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Comparison of antagonist potencies at pre- and post-synaptic $GABA_B$ receptors at inhibitory synapses in the CA1 region of the rat hippocampus

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1 Synaptic activation of γ-aminobutyric acid $(GABA)_B$ receptors at GABA synapses causes (a) postsynaptic hyperpolarization mediating a slow inhibitory postsynaptic potential/current (IPSP/C) and (b) presynaptic inhibition of GABA release which depresses IPSPs and leads to paired-pulse widening of excitatory postsynaptic potentials (EPSPs). To address whether these effects are mediated by pharmacologically identical receptors the effects of six $GABA_B$ receptor antagonists of widely ranging potencies were tested against each response.

2 Monosynaptic $IPSP_B$s were recorded in the presence of $GABA_A$, AMPA/kainate and NMDA receptor antagonists. All $GABA_B$ receptor antagonists tested depressed the $IPSP_B$ with an IC_{50} based rank order of potency of CGP55679 ⩾ CGP56433 = CGP55845A = CGP52432 > CGP51176 > CGP36742.

3 Paired-pulse EPSP widening was recorded as an index of paired-pulse depression of GABA-mediated IPSP/Cs. A similar rank order of potency of antagonism of paired-pulse widening was observed to that for $IPSP_B$ inhibition.

4 Comparison of the IC_{50} values for $IPSP_B$ inhibition and paired-pulse EPSP widening revealed a close correlation between the two effects in that their IC_{50}s lay within the 95% confidence limits of a correlation line that described IC_{50} values for inhibition of paired-pulse EPSP widening that were 7.3 times higher than those for $IPSP_B$ inhibition.

5 Using the compounds tested here it is not possible to assign different subtypes of $GABA_B$ receptor to pre- and post-synaptic loci at GABAergic synapses. However, 5–10 fold higher concentrations of antagonist are required to block presynaptic as opposed to postsynaptic receptors when these are activated by synaptically released GABA.

Keywords: γ-Amino-butyric acid (GABA); $GABA_B$ receptor subtypes; $GABA_B$ receptor antagonists; late IPSP; paired-pulse widening

Abbreviations: aCSF, artificial cerebrospinal fluid; EPSP, Excitatory postsynaptic potential; GABA, γ-amino-butyric acid; IPSP/C, Inhibitory postsynaptic potential/current

Introduction

γ-Amino-butyric acid $(GABA)_B$ receptors are present at pre- and post-synaptic loci in highly diverse regions of the vertebrate central nervous system (Bowery, 1993). Postsynaptically, $GABA_B$ receptors activate an inwardly rectifying potassium conductance which hyperpolarizes the neurone (Gähwiler & Brown, 1985). Presynaptically, $GABA_B$ receptors inhibit the release of numerous neurotransmitters (e.g., GABA, glutamate, noradrenaline, 5-HT, substance P, cholecystokinin and somatostatin) through a number of potential mechanisms (e.g., enhancement of certain potassium conductances (Gage, 1992), inhibition of voltage-gated calcium conductances (Campbell *et al*., 1993; Pfrieger *et al*., 1994; Wu & Saggau, 1995) and inhibition of release machinery *per se* (Thompson *et al*., 1993)). The differential localization of $GABA_B$ receptors raises the possibility that $GABA_B$ receptors at different loci are pharmacologically distinguishable. The importance of this is that it provides the potential to develop drugs that are specifically targeted at regulating the putative inhibitory or disinhibitory roles that these different populations of $GABA_B$ receptors fulfil within the CNS.

The recent development of a series of $GABA_B$ receptor antagonists with widely ranging potencies (Froestl *et al*., 1995; Froestl & Mickel, 1997) has now enabled this possibility to be examined. To date, this has been performed most extensively using neurochemical methods combined with quantitative pharmacological analysis to examine the presynaptic $GABA_B$ receptors that inhibit glutamate, GABA and somatostatin release. The findings of these studies have been contradictory between groups. Thus, some studies have suggested the existence of multiple $GABA_B$ receptor subtypes, each selectively inhibiting the release of specific neurotransmitters (e.g., Bonanno & Raiteri, 1993b). Other reports have failed to observe such a distinction (e.g., Waldmeier *et al*., 1994). Likewise, there is controversy as to whether or not postsynaptic $GABA_B$ receptors exist as two distinct $GABA_B$ receptor subtypes (Pham & Lacaille, 1996). However, in both sets of studies $GABA_B$ receptors have been activated predominantly using selective agonists and not by way of endogenously released GABA. As such, the question still remains as to whether pharmacologically distinguishable $GABA_B$ receptors are activated by synaptically released GABA. The answer to this question is important as it will provide a better understanding of the normal physiological and pathological roles that $GABA_B$ receptors play *in vivo*,

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most notably in terms of their effects on mnemonic processing (Olpe & Karlsson, 1990; Davies *et al*., 1991; Mott & Lewis, 1991; Mondadori *et al*., 1993; Olpe *et al*., 1993b) and absence epilepsy (Liu *et al*., 1992; Hosford *et al*., 1992). Ultimately, this may lead to the development of a new generation of compounds that through targeting of specific $GABA_B$ receptor populations may be more efficacious in treating specific disease states.

The purpose of this study, therefore, was to examine, at GABAergic synapses, the pharmacology of those $GABA_B$ receptors located pre- and post-synaptically that are activated by synaptically released GABA. In this respect, in the CA1 region of the hippocampus, physiological activation of postsynaptic $GABA_B$ receptors results in the late inhibitory postsynaptic potential ($IPSP_B$) (Dutar & Nicoll, 1998a; Soltesz *et al*., 1988; Otis *et al*., 1993; Solís & Nicoll, 1992) whereas activation of presynaptic $GABA_B$ receptors results in paired-pulse depression of synaptic inhibition (i.e. a $GABA_B$ autoreceptor effect: Thompson & Gähwiler, 1989; Deisz & Prince, 1989; Davies *et al*., 1990; 1991; Olpe *et al*., 1994) which causes paired-pulse widening of synaptic excitation (Nathan *et al*., 1990; Nathan & Lambert, 1991; Davies & Collingridge, 1996). By determining the IC_{50} values for antagonism of the late IPSP, and comparing these with those for antagonism of paired-pulse widening of EPSPs for six structurally different $GABA_B$ receptor antagonists, we have attempted to address whether synaptically activated pre- and post-synaptic $GABA_B$ receptors at GABAergic synapses can be differentiated pharmacologically.

Methods

Biological preparation

Experiments were performed on hippocampal slices obtained from Wistar rats (3–5 weeks old) as described previously (Davies *et al*., 1990). In brief, animals were cervically dislocated or anaesthetized using halothane (3–5%) and subsequently decapitated in accordance with U.K. Home Office or Swiss Government guidelines. The brain was removed rapidly and the hippocampus left *in situ* or dissected free. Transverse brain slices (400 μm thick) containing hippocampus, or hippocampal slices *per se*, were cut using either a Campden virboslicer or Sorval® tissue chopper. Where necessary the hippocampal region was dissected free from other surrounding brain areas. Area CA3 was subsequently removed from all freed hippocampal slices and two of the resultant CA3-ectomized hippocampal slices immediately transferred to an interface recording chamber maintained at 30–32°C. Here slices rested on a nylon mesh at the interface of a warmed perfusing artificial cerebrospinal fluid containing either (mM): NaCl 124; KCl 3.0; $NaHCO_3$ 26; $CaCl_2$ 2.0; $MgSO_4$ 1; D-glucose 10; NaH_2PO_4 1.25, or NaCl 120; KCl 2.5; $NaHCO_3$ 30; $CaCl_2$ 2.5; $MgSO_4$ 2; D-glucose 10; KH_2PO_4 1.2, bubbled with a 95% O_2/5% CO_2 mixture. No differences were observed between experiments performed in either solution. Spare slices were stored submerged and oxygenated at room temperature for later use.

Electrophysiological recording

Intracellular recordings were obtained from neurones in stratum pyramidale using glass microelectrodes (60–120 MΩ) filled with potassium methylsulphate (2 M) connected to an Axoclamp-2A amplifier used in discontinuous current-clamp or bridge mode (Axon Instruments, Foster City, CA, U.S.A.). Spike frequency adaptation and input resistance of pyramidal cells were routinely measured throughout each experiment by passing current pulses (amplitude $\pm$0.1–0.5 nA, duration 300–700 ms) through the intracellular recording electrode every 30–120 s to depolarize or hyperpolarize the neurone, respectively. In all experiments 6-nitro-7-sulphamoylbenzo-[f]-quinoxaline-2,3-dione (NBQX, 3 μM) or 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 10 μM), and D-(E)-2-amino-4-methyl-5-phospho-3-pentanoic acid (CGP 40116; 50 μM) or D-2-amino-5-phosphonopentanoate (D-AP5, 50 μM) were present in the perfusing medium to block all ionotropic glutamate receptor-mediated synaptic transmission. In certain experiments picrotoxin was used to block all $GABA_A$ receptor-mediated synaptic inhibition so that pure $IPSP_B$s could be isolated. Monosynaptic biphasic IPSPs and pure $IPSP_B$s were evoked by delivering a single constant current stimulus (40–140 μA, 0.02–0.2 ms pulse width) using bipolar nickel/chromium or stainless steel stimulating electrodes placed in stratum radiatum close to the recorded neurone, within 500 μm laterally but half to two-thirds the distance down the apical dendritic tree. To quantify the effects of drugs synaptic responses were compared before and after drug treatment at a fixed membrane potential. This was achieved by injecting DC to compensate for any spontaneous membrane potential fluctuations. In all experiments stimulus strengths were set to activate maximal $IPSP_B$s at membrane potentials between -62 and -65 mV. The effect of each concentration of the antagonists used was quantified in terms of the percentage reduction of $IPSP_B$ peak amplitude after a 20 min antagonist application, relative to a 20 min baseline period.

Paired-pulse widening of EPSPs was studied using extracellular recording techniques. Extracellular recordings of glutamate-mediated field excitatory postsynaptic potentials (EPSPs) were obtained from stratum radiatum with a NaCl (4 M) filled microelectrode (2–5 MΩ). Synaptic responses were evoked by paired pulse stimulation (2–10 V, 20 μs pulse width) delivered at a fixed interval of 100–200 ms every 30 s to the Schaffer collateral-commissural fibres in the lower third of stratum radiatum using a bipolar stimulating electrode. The magnitude of antagonism of paired-pulse widening of field EPSPs induced by each concentration of the antagonists was calculated using the half widths of the field EPSPs as follows:

$$\frac{t_{\frac{1}{2}}\text{2nd EPSP}}{\text{(control)}} - \frac{t_{\frac{1}{2}}\text{2nd EPSP}}{\text{(antagonist)}} = X$$

$$\frac{t_{\frac{1}{2}}\text{2nd EPSP}}{\text{(control)}} - \frac{t_{\frac{1}{2}}\text{1st EPSP}}{\text{(control)}} = Y$$

The ratio X : Y was then converted to a percentage. Thus, 100% inhibition of paired-pulse widening of EPSPs occurred when the concentration of antagonist was sufficient to cause the second field EPSP half width to be equal to the half width of the first EPSP in the presence of the antagonist (which is equal to the half width of the first EPSP in control conditions).

Drugs

Drugs were administered by addition to the superfusing medium and were applied for a sufficient period (15–20 min) to allow their full equilibration. Picrotoxin, was obtained from Sigma. AP5, CNQX and NBQX were purchased from Tocris-Cookson. D-(E)-2-amino-4-methyl-5-phospho-3-pentanoic acid (CGP 40116), 3-aminopropyl-*n*-butyl-phosphinic acid (CGP 36742), 3-amino-2-(*R*)-hydroxypropyl-cyclohexyl-methyl-phosphinic acid hydrochloride (CGP 51176A), [3-

[[(3,4-dichlorophenyl) methyl] amino] propyl] -diethoxymethyl-phosphinic acid (CGP 52432), [2-(*S*)-hydroxy-3-[[1-(*S*)-(3,4,5-trimethoxyphenyl) - ethyl] amino] propyl] - cyclohexylmethyl-phosphinic acid (CGP 55679), [3-[1-(*S*)-[[3-(cyclohexylmethyl)-hydroxyphosphinyl] - 2 - (*S*) - hydroxypropyl] amino]ethyl]-benzoic acid (CGP 56433), and [1-(*S*)-3,4-dichlorophenyl)ethyl] amino-2-(S)-hydroxypropyl-benzyl-phosphinic acid (CGP 55845A) were synthesized *de novo* by the Chemistry Department at Novartis Pharma AG, Basle, Switzerland. Each drug was dissolved in distilled water or equimolar NaOH at 100–1000 times its final bath applied concentration and was stored frozen until just prior to experimental use. *n* signifies the number of times each drug was tested, which was the same as the number of slices tested. Each slice was obtained from a separate rat.

Results

Postsynaptic $GABA_B$ receptors

In a first series of experiments postsynaptic $GABA_B$ receptors were activated physiologically. Thus, either a monosynaptic biphasic IPSP comprising a $GABA_A$ receptor-mediated IPSP ($IPSP_A$) followed by a $GABA_B$ receptor-mediated IPSP ($IPSP_B$), or an isolated monosynaptic $IPSP_B$, were evoked in a CA1 pyramidal neurone in the presence of the excitatory amino acid antagonists AP5 or CGP 40116 (50 μM) and CNQX (10 μM) or NBQX (3 μM) to block fast glutamatergic synaptic excitation (Davies *et al*., 1990). Of the six structurally diverse compounds (Figure 1) tested all abolished $IPSP_B$s without substantially affecting $IPSP_A$s. The concentration response relationship for the inhibition of the $IPSP_B$ for each antagonist paralleled those of the other compounds (Figure 2). The respective IC_{50} values for each compound are provided in Table 1.

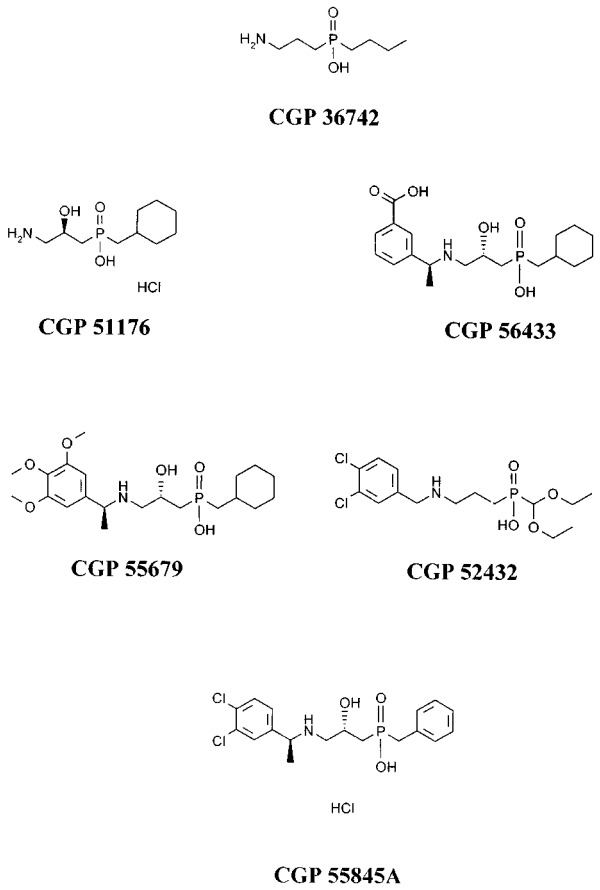

Figure 1 Structures of $GABA_B$ receptor antagonists.

$GABA_B$ autoreceptors

In a second series of experiments the effectiveness of $GABA_B$ receptor antagonists to block $GABA_B$ autoreceptors activated by synaptically released GABA was evaluated. To do this the effects of $GABA_B$ receptor antagonists on paired-pulse widening of AMPA receptor-mediated field EPSPs was tested as this (a) has been reported to be a direct consequence of $GABA_B$ autoreceptor-mediated paired-pulse depression of GABA-mediated synaptic inhibition (Nathan *et al*., 1990) and (b) provides a potentially more efficient method for quantitative evaluation of $GABA_B$ autoreceptor pharmacology than studying paired-pulse depression of IPSCs. To confirm that this was the case and, further, to establish whether this experimental approach was a fair quantitative

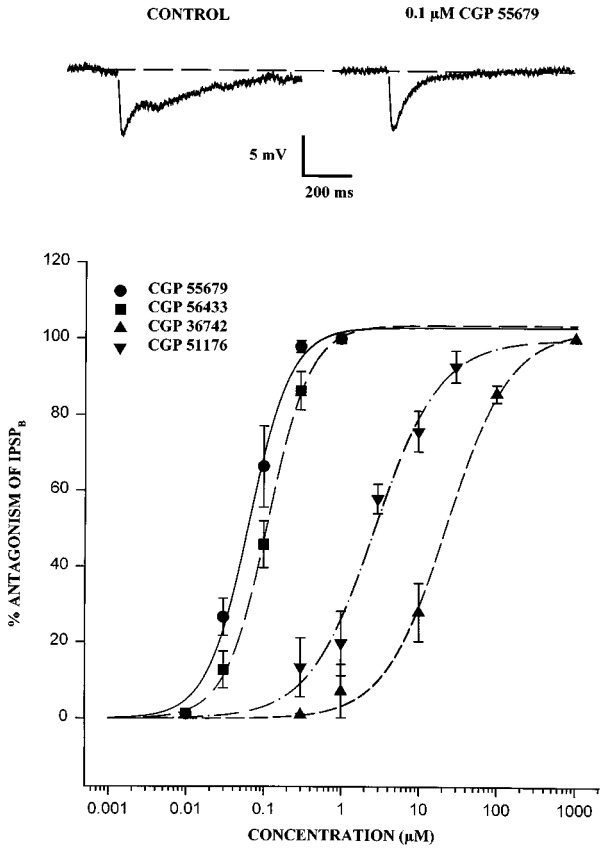

Figure 2 Comparison of the potency of different $GABA_B$ receptor antagonists to block $IPSP_B$. Synaptic traces are monosynaptically-activated biphasic IPSPs. Bath application of 0.1 μM CGP 55679 for 15–25 min depressed the $IPSP_B$ without substantially affecting the $IPSP_A$. Each trace is an average of four consecutive IPSPs and stimulus artefacts have been blanked for clarity. The membrane potential of the neurone was −63 mV. The graph shows plots of the percentage inhibition of $IPSP_B$ *versus* antagonist concentration for four of the antagonists tested. Data were fitted to the logistic expression $Y = M(X^P/[X^P + K^P])$ where Y is the percentage inhibition of $IPSP_B$, X is the antagonist concentration, M is the unconstrained maximum effect, K is the concentration of antagonist producing 50% inhibition (i.e. IC_{50}) and the power P determines the slope of the curve (Barlow & Blake, 1989). Symbols represent mean values and bars standard errors of the mean where these are larger than the symbols.

representation of the activity of compounds at $GABA_B$ autoreceptors we performed two sets of experiments.

In the first we demonstrated that $GABA_B$ receptor antagonists abolished paired-pulse widening of field EPSPs whether these were recorded in the presence, or absence, of CGP 40116 or D-AP5 to block any activation of NMDA receptor-mediated EPSPs (Figure 3a). Two observations suggested that the effects of the antagonists were due to their block of paired-pulse depression of $IPSP_A$s: Firstly, abolition of $IPSP_A$s using picrotoxin enhanced the duration of the first field EPSP in the pair and thereby occluded paired-pulse widening of field EPSPs since both EPSPs were no longer constrained by this synaptic potential ($n=3$; Figure 3b). Secondly, $GABA_B$ receptor antagonists had no additional effect on field EPSPs evoked by paired-pulse stimulation in the presence of this $GABA_A$ receptor antagonist ($n=3$; Figure 3b).

In a second series of experiments we used single electrode voltage-clamp recording in the presence of AP5 (40 μM) and CNQX (20 μM) to record monosynaptic biphasic inhibitory postsynaptic currents (IPSCs) (Davies *et al*., 1990). We compared the concentration response relationship for the antagonism of paired-pulse depression of IPSCs recorded under these conditions with that for antagonism of paired-pulse widening of EPSPs. When two stimuli were delivered 50–1000 ms apart there was a marked reduction in the IPSC evoked by the second stimulus, i.e. paired-pulse depression occurred. This depression was maximal at an interstimulus interval of 100–200 ms and has previously been shown to result from activation of $GABA_B$ autoreceptors (Davies *et al*., 1990; Nathan & Lambert, 1991; Davies & Collingbridge, 1993). CGP 55845A (0.03–10 μM) inhibited the late component of the IPSC evoked by both stimuli and reversed paired-pulse depression of the early $GABA_A$ receptor-mediated IPSC ($n=4$). As illustrated in Figure 4 the two relationships closely paralleled each other although that for inhibition of $IPSP_B$ was shifted to the left by a factor of approximately 7. In addition, the concentration response relationship for the antagonism of the $IPSC_B$ by CGP 55845A matched that for its antagonism of $IPSP_B$, and the concentration response relationship for antagonism of paired-pulse depression of IPSCs mirrored almost exactly that for inhibition of paired-pulse widening of field EPSPs (Figure 4).

Table 1 Comparison of IC_{50} values for antagonism of $IPSP_B$s and paired-pulse widening (PPW) of EPSPs

Compound	*$IPSP_B$ IC_{50} (μM)*	*PPW of EPSP IC_{50} (μM)*	*Ratio EPSP/ $IPSP_B$*
CGP 55679	0.06±0.01	0.24±0.03	4.0
CGP 56433	0.11±0.01	0.28±0.11	2.5
CGP 55845A	0.11±0.01	0.74±0.05	6.7
CGP 52432	0.12±0.01	0.68±0.13	5.7
CGP 51176A	3.0±1.0	9.0±6.0	3.0
CGP 36742	23.0±4.0	239±104	10.4

Based on these two series of experiments, therefore, it is reasonable to suggest that an analysis of the effects of $GABA_B$ receptor antagonists on paired-pulse widening of field EPSPs provides an accurate measurement of the activity of these compounds at physiologically activated $GABA_B$ autoreceptors. As such, we studied next the ability of a range of antagonists to inhibit this effect. As was the case for $IPSP_B$, every antagonist tested inhibited paired-pulse widening of EPSPs. Again, the concentration response relationships all

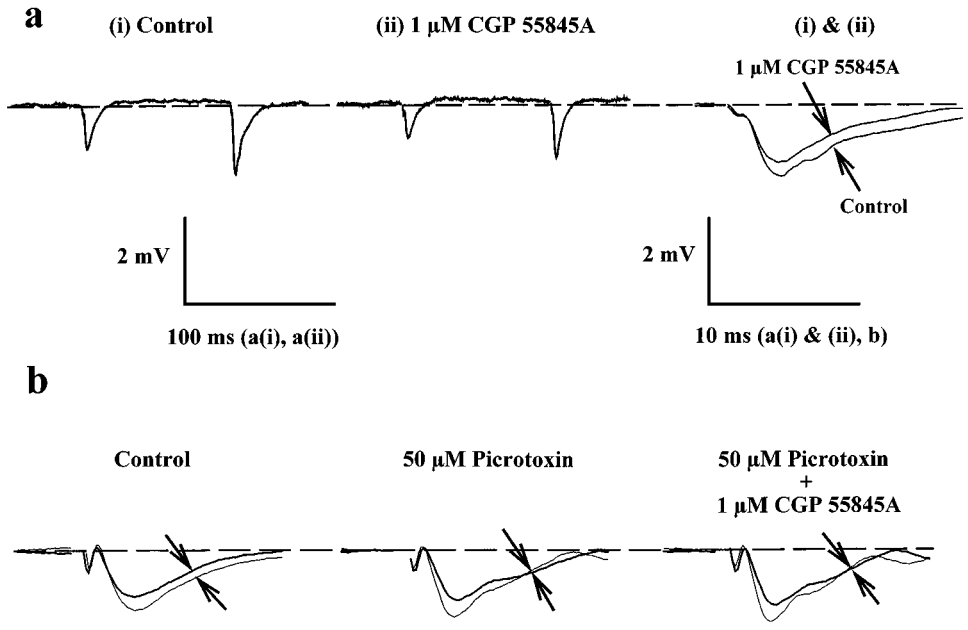

Figure 3 Paired-pulse widening of field EPSPs is an accurate model for studying the pharmacology of $GABA_B$ autoreceptors. In (a(i)) synaptic traces are extracellularly recorded field EPSPs evoked by a pair of stimuli delivered 100 ms apart in control medium containing 50 μM CGP 40116. Note that the duration of the second field EPSP is longer than that of the first EPSP of the pair. (a(ii)) shows the corresponding responses evoked by the same stimulation protocol in the presence of 1 μM CGP 55845A. Note that under these conditions the durations of the first and second field EPSPs are similar. The far right hand trace is a superimposition of the second field EPSPs illustrated in (i) and (ii) plotted on a faster time base to illustrate the difference in durations of the second field EPSPs evoked in control and in CGP 55845A-containing medium. In (b) superimposed traces are the first (thick line) and second (thin line) field EPSPs of a pair of field EPSPs evoked by a pair of stimuli delivered 200 ms apart in control medium containing 50 μM CGP 40116, in the additional presence of 50 μM picrotoxin and in the additional combined presence of 50 μM picrotoxin and 1 μM CGP 55845A. Note that in control medium the second EPSP is wider than the first whereas in the presence of picrotoxin or the combination of picrotoxin and CGP 55845A the width of each EPSP was identical. Note also that the far right hand responses are pure non-NMDA receptor-mediated field EPSPs and that the increase in amplitude of the second field EPSP in the pair results from paired-pulse facilitation of glutamate release.

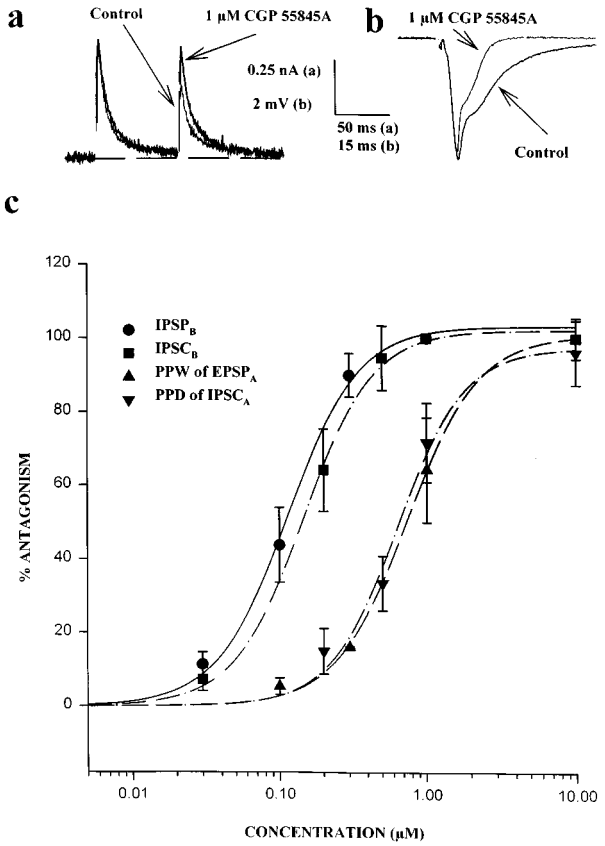

Figure 4 Comparison of the potency of CGP 55845A to inhibit paired-pulse depression of IPSCs with its potency to inhibit paired-pulse widening of field EPSPs. In (a) synaptic traces represent monosynaptic IPSCs evoked by two stimuli delivered 100 ms apart in the presence of D-AP5 (50 μM) and CNQX (10 μM) superimposed on the corresponding responses evoked in the presence of these antagonists plus 1 μM CGP 55845A. Note that in control medium the second IPSC of the pair is reduced compared to the first IPSC and that in the presence of CGP 55845A the peak amplitude of the second IPSC is greatly enhanced such that it now approaches the size of the first IPSC of the pair. In this particular example there is little or no activation of an IPSC$_B$ and the cell was held at a membrane potential of -61 mV. In (b) synaptic responses represent the second field EPSP of a pair of EPSPs evoked by two stimuli delivered 200 ms apart in control and in CGP 55845A-containing medium. Note that in the presence of the GABA$_B$ receptor antagonist the field EPSP is much narrower. The graph in (c) illustrates the concentration response relationships for CGP 55845A-induced antagonism of the IPSP$_B$, the IPSC$_B$ paired-pulse widening of the field EPSP and paired-pulse depression of IPSC$_A$. Each data plot was fitted to the logistic expression $Y = M(X^P/[X^P + K^P])$ as described in Figure 2. Note the close correlation between antagonism of paired-pulse widening of EPSP$_A$ and paired-pulse depression of IPSC$_A$.

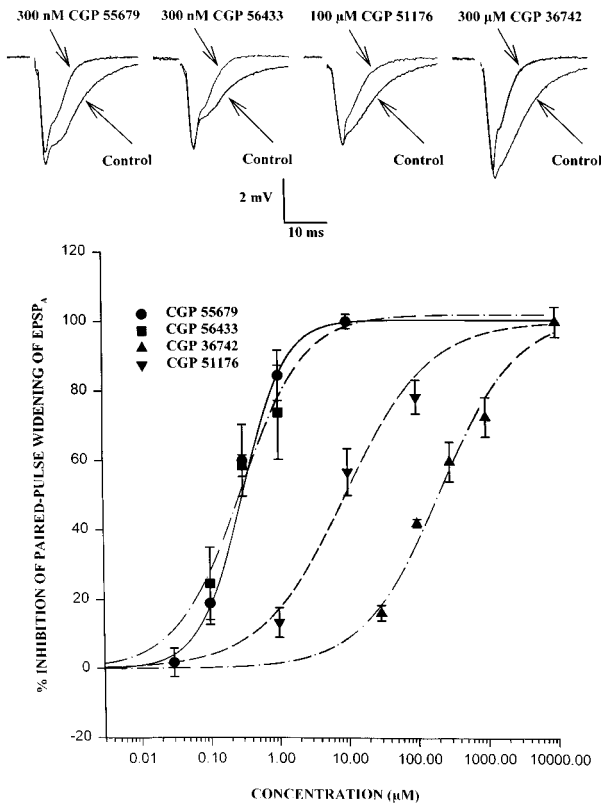

Figure 5 Comparison of the potency of different GABA$_B$ receptor antagonists to inhibit paired-pulse widening of field EPSPs. Each of the four superimposed synaptic traces represent the second field EPSP of a pair evoked by two stimuli delivered 200 ms apart in control medium containing 50 μM CGP 40116 superimposed on the corresponding second field EPSP evoked in the presence of the concentration of antagonist indicated. The graph shows plots of the percentage inhibition of paired-pulse widening of field EPSPs *versus* antagonist concentration for four of the antagonists tested. Data were fitted to the logistic expression $Y = M(X^P/[X^P + K^P])$ as described in Figure 2.

paralleled each other and showed similar maximum effects (Figure 5). The rank order of antagonism was the same as that for the IPSP$_B$ although the individual IC$_{50}$ values were higher. For clarity, a comparison of IC$_{50}$ values for antagonism of paired-pulse widening of EPSPs and inhibition of IPSP$_B$s are given in Table 1.

Comparison of antagonist potency at pre- and post-synaptic GABA$_B$ receptors

Finally, we compared the IC$_{50}$ values of these six compounds to inhibit the IPSP$_B$ and paired-pulse widening of field EPSPs with those we have previously calculated for phaclofen, 2-hydroxy-saclofen and CGP 35348 (Davies & Collingridge, 1993; Davies *et al*., 1993). As illustrated in Figure 6 there was a good correlation between IC$_{50}$ values for each antagonist to inhibit physiologically activated pre- and post-synaptic GABA$_B$ receptors. Thus, the mean IC$_{50}$ values for the two effects all lay very close to, or within, the 95% confidence limits of a linear regression line that described IC$_{50}$ values for inhibition of paired-pulse widening of EPSPs that were 7.3 times higher than those for antagonism of IPSP$_B$.

Discussion

The present data demonstrate that, like phaclofen, 2-hydroxy-saclofen and CGP 35348, six additional phosphinic acid derivatives of GABA or corresponding N-substituted analogues (i.e. CGP 36742, CGP 51176A, CGP 55845A, CGP 52432, CGP 56433 and CGP 55679; Froestl *et al*., 1992; 1995; Froest & Mickel, 1997) are GABA$_B$ receptor antagonists which are capable of antagonizing both pre- and post-synaptic GABA$_B$ receptors at GABA-mediated synapses in the CA1 region of the rat hippocampus. The rank order of potency for this series of antagonists at both populations of GABA$_B$ receptor is identical and fits well with the rank order of potency that can be generated based on the calculated pK$_i$ values for these antagonists obtained from binding studies (Olpe *et al*., 1993a; Waldmeier *et al*., 1994; Froestl *et al*., 1995). Thus, for example, CGP 36742 is approximately 100 fold less

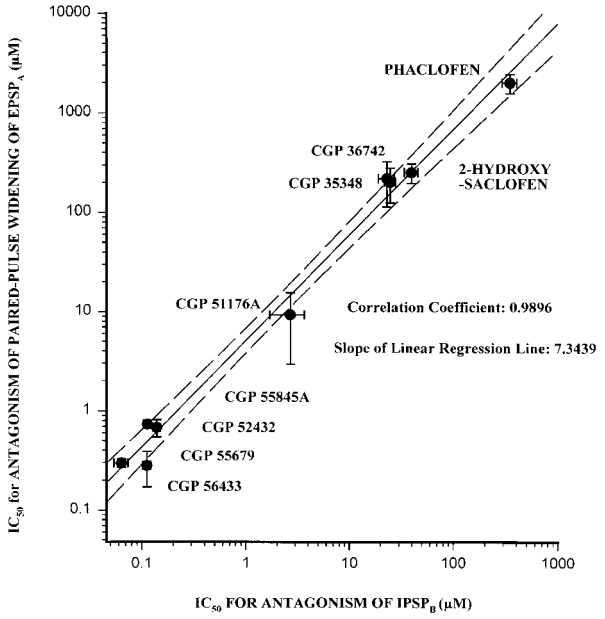

Figure 6 Comparison of IC_{50} values for antagonism of $IPSP_B$ and paired-pulse widening of $EPSP_A$ for different $GABA_B$ receptor antagonists. The graph shows a plot of the IC_{50} value for antagonism of paired-pulse widening of field EPSPs *versus* the corresponding IC_{50} value for antagonism of the $IPSP_B$ for nine different $GABA_B$ receptor antagonists. The line drawn through the data points is a least squares fit regression line and the dashed line bordering it the 95% confidence limits of the fit.

potent than CGP 55679, using both electrophysiological and ligand-binding techniques, irrespective of the population of $GABA_B$ receptor tested. Functionally, the only difference between antagonism at pre- and post-synaptic $GABA_B$ receptors is the approximate 7–8 fold higher concentrations that are required to block presynaptic receptors as opposed to postsynaptic receptors, irrespective of the antagonist used.

At first glance, this concentration difference might point to a difference between pre- and post-synaptic $GABA_B$ receptors. Such a situation might be resolved by comparing calculated K_D values for each anatagonist at these different populations. However, this approach may not provide the appropriate information regarding receptors that are activated physiologically because, firstly, this approach will activate both synaptic and extrasynaptic receptors and, secondly, it has been suggested that two pharmacologically distinct $GABA_B$ receptors exist postsynaptically (Pham & Lacaille, 1996), and it is unclear (1) as to which of these are activated by synaptically released GABA, or, indeed (2) whether both are activated simultaneously. Strongest support for heterogeneity of $GABA_B$ receptors, and in particular amongst those which are expressed presynaptically, has come principally from neurochemical release studies. In this respect, Bonanno & Raiteri (1993b) have suggested the existence of at least four separate $GABA_B$ receptor subtypes controlling the release of different neurotransmitters from cortical synaptosomes and K^+-stimulated brain slices (Bonanno & Raiteri, 1993a; Lanza *et al*., 1993; Fassio *et al*., 1994). This subclassification is based on the differential susceptibility of separate $GABA_B$ receptor populations to the agonists baclofen and 3-aminopropylphosphinic acid (3-APPA) and the antagonists phaclofen, CGP 35348 and CGP 52432. Whilst it is difficult to make direct comparisons between the data presented here and that generated using baclofen or 3-APPA to activate $GABA_B$ receptors, it is interesting that our data for both pre- and postsynaptic $GABA_B$ receptors at GABA-mediated synapses fit best with a subtype that is sensitive to all three antagonists mentioned above and, therefore, suggest that these receptors are similar to those that control the cortical release of somatostatin but not GABA or glutamate (Bonanno & Raiteri, 1993b). However, the subclassification of $GABA_B$ receptors suggested by Bonanno & Raiteri (1993b) is not universally accepted, most notably because their observations have not been repeated by others using electrically stimulated release in cortical and dorsal horn slices (Waldmeier *et al*., 1994; Teoh *et al*., 1996). In fact, quantitative pharmacological analysis of the antagonism of baclofen-induced inhibition of electrically-induced GABA and glutamate release revealed that K_D values for $GABA_B$ receptor antagonists were similar to those calculated from radioligand binding studies in the same laboratories (Waldmeier *et al*., 1994), a situation echoed using electrophysiological approaches in the present study. Based on these data and the premise that radioligand binding does not discriminate between pre- and post-synaptic $GABA_B$ receptors it was proposed that either (a) each antagonist does not differentiate between possible $GABA_B$ receptor subtypes or (b) all antagonists are specific for a particular $GABA_B$ receptor subtype and that compounds that activate/antagonize other $GABA_B$ receptor subtypes have yet to be developed. That said, in the dorsal horn CGP 56999A potently antagonized $GABA_B$ receptors controlling the release of GABA and substance P without affecting those which regulate the release of glutamate (Teoh *et al*., 1996) raising the possibility that pharmacologically distinguishable $GABA_B$ receptors do exist in the CNS. If so, it is unlikely that it will be possible to allocate a particular pharmacological subtype to the $GABA_B$ heteroreceptor on glutamate terminals, another to the heteroreceptor on somatostatin terminals, another to the $GABA_B$ autoreceptor and so on. Indeed, one study using CGP 56999A in the cortex has already failed to demonstrate a differential effect of this antagonist on the $GABA_B$ autoreceptors as opposed to heteroreceptors on glutamate terminals (Waldmeier *et al*., 1994). In the absence of an exhaustive study to assess the activity of CGP 56999A at all $GABA_B$ receptor populations in the CNS these data raise the intriguing possibility that the pattern of expression of a CGP 56999A-insensitive $GABA_B$ receptor may be regionally restricted to the spinal cord as opposed to other CNS areas.

Previous electrophysiological studies examining the pharmacology of $GABA_B$ receptors have been equally contentious with, to date, different laboratories claiming differences between $GABA_B$ receptors in the neocortex and hippocampus (Deisz *et al*., 1993; Dutar & Nicoll, 1998b) whilst others reporting no difference in the striatum and hippocampus (Seabrook *et al*., 1990; Thompson & Gähwiler, 1992). It could be argued on the basis of the present electrophysiological data that different $GABA_B$ receptors exist at pre- and post-synaptic loci at GABAergic synapses in the hippocampal CA1 region. However, it is equally feasible that both populations of $GABA_B$ receptors are pharmacologically identical. In this respect, the rank order of antagonism of pre- and post-synaptic $GABA_B$ receptors is similar and the 7–10 fold concentration difference to antagonize the two populations exists because either (1) each compound tested is a competitive antagonist of $GABA_B$ receptors and $GABA_B$ autoreceptors encounter a higher concentration of synaptically released GABA than do postsynaptic $GABA_B$ receptors even under circumstances where the amount of GABA initially released is the same (e.g., in response to single shock stimulation), or (2) at the presynaptic site $GABA_B$ receptors couple to different effector systems for which there is greater receptor reserve/coupling efficiency. With respect to the former situation there

is, at present, no report of the relative concentrations of synaptically released GABA at pre- and post-synaptic $GABA_B$ receptors at GABAergic synapses in the hippocampus. However, it appears that (a) $GABA_B$ autoreceptors may be outside the range of synaptically released GABA since despite their presence in hippocampal cultures they do not appear to account for paired-pulse depression of IPSCs (Yoon & Rothman, 1991; Wilcox & Dichter, 1994) and (b) $GABA_B$ autoreceptors are not saturated in slices since experimental manipulations that increase the concentration and/or availability of GABA enhance paired-pulse depression of IPSCs (Roepstorff & Lambert, 1994). In contrast, numerous studies have suggested differences in the transduction mechanisms coupled to pre- and post-synaptic $GABA_B$ receptors at these synapses (Scherer *et al*., 1988; Lambert & Wilson, 1993; Pitler & Alger, 1994; Thompson & Gähwiler, 1992). Thus, postsynaptic $GABA_B$ receptors are generally agreed to couple directly to an inwardly rectifying potassium conductance *via* a pertussis toxin sensitive G-protein (e.g. Gähwiler & Brown, 1985; Dutar & Nicoll, 1998a,b) whereas $GABA_B$ autoreceptors are believed to couple to alternative transduction mechanisms. In this respect, it has been suggested that $GABA_B$ autoreceptors may enhance an A-type K^+ current (Gage, 1992), inhibit an N-type Ca^{2+} current (Doze *et al*., 1995; Lambert & Wilson, 1996) or inhibit the release machinery *per se*. Whichever presynaptic mechanism is correct the observation that (−)-baclofen depresses GABA-mediated synaptic responses at lower concentrations than are required to cause postsynaptic hyperpolarization (Davies *et al*., 1990) points to more effective presynaptic receptor-effector coupling compared to that postsynaptically, assuming identical $GABA_B$ receptors at both loci. The recent cloning of two distinct $GABA_B$ receptor subunits, however, still raises the possibility that pre- and postsynaptic receptors may be pharmacologically distinct (Kaupmann *et al*., 1997, 1998; White *et al*., 1998; Jones *et al*., 1998). That said, the observation that $GABA_BR_2$ itself exhibits limited binding of existing $GABA_B$ receptor antagonists suggests that both pre- and postsynaptic receptors at GABAergic synapses contain a $GABA_BR_1$ subunit (i.e. either of the splice variants $GABA_BR_{1a}$ and $GABA_BR_{1b}$). Indeed, pharmacologically, a comparison of the rank order of potency of antagonists to block each of the homomeric $GABA_BR_1$ receptors, when expressed in COS-7 cells and activated by (−)-baclofen, revealed a similar rank order of potency to that for antagonism of the native receptors studied here (Kaupmann *et al*., 1997). In addition, quantitative pharmacological evaluation of the cloned $GABA_BR_{1a}$ and $GABA_BR_{1b}$ receptors revealed little differences between the two receptors. However, that is not to say that $GABA_BR_2$ subunits do not exist in pre- and postsynaptic $GABA_B$ receptors at GABAergic synapses. Indeed, it is likely that both receptors are hetero-oligomeric complexes since these receptors when expressed in cell-lines exhibit closer binding affinities to those of native receptors and more effectively activate inwardly rectifying potassium channels which mediate postsynaptic hyperpolarization (Kaupmann *et al*., 1998; White *et al*., 1998; Jones *et al*., 1998). This does not preclude pharmacological differences between pre- and postsynaptic $GABA_B$ receptors since existing antagonists do not bind with high affinity to the $GABA_BR_2$ subunit and $GABA_BR_1$ splice variants differ in their extracellular N-terminal region opening up the possibility of differential modulation by specific ligands that, unlike the antagonists developed to date, do not necessarily interact directly with the GABA binding site. In addition, it might be surprising if further subtypes of $GABA_B$ receptor are not discovered in the future since the homologous metabotropic glutamate receptor consists of eight different subtypes, six which couple to the same G proteins (i.e. $G_{i/o}$) as $GABA_B$ receptors.

Concluding remarks

Whilst on the basis of the compounds tested in the present study it is not possible to ascribe different $GABA_B$ receptor subtypes to pre- and post-synaptic loci at GABAergic synapses it is clear that substantially higher concentrations of each antagonist are required to block $GABA_B$ autoreceptors than postsynaptic $GABA_B$ receptors. These data when extrapolated to a clinical context and taken in conjunction with the calculated concentrations of antagonist that reach the brain following i.v. administration would suggest that the predominant effect of these drugs would be to block the effects of postsynaptic receptors as opposed to autoreceptors. The exact outcome of such a balance of antagonism is difficult to assess because of the complexity of neuronal circuits and the possibility that in brain regions other than the CA1 area there may be less, or even more, clearcut differential antagonism of separate populations of $GABA_B$ receptors (Deisz *et al*., 1993). However, on a simplistic level, it might be envisaged that $GABA_B$ receptor antagonists, through their preferential blockade of postsynaptic as opposed to presynaptic $GABA_B$ receptors, might enable increased excitability in the hippocampus particularly during periods of repetitive afferent activity, as for example might occur during learning, and that this might account for their cognitive enhancing properties (Mondadori *et al*., 1993).

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References

BARLOW, R.B. & BLAKE, J.F. (1989). Hill coefficients and the logistic equation. *TIPS,* **10,** 2657–2666.

BONANNO, G. & RAITERI, M. (1993a). γ-Aminobutyric acid (GABA) autoreceptors in rat cerebral cortex and spinal cord represent pharmacologically distinct subtypes of the $GABA_B$ receptor. *J. Pharmacol. Exp. Ther.,* **265,** 765–770.

BONANNO, G. & RAITERI, M. (1993b). Multiple $GABA_B$ receptors. *TIPS,* **14,** 259–261.

BOWERY, N.G. (1993). $GABA_B$ receptor pharmacology. *Ann. Rev. Pharmacol. Toxicol.,* **33,** 109–147.

CAMPBELL, V., BERROW, N. & DOLPHIN, A.C. (1993). $GABA_B$ receptor modulation of Ca^{2+} currents in rat sensory neurones by the G-protein G_o: antisense oligonucleotide studies. *J. Physiol.,* **470,** 1–11.

DAVIES, C.H. & COLLINGRIDGE, G.L. (1993). The physiological regulation of synaptic inhibition by $GABA_B$ autoreceptors in rat hippocampus. *J. Physiol.,* **472,** 245–265.

DAVIES, C.H. & COLLINGRIDGE, G.L. (1996). Regulation of EPSPs by the synaptic activation of $GABA_B$ autoreceptors in rat hippocampus. *J. Physiol.,* **496,** 451–470.

DAVIES, C.H., DAVIES, S.N. & COLLINGRIDGE, G.L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J. Physiol.,* **424,** 513–531.

DAVIES, C.H., POZZA, M.F. & COLLINGRIDGE, G.L. (1993). CGP 55845A: A potent antagonist of $GABA_B$ receptors in the CA1 region of rat hippocampus. *Neuropharmacol.,* **32,** 1071–1073.

DAVIES, C.H., STARKEY, S.J., POZZA, M.F. & COLLINGRIDGE, G.L. (1991). $GABA_B$ autoreceptors regulate the induction of LTP. *Nature,* **349,** 609 – 611.

DEISZ, R.A., BILLARD, J.M. & ZIEGLGÄNSBERGER, W. (1993). Pre- and postsynaptic $GABA_B$ receptors of rat neocortical neurons differ in their pharmacological properties. *Neurosci. Lett.,* **154,** 209 – 212.

DEISZ, R.A. & PRINCE, D.A. (1989). Frequency-dependent depression of inhibition in guinea-pig neocortex *in vitro* by $GABA_B$ receptor feed-back on GABA release. *J. Physiol.,* **412,** 513 – 542.

DOZE, V.A., COHEN, G.A. & MADISON, D.V. (1995). Calcium channel involvement in $GABA_B$ receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *J. Neurophysiol.,* **74,** 43 – 53.

DUTAR, P. & NICOLL, R.A. (1988a). A physiological role for $GABA_B$ receptors in the central nervous system. *Nature,* **332,** 156 – 158.

DUTAR, P. & NICOLL, R.A. (1988b). Pre- and post synaptic $GABA_B$ receptors in the hippocampus have different pharmacological properties. *Neuron,* **1,** 585 – 591.

FASSIO, A., BONANNO, G., CAVAZZANI, P. & RAITERI, M. (1994). Characterization of the GABA autoreceptor in human neocortex as a pharmacological subtype of the $GABA_B$ receptor. *Eur. J. Pharmacol.,* **263,** 311 – 314.

FROESTL, W. & MICKEL, S.J. (1997). Chemistry of $GABA_B$ modulators. In: *The GABA receptors*. ed. Enna, S.J. & Bowery, N.G. pp. 271 – 296. New Jersey, Humana Press Inc.

FROESTL, W., MICKEL, S.J., VON SPRECHER, G., BITTIGER, H. & OLPE, H.R. (1992). Chemistry of new $GABA_B$ antagonists. *Pharmacol Comm.,* **2,** 52 – 56.

FROESTL, W., MICKEL, S.J., VON SPRECHER, G., DIEL, P.J., HALL, R.G., MAIER, L., STRUB, D., MELILLO, V., BAUMANN, P.A., BERNASCONI, R., GENTSCH, C., HAUSER, K., JAEKEL, J., KARLSSON, G., KLEBS, K., MAÎTRE, L., MARESCAUX, C., POZZA, M.F., SCHMUTZ, M., STEINMANN, M.W., VAN RIEZEN, H., VASSOUT, A., MONDADORI, C., OLPE, H.-R., WALDMEIER, P.C. & BITTIGER, H. (1995). Phosphinic acid analogues of GABA. 2. Selective, orally active $GABA_B$ antagonists. *J. Med. Chem.,* **38,** 3313 – 3331.

GAGE, P.W. (1992). Activation and modulation of neuronal K^+ channels by GABA. *TINS,* **15,** 46 – 51.

GÄHWILER, B.H. & BROWN, D.A. (1985). $GABA_B$ receptor-activated K^+ current in voltage clamped CA3 pyramidal cells in hippocampal cultures. *PNAS,* **82,** 1558 – 1562.

HOSFORD, D.A., CLARK, S., CAO, Z., WILSON, JR W.A., LIN, F., MORRISSETT, R.A. & HUIN, A. (1992). The role of $GABA_B$ receptor activation in absence seizures of lethargic (*lh*/*lh*) mice. *Science,* **257,** 398 – 401.

JONES, K.A., BOROWSKY, B., TAMM, J.A., CRAIG, D.A., DURKIN, M.M., DAI, M., YAO, W.-J., JOHNSON, M., GUNWALDSEN, C., HUANG, L.-Y., TANG, C., SHEN, Q., SALON, J.A., MORSE, K., LAZ, T., SMITH, K.E., NAGARATHNAM, D., NOBLE, S.A., BRANCHEK, T.A. & GERALD, C. (1998). $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_B$R1 and $GABA_B$R2. *Nature,* **396,** 674 – 678.

KAUPMANN, K., HUGGEL, K., HEID, J., FLOR, P.J., BISCHOFF, S., MICKEL, S.J., MCMASTER, G., ANGST, C., BITTIGER, H., FROESTL, W. & BETTLER, B. (1997). Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature,* **386,** 239 – 246.

KAUPMANN, K., MALITSHECK, B., SCHULER, V., HEID, J., FROESTL, W., BECK, P., MOSBACHER, J., BISCHOFF, S., KULIK, A., SHIGEMOTO, R., KARSCHIN, A. & BETTLER, B. (1998). $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. *Nature,* **396,** 683 – 687.

LAMBERT, N.A. & WILSON, W. (1993). Discrimination of post and presynaptic $GABA_B$ receptor-mediated responses by tetrahydroaminoacridine in area CA3 of the rat hippocampus. *J. Neurophysiol.,* **69,** 630 – 635.

LAMBERT, N.A. & WILSON, W. (1996). High-threshold Ca^{2+} currents in rat hippocampal interneurones and their selective inhibition by activation of $GABA_B$ receptors. *J. Physiol.,* **492,** 115 – 128.

LANZA, M., FASSIO, A., GEMIGNANI, A., BONANNO, G. & RAITERI, M. (1993). CGP 52432: A novel and selective $GABA_B$ autoreceptor antagonist in rat cerebral cortex. *Eur. J. Pharmacol.,* **237,** 191 – 195.

LIU, Z., VERGNES, M., DEPAULIS, A. & MARESCAUX, C. (1992). Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat. *Neurosci,* **48,** 87 – 93.

MONDADORI, C., JAEKEL, J. & PREISWERK, G. (1993). CGP 36742. The first orally active $GABA_B$ blocker improves the cognitive performance of mice rats and rhesus monkeys. *Behav. Neurol. Biol.,* **60,** 62 – 68.

MOTT, D.D. & LEWIS, D.V. (1991). Facilitation of the induction of long-term potentiation by $GABA_B$ receptors. *Science,* **252,** 1420 – 1423.

NATHAN, T., JENSEN, M.S. & LAMBERT, J.D.C. (1990). $GABA_B$ receptors play a major role in paired-pulse facilitation in area CA1 of the rat hippocampus. *Brain Res.,* **531,** 55 – 65.

NATHAN, T. & LAMBERT, J.D.C. (1991). Depression of the fast IPSP underlies paired-pulse facilitation in area CA1 of the rat hippocampus. *J. Neurophysiol.,* **66,** 1704 – 1715.

OLPE, H.-R. & KARLSSON, G. (1990). The effects of baclofen and two $GABA_B$-receptor antagonists on long-term potentiation. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **342,** 194 – 197.

OLPE, H.-R., STEINMANN, M.W., FERRAT, T., POZZA, M.F., GREINER, K., BRUGGER, F., FROESTL, F., MICKEL, S.J. & BITTIGER, H. (1993a). The actions of orally active $GABA_B$ receptor antagonists on GABAergic transmission in vivo and in vitro. *Eur. J. Pharmacol.,* **233,** 179 – 186.

OLPE, H.-R., STEINMANN, M.W., GREINER, K. & POZZA, M.F. (1994). Contribution of presynaptic GABA-B receptors to paired-pulse depression of GABA-responses in the hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **349,** 473 – 477.

OLPE, H.-R., WÖRNER, W. & FERRAT, T. (1993b). Stimulation parameters determine role of $GABA_B$ receptors in long-term potentiation. *Experientia,* **49,** 542 – 546.

OTIS, T.S., DE KONINCK, Y. & MODY, I. (1993). Characterization of synaptically elicited $GABA_B$ responses using patch-clamp recordings in rat hippocampal slices. *J. Physiol.,* **463,** 391 – 407.

PFRIEGER, F.W., GOTTMANN, K. & LUX, H.D. (1994). Kinetics of $GABA_B$ receptor-mediated inhibition of calcium currents and excitatory synaptic transmission in hippocampal neurons *in vitro*. *Neuron,* **12,** 97 – 107.

PHAM, T.M. & LACAILLE, J.-C. (1996). Multiple postsynaptic actions of GABA via $GABA_B$ receptors on CA1 pyramidal cells of rat hippocampal slices. *J. Neurophysiol.,* **76,** 69 – 80.

PITLER, T.A. & ALGER, B.E. (1994). Differences between presynaptic and postsynaptic $GABA_B$ mechanisms in rat hippocampal pyramidal cells. *J. Neurophysiol.,* **72,** 2317 – 2327.

ROEPSTORFF, A. & LAMBERT, J.D.C. (1994). Factors contributing to the decay of the stimulus-evoked IPSC in rat hippocampal CA1 neurons. *J. Neurophysiol.,* **72,** 2911 – 2926.

SCHERER, R.W., FERKANY, J.W. & ENNA, S.J. (1988). Evidence for pharmacologically distinct subsets of $GABA_B$ receptors. *Brain Res. Bull.,* **21,** 439 – 443.

SEABROOK, G.R., HOWSON, W. & LACEY, M.G. (1990). Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic $GABA_B$ receptors on neurones in rat brain slices. *Br. J. Pharmacol.,* **101,** 949 – 957.

SOLÍS, J.M. & NICOLL, R.A. (1992). Pharmacological characterization of $GABA_B$-mediated responses in the CA1 region of the rat hippocampal slice. *J. Neurosci.,* **12,** 3466 – 3472.

SOLTESZ, I., HABY, M., LERESCHE, N. & CRUNELLI, V. (1988). The $GABA_B$ antagonist phaclofen inhibits the late K^+-dependent IPSP in cat and rat thalamic and hippocampal neurones. *Brain Res.,* **448,** 351 – 354.

TEOH, H., MALCANGIO, M. & BOWERY, N.G. (1996). GABA, glutamate and substance P-like immunoreactivity release: effects of novel $GABA_B$ antagonists. *Br. J. Pharmacol.,* **118,** 1153 – 1160.

THOMPSON, S.M., CAPOGNA, M. & SCANZIANI, M. (1993). Presynaptic inhibition in the hippocampus. *TINS,* **16,** 222 – 227.

THOMPSON, S.M. & GÄHWILER, B.H. (1989). Activity-dependent disinhibition. III. Desensitization and $GABA_B$ receptor-mediated presynaptic inhibition in the hippocampus *in vitro*. *J. Neurophysiol.,* **61,** 524 – 533.

THOMPSON, S.M. & GÄHWILER, B.H. (1992). Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus *in vitro*. *J. Physiol.,* **451,** 329 – 345.

WALDMEIER, P.C., WICKI, P., FELDTRAUER, J.-J., MICKEL, S.J., BITTIGER, H. & BAUMANN, P.A. (1994). GABA and glutamate release affected by $GABA_B$ receptor antagonists with similar potency: no evidence for pharmacologically different presynaptic receptors. *Br. J. Pharmacol.,* **113,** 1515 – 1521.

WHITE, J.H., WISE, A., MAIN, M.J., GREEN, A., FRASER, N.J., DISNEY, G.H., BARNES, A.A., EMSON, P., FOORD, S.M. & MARSHALL, F.H. (1998). Heterodimerization is required for the formation of a functional $GABA_B$ receptor. *Nature,* **396,** 679 – 682.

WILCOX, K.S. & DICHTER, M.A. (1994). Paired-pulse depression in cultured hippocampal neurons is due to a presynaptic mechanism independent of $GABA_B$ autoreceptor activation. *J. Neurosci.,* **14,** 1775 – 1788.

WU, L.-G., SAGGAU, P. (1995). $GABA_B$ receptor-mediated presynaptic inhibition in guinea-pig hippocampus is caused by a reduction of presynaptic Ca^{2+} influx. *J. Physiol.,* **485,** 649 – 657.

YOON, K.-W. & ROTHMAN, S.M. (1991). The modulation of rat hippocampal synaptic conductances by baclofen and gamma-aminobutyric acid. *J. Physiol.,* **442,** 377 – 390.

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