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GABA, glutamate and substance P-like immunoreactivity release: effects of novel $GABA_B$ antagonists

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1 The effects of various GABA receptor ligands on the electrically-evoked release of endogenous GABA, glutamate and substance P-like immunoreactivity from the dorsal horn of rat isolated spinal cord were examined.

2 Exogenous GABA (10-300 μM) significantly decreased the evoked, but not basal, release of endogenous glutamate in a concentration-dependent manner. The $GABA_A$ agonist, isoguvacine (1-100 μM), failed to decrease the release of glutamate although it did reduce the release of GABA. Baclofen (0.1-1000 μM), the $GABA_B$ agonist, reduced the release of GABA and glutamate in a stereospecific and concentration-dependent manner.

3 The actions of five $GABA_B$ antagonists on these release systems were compared. CGP36742, CGP52432, CGP55845A and CGP57250A significantly increased the evoked release of GABA and glutamate. They also reversed the effects of (-)-baclofen in a concentration-dependent manner. On the other hand, while CGP56999A had no effect on glutamate release, it was an effective antagonist of the baclofen-induced inhibition of GABA and substance P release.

4 These results suggest that $GABA_B$ receptors on nerve terminals within the dorsal horn spinal cord may be heterogeneous. However, this is based solely on the data obtained with CGP56999A which affected only GABA and substance P, but not glutamate, release.

Keywords: $GABA_B$ antagonists; GABA; glutamate; substance P-like immunoreactivity; spinal cord; CGP36742; CGP52432; CGP55845A; CGP57250A; CGP56999A

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory transmitter in the central nervous system (CNS) and has been implicated in both pre- and postsynaptic transmission in the spinal cord (see Bowery, 1993). GABA exerts its actions via $GABA_A$ and $GABA_B$ receptors (Hill & Bowery, 1981; Bowery *et al.*, 1983) which have very different profiles. Within the spinal cord, activation of $GABA_A$ receptors on presynaptic terminals results in the opening of chloride channels providing the basis for primary afferent depolarization (PAD) and presynaptic inhibition (Curtis *et al.*, 1971). On the other hand, $GABA_B$ receptors mediate an increase in potassium or a decrease in calcium conductance, either of which could produce a decrease in neurotransmitter release (Bowery *et al.*, 1980; Conzelmann *et al.*, 1986; Pittaluga *et al.*, 1987; Waldmeier *et al.*, 1988a,b; Bonanno *et al.*, 1989a; 1991; Malcangio & Bowery, 1993; Santiago *et al.*, 1993). Both types of GABA receptors are located on the Aδ and C afferent fibres (Singer & Placheta, 1980; Désarmenien *et al.*, 1984; Price *et al.*, 1984) which terminate predominantly in the substantia gelatinosa (Cervero & Iggo, 1980; Brown, 1982) suggesting that this inhibitory amino acid and its receptors may be involved in the modulation of nociception. In support of this theory, exogenous GABA and GABA receptor agonists have been reported to inhibit the release of putative nociceptive neurotransmitters and neuropeptides from central nerve endings (Kangrga *et al.*, 1991; Benoliel *et al.*, 1992; Malcangio & Bowery, 1993; Pende *et al.*, 1993). Moreover, agents acting on GABA receptors have been demonstrated in several behavioural models to alter nociceptive sensitivity (Cutting & Jordan, 1975; Wilson & Yaksh, 1978; Levy & Proudfit, 1979; Hwang & Wilcox, 1989; Malcangio *et al.*, 1991).

Glutamate (Glu) is believed to be the major excitatory synaptic transmitter in the CNS (Watkins & Evans, 1981; Fagg & Foster, 1983) and there is much evidence pointing to it being an important neuromediator in the spinal cord (Mayer & Westbrook, 1987; Kangrga *et al.*, 1990; 1991; Kangrga & Randić, 1991). This amino acid is co-localized in the small diameter primary afferents with substance P (SP), another proposed mediator of nociception (De Biasi & Rustioni, 1988) and it has been postulated that these two agents may act as co-transmitters in the dorsal horn. Stimulation of dorsal root ganglia and dorsal roots increases Glu release from organotypic dorsal root ganglia cultures and hemisected dorsal spinal cord slices (Kangrga *et al.*, 1990; 1991; Kangrga & Randić, 1991; Jeftinija *et al.*, 1991; Teoh *et al.*, 1995b). Elevated levels of Glu are also observed in the cerebrospinal fluid of the spinal cord during noxious stimulation (Smullin *et al.*, 1990; Sorkin & McAdoo, 1993). Behavioural studies have demonstrated that Glu agonists and antagonists can respectively cause/potentiate and reduce/alleviate nociception (Aanonsen & Wilcox, 1987; Follenfant & Nakamura-Craig, 1992; Ren *et al.*, 1992; McGowan & Hammond, 1993; Mao *et al.*, 1994). It is plausible, therefore, that analgesics, which modify the transmission of nociceptive impulses, may do so by reducing the activity of this excitatory system.

In the present work, we have utilized a superfusion system and various novel $GABA_B$ receptor ligands with affinities ranging from the low nanomolar to micromolar range to characterize further the $GABA_B$ receptors in the spinal cord. Concomitantly, we sought to provide a better understanding of the role that the $GABA_B$ receptor(s) plays in the modulation of Glu and substance P-like immunoreactivity (SP-LI) release. Preliminary results have been communicated to the British Pharmacological Society (Teoh *et al.*, 1995a) and the International Society of Neurochemistry (Teoh *et al.*, 1996a).

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Methods

Release experiments

Hemisected lumbosacral dorsal horn slices were obtained as described previously (Malcangio & Bowery, 1993; Teoh *et al.*, 1995b). Briefly, adult male Wistar rats (≈250 g) were decapitated and the lumbosacral segments of the spinal cords removed. Slices (≈200–300 μm thick, ≈2 cm long) were cut in cold oxygenated Krebs-bicarbonate solution. Each slice, with a pair of intact dorsal roots attached to either side of the cord, was placed in the central division of a three-compartment bath. The roots were positioned on bipolar platinum electrodes in the lateral compartments and immersed in mineral oil (Aldrich Chemical Co. Ltd., UK).

The slices were continuously superfused at 1 ml min^{-1} with oxygenated (95% O_2 + 5% CO_2) Krebs-bicarbonate solution for 1 h at room temperature. This solution contained (in mM): NaCl 118, KCl 4, KH_2PO_4 1.2; $NaHCO_3$ 25; $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11. In some experiments, following the preliminary incubation, the bathing solution was replaced with a modified Krebs-bicarbonate solution containing 0.1% bovine serum albumin, 100 μM captopril, 1 μM phosphoramidon, 20 μg ml^{-1} bacitracin and 6 μM dithiothreitol to prevent the metabolic breakdown of SP. Consecutive 3 min (amino acids) and 8 min (SP-LI) samples of superfusate were then obtained prior to, during and after drug application and/or electrical stimulation of dorsal roots. In the amino acid studies, three fractions were obtained before a 20 V, 0.5 ms stimulus at 1 Hz was applied for 3 min. This was repeated after an interval of 9 min. Samples were maintained at −80°C until they were derivatized and analysed chemically. Only one 8 min electrical pulse (all other parameters as above) was applied during collection of the fourth of seven fractions in the SP-LI experiments.

Chemical analysis

GABA and Glu contents in the superfusate samples were determined by high performance liquid chromatography (h.p.l.c.) coupled with fluorescence detection (Lindroth & Mopper, 1979). Pre-column derivatization of amino acids was achieved with an *o*-phthaldialdehyde (OPA)-β-mercaptoethanol reagent. All separations were performed at a flow rate of 1 ml min^{-1} on a reversed-phased C_{18} Microsorb column (150 × 4.6 mm) in conjunction with the appropriate guard column (both from Ranin Instruments Co. Inc., U.S.A.). A 40 min pH 5.5 phosphate-methanol/methanol gradient cycle was used for satisfactory separation. On occasions, the GABA peak was difficult to detect or distinguish as the limit of detection for GABA in this set-up was about 100 fmol/20 μl. To ensure that the correct peak was used for analysis, the samples were 'spiked' with a known concentration of GABA and reanalysed twice on the h.p.l.c. system. Two samples containing only this known concentration of GABA in the superfusion medium were also analysed. The differences in the concentrations obtained from the 'spiked' samples and the 'pure' ones were used as the final values for analysis. SP-LI in each superfusate sample was measured by radioimmunoassay using the scintillation proximity assay bead technique (Amersham International, U.K.) as described previously (Malcangio & Bowery, 1993)

Expression of results and statistics

For analyses, the mean concentrations of GABA and Glu measured in the first three samples were taken to be the basal level. The levels of these two amino acids in the following fractions were then expressed as a percentage of this basal level. The first stimulated response in each case was identified as S1 and the second as S2. The relationship between them was then expressed as an S2/S1 ratio. SP-LI results were calculated as fmol released by electrical stimulation after subtracting the basal outflow. The data shown in the graphs are expressed as mean ± s.e.mean and statistical significance was obtained by comparing the results in each experiment with the appropriate controls using the Mann-Whitney 'U' test.

Chemicals

(−)-Bicuculline methobromide and γ-aminobutyric acid (GABA) were obtained from Sigma Chemicals Co. Ltd., Poole, Dorset, U.K. Isoguvacine.HCl was from Cambridge Research Biochemicals, Cheshire, U.K. Antiserum raised against substance P was from Amersham, U.K. The following were from CIBA-Geigy, Basel, Switzerland: (+)- and (−)-baclofen, CGP36742 (3-aminopropyl-n-butyl phosphonic acid), CGP55845A (3-[1-(S)-(3,4-dichlorophenyl) ethyl] amino-2(S)hydroxypropyl-*P*-benzyl-phosphonic acid), CGP52432 ([3-[[(3,4-dichlorophenyl)methyl]amino]propyl]-diethoxymethyl phosphonic acid), CGP56999A ([3-{[1-(**R**)-(3-carboxyphenyl)ethyl]amino}-2-(**S**)-hydroxy-propyl]-cyclohexyl-methyl-phosphonic acid) and CGP57250A ([3-(1-(**R**)-[[3-(diethoxymethyl)hydroxyphosphinyl]− 2-(**S**)-hydroxypropyl]-amino]ethyl]-benzoic acid). All other chemicals were of AnalaR grade and obtained from BDH Chemicals, Poole, U.K.

Results

Electrically-evoked release of GABA and Glu

Basal concentrations of GABA and Glu in the superfusate samples were respectively 151.44 ± 10.6 nM (n = 3) and 234.48 ± 34.8 nM (n = 6). The two periods of electrical stimulation significantly increased the release of both amino acids which rapidly returned to basal levels. During S_1, the concentration of GABA increased to 243.26 ± 5.9 nM whilst the Glu concentration rose to 510.71 ± 38.9 nM. The levels in S_2 were lower for both GABA and Glu resulting in mean S_2/S_1 values of 0.64 ± 0.02 and 0.72 ± 0.03 respectively. The mean pulse current passing during the stimulation period was 11.47 ± 1.57 mA (n = 15).

Effects of established GABA$_A$ and GABA$_B$ receptor ligands

GABA (10–300 μM) produced no effect on the basal outflow of Glu. However, the electrically-evoked release of Glu was

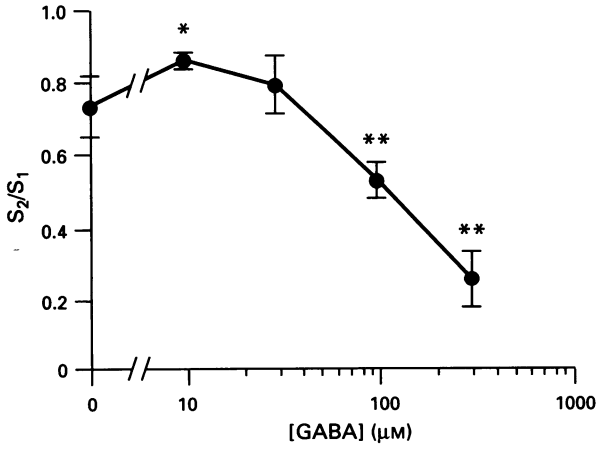

Figure 1 Effect of exogenous GABA on the electrically-evoked release of glutamate (Glu) (●) from the hemisected dorsal rat spinal cord. GABA was added to the superfusion medium 1 min prior to and during the second stimulation (n = 3 for each data point). Mean basal level of Glu in these slices was 210.74 ± 22.9 nM. *P < 0.05, **P < 0.01 compared with the control value (n = 6; Mann-Whitney 'U' test). Data are mean ± s.e.mean.

reduced in a concentration-dependent manner ($n=12$; Figure 1). At 300 μM GABA, the S_2/S_1 ratio for Glu release was $0.25\pm0.08\%$ with an IC_{50} value of 78.38 μM.

The $GABA_A$ agonist, isoguvacine (1–100 μM), appeared to decrease the S_2/S_1 ratio for both GABA and Glu. However, this effect reached statistical significance for GABA only at 100 μM and not at all for Glu (Figure 2). Concomitant administration of the $GABA_A$ antagonist, bicuculline, reversed the effect of isoguvacine on stimulated GABA release. Bicuculline alone did not significantly affect the S_2/S_1 ratio for either GABA or Glu.

(−)-Baclofen (0.1–1000 μM), the $GABA_B$ agonist, concentration-dependently inhibited the evoked, but not basal, release of GABA and Glu (Figure 3). These effects of baclofen were stereospecific. In the presence of the (+)-enantiomer the S_2/S_1 ratios produced for GABA and Glu were not significantly different from control. (−)-Baclofen at 30 μM produced 40% (GABA) and 50% (Glu) inhibition of release. These responses were just submaximal and thus this concentration was used subsequently for examining the effects of antagonists.

Effects of novel $GABA_B$ receptor antagonists

The effects of the following $GABA_B$ receptor antagonists: CGP36742, CGP52432, CGP55845A, CGP56999A and CGP57250A, on the stimulated release of GABA and Glu were examined. None of them (0.01–30 μM) affected the basal release of GABA or Glu (data not shown). CGP36742, CGP52432, CGP55845A and CGP57250A (1–30 μM) significantly increased the evoked release of GABA and Glu as well as reversing the inhibitory effect of (−)-baclofen on their outflow ($P \leq 0.05$; see Figures 4 and 5) CGP56999A had no significant effect on the stimulated release of Glu in the presence or absence of (−)-baclofen (Figure 4). However, CGP56999A (0.01–10 μM) reversed the inhibitory action of (−)-baclofen on the evoked release of GABA ($P<0.05$; see Figure 5b).

A submaximal concentration of (−)-baclofen (10 μM) significantly reduced the evoked release of SP-LI as reported previously (Malcangio & Bowery, 1993). On its own, CGP56999A (100 μM) failed to alter the stimulated response but it did reverse the inhibitory actions of 10 μM (−)-baclofen (Figure 6). This concentration of the antagonist was ineffective against the stimulated release of glutamate (data not shown).

Discussion

It seems likely that the electrically-evoked release of GABA and Glu in this preparation is primarily of neuronal origin since in both cases the release was tetrodotoxin-sensitive and calcium-dependent (Teoh *et al.*, 1995b). However, a contribution from glial cells cannot be ignored (Parpura *et al.*, 1994). Results obtained from bath-superfusion of the vanyllilnoneamide neurotoxin, capsaicin, have indicated that a

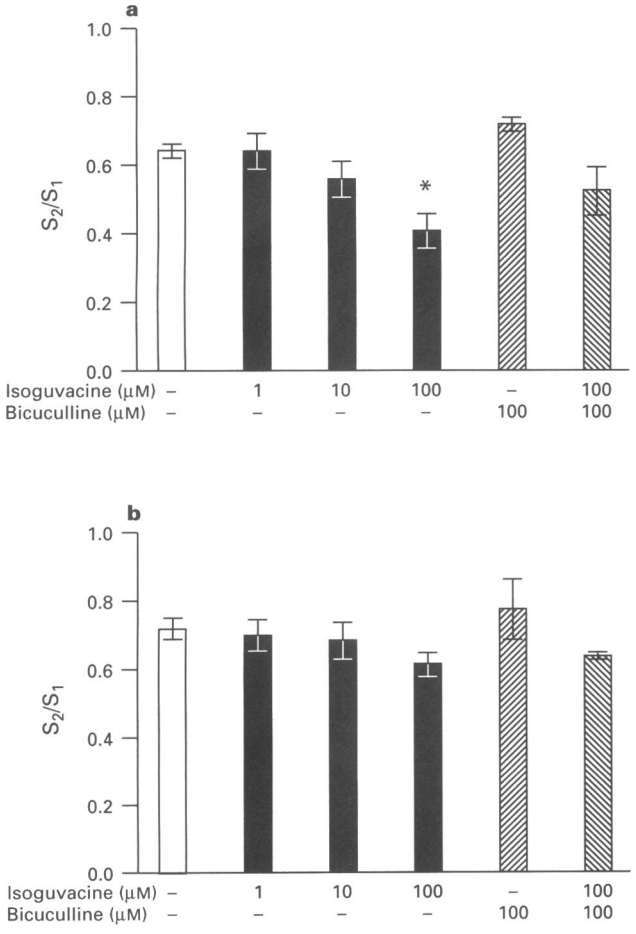

Figure 2 Effect of isoguvacine and bicuculline on the electrically-evoked release of (a) GABA and (b) glutamate (Glu). Drugs were added to the superfusing medium 1 min before and during the second 3 min stimulation. Basal outflow was 163.33 ± 16.2 nM and 218.33 ± 16.2 nM for GABA and Glu respectively ($n=3$ per drug concentration). Data are means ± s.e.mean. *$P<0.05$ (compared with control data using the Mann-Whitney 'U' test)

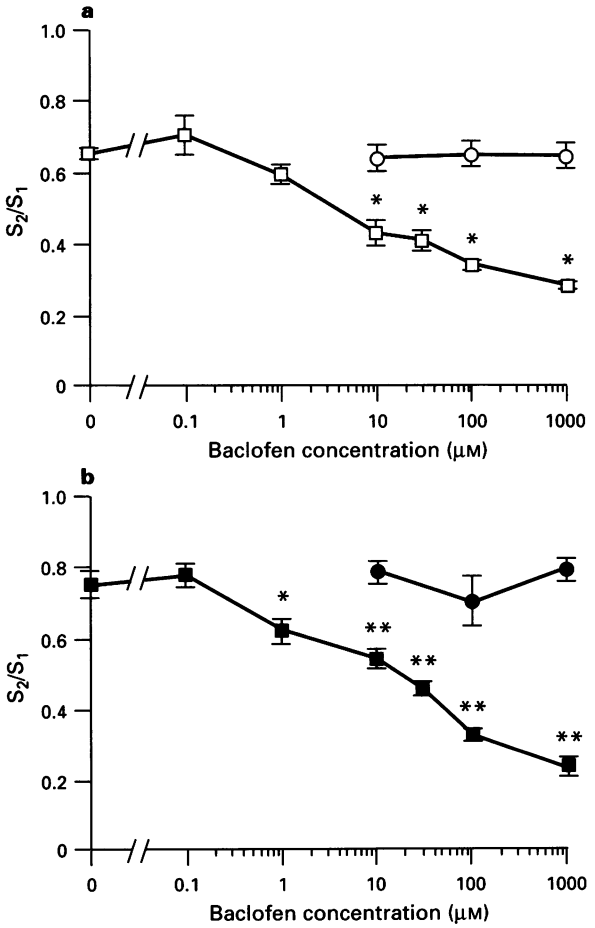

Figure 3 Stereospecific effects of baclofen on the electrically-evoked release of (a) GABA and (b) glutamate (Glu). Slices were superfused with either (+)-baclofen (○, ●; $n=3-6$) or (−)-baclofen (□, ■; $n=3$) 1 min prior to and during the second stimulation. Basal outflow was 198.33 ± 20.1 nM for GABA and 227.51 ± 39.4 nM for Glu in these slices. Values are mean ± s.e.mean. *$P<0.05$; ** $P<0.01$ (tested against the control responses with the Mann-Whitney 'U' test).

significant proportion of the detected Glu and SP-LI originates from capsaicin-sensitive fibres while the source of GABA is likely to be the intrinsic dorsal horn neurones (Malcangio & Bowery, 1993; Teoh *et al.*, 1996b). Hence it is tempting to suggest that a significant proportion of the Glu and SP-LI released upon electrical stimulation arises from the nociceptive-sensitive primary afferents which terminate in the superficial dorsal horn (Cervero & Iggo, 1980; Brown, 1982) where they receive axoaxonal synaptic inputs from intrinsic GABAergic terminals (Barber *et al.*, 1978).

The inhibitory effect of GABA on the evoked release of Glu was mimicked by the prototypical GABA$_B$ agonist, (−)-baclofen, which also concentration-dependently inhibited the stimulated release of GABA. Neither the GABA$_A$ agonist, isoguvacine, nor the GABA$_A$ antagonist, bicuculline, had any significant effect on the basal or evoked release of Glu. Although the apparent increase in the Glu S_2/S_1 ratio observed with bicuculline alone was not significant, the possibility of this drug exerting a disinhibitory effect cannot be dismissed since such a phenomenon has been documented for neurones of the substantia gelatinosa (Magnuson & Dickenson, 1991). The stereoselective effect of baclofen on evoked Glu release coupled with the antagonism of the (−)-baclofen action observed with CGP36742, CGP52432, CGP55845A and CGP57250A further support the concept that Glu release is under the influence of GABA$_B$ heteroreceptors. Taken together, these observations

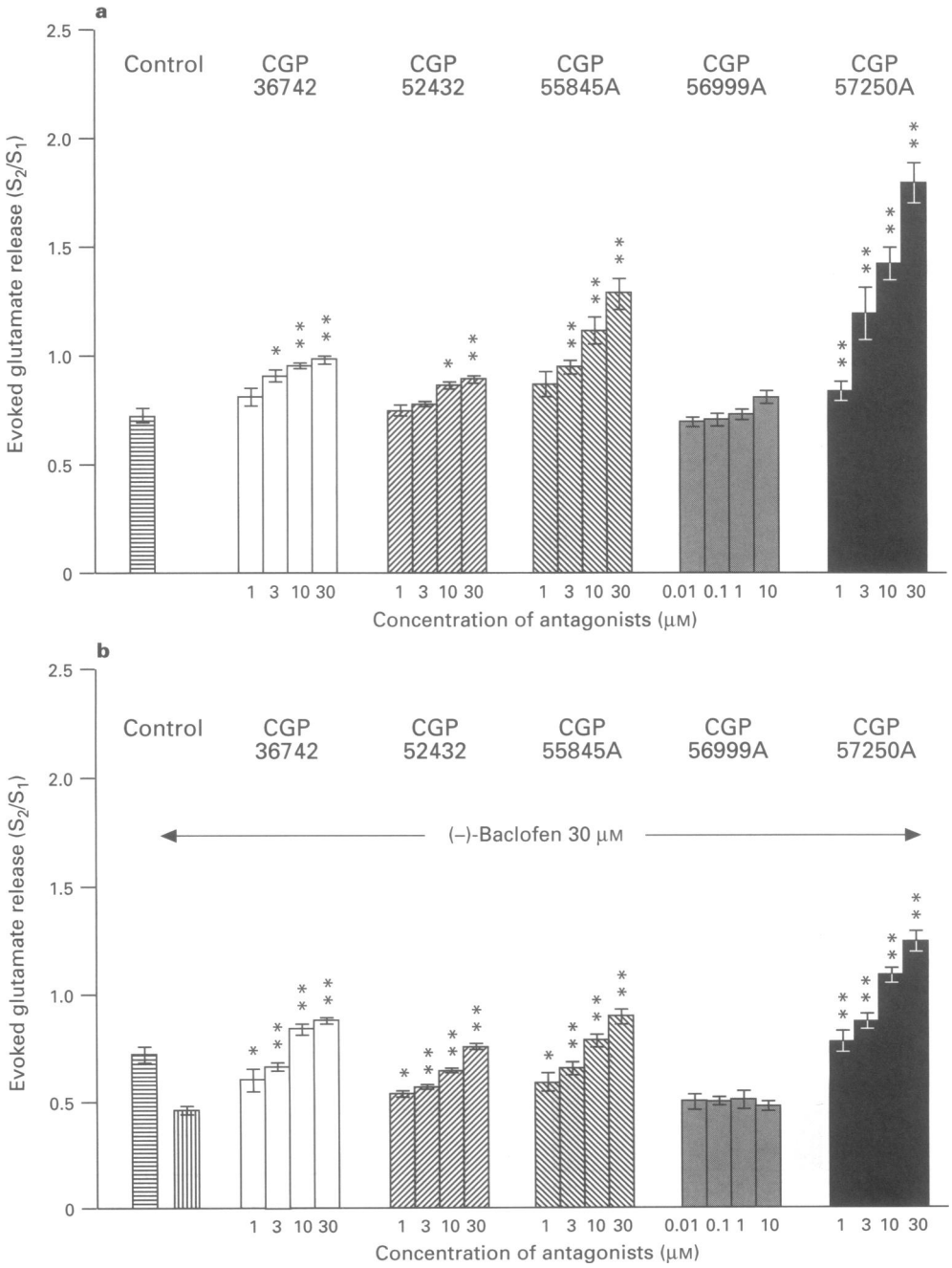

Figure 4 (a) Effects of CGP36742, CGP52432, CGP55845A, CGP56999A and CGP57250A on the S_2/S_1 ratio for glutamate (Glu) release. Antagonists were added to the superfusing medium 6 min prior to and during the second stimulation. Drug effects were compared with the control responses. (b) Antagonism by these antagonists on the inhibitory (−)-baclofen effect on Glu release. All data are mean ± s.e.mean ($n = 6-7$ in each case). Basal levels of Glu in these slices were 247.43 ± 25.3 nM ($n = 192$). The effects of each concentration of antagonist was compared with the (−)-baclofen responses ($n = 7$). * $P < 0.05$; ** $P < 0.01$ (Mann-Whitney 'U' test).

indicate that only GABA$_B$ receptors are involved in the regulation of Glu release in the dorsal horn and are in agreement with those previously reported (Kangrga *et al.*, 1991). The same was true for SP (Malcangio & Bowery, 1993).

The potentiation of the S_2/S_1 ratios for Glu by these four antagonists in the absence of (−)-baclofen would suggest that under physiological conditions, the GABA$_B$ heteroreceptors in the spinal cord may be activated by some form of endogenous inhibitory tone. This is in agreement with the observations of Blake *et al.*(1994) who showed that the GABA$_B$ antagonists, CGP35348 (*p*-3-aminopropyl-*p*-diethoxymethyl-phosphonic acid; Bittiger *et al.*, 1993) and CGP55845, in the absence of exogenous GABA$_B$ agonists, enhanced the potassium stimulated inward synaptic currents in dorsal root neurones.

During electrical stimulation of dorsal roots in our cord preparation, we would estimate that the concentration of GABA in the superfusion solution is in the region of 10 μM which is below the apparent threshold (50 μM) for inhibition of Glu release (see Figure 1). However, the concentration of GABA in the immediate vicinity of the GABA$_B$ receptor, during nerve stimulation may be much higher, particularly since no attempts to reduce the inactivation of the released GABA were made in our experiments. Nevertheless, the possibility remains that the GABA$_B$ antagonists are acting in a non-specific manner to enhance the levels of glutamate and GABA but this seems unlikely in view of the inert nature of the compounds when tested in a variety of biological assay systems (W. Froestl & H. Bittiger, personal communication). It seems

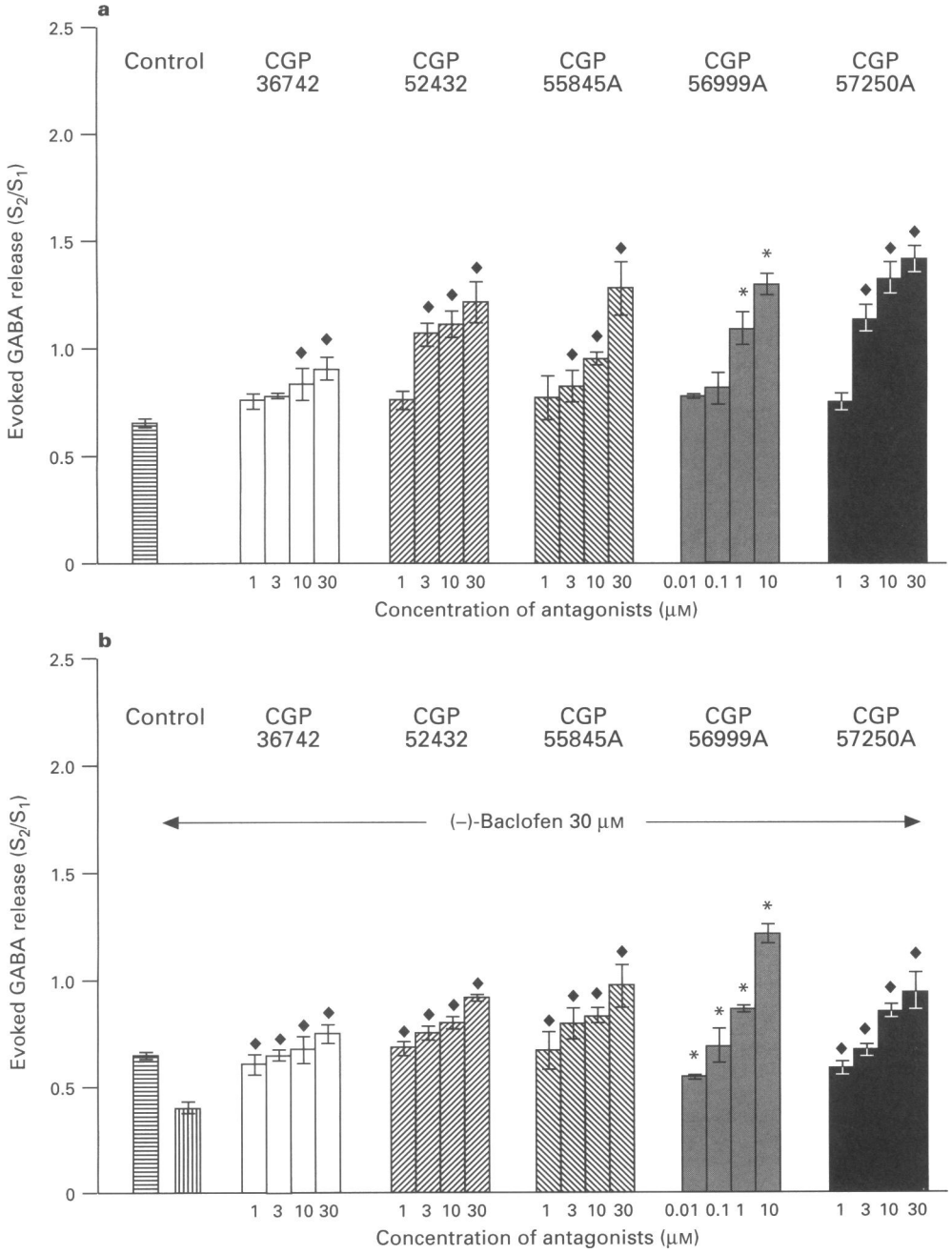

Figure 5 (a) Effects of CGP36742, CGP52432, CGP55845A, CGP56999A and CGP57250A on the S_2/S_1 ratio for GABA release. Antagonists were added to the superfusing medium 6 min prior to and during the second stimulation. Drug effects were compared with the control responses. (b) Antagonism by these antagonists on the inhibitory (−)-baclofen effect on GABA release. All data are mean ± s.e.mean ($n = 3-4$ in each case). Basal levels of GABA in these slices were 153.20 ± 13.9 nM ($n = 96$). The effects of each concentration of antagonist was compared with the (−)-baclofen responses ($n = 3$). ◆$P = 0.05$; *$P < 0.05$ (Mann-Whitney 'U' test).

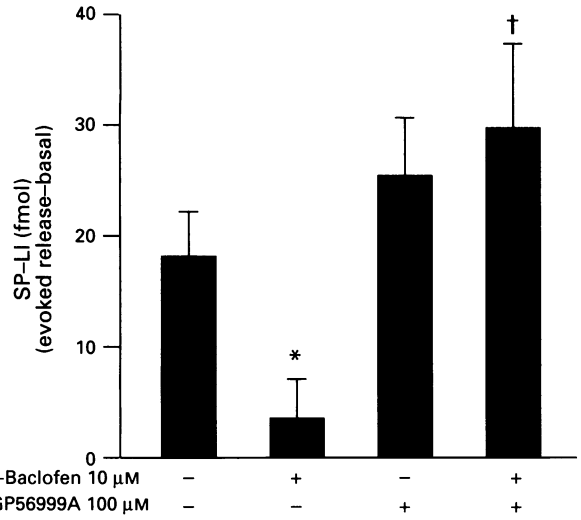

Figure 6 Effect of (−)-baclofen and CGP56999A on the electrically-evoked release of substance P-like immunoreactivity (SP-LI). Drugs were superfused 5 min prior to and during stimulation. All data are means ± s.e.mean ($n = 3-4$ in each case). Basal levels of SP-LI in these slices were 15.9 ± 2.2 fmol 8 min^{-1} sample ($n = 16$). *$P < 0.05$ when compared with the control response; †$P < 0.05$ when compared with the (−)-baclofen response (Mann-Whitney 'U' test).

more probable that the local concentration of endogenous GABA in the immediate vicinity of the GABA$_B$ receptor is much greater than 10 μM after primary afferent stimulation.

Since Glu is postulated to be involved in nociceptive transmission (see Introduction), it is possible that the anti-nociceptive actions exhibited by GABA and its analogues (see Sawynok, 1987) could be attributed in part to its inhibitory effects on the glutamatergic system as observed here. This could occur in conjunction with or in addition to the GABAergic modulation of SP release that has already been demonstrated in this preparation (Malcangio & Bowery, 1993; present paper). Indeed, several animal models have indicated that baclofen-induced antinociception is not limited to supraspinal control (Proudfit & Levy, 1978; Levy & Proudfit, 1979; Liebman & Pastor, 1980) and probably involves a spinal component (Wilson & Yaksh, 1978; Liebman & Pastor, 1980). Interestingly, the antinociceptive responses elicited by baclofen are also stereoselective (Wilson & Yaksh, 1978; Aley & Kulkarni, 1991) and reversible by the GABA$_B$ antagonist, CGP35348 which has a lower binding affinity than the GABA$_B$ antagonists studied in the present paper (Aley & Kulkarni, 1991; Malcangio *et al.*, 1991; McGowan & Hammond, 1993).

There is evidence that GABA$_B$ receptors are heterogeneous (Kerr *et al.*, 1987; Dutar & Nicoll, 1988). Recent studies suggest that the autoreceptor for GABA is of the GABA$_B$ class (Pittaluga *et al.*, 1987; Bonanno *et al.*, 1989a,b; Waldmeier *et al.*, 1988b; Baumann *et al.*, 1990) although there have been some indications that there may also be GABA$_A$ autoreceptors (Mitchell & Martin, 1978; Brennan *et al.*, 1981; Anderson & Mitchell, 1985). Using different GABA$_B$ receptor antagonists (phaclofen, CGP35348 and CGP52432), Raiteri and colleagues have further postulated that not only is there heterogeneity amongst the GABA$_B$ autoreceptors in different parts of the rat CNS (Raiteri *et al.*, 1989; Bonanno & Raiteri, 1993) but that the presynaptic GABA$_B$ heteroreceptors on Glu, somatostatin and cholecystokinin terminals are also different from each other (Bonanno & Raiteri, 1992; Lanza *et al.*, 1993; Gemignani *et al.*, 1994). In contrast, Waldmeier *et al.*(1994) failed to distinguish between the various groups of receptors when they used a wider range of structurally related drugs in a different release preparation.

In the present study, we were unable to demonstrate any selectivity of CGP36742, CGP52432, CGP55845A and CGP57250A for GABA$_B$ sites controlling the release of GABA and Glu in the rat dorsal horn. We have previously reported that CGP35348 and CGP36742 antagonized the inhibitory effect that (−)-baclofen had on the electrically-evoked release of SP-LI (Malcangio & Bowery, 1993). The data obtained in this study with CGP52432 is therefore in contrast to those described by Raiteri and colleagues (Bonanno & Raiteri, 1992; Lanza *et al.*, 1993). CGP56999A, on the other hand, antagonized the (−)-baclofen-induced inhibition of GABA and SP-LI release whilst not affecting that of Glu. This finding therefore supports the possible distinction between the GABA$_B$ autoreceptor and the GABA$_B$ heteroreceptor(s) which modulate(s) Glu and SP release in the spinal cord dorsal horn. It is important to note, however, that the classification of GABA$_B$ receptors into subtypes (Bonanno & Raiteri, 1993) did not involve CGP56999A and that Waldmeier and colleagues (1994) were unable to differentiate between the effects that CGP56999A had on the GABAergic and glutamatergic systems. The reasons for the discrepancies between our results and those described previously are not known. It is possible that the different tissues (spinal cord slice as opposed to cortical slices and synaptosomes) and stimulatory conditions could be of fundamental importance. Our results further indicate that CGP56999A is a potent antagonist at the autoreceptor but appears to lack any influence on the control of Glu release (heteroreceptors).

In conclusion, we have demonstrated that activation of sensory nerves results in the enhanced release of GABA, Glu and SP-LI. The release of Glu and SP-LI from the primary afferents is baclofen-sensitive suggesting that the antinociceptive properties of baclofen could be exerted at the spinal level via inhibition of Glu and/or SP release. Our results also provide marginal support for the suggestion that GABA$_B$ receptors regulating the release of GABA and Glu in the region of the superficial dorsal horn may be different from each other. If indeed, such heterogeneity exists, it augurs well for the potential development of receptor-selective drugs.

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