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Reducing effect of the positive allosteric modulators of the $GABA_B$ receptor, CGP7930 and GS39783, on alcohol intake in alcohol-preferring rats

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Abstract

The γ-aminobutyric $acid_B$ ($GABA_B$) receptor full agonists, baclofen and CGP44532, have been found to suppress different aspects of alcohol drinking behavior, including acquisition and maintenance, in selectively bred Sardinian alcohol-preferring (sP) rats. The present study was designed to assess whether this capability extends to the recently synthesized, positive allosteric modulators of the $GABA_B$ receptor, 2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and *N*,*N*′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783). In the "acquisition" experiments, CGP7930 (0, 25, 50 and 100 mg/kg; i.g.) and GS39783 (0, 6.25, 12.5 and 25 mg/kg; i.g.) were administered for 5 consecutive days to alcohol-naive sP rats. In the "maintenance" experiments, (0, 50 and 100 mg/kg; i.g.) and GS39783 (0, 50 and 100 mg/kg; i.g.) were administered for 5 consecutive days to alcohol-experienced sP rats. Alcohol intake was evaluated under the standard, homecage 2-bottle "alcohol (10%, *v*/*v*) vs water" regimen with unlimited access for 24 h/day. Both CGP7930 and GS39783 dose-dependently suppressed the acquisition of alcohol drinking behavior. In the "maintenance" experiments, CGP7930 and GS39783 reduced daily alcohol intake by 30–40% only at the highest dose when compared to vehicle-treated rats; this effect tended to vanish on continuing treatment. The results of the present study suggest that positive allosteric modulation of the $GABA_B$ receptor produced an effect on alcohol drinking behavior similar to that produced by $GABA_B$ receptor full agonists. These data also suggest that positive allosteric modulation of the $GABA_B$ receptor may constitute a potential strategy for developing new drugs for treating alcohol dependence.

Keywords: Positive allosteric modulation; $GABA_B$ receptor; CGP7930; GS39783; Baclofen; Alcohol drinking behavior; Sardinian alcohol-preferring (sP) (Rat)

1. Introduction

Accumulating evidence suggests that agonists at the γ-aminobutyric $acid_B$ ($GABA_B$) receptor may decrease alcohol consumption and alcohol-motivated behaviors in laboratory rodents. Specifically, the $GABA_B$ receptor full agonists, baclofen and CGP44532, suppressed the acquisition (Colombo et al., 2002) and maintenance (Colombo et al., 2000; Perfumi et al., 2002; Stromberg, 2004) of alcohol drinking behavior in rats as well as the extra-amount of alcohol consumed by rats after a period of alcohol abstinence (Colombo et al., 2003a; Carai et al., 2005). Consistently, both baclofen and the other $GABA_B$ receptor full agonist, SKF 97541, decreased the oral self-administration of alcohol in rats and mice tested under different operant procedures (Anstrom et al., 2003; Janak and Gill, 2003; Besheer et al., 2004; Maccioni et al., in press). Baclofen also suppressed the motivational or appetitive properties of alcohol, measured in rats by the extinction responding procedure (Colombo et al., 2003b). Preliminary clinical surveys apparently generalized to human alcoholics the results of the above rodent studies. Indeed, it has been found that baclofen decreased the number of daily alcoholic drinks, promoted abstinence from alcohol and reduced craving for alcohol in alcohol-dependent subjects (Addolorato et al., 2000, 2002; Flannery et al., 2004; see also Ameisen, 2005).

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The recent synthesis of two in vivo effective, positive allosteric modulators of the $GABA_B$ receptor, 2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) (Urwyler et al., 2001) and *N*,*N*′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) (Urwyler et al., 2003), offers a novel means for pharmacological investigations on the $GABA_B$ receptor, including its role in the control of alcohol drinking behavior and alcohol reinforcement. CGP7930 and GS39783 have been found to bind to a site of the $GABA_B$ receptor which is topographically distinct from that of the neurotransmitter GABA; their binding results in an increased affinity of the $GABA_B$ receptor for GABA and full agonists and in a subsequent synergistic potentiation of their effects (Urwyler et al., 2001, 2003, 2005). Accordingly, CGP7930 and GS39783 (a) increased the potency and efficacy of GABA in [^{35}S]GTPγS binding assays (Urwyler et al., 2001, 2003) and (b) potentiated the sedative/hypnotic effect of baclofen and the other $GABA_B$ receptor agonist, γ-hydroxybutyric acid, in mice (Carai et al., 2004). Importantly, CGP7930 and GS39783 are expected to possess a better side-effect profile than full agonists, since they activate the system only in the presence of GABA (e.g.: Cryan et al., 2004; Mombereau et al., 2004; Smith et al., 2004).

Since CGP7930 and GS39783 enhance the function of the $GABA_B$ receptor, we hypothesized that administration of these compounds would decrease alcohol drinking behavior in a manner comparable to that of the $GABA_B$ receptor full agonists. This hypothesis was examined in the present study testing the effect of CGP7930 and GS39783 on acquisition and maintenance of alcohol drinking behavior in selectively bred Sardinian alcohol-preferring (sP) rats. Rats of the sP line apparently constitute an appropriate animal model for the proposed investigation, since different aspects of their alcohol drinking behavior, including acquisition and maintenance, have been found to be modified by activation of the $GABA_B$ receptor. Indeed, the repeated administration of baclofen and CGP44532 has been observed to suppress alcohol intake in sP rats during both acquisition and maintenance phases (Colombo et al., 2000, 2002).

2. Materials and methods

All 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".

2.1. Animals

Male sP rats, from the 59th and 60th generations and 75-days-old at the start of the study, were used. Rats weighed approximately 400 g in the "acquisition" experiments and approximately 500 g in the "maintenance" experiments. Rats derived from a population of sP rats which underwent caesarian derivation at Charles River (Lyon, France) for production of Specific Pathogen Free individuals. Rats were individually housed in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12 : 12 h light-dark cycle (lights on at 23:00), at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Rats were extensively habituated to handling and i.g. injection. Standard rat chow (Mucedola, Settimo Milanese, Italy) was always available.

2.2. Drugs

CGP7930 (Novartis, Basel, Switzerland) was suspended in a mixture containing Cremophor EL (80%) and 1,2-propandiol (20%). GS39783 (Novartis, Basel, Switzerland) was suspended in distilled water with a few drops of Tween 80. Both drugs were administered by gavage; the infusion volume was 1.82 and 2 ml/kg in the CGP7930 and GS39783 experiments, respectively.

2.3. 2-Bottle choice regimen

Alcohol was offered under the standard, homecage 2-bottle choice regimen between the alcohol solution (10% in tap water, *v*/*v*) and tap water with unlimited access for 24 h/day. Bottles were refilled every day with fresh solution and their left-right positions interchanged at random to avoid development of position preference. Alcohol, water and food intake was monitored by weighing the bottles and food pellets (0.1 g accuracy) once daily immediately before the start of the dark phase.

2.4. "Acquisition" experiments

Two independent experiments (one with CGP7930 and one with GS39783) were conducted. Rats were alcohol-naive before the start of the study. In both experiments, rats were divided into four groups ($n = 10$), matched for body weight. Alcohol presentation was initiated at the start of the dark phase of day 1. Rats received their first treatment with CGP7930 (0, 25, 50 and 100 mg/kg) or GS39783 (0, 6.25, 12.5 and 25 mg/kg) 60 min before alcohol presentation. Drug administration was repeated once a day (60 min before lights off) for 5 consecutive days. Drug dose-ranges were chosen on the basis of the results of preliminary experiments, as to produce the maximal effect on alcohol intake without inducing any sedative effect. Daily alcohol, water and food intake was recorded throughout the 5 days of treatment as well as for an additional 7 (CGP7930 experiment) and 24 (GS39783 experiment) days after termination of treatment.

2.5. "Maintenance" experiments

Two independent experiments (one with CGP7930 and one with GS39783) were conducted. Rats were exposed to the 2-bottle "alcohol vs water" regimen for 4 consecutive weeks before the start of the drug treatments (alcohol-experienced rats). In both experiments, rats were divided into three groups ($n = 7–8$), matched for body weight as well as alcohol and water intake during the last 3 days preceding the start of each experiment. Rats were treated with CGP7930 (0, 50 and 100 mg/kg) or GS39783 (0, 50 and 100 mg/kg) once a day, 60 min before lights off, for 5 consecutive days. Recording of daily alcohol,

water and food intake was performed throughout the 5 days of treatment as well as for an additional 2 (CGP7930 experiment) and 6 (GS39783 experiment) days after termination of treatment.

2.6. Statistical analysis

Data concerning daily alcohol, water, total fluid (i.e., the sum of alcohol solution and water consumed) and food intake during the 5 day treatment period as well as the post-treatment period of each experiment were expressed in g/kg, ml/kg, ml/kg and g/kg, respectively, and evaluated by separate 2-way (drug treatment; day) analyses of variance (ANOVAs) with repeated measures on the factor day.

3. Results

3.1. "Acquisition" experiments

In both "acquisition" (alcohol-naive rats) experiments, mean daily alcohol intake in vehicle-treated rats rose to 5–6 g/kg/day [i.e., the amount of alcohol usually consumed daily by sP rats (e.g., Colombo et al., 2002)] within 4–5 days of the start of exposure to the 2-bottle choice (Figs. 1 and 2, panels A).

The repeated administration of CGP7930 dose-dependently reduced daily alcohol intake, resulting in a marked delay of the acquisition of alcohol drinking [$F(3,144)=10.44$, $P<0.00005$] (Fig. 1, panel A); in the rat group treated with 100 mg/kg CGP7930, mean daily alcohol intake was steadily lower than 1 g/kg throughout the 5 day treatment period. Reduction in alcohol intake in CGP7930-treated rats was associated with a compensatory, higher daily consumption of water [$F(3,144)=12.80$, $P<0.0001$] (Fig. 1, panel B), so that daily total fluid intake was not altered by treatment with CGP7930 [$F(3,144)=0.32$, $P>0.05$] (Fig. 1, panel C). Daily food intake was higher in CGP7930-than vehicle-treated rats [$F(3,144)=13.75$, $P<0.00001$] (Fig. 1, panel D).

Following completion of treatment, daily alcohol intake in all rat groups treated with CGP7930 increased progressively, reaching control values within 7 days [$F(3,216)=3.22$, $P<0.05$] (Fig. 1, panel A). Consistently, daily water intake in CGP7930-treated rats progressively diminished [$F(3,216)=3.28$, $P<0.05$] (Fig. 1, panel B). Finally, neither daily total fluid intake [$F(3,216)=2.03$, $P>0.05$] (Fig. 1, panel C) nor daily total food intake [$F(3,216)=2.10$, $P>0.05$] (Fig. 1, panel D) differed among rat groups.

Similar to the effect produced by CGP7930, the repeated administration of GS39783 resulted in a dose-dependent reduction in daily alcohol intake and a subsequent suppression of the acquisition of alcohol drinking [$F(3,144)=7.77$, $P<0.0005$] (Fig. 2, panel A); in the rat groups treated with 12.5 and 25 mg/kg GS39783, mean daily alcohol intake was steadily lower than 1 g/kg throughout the 5 day treatment period. Daily water intake was higher in GS39783- than vehicle-treated rats [$F(3,144)=8.84$, $P<0.0005$] (Fig. 2, panel B), so that daily total fluid intake was not altered by treatment with GS39783 [$F(3,144)=0.23$, $P>0.05$] (Fig. 2, panel C). Daily food intake was higher in GS39783- than vehicle-treated rats [$F(3,144)=5.83$, $P<0.005$] (Fig. 2, panel D).

Following completion of treatment, daily alcohol intake in 6.25, 12.5 and 25 mg/kg GS39783-treated rats returned to control values within 9, 15 and 24 days, respectively [$F(3,828)=5.76$, $P<0.005$] (Fig. 2, panel A). Daily water intake in GS39783-treated animals progressively diminished [$F(3,828)=7.97$, $P<0.0005$] (Fig. 2, panel B), so that daily total fluid intake was similar among rat groups [$F(3,828)=0.58$, $P>0.05$] (Fig. 2, panel C). Daily food intake was initially higher in GS39783-treated rats and reached control values on the subsequent days [$F(3,828)=3.80$, $P<0.05$] (Fig. 2, panel D).

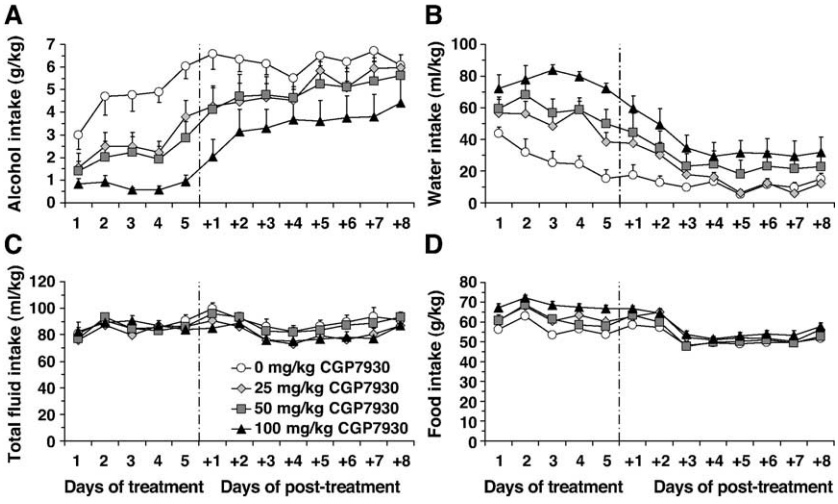

Fig. 1. Effect of the repeated administration of the positive allosteric modulator of the $GABA_B$ receptor, CGP7930, on acquisition of alcohol drinking behavior in alcohol-naive Sardinian alcohol-preferring (sP) rats. CGP7930 (0, 25, 50 and 100 mg/kg; i.g.) was administered once daily (60 min before lights off) for 5 consecutive days. Alcohol (10%, *v*/*v*) and water were offered, under the homecage 2-bottle choice regimen with unlimited access for 24 h/day, starting 60 min after the first treatment with CGP7930. Food pellets were always available. Alcohol, water and food intake was monitored once a day immediately before lights off. The dashed line indicates the completion of the 5 day treatment period and the start of the post-treatment period. Each point is the mean±S.E.M. for $n=10$ rats.

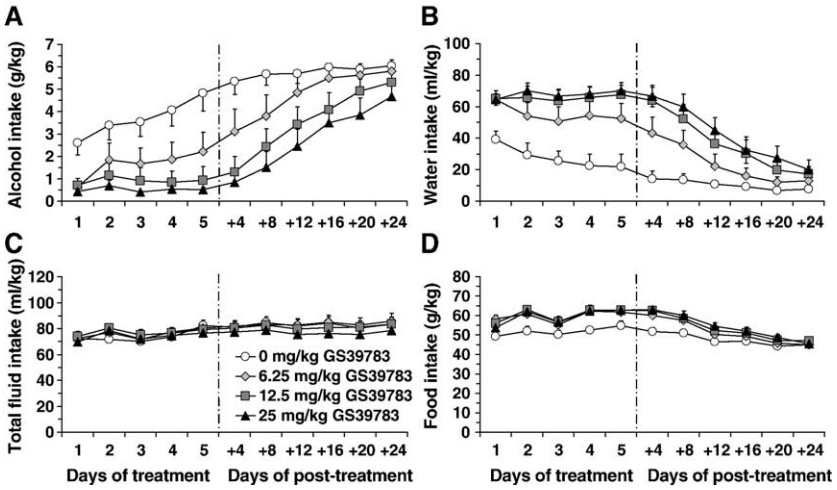

Fig. 2. Effect of the repeated administration of the positive allosteric modulator of the $GABA_B$ receptor, GS39783, on acquisition of alcohol drinking behavior in alcohol-naive Sardinian alcohol-preferring (sP) rats. GS39783 (0, 6.25, 12.5 and 25 mg/kg; i.g.) was administered once daily (60 min before lights off) for 5 consecutive days. Alcohol (10%, *v*/*v*) and water were offered, under the homecage 2-bottle choice regimen with unlimited access for 24 h/day, starting 60 min after the first treatment with GS39783. Food pellets were always available. Alcohol, water and food intake was monitored once a day immediately before lights off. The dashed line indicates the completion of the 5 day treatment period and the start of the post-treatment period. Each point is the mean±S.E.M. for $n=10$ rats.

3.2. "Maintenance" experiments

In both "maintenance" (alcohol-experienced rats) experiments, mean daily alcohol intake in vehicle-treated rats averaged approximately 6 g/kg. The repeated administration of 100 mg/kg CGP7930 resulted in a reduction, by 30–40% when compared to vehicle-treated rats, in daily alcohol intake [$F(2,72)=4.07$, $P<0.05$] (Fig. 3, panel A); however, the reducing effect of 100 mg/kg CGP7930 was maximal on the first 2 days and tended to decrease on continuing treatment. A compensatory increase in daily water intake [$F(2,72)=5.67$, $P<0.05$] (Fig. 3, panel B) left daily total fluid intake virtually unchanged [$F(2,72)=0.62$, $P>0.05$] (Fig. 3, panel C). CGP7930 administration also produced a marked increase in food intake, which lasted for the 5 days of the treatment period [$F(2,72)=5.66$, $P<0.05$] (Fig. 3, panel D). Neither daily alcohol [$F(2,18)=0.42$, $P>0.05$] (Fig. 3, panel A), water [$F(2,18)=0.65$, $P>0.05$] (Fig. 3, panel B), total fluid [$F(2,18)=0.98$, $P>0.05$] (Fig. 3, panel C) nor food [$F(2,18)=0.25$, $P>0.05$] (Fig. 3, panel D) intake differed among rat groups on the 2 days of the post-treatment period.

Similarly to CGP7930 effect, the repeated administration of 100 mg/kg GS39783 reduced daily alcohol intake by 30–40% when compared to vehicle-treated rats [$F(2,76)=6.03$, $P<0.01$] (Fig. 4, panel A); this effect was however evident solely on the first 2 days of treatment. An increase in daily water intake

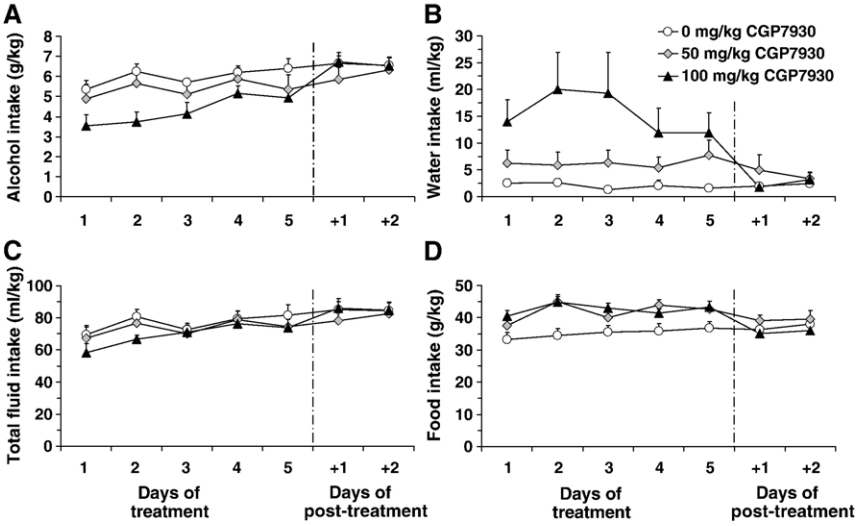

Fig. 3. Effect of the repeated administration of the positive allosteric modulator of the $GABA_B$ receptor, CGP7930, on maintenance of alcohol drinking behavior in alcohol-experienced Sardinian alcohol-preferring (sP) rats. CGP7930 (0, 50 and 100 mg/kg; i.g.) was administered once daily (60 min before lights off) for 5 consecutive days. Alcohol (10%, *v*/*v*) and water were offered under the homecage 2-bottle choice regimen with unlimited access for 24 h/day. Food pellets were always available. Alcohol, water and food intake was monitored once a day immediately before lights off. The dashed line indicates the completion of the 5 day treatment period and the start of the post-treatment period. Each point is the mean±S.E.M. for $n=7$ rats.

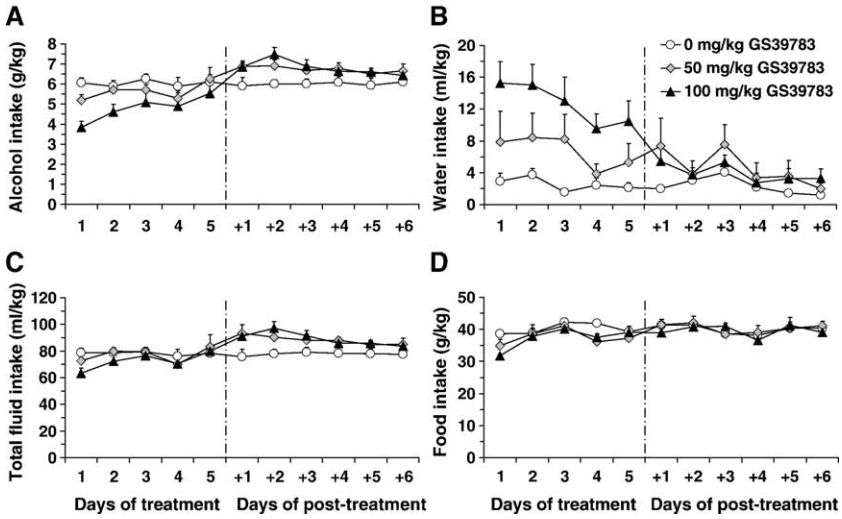

Fig. 4. Effect of the repeated administration of the positive allosteric modulator of the $GABA_B$ receptor, GS39783, on maintenance of alcohol drinking behavior in alcohol-experienced Sardinian alcohol-preferring (sP) rats. GS39783 (0, 50 and 100 mg/kg; i.g.) was administered once daily (60 min before lights off) for 5 consecutive days. Alcohol (10%, *v*/*v*) and water were offered under the homecage 2-bottle choice regimen with unlimited access for 24 h/day. Food pellets were always available. Alcohol, water and food intake was monitored once a day immediately before lights off. The dashed line indicates the completion of the 5 day treatment period and the start of the post-treatment period. Each point is the mean±S.E.M. for $n=7–8$ rats.

[$F(2,76)=9.12$, $P<0.005$] (Fig. 4, panel B) compensated the reduction in alcohol intake, so that total fluid intake was unchanged [$F(2,76)=0.82$, $P>0.05$] (Fig. 4, panel C). Treatment with GS39783 did not alter daily food intake [$F(2,76)=2.71$, $P>0.05$] (Fig. 4, panel D). Following completion of treatment, daily alcohol intake was higher in GS39783- than vehicle-treated rats during the first 2 days of the post-treatment period [$F(2,95)=3.84$, $P<0.05$] (Fig. 4, panel A). No group differences were observed in daily water [$F(2,95)=0.78$, $P>0.05$] (Fig. 4, panel B) and food [$F(2,95)=0.11$, $P>0.05$] (Fig. 4, panel D) intake. As a consequence of the increase in alcohol intake and lack of change in water intake, daily total fluid intake was seen to be higher in GS39783- than vehicle-treated rats [$F(2,95)=4.40$, $P<0.05$] (Fig. 4, panel C).

4. Discussion

The results of the "acquisition" experiments indicate that the repeated, daily administration of the positive allosteric modulators of the $GABA_B$ receptor, CGP7930 and GS39783, dose-dependently prevented the acquisition of alcohol drinking behavior in alcohol-naive sP rats. Indeed, the daily mean of alcohol intake in the rat groups treated with 100 mg/kg CGP7930 or 12.5–25 mg/kg GS39783 was steadily lower than 1 g/kg throughout the 5 day treatment period; conversely, daily alcohol intake in the vehicle-dosed rat groups rapidly rose to the standard amount of 5–6 g/kg.

The results of the "maintenance" experiments indicate that the repeated, daily administration of CGP7930 and GS39783 produced a dose-dependent reduction in daily alcohol intake in alcohol-experienced sP rats. During the first days of treatment, the daily mean of alcohol intake in the rat groups treated with 100 mg/kg CGP7930 or 100 mg/kg GS39783 was 30–40% lower than that recorded in vehicle-dosed rats. In both experiments, a tendency toward a relatively rapid development of tolerance to the reducing effect of CGP7930 and GS39783 on alcohol intake was observed on continuing treatment.

When the results of the two series of experiments ("acquisition" and "maintenance") were compared, CGP7930 and GS39783 were revealed to be more potent and effective in reducing acquisition than maintenance of alcohol drinking behavior of sP rats. This differential profile has been observed in sP rats with drugs of different classes, including baclofen (Colombo et al., 2000, 2002). We hypothesize that this differential efficacy may be secondary to alterations, induced by chronically consumed alcohol, in the function of the receptor systems mediating alcohol effects; these neuroadaptative processes might result in a decreased potency and efficacy of the ligands. Alternatively, alcohol drinking in alcohol-experienced, but not-naive, rats may occur in part as a conditioned response to some external stimuli, such as the start of the dark phase of the light–dark cycle, and this response might be modestly sensitive to pharmacological treatment.

CGP7930 and GS39783 displayed comparable potency and efficacy when tested in the "maintenance" procedure. Indeed, only the 100 mg/kg dose of both drugs exerted a significant reduction in alcohol intake; this reduction resulted as being of similar magnitude and duration. Vice versa, GS39783 resulted to be more potent than CGP7930 in the "acquisition" experiment, as indicated by the fact that the suppressing effect of 100 mg/kg CGP7930 on daily alcohol intake was reproduced by a dose of GS39783 as low as 12.5 mg/kg. These results suggest the specific effectiveness of GS39783 in blocking the disclosure and acquisition, rather than their experience when already consolidated, of the central effect of alcohol underlying alcohol drinking behavior in sP rats.

The results of the present study closely replicate those previously collected with the $GABA_B$ receptor full agonists, baclofen and CGP44532. Indeed, the latter drugs dose-dependently suppressed the acquisition of alcohol drinking

behavior in alcohol-naive sP rats (Colombo et al., 2002). Further, chronic administration of baclofen reduced, up to 40–45% compared to control rats, daily alcohol drinking in alcohol-experienced sP rats; the magnitude of this effect tended to decrease on treatment continuation (Colombo et al., 2000).

Taken together, the above results suggest that activation of the $GABA_B$ receptor—either by means of full agonists or positive allosteric modulators—resulted in a reduced acquisition and experience of the psychopharmacological effects of alcohol that sustain alcohol drinking behavior in sP rats. The results of the present study suggest that CGP7930 and GS39783 potentiated the action of endogenously released GABA at the $GABA_B$ receptor to an extent comparable to the activating effect exerted on the receptor by the full agonists, baclofen and CGP44532.

Administration of CGP7930 and GS39783 stimulated food intake. This effect was particularly evident in both experiments testing CGP7930 ("acquisition" and "maintenance") and in the "acquisition" experiment testing GS39783. This increase in food intake may be explained as a compensation, in terms of caloric intake, for the reduced supply provided by alcohol, the intake of which was decreased by treatment with CGP7930 and GS39783. These data are also consistent with a number of experimental reports indicating that the activation of the $GABA_B$ receptor by low to moderate doses of baclofen stimulated food intake in rats tested under multiple experimental procedures (e.g.: Ebenezer and Pringle, 1992; Ebenezer, 1995; Stratford and Kelley, 1997; Ward et al., 2000; Higgs and Barber, 2004; Ebenezer and Patel, 2004).

In terms of the mechanism of action by which full agonists and positive allosteric modulators at the $GABA_B$ receptor exert their reducing effect on alcohol drinking behavior, we hypothesize the involvement of the $GABA_B$ receptors located in the mesolimbic dopamine "reward" system. Specifically, the pharmacological activation of the $GABA_B$ receptors located in the ventral tegmental area (Bowery et al., 1987), the area where mesolimbic dopamine neurons originate, may inhibit alcohol-induced stimulation of mesolimbic dopamine transmission, i.e. the likely neurochemical substrate underlying alcohol reinforcement and reward (see Weiss and Porrino, 2002). In agreement with this hypothesis, the decreasing effect of baclofen on alcohol intake in sP rats was associated with a reduction in alcohol-stimulated dopamine release in the shell of the nucleus accumbens (the area where mesolimbic dopamine neurons project their axons) (Colombo et al., 2004); further, a recent study demonstrated that $GABA_B$ receptors located in the ventral tegmental area underwent positive allosteric modulation by CGP7930 (Chen et al., 2005).

In a recent study, both CGP7930 and GS39783 have been found to reduce the intravenous self-administration of cocaine in rats (Smith et al., 2004). These data constitute further important pieces of similarity between the anti-addictive profile of $GABA_B$ receptor full agonists and that of the positive allosteric modulators of the $GABA_B$ receptor. Indeed, baclofen and CGP44532 have been repeatedly reported to suppress the self-administration of different abusive drugs, including cocaine (see Brebner et al., 2002; Cousins et al., 2002). The data on the suppressive effect of CGP7930 and GS39783 on cocaine self-administration in rats (Smith et al., 2004), together with those of the present study, indicating that CGP7930 and GS39783 reduced alcohol drinking behavior in alcohol-preferring rats, suggest that the positive allosteric modulation of the $GABA_B$ receptor may serve as a novel strategy for developing new drugs for treating drug addiction and alcoholism; these drugs might theoretically possess a superior side-effect profile when compared to the $GABA_B$ receptor full agonists (Smith et al., 2004).

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