CGP 35348, a new GABAB antagonist, prevents antinociception and muscle-relaxant effect induced by baclofen

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1 CGP 35348, a new GABAB antagonist, was examined on antinociception induced by ($\pm$)-baclofen by use of the hot plate and writhing tests in mice and the paw pressure test in rats. CGP 35348 was also studied in mice on ($\pm$)-baclofen-induced impairment of rota-rod performance.

2 CGP 35348, injected either i.p. (60–100 mg kg^{-1} in mouse) or intracerebroventricularly (i.c.v.) (0.5–2.5 μg per mouse; 25 μg per rat) prevented ($\pm$)-baclofen-induced antinociception.

3 CGP 35348 did not modify oxotremorine- and morphine-induced antinociception in mice and rats.

4 CGP 35348 (2.5 μg i.c.v. per mouse) also prevented ($\pm$)-baclofen-induced impairment of the rota-rod test.

5 Two other GABAB antagonists, phaclofen (50 μg i.c.v. per mouse) and 2-OH-saclofen (2.5 μg–10 μg i.c.v. per mouse) did not modify ($\pm$)-baclofen-induced antinociception.

7 These results suggest that, at present, CGP 35348 is the only compound able to antagonize ($\pm$)-baclofen-induced antinociception.

Keywords: Analgesia; GABAB-antagonism; 2-OH-saclofen; CGP 35348; baclofen; muscle relaxation

Introduction

The antinociceptive effect of systemically administered baclofen has been well documented in rodents (Cutting & Jordan, 1975; Levy & Proudfit, 1977; Bartolini et al., 1981; Hill et al., 1981; Sawynok & La Bella 1982; Vaught et al., 1985) and has also been observed in man (Corli et al., 1984). Baclofen exerts an antinociceptive effect which is not reduced by GABAA (Bartolini et al., 1981; Sawynok & La Bella, 1982), opioid (Levy & Proudfit, 1979; Bartolini et al., 1981) or muscarinic receptor antagonists (Bartolini et al., 1981). It has been suggested that baclofen antinociception has both supraspinal and spinal components (reviewed by Sawynok, 1987) and catecholamines and substance P may play some role in the antinociceptive effect (Sawynok 1983; 1989; Sawynok et al., 1984; Sawynok & Dickson, 1985b; Hwang & Wilcox, 1989).

Although some compounds, namely (+)-baclofen, δ-aminovaleric acid and phaclofen have been reported to prevent ($\pm$)-baclofen-induced antinociception, their lack of brain penetration and low potency have hampered in vivo studies (Bowery, 1989). In fact, (+)-baclofen and δ-aminovaleric acid only inhibit the antinociception if injected intrathecally at doses at least 20 times higher than those of (−)-baclofen (Sawynok & Dickson, 1985a; Sawynok, 1986).

Similarly, phaclofen was only able to antagonize the antinociception when administered intracerebroventricularly (Giuliani et al., 1988).

It appeared, therefore, worthwhile to investigate whether CGP 35348 (3-aminopropyl-diethoxymethyl-phosphinic acid) and 2-hydroxysaclofen (3-amino-2-(4-chlorphenyl)-2-hydroxy-propylsulfonic acid), two new GABAB antagonists described respectively by Olpe et al. (1990) and by Kerr et al. (1988), were more effective antagonists of baclofen antinociception than phaclofen.

The effect of CGP 35348 on baclofen-induced muscle relaxant activity (Levy & Proudfit, 1977; 1979; Wilson & Yash, 1978; Sawynok & La Bella, 1982; Hammond & Drower, 1984) has also been investigated.

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Methods

Male Swiss-Webster mice (25–30 g) and Wistar rats (120–150 g) were used. Fifteen mice or five rats were housed per cage. The cages were brought into the experimental room 24 h before the experiment for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum.

Intracerebroventricular (i.c.v.) injection was performed during short ether anaesthesia, isotonic saline being used as solvent according to the method described by Haley & McCormick (1957). Briefly, during anaesthesia, mice or rats were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle attached to a 10 μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of mouse and 4 mm into the brain of rat, where 5 μl (for mouse) or 10 μl (for rat) of solution were injected. The injection site was 1 mm (for mouse) or 2 mm (for rat) to the right or left of the midline along a line drawn through the anterior base of the ears.

To ascertain the exact point into which drugs were administered, some mice or rats were injected i.c.v. with 5 μl or 10 μl of diluted 1:10 Indian ink and their brains were examined macroscopically after sectioning.

Hot plate

Mice were placed inside a stainless steel container thermostatically set at 52.5 $\pm$ 0.1°C in a precision water-bath from KW Mechanical Workshop, Siena Italy. The reaction time(s) was measured with a stop-watch before and at various times after treatment. The endpoint used was the licking of the fore or hind paws. Those mice which scored below 12 and over 18 s in the pretest were rejected. An arbitrary cut-off time of 45 s was adopted.

Paw pressure (Randall-Selitto)

The nociceptive threshold was determined with an analgesimeter (Ugo Basile, Varese, Italy) in rats. The instrument exerts

a force, expressed in g, which is applied at a constant rate with a cone-shaped pusher on the upper surface of the normal rat hind paw. The force was continuously monitored by a pointer moving along a linear scale. The pain threshold was given by the force which induced initial struggling of the rat. Those rats which scored below 60 g or over 120 g during pretests were rejected. An arbitrary cut-off value of 300 g was adopted.

Writhing

Mice were injected i.p. with 0.6% aqueous solution of acetic acid (10 ml kg^{-1}). The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Rota-rod

Mice were tested on a rota-rod treadmill (Ugo Basile, Varese, Italy). The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-slippery surface. This rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod. One day before the test, the animals were trained twice. On the day of the test only mice able to stay balanced on the rotating rod between 70 and 120 s (cut-off time) were selected. The performance time was measured before and at various times after treatment.

Drugs and reagents

The following drugs were used: ($\pm$)-baclofen (β-p-chloro-phenyl GABA) and CGP 35348 (3-aminopropyl-diethoxy-methyl-phosphinic acid) (CIBA-GEIGY), phaclofen (β-p-chlorophenyl-3-aminopropylphosphonic acid) and 2-hydro-xysaclofen (3-amino-2-(4-chlorphenyl)-2-hydroxypropyl-sulphonic acid) (Tocris), oxotremorine (Sigma), morphine hydrochloride (Carlo Erba). All drugs were dissolved in iso-tonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml kg^{-1} by either s.c. or i.p. injection.

Statistical analysis

Significant differences were determined by analysis of variance (ANOVA), after Cochran's and Bartlett's tests had shown homogeneity of variances. Multiple comparisons with appropriate controls were made by ANOVA followed by the multiple range test for least significant differences (LSD). Means with 95% confidence intervals that did not overlap were considered significantly different (for statistical methods used, see Snedecor & Cochran, 1973). All data were analyzed by the programme STATGRAPHICS (1986, STSC Inc. USA).

Results

Effect of CGP 35348, 2-hydroxysaclofen and phaclofen on ($\pm$)-baclofen-induced antinociception in mice and rats

Pretreatment of mice by systemically administered CGP 35348 inhibited the increase in paw licking latency induced by ($\pm$)-baclofen in the hot plate test. Intraperitoneally injected CGP 35348 was ineffective at 30 mg kg^{-1} while at 60 mg kg^{-1} it significantly reduced ($\pm$)-baclofen antinociception which was abolished at the dose of 100 mg kg^{-1} (Table 1). All three doses of CGP 35348 alone did not modify paw licking latency of mice with respect to controls (Table 1).

Similarly, i.c.v. injection of CGP 35348 in mice caused a dose-dependent antagonism of ($\pm$)-baclofen-induced anti-nociception in the hot plate test (Table 2). CGP 35348 at the lowest dose of 0.1 μg i.c.v., failed to modify significantly the antinociceptive effect of ($\pm$)-baclofen whereas 0.5 μg and 2.5 μg reduced or abolished, respectively, the response to ($\pm$)-baclofen.

Mice treated with CGP 35348 2.5 μg i.c.v. appeared excited and their motility was significantly increased compared to controls (data not shown). The excitability was accompanied by a decreased paw licking latency in the hot plate test (Table 2). Higher doses of CGP 35348 were not tested in the hot plate assay since alterations in the behaviour of the mice were even more evident. A dose of 10 μg per mouse induced hyper-motility and scratching while 25 μg produced seizures.

The effect of 2-hydroxy-saclofen was examined at doses of 2.5, 10 and 17.5 μg i.c.v. per mouse. However only 2.5 and 10 μg were used in the hot plate test in mice since 17.5 μg induced salivation and passivity. In contrast to CGP 35348, neither 2.5 nor 10 μg 2-hydroxy-saclofen antagonized the effect of baclofen on licking latency, but rather it was increased (Table 2). Similarly, phaclofen (50 μg per mouse i.c.v.) failed to antagonize the effect of baclofen (Table 2). This dose of pha-clofen was the highest possible to be injected without impairing the overall behaviour of the mouse.

CGP 35348, injected both i.c.v. and systemically, did not modify oxotremorine- or morphine-induced increases in paw

Table 1 Effect of systemically injected CGP 35348 on ($\pm$)-baclofen activity on paw licking latency in the hot plate test (52.5°C)

Pretreatment (mg kg^{-1} i.p.)	Treatment (mg kg^{-1} s.c.)	n	Licking latency in mice (s) before pretreatment	after treatment (min) 30	45
Saline 10 ml	Saline 10 ml	20	14.1 ± 0.4	14.9 ± 0.7	14.6 ± 0.6
CGP 35348 30	Saline 10 ml	10	15.7 ± 1.1	14.2 ± 1.2	14.4 ± 0.7
CGP 35348 60	Saline 10 ml	10	14.8 ± 0.7	14.8 ± 1.8	15.6 ± 1.2
CGP 35348 100	Saline 10 ml	10	16.1 ± 0.6	14.5 ± 1.3	15.5 ± 1.4
Saline 10 ml	Baclofen 4	25	14.4 ± 0.8	30.9 ± 2.4*	33.0 ± 2.1*
CGP 35348 30	Baclofen 4	10	14.5 ± 0.8	26.7 ± 3.3*	32.9 ± 2.7*
CGP 35348 60	Baclofen 4	8	13.0 ± 1.7	19.0 ± 1.5*	25.9 ± 3.6*
CGP 35348 100	Baclofen 4	10	13.8 ± 0.9	12.9 ± 1.6	17.2 ± 1.4

Pretreatment injection was made 5 min before the 'treatment' drug injection. Significant differences were determined by ANOVA followed by LSD multiple range test: * $P < 0.05$ versus S/S controls.

Table 2 Effect of CGP 35348, phaclofen and 2-OH-saclofen i.c.v. injected, on ($\pm$)-baclofen activity on paw licking latency in the hot plate test (52.5°C)

Pretreatment μg i.c.v.	Treatment mg kg^{-1} s.c.	n	Licking latency in mice (s)		
			before pretreatment	after treatment (min) 30	45
Saline 5 μl	Saline 10 ml	18	14.5 $\pm$ 0.6	14.6 $\pm$ 1.6	15.6 $\pm$ 1.6
CGP 35348 0.1	Saline 10 ml	10	14.2 $\pm$ 1.2	15.5 $\pm$ 1.1	14.0 $\pm$ 1.5
CGP 35348 0.5	Saline 10 ml	10	14.7 $\pm$ 0.8	13.5 $\pm$ 0.9	14.6 $\pm$ 0.9
CGP 35348 2.5	Saline 10 ml	15	14.5 $\pm$ 0.6	12.0 $\pm$ 0.5*	12.0 $\pm$ 0.9*
2-OHsaclofen 2.5	Saline 10 ml	10	13.5 $\pm$ 0.9	13.9 $\pm$ 1.6	14.2 $\pm$ 1.7
2-OHsaclofen 10	Saline 10 ml	10	14.3 $\pm$ 0.7	17.2 $\pm$ 1.5	18.4 $\pm$ 2.8
Phaclofen 50	Saline 10 ml	19	13.4 $\pm$ 0.4	16.4 $\pm$ 1.6	14.7 $\pm$ 2.0
Saline 5 μl	Baclofen 4	20	13.4 $\pm$ 0.9	30.8 $\pm$ 2.6*	32.3 $\pm$ 2.1*
CGP 35348 0.1	Baclofen 4	10	14.6 $\pm$ 0.9	25.6 $\pm$ 3.1*	27.9 $\pm$ 3.1*
CGP 35348 0.5	Baclofen 4	10	14.4 $\pm$ 0.8	17.4 $\pm$ 1.8	23.3 $\pm$ 2.7*
CGP 35348 2.5	Baclofen 4	20	12.6 $\pm$ 1.1	13.0 $\pm$ 1.7	14.6 $\pm$ 1.5
2-OHsaclofen 2.5	Baclofen 4	10	14.0 $\pm$ 0.8	41.3 $\pm$ 1.9*†	36.7 $\pm$ 2.6*†
2-OHsaclofen 10	Baclofen 4	10	13.8 $\pm$ 0.6	40.9 $\pm$ 2.1*†	41.2 $\pm$ 1.9*†
Phaclofen 50	Baclofen 4	46	14.3 $\pm$ 0.4	29.1 $\pm$ 1.9*	32.4 $\pm$ 1.9*

All pretreatment drugs were injected 5 min prior to the 'treatment' injection. Significant differences were determined by ANOVA followed by LSD multiple range test. *$P < 0.05$ versus S/S controls. † $P < 0.05$ versus S/baclofen group.

licking latency detected 15, 30 and 45 min after injection (Table 3).

CGP 35348, administered i.c.v., was tested against ($\pm$)-baclofen in two other classical models of antinociception.

Figure 1 shows results obtained in the acetic acid-induced writhing test, in which CGP 35348 (2.5 μg per mouse) antagonized the ($\pm$)-baclofen-induced decrease in the number of stretches while 2-hydroxy-saclofen (2.5 μg i.c.v. per mouse)

Table 3 Lack of antagonism by CGP 35348 of effect of oxotremorine and morphine on paw licking latency in the hot plate test (52.5°C)

Pretreatment	Treatment (mg kg^{-1} s.c.)	n	Licking latency in mice (s)	
			before pretreatment	after treatment (min) 30
Saline i.p.	Saline s.c.	20	14.1 $\pm$ 0.4	14.9 $\pm$ 0.7
Saline i.p.	Morphine 10	10	12.9 $\pm$ 0.7	32.9 $\pm$ 2.9*
CGP 35348 100 mg kg^{-1} i.p.	Morphine 10	10	13.1 $\pm$ 0.7	33.3 $\pm$ 1.9*
Saline i.p.	Oxotremorine 0.1	10	13.2 $\pm$ 0.6	39.4 $\pm$ 0.6*
CGP 35348 100 mg kg^{-1} i.p.	Oxotremorine 0.1	10	13.5 $\pm$ 0.6	32.2 $\pm$ 1.9*
Saline i.c.v.	Saline s.c.	18	14.5 $\pm$ 0.6	14.6 $\pm$ 1.6
Saline i.c.v.	Morphine 10	10	13.6 $\pm$ 1.1	40.0 $\pm$ 4.8*
CGP 35348 2.5 μg i.c.v.	Morphine 10	10	13.3 $\pm$ 0.8	39.2 $\pm$ 2.3*
Saline i.c.v.	Oxotremorine 0.1	10	14.2 $\pm$ 0.7	34.2 $\pm$ 2.3*
CGP 35348 2.5 μg	Oxotremorine 0.1	10	13.3 $\pm$ 0.6	40.6 $\pm$ 1.9*

All pretreatment drugs were injected 5 min prior to the 'treatment' injection. The licking latency scores were obtained at the time of maximum effect for both antinociceptive substances. Significant differences were determined by ANOVA followed by LSD multiple range test.
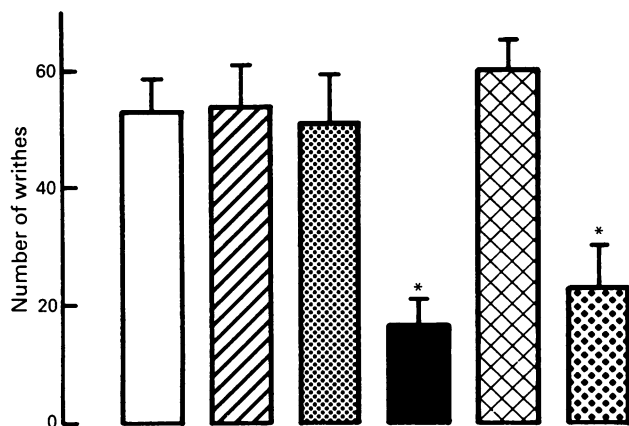
* $P < 0.05$ versus S/S controls.

Figure 1 Effect of CGP 35348 (2.5 μg, i.c.v.) and 2-hydroxysaclofen (2.5 μg, i.c.v.) on ($\pm$)-baclofen (2 mg kg^{-1}, s.c.)-induced inhibition of mice writhing test. Groups were (pretreatment/treatment): saline/ saline (open column); CGP 35348/saline (hatched column); 2-hydroxysaclofen/saline (finely stippled column); saline/($\pm$)-baclofen (solid column); CGP 35348/($\pm$)-baclofen (cross-hatched column); 2-hydroxysaclofen/($\pm$)-baclofen (large stippled column). Pretreatment was performed 5 min before treatment and the writhing test was performed 45 min after treatment. At least 10 mice were used for each group. Bars represent s.e.mean. * $P < 0.05$ versus S/S controls, ANOVA followed by LSD range test.
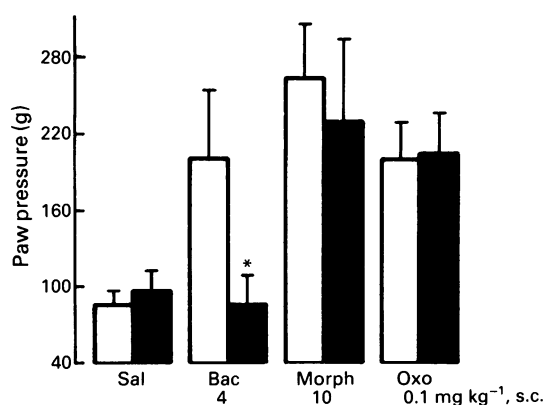
Figure 2 Effect of CGP 35348 (25 μg, i.c.v.) on ($\pm$)-baclofen-(Bac)-, morphine (Morph)- and oxotremorine (Oxo)-induced capacity to withstand increased paw pressure in the rat. I.c.v. pretreatment with saline (open columns) and CGP 35348 (solid columns), was given 5 min before treatment. Values shown were obtained 30 min after saline (Sal)-, baclofen- and oxotremorine-treatment and 15 min after morphine-treatment. At least 6 rats were used for each group. Bars represent s.e.mean. * $P < 0.05$ ANOVA followed by LSD range test.
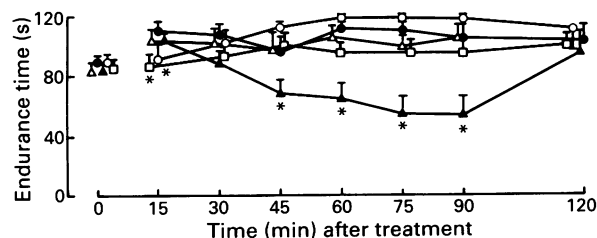

Figure 3 Effect of ($\pm$)-baclofen and CGP 35348 on rota-rod test performance in mice. Groups were treated (i.c.v./s.c.) as follows: saline/ saline ($\bullet$); saline/baclofen 4 mg kg^{-1} ($\triangle$); saline/baclofen 6 mg kg^{-1} ($\blacktriangle$); CGP 35348 2.5 μg/saline ($\bigcirc$); CGP 35348 2.5 μg/baclofen 6 mg kg^{-1} ($\square$). All pretreatment drugs were injected 5 min prior to the 'treatment' injection. Endurance time of mice on the treadmill was measured before treatment and then starting 15 min after treatment up to 120 min. At least ten mice were used for each group. * $P < 0.05$ versus saline controls by ANOVA followed by LSD range test. Bars represent s.e.mean.

failed to alter the frequency. The stretch frequency was not modified by treatment with CGP 35348 or 2-hydroxy-saclofen alone. Antagonism of the effect of ($\pm$)-baclofen (2 mg kg^{-1}, s.c.) by systemically injected CGP 35348 (60 mg kg^{-1}, i.p., 5 min before baclofen) was also confirmed in this test performed 30 min after baclofen (pretreatment/treatment: number of writhes mean $\pm$ s.e.mean; saline i.p./saline s.c.: 51 ± 6.8; CGP 35348 i.p./saline s.c.: 51.1 ± 10.8; CGP 35348 i.p./ baclofen s.c.: 55.9 ± 7.5; saline i.p./baclofen s.c.: $25.5^* \pm 11.9$; $n = 10$; * $P < 0.05$ versus S/S controls).

Figure 2 shows results obtained with the rat in the paw-pressure test. ($\pm$)-Baclofen (4 mg kg^{-1}, s.c.) elevated the ability of the rat to withstand paw pressure and this effect was antagonized by CGP 35348 (25 μg per rat) pretreatment. However, CGP 35348 did not change the morphine- or oxotremorine-induced capacity to withstand increased paw pressure.

Effect of CGP 35348 on ($\pm$)-baclofen-induced muscle-relaxation in mice

In addition to its antinociceptive activity, ($\pm$)-baclofen is an effective muscle-relaxant and this property could contribute to the results obtained in the antinociception models. However, in the antinociception experiments, the maximum doses of ($\pm$)-baclofen and CGP 35348 were 4 mg kg^{-1} and 2.5 μg i.c.v. per mouse respectively. Data shown in Figure 3 show that the endurance time on the rota-rod for mice injected with an antinociceptive dose of ($\pm$)-baclofen (4 mg kg^{-1}) was not altered with respect to controls for up to 120 min. Similarly, CGP 35348 injected i.c.v. at a dose of 2.5 μg, did not alter the rota-rod endurance time from 30 to 120 min after injection (Figure 3).

A decrease in the mouse endurance time on the rota-rod was induced by ($\pm$)-baclofen at a dose of 6 mg kg^{-1} which started 45 min after subcutaneous injection and persisted unchanged for 90 min but disappeared after 120 min (Figure 3).

CGP 35348 (2.5 μg i.c.v. per mouse) injected 5 min before ($\pm$)-baclofen (6 mg kg^{-1}) prevented this decrease in the rota-rod endurance time induced by ($\pm$)-baclofen which was detected for up to 120 min (Figure 3).

Discussion

The present results demonstrate that the GABA-analogue CGP 35348, antagonized ($\pm$)-baclofen-induced antinociception in mice and in rats as well as the ($\pm$)-baclofen muscle relaxant effect in mice. The effect of CGP 35348 appears to be specific to GABA$_B$ receptors since the antagonist was not able to modify morphine- or oxotremorine-induced antinociception.

Two other putative GABA$_B$ antagonists, phaclofen and 2-hydroxysaclofen, which were used for comparative purposes, failed to prevent the antinociceptive effect of ($\pm$)-baclofen. Antagonism of ($\pm$)-baclofen antinociception by CGP 35348 was obtained by both systemic and i.c.v. administration.

CGP 35348 efficacy after i.c.v. administration supports the contention that ($\pm$)-baclofen-induced antinociception is centrally mediated (Liebman & Pastor, 1980; Sawynok, 1987). CGP 35348 antagonism of ($\pm$)-baclofen antinociception occurred even after i.p. administration revealed the ability of this molecule to cross the blood-brain barrier although high doses (60–100 mg kg^{-1}) were necessary. These results are in agreement with Bittiger *et al.* (1990) who reported that CGP 35348 at doses of 30 mg kg^{-1} i.v. and 100 mg kg^{-1} i.p. was able to inhibit the depressant effect of cortically applied baclofen and to reverse the shift in the EEG induced by baclofen in freely moving rats.

The ability of CGP 35348 to cross the blood-brain barrier is a major advance by comparison with other putative GABA$_B$ antagonists. (+)-Baclofen and δ-aminovaleric acid have been

reported to antagonize partially the effects of (−)-baclofen, when injected intrathecally at high doses (10 μg) (Sawynok & Dickson, 1985a; Sawynok 1986). Similarly phaclofen was described as an antagonist of (±)-baclofen antinociception when injected i.c.v. at high doses (12.5–50 μg per mouse), being without effect after intraperitoneal administration (Giuliani et al., 1988). These in vivo doses of phaclofen are almost 20 times higher than those of CGP 35348 used in the present work. Interestingly, a similar ratio of potency between phaclofen and CGP 35348 in antagonizing the (−)-baclofen effect has been reported in vitro in the spinal cord and the hippocampus (Olpe et al., 1990).

However, in our study, phaclofen injected intracerebroventricularly was unable to prevent (±)-baclofen-induced antinociception. Discrepancies in the activity of phaclofen as a GABA_B receptor antagonist have already been noted by others since earlier results showing phaclofen as a GABA_B antagonist (Kerr et al., 1987; Dutar & Nicoll, 1988) have not been substained (Robinson et al., 1989; Stirling et al., 1989; Wang & Dun, 1990). However the low potency of phaclofen may be the main cause of the contrasting results present in the literature (Bowery, 1989).

2-Hydroxysaclofen, although reported as more potent than phaclofen (Kerr et al., 1988) was unable to prevent (±)-baclofen-induced antinociception in our experimental conditions even when injected directly into the cerebral ventricles. In the hot plate test, 2-hydroxysaclofen augmented the antinociceptive action of baclofen.

Among the three GABA_B antagonists tested, CGP 35348 was the only one able to antagonize (±)-baclofen antinociception. CGP 35348 given i.c.v. but not i.p., induced a decrease in paw licking latency in the hot plate test. This effect of CGP 35348 does not seem to be due to an hyperalgesic action since neither in the mouse writhing test nor in the rat paw pressure test did CGP 35348 have any effect by itself. It seems likely that the decreased licking latency in the hot plate test may depend on an increased excitability of mice treated with CGP 35348 i.c.v. injected.

It is well known that (±)-baclofen has a muscle-relaxant effect in addition to its antinociceptive effect (Levy & Proudfit, 1977; 1979; Wilson & Yash, 1978; Sawynok & La Bella, 1982; Hammond & Drower, 1984). These two effects are not related since antinociception occurs at smaller doses of (±)-baclofen but CGP 35348, injected directly into cerebral ventricles, was also able to antagonize (±)-baclofen-induced motor incoordination. These results not only confirm CGP 35348 antagonism of muscle-relaxation induced by (±)-baclofen (already shown by administering CGP 35348 i.p. by Olpe et al. (1990)), but also demonstrate that this effect, like antinociception, is centrally mediated.

A comparison of the activities of phaclofen, 2-hydroxysaclofen and CGP 35348, has demonstrated that CGP 35348 is the most active GABA_B antagonist in vivo, and thus will be a valuable tool for the investigation of the physiological role of GABA_B receptors.

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