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European Journal of Pharmacology 498 (2004) 119–123

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Suppressing effect of the cannabinoid CB_1 receptor antagonist, SR 141716, on alcohol's motivational properties in alcohol-preferring rats

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Received 15 January 2004; received in revised form 22 April 2004; accepted 13 July 2004
Available online 12 August 2004

Abstract

Administration of the cannabinoid CB_1 receptor antagonist, SR 141716 [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide], has been reported to reduce alcohol intake and alcohol self-administration in different models of excessive alcohol consumption, including the selectively bred Sardinian alcohol-preferring (sP) rats. The present study investigated whether SR 141716 was also capable of decreasing, in this rat line, alcohol's motivational properties. Extinction responding for alcohol, defined as the maximal number of lever responses reached in the absence of alcohol in rats trained to lever-press for alcohol, was used as index of alcohol's motivational properties. Rats were initially trained to lever-press for oral alcohol (15%, v/v) under a fixed ratio (FR) schedule of FR4. Once self-administration behavior was established, extinction sessions were conducted. SR 141716 (0, 0.3, 1 and 3 mg/kg; i.p.) was acutely administered before extinction sessions. In order to assess the specificity of SR 141716 action on extinction responding for alcohol, a separate group of sP rats was trained to lever-press for a 3% (w/v) sucrose solution under an FR4 schedule. SR 141716 administration produced a dose-dependent, virtually complete suppression of extinction responding for alcohol. In contrast, extinction responding for sucrose was not significantly altered by treatment with SR 141716. Further to the consummatory aspects, these results also extend the suppressing effect of SR 141716 to the appetitive aspects of alcohol drinking behavior in sP rats. The results also implicate the cannabinoid CB_1 receptor in the neural substrate mediating alcohol's motivational properties in this rat line.

Keywords: Cannabinoid CB_1 receptor antagonist, SR 141716; Extinction responding for alcohol; Sardinian alcohol-preferring (sP), rat

1. Introduction

Accumulating experimental data suggest that the cannabinoid CB_1 receptor may constitute a novel player among the receptor systems controlling alcohol-related behaviors, and particularly alcohol intake and alcohol self-administration. Beside the investigations demonstrating the promoting effect of cannabinoid CB_1 receptor agonists on alcohol intake and alcohol's motivational properties (Gallate et al., 1999; Colombo et al., 2002; Wang et al., 2003), most of the work in the field has been focused on the anti-alcohol properties of the cannabinoid CB_1 receptor antagonist, SR 141716 [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide]. Its administration has indeed been found to suppress acquisition and maintenance of alcohol drinking behavior under the home-cage two-bottle "alcohol vs. water" choice and relapse-like drinking, represented by the alcohol deprivation effect (i.e., the temporary increase in alcohol intake after a period of deprivation from alcohol), in selectively bred Sardinian alcohol-preferring (sP) rats (Colombo et al., 1998; Serra et al., 2001; Serra et al., 2002). Similarly, SR 141716 administration has been reported to reduce alcohol intake under the two-bottle choice paradigm in alcohol-consuming

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0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.069

C57BL/6 mice (Arnone et al., 1997) and Wistar rats (Lallemand et al., 2001). Consistently, SR 141716 suppressed the oral self-administration of alcohol in Long Evans and Wistar rats tested under operant procedures (Freedland et al., 2001; Hungund et al., 2002). In close agreement with the above results, CB_1 receptor knockout mice tested under the two-bottle choice paradigm displayed significantly lower levels of alcohol preference and consumption in comparison to the wild-type mice (Hungund et al., 2003; Poncelet et al., 2003; Wang et al., 2003; Naassila et al., 2004; see, however, Racz et al., 2004).

The above-mentioned work with sP rats has been mainly focused on the effect of SR 141716 on some consummatory aspects of alcohol drinking behavior of this line of alcohol-preferring rats. The present study was designed to investigate whether SR 141716 could also affect, in this rat line, the appetitive or motivational properties of alcohol. To this aim, we evaluated the effect of SR 141716 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 a reliable index of the rat motivation to consume alcohol (Samson et al., 2001; Samson et al., 2003); further, it has recently been characterized as a remarkable phenomenon in sP rats trained to self-administer alcohol (Vacca et al., 2002).

The working hypothesis of the present study was that SR 141716 could reduce the extinction responding for alcohol in sP rats, consistently with its decreasing effect on alcohol intake in this rat line. Should this prediction be confirmed, the results of the present study would be consistent with recent experimental evidence demonstrating the ability of SR 141716 to decrease the probability of completion of response requirement for alcohol (another measure of alcohol's motivational properties) in Long Evans and Wistar rats trained to lever-press to gain access to alcohol (Gallate and McGregor, 1999; Freedland et al., 2001).

In the present study, the specificity of the effect of SR 141716 on the extinction responding for alcohol was assessed evaluating its effect also on the extinction responding for a sucrose solution in sP rats.

2. Materials and methods

2.1. Animals

Male sP rats, approximately 4 months old at the start of the study, were used. Rats were 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 an animal facility with inverted 12:12 h light–dark cycle, constant temperature of 22±2 °C and relative humidity of approximately 60%. Standard rat chow (Mucedola, Settimo Milanese, MI, Italy) and water were always available. Rats were extensively habituated to handling and i.p. injection.

2.2. Experimental procedure

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. Experimental 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 divided into two groups. One group of rats (n=7) 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 4 consecutive days. Subsequently, over 22 consecutive sessions, sucrose concentration was progressively diminished to 0% while alcohol concentration was progressively increased to 15% (v/v). A fixed ratio (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 15% alcohol concentration were maintained from then onwards (maintenance phase).

The second group of rats (n=8) 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 onwards (maintenance phase).

Alcohol and sucrose intake was measured by weighing the fluid reservoir before and after each self-administration session (0.1-g accuracy). Alcohol intake was expressed in g/kg pure alcohol; sucrose intake was expressed in ml/kg sucrose solution.

After approximately 25 self-administration sessions of the maintenance phase (when number of lever-presses and intake of alcohol and sucrose were constant among sessions), 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 (they replaced the Saturday sessions of self-administration) following five consecutive self-administration sessions. Each rat was exposed to four extinction sessions. 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 fluid reservoir was however filled and located inside the chamber, to enable the rat to smell the fluid. After each extinction session, alcohol and sucrose self-administration rapidly recovered to baseline levels.

SR 141716 (Sanofi-Synthelabo, Montpellier, France) was suspended in saline with a few drops of Tween 80 and injected i.p. (injection volume: 1 ml/kg) at the doses of 0, 0.3, 1 and 3 mg/kg 20 min before the start of the extinction session. All four doses of SR 141716 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 SR 141716 treatment were analyzed by one-way analyses of variance (ANOVAs) with repeated measures. Data on the effect of SR 141716 on extinction responding for alcohol or sucrose were analyzed by one-way ANOVAs with repeated measures, followed by the Newman–Keuls test for multiple comparisons.

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".

3. Results

All rats acquired and maintained alcohol or sucrose self-administration. No differences in (a) number of lever presses and (b) amount of alcohol or sucrose consumed over the Friday self-administration sessions which preceded extinction sessions were recorded among the subgroups subsequently treated with the different doses of SR 141716 (Table 1).

Pretreatment with SR 141716 resulted in a dose-dependent suppression of extinction responding for alcohol [$F(3,18)=6.5746$, $P<0.005$] (Fig. 1, top panel). When compared to values of vehicle-treated rats, extinction responding for alcohol in the rat groups treated with 0.3, 1 and 3 mg/kg SR 141716 was 28%, 57% and 88% lower, respectively. In the 3 mg/kg SR 141716-dosed group, 4/7 rats completely avoided to perform any press on the lever (0/7 in the vehicle-treated group).

Table 1
Number of lever-presses and intake of alcohol or sucrose solution during the last self-administration session before the extinction sessions with SR 141716 in Sardinian alcohol-preferring (sP) rats trained to oral self-administer alcohol or sucrose

	SR 141716 (mg/kg)			
	0	0.3	1	3
Number of responses	136.2±28.2	140.7±33.9	128.9±21.5	132.4±31.1
Alcohol intake (g/kg)	0.65±0.12	0.67±0.15	0.64±0.12	0.61±0.12
Number of responses	572.9±52.5	579.6±62.6	598.6±63.5	606.5±51.8
Sucrose intake (ml/kg)	24.3±2.3	24.4±2.7	25.1±2.7	25.6±2.2

Self-administration sessions lasted 30 min; alcohol (15%, v/v) or sucrose (3%, w/v) were available under a fixed ratio (FR) schedule of FR4. Alcohol intake is expressed in g/kg pure alcohol. Sucrose intake is expressed in ml/kg sucrose solution. Each value is the mean±S.E.M. of $n=7$ in the "alcohol" group and $n=8$ in the "sucrose" group.

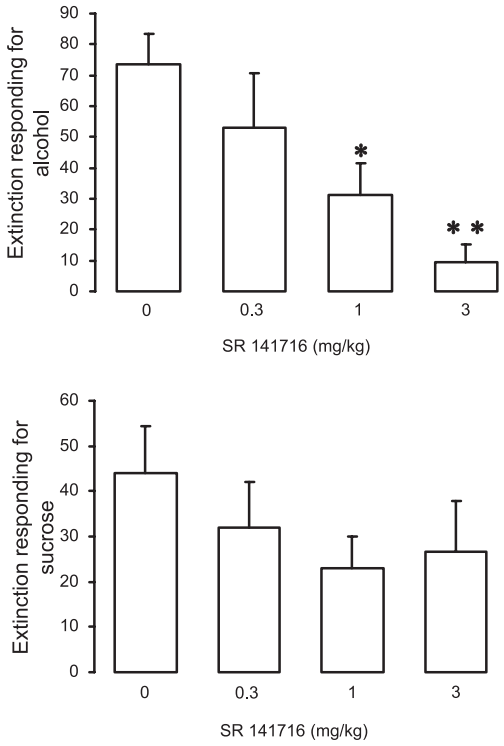

Fig. 1. Effect of the acute administration of SR 141716 on the extinction responding for alcohol (top panel) or sucrose (bottom panel) in Sardinian alcohol-preferring (sP) rats trained to lever-press for oral 15% (v/v) alcohol or 3% (w/v) sucrose under a fixed ratio (FR) schedule of FR4. Extinction responding 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 stabilized. SR 141716 was injected i.p. 20 min before the start of the extinction session. Each bar is the mean±S.E.M. of $n=7$ in the "alcohol" group and $n=8$ in the "sucrose" group. *$P<0.05$ and **$P<0.005$ with respect to vehicle-treated rats (Newman–Keuls test).

In contrast, pretreatment with SR 141716 failed to significantly alter extinction responding for sucrose [$F(3,21)=0.8028$, $P>0.05$] (Fig. 1, bottom panel).

4. Discussion

Acute administration of the cannabinoid CB_1 receptor antagonist, SR 141716, resulted in a dose-dependent suppression of extinction responding for alcohol, a measure of the strength of the appetitive or motivational properties of alcohol, in alcohol-preferring sP rats. After injection of 3 mg/kg SR 141716, extinction responding for alcohol was virtually completely suppressed (Fig. 1, top panel). These results suggest that the cannabinoid CB_1 receptor is likely part of the neural substrate mediating the motivational attributes of alcohol in sP rats; they also suggest that the reducing effect of SR 141716 on the consummatory aspects

of alcohol drinking behavior in sP rats (Colombo et al., 1998; Serra et al., 2001; Serra et al., 2002) is likely secondary to a reduction in the motivation to consume alcohol.

The results of the present study are in line with those reported by Gallate and McGregor (1999) and Freedland et al. (2001), who demonstrated that the acute administration of SR 141716, at dose-ranges comparable with that used in the present study, decreased the probability of response requirement completion for access to alcohol - another procedure for measuring the appetitive strength of alcohol - in unselected rats trained to orally self-administer alcohol under operant procedure.

Importantly enough, the present results also indicate that the suppressing effect of SR 141716 on extinction responding was specific for alcohol, since treatment with SR 141716 did not affect extinction responding for an alternative reinforcer such as a 3% sucrose solution (Fig. 1, bottom panel). In the previous studies investigating the effect of SR 141716 on alcohol's motivational properties, the separation between the anti-motivational properties of the drug for alcohol and a different reinforcer was less clear (Gallate and McGregor, 1999; Freedland et al., 2001). Specifically, treatment with SR 141716 affected to some extent both the breakpoint for a "near-beer" solution (an alcohol-free beverage tasting similarly to the alcohol-containing beverage) (Gallate and McGregor, 1999) and the probability of response requirement completion for access to a 3% sucrose solution (Freedland et al., 2001). The greater specificity of the suppressing effect of SR 141716 on alcohol's motivational properties observed in the present study, when compared to that reported in the studies by Gallate and McGregor (1999) and Freedland et al. (2001), might depend upon differences in the methodologies used to measure the rat motivation to consume alcohol as well as in the rat strains. With regard to the latter possibility, selectively bred alcohol-preferring sP rats might possess, when compared to unselected Long Evans and Wistar rats, a greater sensitivity to the suppressing effect of SR 141716 on the appetitive strength of alcohol.

With regard to the possible mechanism of action by which SR 141716 exerts its suppressing effect on alcohol consumption and alcohol's motivational properties, recent microdialysis experiments demonstrated that behaviorally relevant doses of SR 141716 suppressed alcohol-stimulated dopamine release in the nucleus accumbens of rats and mice (Cohen et al., 2002; Hungund et al., 2003); accordingly, alcohol-induced stimulation of dopamine release in the nucleus accumbens was completely absent in CB_1 receptor knockout mice (Hungund et al., 2003). Different lines of experimental evidence suggest that mesolimbic dopamine neurons are involved in the mediation of alcohol intake and reinforcement (see Weiss and Porrino, 2002). The results of the above-mentioned microdialysis studies led to hypothesize that SR 141716 may remove an inhibitory cannabinoidergic tone on the GABA interneurons controlling the mesolimbic dopamine neurons, resulting in the suppression of dopamine-mediated alcohol-reinforced and -motivated behaviors.

In summary, the results of the present study indicate that the cannabinoid CB_1 receptor antagonist, SR 141716, is capable to specifically suppress the appetitive attributes of alcohol in sP rats; these results, together with those of previous investigations demonstrating the reducing effect of SR 141716 on some consummatory aspects of alcohol drinking behavior in sP rats, strengthen the hypothesis that the cannabinoid CB_1 receptor plays an important role in alcohol-related behaviors in this rat line.

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