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ORIGINAL INVESTIGATION

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Baclofen suppresses motivation to consume alcohol in rats

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Abstract *Rationale:* Recent studies demonstrated that treatment with the γ-aminobutyric acid $(GABA)_B$ receptor agonist baclofen reduced alcohol intake in selectively bred Sardinian alcohol-preferring (sP) rats tested under the home-cage, two-bottle choice regimen. *Objectives:* The present study investigated the effect of baclofen on the appetitive, rather than consummatory, aspects of alcohol ingestion in sP rats. *Methods:* Rats were trained to lever-press for oral alcohol (10%, v/v) or sucrose (3%, w/v) under a fixed-ratio schedule of 4. Once self-administration behavior was established, alcohol intake averaged approximately 0.7 g/kg over the 30-min session. Subsequently, the effect of the acute administration of baclofen (0, 1, 2 and 3 mg/kg, i.p.) on the extinction responding for alcohol and sucrose (defined as the maximal number of lever responses reached in the absence of reinforcement and used as index of motivation to consume alcohol and sucrose) was evaluated. *Results:* All doses of baclofen produced a marked suppression of extinction responding for alcohol. Conversely, only the 3-mg/kg baclofen dose significantly affected extinction responding for sucrose. A separate open-field test indicated that baclofen (0, 1, 2 and 3 mg/kg, i.p.) did not affect spontaneous motor activity in sP rats. *Conclusions:* These results suggest that baclofen may specifically reduce the motivational properties of alcohol; further, these results are in agreement with the recently reported anti-craving potential of baclofen in alcoholics.

Keywords $GABA_B$ receptor · Baclofen · Extinction responding for alcohol · Sardinian alcohol-preferring (sP) rats

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Introduction

Accumulating lines of experimental evidence suggest that pharmacological stimulation of the γ-aminobutyric acid $(GABA)_B$ receptor may reduce the reinforcing properties of different drugs of abuse. Indeed, administration of the $GABA_B$ receptor agonist baclofen has been found to suppress the self-administration of cocaine, heroin and nicotine in rats tested under multiple experimental procedures (Cousins et al. 2002; Brebner et al. 2002).

Baclofen has also been reported to suppress acquisition (Colombo et al. 2002) and maintenance (Colombo et al. 2000) of alcohol drinking behavior as well as relapse-like drinking (Colombo et al. 2003) in Sardinian alcohol-preferring (sP) rats tested under the home-cage, two-bottle choice regimen. The results of a recently completed double-blind, placebo-controlled survey suggest that the anti-alcohol effects of baclofen observed in sP rats may generalize to human alcoholics. Indeed, the daily administration of relatively low doses of baclofen resulted in a marked suppression in the number of daily alcohol drinks as well as in a significant reduction in the compulsive and obsessive components of alcohol craving in baclofen-treated patients when compared with placebo-treated subjects (Addolorato et al. 2002).

While the previous experiments with sP rats focused on the effect of baclofen treatment on some consummatory aspects of alcohol ingestive behavior, the present study was designed to investigate whether baclofen may affect, in this rat line, the appetitive or motivational properties of alcohol. Specifically, the present study evaluated the effect of baclofen on the extinction responding for alcohol, i.e., the maximal amount of

"work" that rats trained to lever-press for alcohol were willing to perform to obtain alcohol. Extinction responding has been proposed to represent an index of the rat motivation to consume alcohol or, in other words, of the appetitive strength of alcohol (Samson et al. 2001; Vacca et al. 2002). The specificity of the effect of baclofen on the motivation to consume alcohol was assessed, evaluating its effect also on the extinction responding for a sucrose solution in sP rats.

Materials and methods

Animals

Male sP rats, approximately 3 months old at the start of the study, were used. Rats were derived from a population of sP rats that underwent caesarian derivation at Charles River (Lyon, France) for production of specific pathogen-free individuals. Rats were individually housed in an animal facility with an inverted 12-h/12-h light/dark cycle, constant temperature of 22±2°C and relative humidity of approximately 60%. Standard rat chow and water were always available, except as noted. Rats were extensively habituated to handling and i.p. injection.

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 ethics committee of the University of Cagliari.

Experimental procedure

Operant experiments

Self-administration and extinction sessions were conducted in modular chambers (Med Associates, Georgia, VT, USA) located in sound-attenuated cubicles. Each chamber contained one response lever and one liquid dipper (0.1-ml cup). Dipper presentation was associated with flashing of a green light positioned above the lever. The cup dipper remained exposed until the rat reached the required fixed-ratio (FR) schedule (see below) again. Self-administration sessions lasted 30 min and were conducted 6 days per week (Monday to Saturday) during the dark phase of the light/dark cycle.

Rats were separated into two groups. One group of rats (n=8) was initiated to lever press for alcohol using the sucrose fading procedure (Samson 1986). Initially, rats of this group were shaped to lever press for sucrose (20%, w/v in water) for four consecutive days. Subsequently, over 22 consecutive sessions, sucrose concentration was progressively diminished to 0%, while alcohol concentration was progressively increased to 10% (v/v). A FR schedule of 1 (FR1) was maintained throughout the initiation phase. After completion of the initiation phase, the FR schedule was progressively increased to FR4 over four consecutive sessions. FR4 and 10% alcohol concentration were maintained from then onward (maintenance phase).

The second group of rats (n=7) was trained to lever press for sucrose. Rats were initially shaped under FR1 and 3% (w/v) sucrose. Over 14 consecutive sessions, the FR schedule was progressively increased to FR4. FR4 and 3% sucrose concentration were maintained from then onward (maintenance phase).

Alcohol and sucrose intake was measured by weighing the fluid reservoir before and after each self-administration session (accuracy 0.1 g). Alcohol intake was expressed as grams per kilogram of pure alcohol; sucrose intake was expressed as milliliters per kilogram of sucrose solution.

After approximately 20 self-administration sessions of the maintenance phase, extinction responding for alcohol or sucrose, defined as the maximal number of lever responses reached by each rat in the absence of alcohol or sucrose reinforcement, was determined. Extinction sessions were conducted once a week (on Saturdays) following five self-administration sessions. Each rat was exposed to four extinction sessions. After each extinction session, alcohol and sucrose self-administration rapidly recovered to baseline levels. During extinction sessions, rats were exposed to the operant conditioning chamber for 30 min but lever pressing did not result in any dipper presentation. The alcohol reservoir was, however, located inside the chamber, to enable the rat to smell the alcohol.

Baclofen (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 2 ml/kg saline and injected i.p. at the doses of 0, 1, 2 and 3 mg/kg, 30 min before the start of the extinction session. All four doses of baclofen were tested in each rat of both groups under a latin-square design.

Data on the number of responses and the amount of alcohol or sucrose consumed over the last self-administration sessions preceding the extinction sessions among the rat subgroups subsequently assigned to baclofen treatment were analyzed using one-way analyses of variance (ANOVAs) with repeated measures. Data on the effect of baclofen on extinction responding for alcohol or sucrose were analyzed using one-way ANOVAs with repeated measures, followed by the Scheffé test for multiple comparisons.

Motor activity experiment

A separate experiment tested the effect of baclofen on spontaneous motor activity. In this experiment, male sP rats, of an age comparable to that of the rats tested in the extinction study, were used. Before the experiment, rats were housed individually for a period of time identical to that spent by the rats of the extinction study.

Motor activity was measured using a black plexiglas open-field arena [60×60×35(height) cm], the floor of which was divided in nine equal squares. One of the walls was made of transparent Plexiglas. The apparatus was located in a soundproof, dimly lit room. Rat behavior was monitored by three observers stationed behind a screen 2 m from the apparatus and unaware of rat allocation. Baclofen was dissolved in 2 ml/kg saline and injected acutely at the doses of 0, 1, 2 and 3 mg/kg (i.p.), 30 min before the start of the trial, to independent groups of n=8 rats. Trials lasted 10 min and were conducted during the dark phase of the light/dark cycle. After each trial, the open-field arena was cleaned thoroughly. Rats were tested in a randomized order.

Time spent moving (including ambulation and rearings), number of square crossings (scored any time the rat entered a square with at least one paw) and number of rearings (defined as the rat rising on its hind legs with forelegs not touching the floor) were used as indices of motor activity. Data were analyzed using one-way ANOVAs.

Results

In close agreement with previous observations from this laboratory (Vacca et al. 2002), all rats acquired and maintained alcohol or sucrose self-administration. Table 1 depicts the number of lever presses and the amount of alcohol or sucrose consumed over the Friday self-administration sessions, which preceded extinction sessions. No differences were recorded among the subgroups subsequently assigned to baclofen treatment.

During extinction sessions, extinction responding for alcohol and sucrose in saline-treated rats averaged 54.8±8.4 and 55.6±13.2 (mean±SEM), respectively, suggesting that both alcohol and sucrose were functioning as reinforcers and had comparable motivational capacities in directing lever-pressing behavior in sP rats. Pretreatment

Table 1 Number of lever presses and intake of alcohol or sucrose solution during the last self-administration session before the extinction sessions with baclofen in Sardinian alcohol-preferring (sP) rats trained to oral self-administer alcohol or sucrose. Self-administration sessions lasted 30 min; alcohol (10%, v/v) or sucrose (3%, w/v) were available under a fixed ratio (FR) schedule of FR4. Alcohol intake is expressed as grams per kilogram of pure alcohol. Sucrose intake is expressed as milliliters per kilogram of sucrose solution. Each value is the mean±SEM of n=8 in the "alcohol" group and n=7 in the "sucrose" group

	Baclofen (mg/kg)			
	0	1	2	3
Alcohol				
Number of responses	206.3±44.8	172.8±26.6	180.9±48.7	170.6±36.5
Alcohol intake (g/kg)	0.72±0.16	0.60±0.09	0.63±0.18	0.59±0.12
Sucrose				
Number of responses	472.7±46.8	466.7±37.2	429.9±29.7	484.7±51.8
Sucrose intake (ml/kg)	23.6±2.8	22.8±1.3	21.8±2.5	24.0±3.1

Table 2 Effect of the acute administration of baclofen on three different measures of motor activity in Sardinian alcohol-preferring (sP) rats tested in an open-field arena. Baclofen was injected i.p. 30 min before the start of the open-field test. Each value is the mean±SEM of n=8

	Baclofen (mg/kg)			
	0	1	2	3
Time spent moving (s)	152.3±33.6	123.6±32.2	185.6±33.9	161.5±49.0
Number of square crossings	24.4±9.0	17.8±5.5	35.5±10.0	24.9±8.0
Number of rearings	6.0±1.7	4.6±2.0	9.1±3.2	6.3±2.6

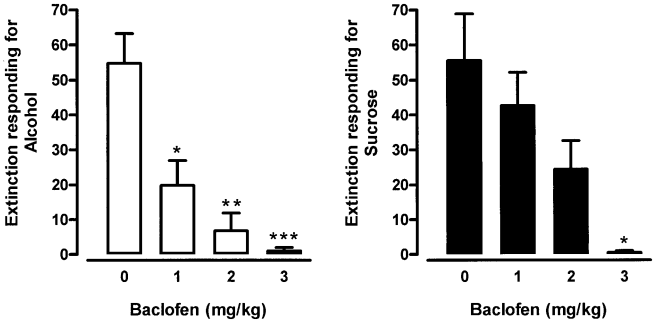

Fig. 1 Effect of the acute administration of baclofen on the extinction responding for alcohol (*left panel*) or sucrose (*right panel*) in Sardinian alcohol-preferring (sP) rats trained to lever press for oral 10% (v/v) alcohol or 3% (w/v) sucrose under a fixed ratio (FR) schedule of FR4. Extinction responding was defined as the maximal number of lever responses performed by each rat in the absence of alcohol or sucrose reinforcement. Extinction sessions were performed once self-administration behavior was well established. Baclofen was injected i.p. 30 min before the start of the extinction session. Each *bar* is the mean±SEM of n=8. *P<0.01, **P<0.001 and ***P<0.0001 with respect to saline-treated rats (Scheffé test)

with baclofen suppressed the extinction responding for alcohol ($F_{3,21}$=15.0191, P<0.0001) (Fig. 1, left panel). Specifically, lever responses in 1, 2 and 3 mg/kg baclofen-treated "alcohol" rats was 64, 88 and 98% lower, respectively, than that recorded in saline-dosed rats. The numbers of rats that completely avoided pressing the lever were zero of eight, three of eight, five of eight and seven of eight in the "alcohol" rat groups receiving 0, 1, 2 and 3 mg/kg baclofen, respectively. Pretreatment with baclofen produced a dose-dependent reduction of extinction responding for sucrose ($F_{3,18}$=6.8868, P<0.005; Fig. 1, right panel). However, post-hoc analysis revealed that only the highest dose of baclofen tested (3 mg/kg) significantly affected extinction responding in the "sucrose" rats. Complete avoidance of lever pressing was observed only in four of eight rats of the 3-mg/kg baclofen group.

In the motor activity experiment, treatment with baclofen affected neither the time spent moving, the number of square crossings nor the number of rearings (Table 2).

Discussion

The results of the present study indicate that acute treatment with the prototype $GABA_B$ receptor agonist baclofen suppressed non-reinforced lever-pressing for alcohol, i.e., an index of motivation to consume alcohol, in selectively bred alcohol-preferring sP rats. All doses of baclofen tested (1, 2 and 3 mg/kg) markedly suppressed extinction responding for alcohol; further, as the dose of baclofen was augmented, an increasing number of rats even avoided making any press on the lever. In order to assess the specificity of the effect of baclofen on the motivation to consume alcohol, a parallel experiment tested in sP rats the effect of baclofen on the extinction responding for an alternative reinforcer such as sucrose. Notably, extinction responding for alcohol and sucrose was strikingly similar in saline-treated rats [54.8±8.4 and 55.6±13.2 (mean±SEM), respectively], suggesting that the alcohol and sucrose solutions used in the present study had comparable appetitive strength in sP rats. However,

only the highest dose of baclofen tested (3 mg/kg) significantly affected extinction responding for sucrose, suggesting that in sP rats baclofen was more potent in reducing the motivation for alcohol than that for sucrose. Finally, the lack of any effect of baclofen on indices of rat motor activity, as revealed by the results of the open-field test, suggests that the observed suppression in lever-pressing was indeed secondary to a reduction in the appetitive strength of alcohol (and, to a lesser extent, that of sucrose) and not to non-specific factors such as sedation or motor impairment.

These results lead to hypothesize the implication of the $GABA_B$ receptor in the neural system mediating alcohol reinforcement. Mesolimbic dopamine neurons, originating in the ventral tegmental area (VTA) and projecting into the nucleus accumbens, have been repeatedly proposed to mediate alcohol reinforcement (Weiss and Porrino 2002). Accordingly, $GABA_B$ receptors located in the VTA (Bowery et al. 1987) might constitute the site of action of the anti-alcohol effects of baclofen: their activation by baclofen would inhibit the enhanced function of mesolimbic dopamine transmission underlying alcohol-reinforced behaviors. A similar mechanism of action has been proposed to explain the attenuating effect of $GABA_B$ receptor agonists on the reinforcing properties of cocaine, heroin and nicotine (Brebner et al. 2002; Cousins et al. 2002).

The results of the present study also suggest that the reducing effect of baclofen on alcohol intake in sP rats (Colombo et al. 2000, 2002) may be secondary to its ability to suppress alcohol motivational properties. In agreement with this hypothesis, a recent double-blind placebo-controlled study found that the suppressing effect of baclofen on alcohol consumption in human alcoholics was accompanied by a significant reduction in the obsessional and compulsive components of alcohol craving (Addolorato et al. 2002). Further, suppression of thinking about and interest for alcohol was observed in alcoholics under treatment with baclofen (Addolorato et al. 2000).

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