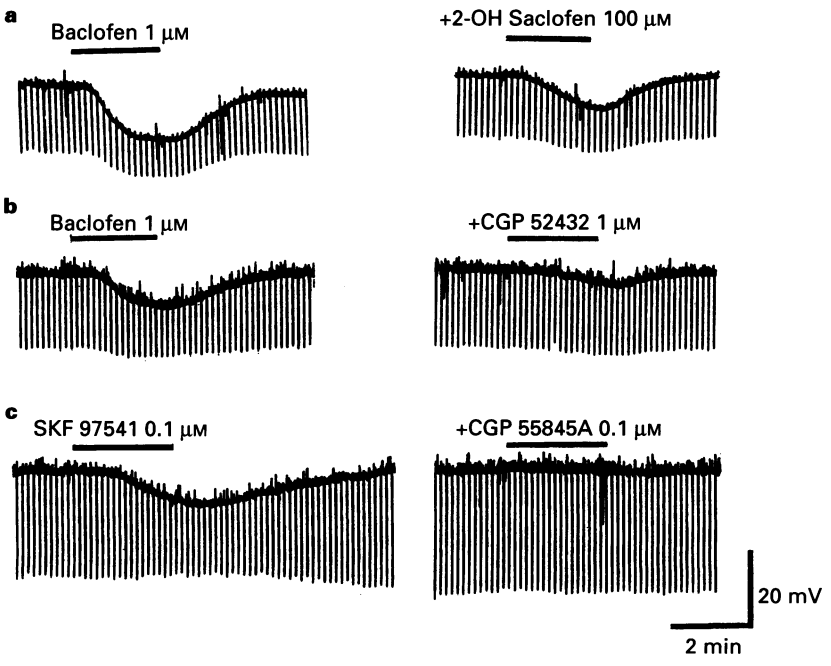

Figure 5 Antagonism of GABA$_B$ receptor-mediated hyperpolarizations by (a) 100 μM 2-OH saclofen, (b) 1 μM CGP 52432 and (c) 0.1 μM CGP 55845A. Records were obtained in control solution (left panels) and in the same cell after 30 min superfusion with antagonist (right panels). In (a) and (b) the agonist (solid bars) was 1 μM baclofen and in (c) it was 0.1 μM SK&F 97541. The electrotonic potentials were elicited by constant current pulses (−0.2 nA, 300 ms, 0.1 Hz); membrane potentials were held constant at (a) −68 mV, (b) −77 mV and (c) −82 mV.

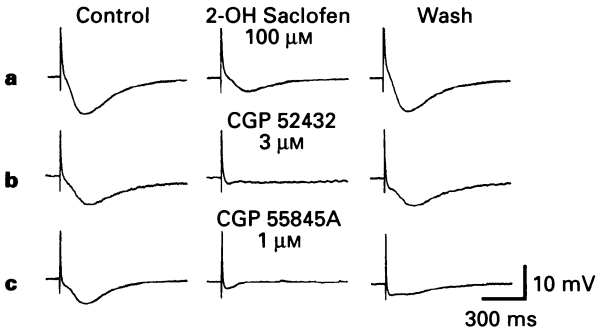

Figure 6 Comparison of the action of (a) 100 μM 2-OH saclofen, (b) 3 μM CGP 52432 and (c) 1 μM CGP 55845A on synaptic potentials evoked by focal stimulation in the septal nucleus (10–20 V; 100 μs; 0.1 Hz). Recordings were made before drug application (left traces), 10 to 15 min after addition of antagonist (middle traces) and after 15 min of washing with drug-free Krebs solution (right traces). Each trace is the digital average of 8 consecutive evoked synaptic responses. The amplitude of the late i.p.s.p. was reduced during application of the 3 GABA$_B$ antagonists. Resting membrane potentials were (a) −80 mV, (b) −71 mV and (c) −86 mV. The peak of the action potential has been cut-off in order to illustrate the late i.p.s.p. at high amplification.

35348, CGP 52432 and CGP 55845A (Waldmeier *et al.*, 1994). Furthermore, the rank order of potency of these compounds (CGP 55845A > CGP 52432 > CPG 35348 > 2-OH-saclofen > CGP 36742) is in excellent agreement with that determined from the inhibition of [^{3}H]-CGP 27492 binding to GABA$_B$ receptors in rat cortical membranes (Bittiger *et al.*, 1990; Olpe *et al.*, 1990; Kerr *et al.*, 1990; Froestl *et al.*, 1992; Waldmeier *et al.*, 1994).

All five GABA$_B$ antagonists selectively reduced the late i.p.s.p. amplitude confirming studies showing that the early i.p.s.p. is mediated via activation of GABA$_A$ receptors while the latter is mediated by GABA$_B$ receptors (Stevens *et al.*, 1987; Hasuo & Gallagher, 1988). The blockade with CGP 36742, CPG 35348, 2-OH-saclofen, CGP 52432 was reversible by washing for 15 min in drug-free Krebs solution as has been previously described for some of these compounds (Lambert *et al.*, 1989; Olpe *et al.*, 1993). However, no complete recovery was observed with CGP 55845A, even after prolonged washing in drug-free Krebs solution. For four of the five antagonists, the concentration that produced a significant rightward shift in the agonist concentration-response curve also significantly inhibited the late i.p.s.p. However, in the case of CGP 55845A (the most potent antagonist), a ten fold higher concentration was required to reduce the i.p.s.p. amplitude. One possible explanation for these results is that CGP 55845A could also act as an antagonist at presynaptic GABA$_B$ receptors to increase glutamate release and subsequent feed forward inhibition or to increase GABA release from the inhibitory interneuronal nerve terminals. Either action would tend to increase synaptic levels of GABA and therefore a higher concentration of CGP 55845A would be needed to displace GABA from the postsynaptic receptors. Thus the present experiments may underestimate the potency of the antagonists as inhibitors of the late i.p.s.p. Karlsson *et al.* (1990) have also reported a difference between the concentration of antagonist required to block agonist-induced responses and late i.p.s.ps. In their study performed on hippocampal slices, CGP 35348 (30–100 μM) abolished the late i.p.s.p. and the membrane hyperpolarization induced by 10 μM baclofen. However, phaclofen (300 μM), another low potency GABA$_B$ antagonist reduced the late i.p.s.p. but had little or no effect on the baclofen-induced hyperpolarization.

These and other differential actions of GABA$_B$ antagonists on GABA$_B$-mediated processes provide limited pharmacological evidence for multiple types of GABA$_B$ receptor in the mammalian central nervous system (Scherer *et al.*, 1988; Bonanno & Raiteri, 1992; Huston *et al.*, 1995; Beck, *et al.*, 1995). For example, it has been reported that CGP 35348 potently antagonized baclofen as an inhibitor of glutamate release, whereas phaclofen was almost ineffective; both compounds antagonized baclofen as an inhibitor of somatostatin release (Bonanno & Raiteri, 1992). In other experiments, Huston *et al.* (1995) showed that multiple calcium channel sub-types are involved in glutamate release from the cerebellum and that they are modulated by baclofen to different degrees. These results were confirmed by the work of Guyon & Leresche (1995). They found that CGP 55845A and CGP 35348 blocked the effect of baclofen on N-type voltage-activated calcium currents in rat thalamocortical neurones. However, CGP 55845A also weakly blocked the action of baclofen on R-type calcium currents whereas CGP 35348 was ineffective. The present experiments do not provide substantial additional evidence for or against the existence of GABA$_B$ receptor subtypes and definitive proof of their existence awaits the cloning and sequencing of this G-protein linked receptor.

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