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Short communication

In vivo effectiveness of CGP7930, a positive allosteric modulator of the $GABA_B$ receptor

Mauro A.M. Carai[a,*], Giancarlo Colombo[b], Wolfgang Froestl[c], Gian Luigi Gessa[a,b]

[a]"*Bernard B. Brodie*" *Department of Neuroscience, University of Cagliari, Viale Diaz 182, I-09126 Cagliari (CA), Italy*
[b]*C.N.R. Institute of Neuroscience, Section of Cagliari, Viale Diaz 182, I-09126 Cagliari (CA), Italy*
[c]*Research Department, Novartis Pharma AG, CH-4002 Basel, Switzerland*

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Abstract

The present study was aimed at assessing the in vivo effectiveness of the positive allosteric modulator of the γ-aminobutyric $acid_B$ ($GABA_B$) receptor, CGP7930 [2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol]. The synergistic potentiation of $GABA_B$ receptor functioning, previously observed in different in vitro assays, has been confirmed in the present work, where pretreatment with CGP7930 (10–170 mg/kg, i.p.) resulted in a marked potentiation of the sedative/hypnotic effect of the $GABA_B$ receptor agonists, baclofen (40 mg/kg, i.p.) and γ-hydroxybutyric acid (500 mg/kg, i.p.), in DBA mice. Pretreatment with the $GABA_B$ receptor antagonist, SCH 50911 [(*S*)-5,5-dimethyl-2-morpholine acetic acid; 100 mg/kg, i.p.], resulted in a complete blockade of the sedative/hypnotic effect of the combination of CGP7930 with either baclofen or γ-hydroxybutyric acid. These results confirm that CGP7930 may constitute an interesting tool for pharmacological studies in the $GABA_B$ receptor field.

Keywords: CGP7930; Positive allosteric modulation of $GABA_B$ receptor; Baclofen; γ-Hydroxybutyric acid (GHB); SCH 50911; DBA, mouse

1. Introduction

Recent studies have provided an initial characterization of the pharmacological profile of CGP7930 [2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol], the first positive allosteric modulator of the γ-aminobutyric $acid_B$ ($GABA_B$) receptor synthesized to date. As an example, these studies demonstrated that CGP7930 (a) potentiated GABA-induced stimulation of GTPγ[^{35}S] binding in $GABA_B$ receptors (Urwyler et al., 2001; Binet et al., 2004), (b) increased the binding affinity of $GABA_B$ receptor agonists (Urwyler et al., 2001), (c) potentiated the effect of GABA on potassium channels (Urwyler et al., 2001), and (d) positively regulated $GABA_B$ receptor-induced stimulation and inhibition of adenylyl cyclase activity in the rat brain (Onali et al., 2003).

The present study was designed to verify whether the potentiating effect of CGP7930 on $GABA_B$ receptor activation (Urwyler et al., 2001; Binet et al., 2004; Onali et al., 2003) was detectable also in an in vivo assay. To this aim, the present study evaluated the capability of doses of CGP7930, comparable to the concentrations tested in the above in vitro studies, to potentiate the sedative/hypnotic effect of the prototype $GABA_B$ receptor agonist, baclofen. The present study also investigated whether pretreatment with CGP7930 potentiated sedation/hypnosis produced by the GABA metabolite, γ-hydroxybutyric acid (also known as GHB) (see Maitre et al., 2002), an effect secondary to activation of the $GABA_B$ receptor (Carai et al., 2001). Finally, the capability of the $GABA_B$ receptor antagonist, SCH 50911 [(*S*)-5,5-dimethyl-2-morpholine acetic acid], to prevent sedation/hypnosis induced by the combination of

* Corresponding author. Tel.: +39 070 302227; fax: +39 070 302076.
E-mail address: macarai@unica.it (M.A.M. Carai).

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CGP7930 with either baclofen or γ-hydroxybutyric acid was evaluated.

2. Materials and methods

The experimental procedures employed in the present study were in accordance with the Italian Law on the "Protection of animals used for experimental and other scientific reasons" and approved by the Ethical Committee of the University of Cagliari.

2.1. Animals

Male DBA mice (Charles River, Calco, LC, Italy), weighing 20–25 g, were used. Mice were housed 20 per cage in standard plastic cages with wood chip bedding under a 12-h artificial light–dark cycle (lights on at 7:00 a.m.), at a constant temperature of 22±2 °C and relative humidity of approximately 60%. Tap water and standard laboratory rodent chow (Mucedola, Settimo Milanese, MI, Italy) were provided ad libitum.

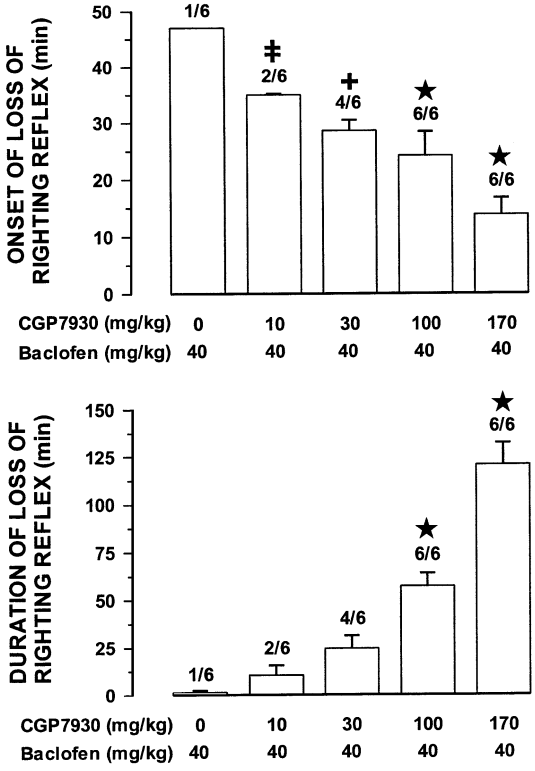

Fig. 1. Potentiation of the sedative/hypnotic effect of the $GABA_B$ receptor agonist, baclofen, by the $GABA_B$ receptor positive modulator, CGP7930, in DBA mice. Top and bottom panels illustrate, respectively, the time required to lose the righting reflex (onset) and duration of loss of righting reflex after administration of CGP7930 and baclofen. CGP7930 was administered i.p. 15 min before the i.p. injection of baclofen. Figures on top of each bar indicate the number of mice which lost the righting reflex over the total number of mice tested. In the top panel, each bar is the mean±S.E.M. of the onset in mice which lost the righting reflex; in the bottom panel, each bar is the mean±S.E.M. of duration of loss of righting reflex in all mice of each group (mice that did not lose the righting reflex were included assigning the value zero). ‡P<0.05, +P<0.005, and ★P<0.0005 in comparison to the "0 mg/kg CGP7930 plus 40 mg/kg baclofen" mouse group.

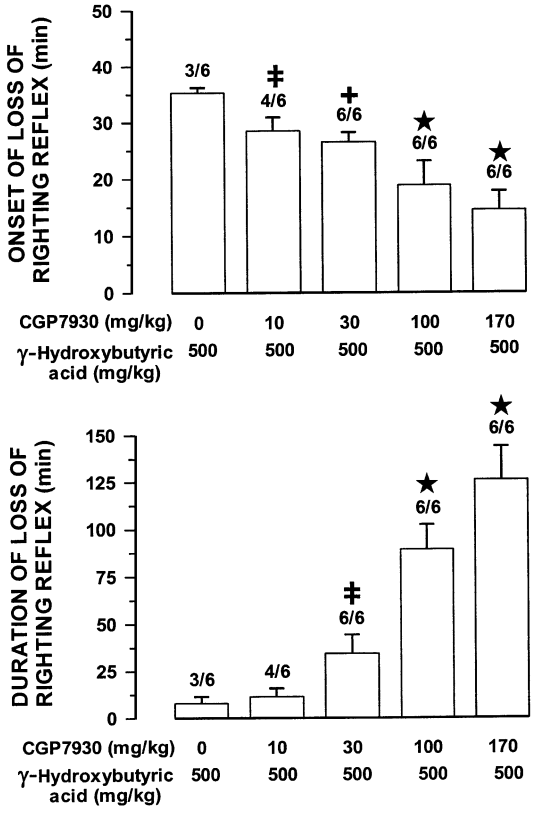

Fig. 2. Potentiation of the sedative/hypnotic effect of the $GABA_B$ receptor agonist, γ-hydroxybutyric acid, by the $GABA_B$ receptor positive modulator, CGP7930, in DBA mice. Top and bottom panels illustrate, respectively, the time required to lose the righting reflex (onset) and duration of loss of righting reflex after administration of CGP7930 and γ-hydroxybutyric acid. CGP7930 was administered i.p. 15 min before the i.p. injection of γ-hydroxybutyric acid. Figures on top of each bar indicate the number of mice which lost the righting reflex over the total number of mice tested. In the top panel, each bar is the mean±S.E.M. of the onset in mice which lost the righting reflex; in the bottom panel, each bar is the mean±S.E.M. of the duration of loss of righting reflex in all mice of each group (mice that did not lose the righting reflex were included assigning the value zero). ‡P<0.05, +P<0.005, and ★P<0.0005 in comparison to the "0 mg/kg CGP7930 plus 500 mg/kg γ-hydroxybutyric acid" mouse group.

2.2. Procedure

In the "CGP7930 plus baclofen" experiment, CGP7930 (0, 10, 30, 100 and 170 mg/kg, i.p.) was administered 15 min before injection of the fixed dose of 40 mg/kg baclofen (i.p.) to groups of n=6 mice. The dose of baclofen was chosen on the basis of previous experiments in order to produce a modest sedative/hypnotic effect (this laboratory, unpublished results). Time of pretreatment with CG7930 was chosen on the basis of the pharmacokinetic profile of the drug (Aichholz R., Blum W., Kühnöl J., Ramstein P., Gertsch W., Imobersteg S., Froestl W., unpublished results) in order to ensure the peak concentration at the time of onset

of baclofen-induced sedation/hypnosis. CGP7930 (Novartis, Basel, Switzerland) was dissolved in 12.5 ml/kg of a 4:1:15 mixture containing Cremophor EL, 1,2-propanediol and distilled water. Baclofen (Sigma-Aldrich, Milan, MI, Italy) was dissolved in 12.5 ml/kg distilled water.

In the "CGP7930 plus γ-hydroxybutyric acid" experiment, CGP7930 (0, 10, 30, 100 and 170 mg/kg, i.p.) was administered 15 min before injection of the fixed dose of 500 mg/kg γ-hydroxybutyric acid (i.p.) to groups of n=6 mice. The dose of γ-hydroxybutyric acid was chosen on the basis of previous experiments in order to produce a modest sedative/hypnotic effect (this laboratory, unpublished results). CGP7930 was prepared as described above. γ-Hydroxybutyric acid (Laboratorio Farmaceutico C.T., Sanremo, IM, Italy) was dissolved in 29.4 ml/kg distilled water (this large volume was chosen to minimize tissue irritation at the injection site).

In blockade experiments, SCH 50911 (100 mg/kg, i.p.) was administered first. CGP7930 (100 mg/kg, i.p.) and either baclofen (40 mg/kg, i.p.) or γ-hydroxybutyric acid (500 mg/kg, i.p.) were administered 15 and 30 min after SCH 50911 injection, respectively. The dose of SCH 50911 was chosen on the basis of previous lines of evidence demonstrating the capability of this drug dose to prevent sedation/hypnosis of different $GABA_B$ receptor agonists, including baclofen and γ-hydroxybutyric acid (Carai et al., 2001, 2004). The dose of CGP7930 was chosen on the basis of the two previous experiments, as being the lowest drug dose producing complete potentiation of both baclofen- and γ-hydroxybutyric acid-induced sedation/hypnosis. SCH 50911 (synthesized according to Blythin et al., 1996) was dissolved in 12.5 ml/kg distilled water. CGP7930, baclofen and γ-hydroxybutyric acid were prepared as described above.

According to the procedure used in previous studies (e.g., Carai et al., 2001, 2004), after baclofen or γ-hydroxybutyric acid injection each mouse was placed on its back once every 60 s until it was unable to right itself within 60 s. The time between drug injection and the start of the 60-s interval when the mouse was unable to right itself was measured as onset of the righting reflex loss. Each mouse was then left undisturbed on its back until it spontaneously regained its righting reflex (determined as having at least three paws under its body). Complete recovery of the righting reflex was defined as the animal being able to turn itself upright twice more within 60 s. If this criterion was not fulfilled, the mouse was left undisturbed until it spontaneously regained its righting reflex. The time between loss and recovery of righting reflex was monitored in each mouse. Observations were conducted by an operator unaware of the drug treatment.

2.3. Statistical analysis

In "CGP7930 plus baclofen" and "CGP7930 plus γ-hydroxybutyric acid" experiments, data on onset and duration (expressed in min) of loss of righting reflex were evaluated by separate one-way analyses of variance (ANOVAs). In blockade experiments, occurrence of lose of righting reflex was evaluated by a Fisher's exact probability test for a 2×2 table; data on duration of loss of righting reflex were evaluated by a paired t-test; data on onset could not be analyzed because no mouse in the SCH 50911-pretreated groups lost the righting reflex.

3. Results

A preliminary experiment showed that no dose of CGP7930 in the 10–170 mg/kg dose range induced any loss of righting reflex in DBA mice (data not shown).

Table 1
Blockade by the $GABA_B$ receptor antagonist, SCH 50911, of the sedative/hypnotic effect produced by the combination of the $GABA_B$ receptor positive allosteric modulator, CGP7930, plus the $GABA_B$ receptor agonist, baclofen (panel A) or γ-hydroxybutyric acid (panel B), in DBA mice

Panel A					
SCH 50911 (mg/kg)	CGP7930 (mg/kg)	Baclofen (mg/kg)	No. of mice that lost the righting reflex over the total no. of mice tested	Onset (min)	Duration (min)
0	100	40	6/6	20.5±1.8	94.3±8.6*
100	100	40	0/6**	ND	0.0±0.0
Panel B					
SCH 50911 (mg/kg)	CGP7930 (mg/kg)	γ-Hydroxybutyric acid (mg/kg)	No. of mice that lost the righting reflex over the total no. of mice tested	Onset (min)	Duration (min)
0	100	500	6/6	21.2±1.6	68.7±8.5*
100	100	500	0/6**	ND	0.0±0.0

In the Onset column, values are the mean±S.E.M. of the onset time of mice which lost the righting reflex (ND, not determined since no mouse lost the righting reflex). In the Duration column, values are the mean±S.E.M. of the duration of loss of righting reflex of all mice of each group (mice that did not lose the righting reflex were included assigning the value zero).

* P<0.001 (t-test).

** P<0.005 (Fisher's exact probability test).

Pretreatment with CGP7930 potentiated the sedative/hypnotic effect of 40 mg/kg baclofen. Indeed, the onset of loss of righting reflex was significantly [$F(4,14)=19.36$, $P<0.00005$] and dose-dependently reduced by CGP7930 administration (Fig. 1, top panel). Consistently, the duration of loss of righting reflex resulted to be significantly [$F(4,25)=36.40$, $P<0.000001$] and dose-dependently increased (Fig. 1, bottom panel). The number of mice that lost the righting reflex was 1/6, 2/6, 4/6, 6/6 and 6/6 in the mouse groups pretreated with 0, 10, 30, 100 and 170 mg/kg CGP7930, respectively.

Similar results were collected in the "CGP7930 plus γ-hydroxybutyric acid" experiment. Indeed, the onset of loss of righting reflex induced by 500 mg/kg γ-hydroxybutyric acid was significantly [$F(4,20)=3.47$, $P<0.000001$] and dose-dependently reduced by CGP7930 administration (Fig. 2, top panel). Consistently, the duration of loss of righting reflex was significantly [$F(4,25)=55.91$, $P<0.000001$] and dose-dependently increased (Fig. 2, bottom panel). The number of mice that lost the righting reflex was 3/6, 4/6, 6/6, 6/6 and 6/6 in the mouse groups pretreated with 0, 10, 30, 100 and 170 mg/kg CGP7930, respectively.

Pretreatment with SCH 50911 completely antagonized the sedative/hypnotic effect produced by the combinations of CGP7930 plus baclofen (Table 1, panel A) and CGP7930 plus γ-hydroxybutyric acid (Table 1, panel B).

4. Discussion

The results of the present study indicate that pretreatment with the positive allosteric modulator of the $GABA_B$ receptor, CGP7930, synergistically increased the sedative/hypnotic effect of the prototype $GABA_B$ receptor agonist, baclofen. All three parameters of baclofen-induced sedation/hypnosis (onset and duration of the loss of righting reflex; proportion of mice losing the righting reflex) were indeed markedly and consistently altered by CGP7930 pretreatment. Similar results were collected when CGP7930 was combined with γ-hydroxybutyric acid, the sedative/hypnotic effect of which has been demonstrated to be mediated by the $GABA_B$ receptor (Carai et al., 2001). As expected, the potentiating effect of CGP7930 on baclofen- and γ-hydroxybutyric acid-induced sedation/hypnosis was completely prevented by the $GABA_B$ receptor antagonist, SCH 50911, indicating the involvement of the $GABA_B$ receptors in this in vivo effect.

Consistently with the pharmacological profile of a receptor positive allosteric modulator, CGP7930—at least in the dose range tested in the present study—failed to produce any sign of sedation on its own.

The results of the present study are in close agreement with those of previous in vitro reports demonstrating the positive allosteric nature of CGP7930 at the $GABA_B$ receptor (Urwyler et al., 2001; Binet et al., 2004; Onali et al., 2003). Recently, Smith et al. (2004) reported that CGP7930 decreased cocaine self-administration in rats and potentiated the decreasing effect of baclofen on this behavior. The results of the present study, extending the data by Smith et al. (2004) to another behavioral effect (sedation/hypnosis) and another $GABA_B$ receptor agonist (γ-hydroxybutyric acid), confirm that CGP7930 is effective in vivo. Taken together, these results (Smith et al., 2004; present study) also suggest that CGP7930 is capable to reach the target brain area(s) and potentiate $GABA_B$-mediated neurotransmission in vivo, being an interesting tool for pharmacological studies in the $GABA_B$ receptor field.

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