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Short communication

Blockade by the cannabinoid CB_1 receptor antagonist, SR 141716, of alcohol deprivation effect in alcohol-preferring rats

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Abstract

The present study investigated the effect of the cannabinoid CB_1 receptor antagonist, SR 141716 (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide), on alcohol deprivation effect (i.e. the temporary increase in alcohol intake after a period of alcohol withdrawal) in Sardinian alcohol-preferring (sP) rats. As expected, alcohol-deprived rats virtually doubled voluntary alcohol intake during the first hour of re-access. Acute administration of SR 141716 (0, 0.3, 1 and 3 mg/kg, i.p.) completely abolished the alcohol deprivation effect. These results suggest that the cannabinoid CB_1 receptor is part of the neural substrate mediating the alcohol deprivation effect and that SR 141716 may possess anti-relapse properties.

Keywords: Cannabinoid CB_1 receptor antagonist, SR 141716; Alcohol deprivation effect; Sardinian alcohol-preferring (sP) rat

1. Introduction

Recent research has shown the ability of the cannabinoid CB_1 receptor antagonist, SR 141716 (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide), to reduce alcohol-motivated behaviors in animal models of alcoholism. Specifically, SR 141716 has been found to block acquisition of alcohol drinking behavior in Sardinian alcohol-preferring (sP) rats (Serra et al., 2001), and to decrease voluntary alcohol intake under the home-cage two-bottle regimen or an operant self administration procedure in alcohol-consuming rats and mice (a possible model of the "active drinking" phase of human alcoholism) (Arnone et al., 1997; Colombo et al., 1998b; Freedland et al., 2001) as well as motivation to consume alcohol in rats (Gallate and McGregor, 1999).

To further characterize the effect of SR 141716 on alcohol intake, the present investigation was designed to assess the effect of the drug on the alcohol deprivation effect, i.e. the temporary increase in voluntary alcohol intake occurring in different animal species after a period of alcohol abstinence, which has been proposed to model the increased alcohol intake and loss of control over alcohol drinking frequently associated with relapse in human alcoholics (see Spanagel and Hölter, 2000). The present study was conducted in sP rats, which have recently been found to display a robust, although transient, alcohol deprivation effect (Agabio et al., 2000). Indeed, in this rat line, prolonged alcohol deprivations resulted in a 50–70% increase in voluntary alcohol intake during the first hour of re-access to alcohol.

2. Materials and methods

2.1. Animals

Male sP rats, from the 50th generation and approximately 75 days 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 standard plastic cages with wood chip bedding.

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The animal facility was under an inverted 12:12 h light–dark cycle (lights on at 23:00 h), at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%.

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.

2.2. Experimental procedure

Rats were continuously offered two bottles containing alcohol (10% v/v, in tap water) and tap water, respectively, for four consecutive weeks. Alcohol and water intakes were monitored once daily by bottle weighing (0.1 g accuracy) immediately before the onset of the dark phase. Bottles were refilled every day with fresh solution and their left–right positions interchanged at random to avoid development of position preference. Food pellets (MIL Morini, San Polo d'Enza, RE, Italy) were always available. Rats were habituated to handling and i.p. injection.

At the end of the 4-week period of access to alcohol and water, rats were divided into two groups ($n = 50$) matched for similar daily alcohol consumption and preference over the last 7 days. One group was deprived of alcohol for 15 consecutive days, during which water was the sole fluid available (alcohol-deprived rats). The second group continued to have unlimited access to alcohol and water (alcohol-nondeprived rats), with exception of the last 6 h before injection of SR 141716, when the alcohol bottle was removed to ensure that blood alcohol levels were equal to zero at the time of the test (this laboratory, unpublished results).

At the end of the 15th day of the deprivation phase, rats of both groups were divided into four subgroups ($n = 12–13$), matched for body weight, and injected i.p. with 0, 0.3, 1 or 3 mg/kg SR 141716 (Sanofi-Synthelabo, Montpellier, France), suspended in 1 ml/kg saline with 0.1% Tween 80. Drug administration occurred 30 min before lights off, when alcohol was re-presented also to the alcohol-deprived rats. Alcohol, water and food intakes were recorded 60 min after lights off.

2.3. Statistical analysis

Data on alcohol and food intakes during the 60-min observation period were expressed in g/kg and analyzed by a two-way (deprivation; treatment) analysis of variance (ANOVA), followed by the Newman–Keuls test for multiple comparisons.

3. Results

ANOVA revealed significant effects of deprivation [$F(1,92) = 23.94$, $P < 0.000001$] and SR 141716 treatment [$F(3,92) = 13.82$, $P < 0.000001$], as well as a significant interaction between the two factors [$F(3,92) = 4.82$, $P < 0.005$] on voluntary alcohol intake in the first hour of the post-deprivation phase (Fig. 1). Alcohol intake was approximately two times higher in vehicle-treated alcohol-deprived rats than alcohol-nondeprived rats, indicative of the development of a robust alcohol deprivation effect. Post hoc analysis showed that all doses of SR 141716 suppressed the alcohol deprivation effect, as indicated by the statistically significant difference between vehicle-treated alcohol-deprived rats and SR 141716-treated alcohol-deprived rats, as well as by the lack of statistically significant difference between alcohol-deprived and -non-deprived rats at each dose of SR 141716. Finally, in alcohol-nondeprived rats, SR 141716 induced a reduction in alcohol intake by approximately 30% when compared to the control group, which however failed to reach statistical significance.

Consistently with the well documented anorectic properties of SR 141716 (Colombo et al., 1998a; Simiand et al., 1998; Freedland et al., 2000; Le Fur et al., 2001; Rowland et al., 2001), 1 and 3 mg/kg SR 141716 produced a significant decrease in food intake [$F(3,92) = 7.45$, $P < 0.0005$], without any difference between alcohol-deprived and -nondeprived rats [$F(1,92) = 0.83$, $P > 0.05$] (data not shown).

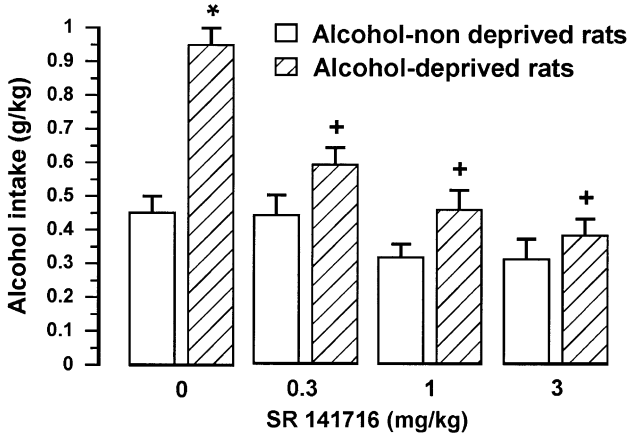

Fig. 1. Effect of the cannabinoid CB_1 receptor antagonist, SR 141716, on alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats. Alcohol-deprived rats were initially allowed to consume alcohol (10%, v/v) and water under the two-bottle free choice regimen with unlimited access for four consecutive weeks and then deprived of alcohol for 15 consecutive days; conversely, alcohol-nondeprived rats had continuous access to alcohol and water (with exception of the last 6 h before SR 141716 injection, when the alcohol bottle was removed to ensure that blood alcohol levels were equal to zero at the time of the test). Thirty minutes before representation of the alcohol bottle (which coincided with lights off), rats of both groups were injected with 0, 0.3, 1 and 3 mg/kg SR 141716. Alcohol intake was registered 60 min after lights off. Each bar is the mean ± S.E.M. of $n = 12–13$. * $P < 0.05$ with respect to alcohol-nondeprived rats receiving 0 mg/kg SR 141716 (Newman–Keuls test); + $P < 0.05$ with respect to alcohol-deprived rats receiving 0 mg/kg SR 141716 (Newman–Keuls test).

4. Discussion

The results of the present study demonstrate that the acute administration of the cannabinoid CB_1 receptor antagonist, SR 141716, suppressed the extra amount of alcohol consumed by selectively bred alcohol-preferring sP rats after a period of alcohol deprivation. These results extend to a model of relapse and loss of control—i.e., the core features of alcohol dependence in humans (see Morse and Flavin, 1992)—the ability of SR 141716 to reduce alcohol intake in animal models of alcoholism. Indeed, previous studies found that SR 141716 blocked acquisition of alcohol drinking behavior in sP rats (Serra et al., 2001), reduced voluntary alcohol intake and self-administration of alcohol in established alcohol-consuming rats and mice (Arnone et al., 1997; Colombo et al., 1998b; Freedland et al., 2001) and motivation to consume alcohol in rats (Gallate and McGregor, 1999).

The neural substrate mediating the alcohol deprivation effect is at present poorly understood. The few studies conducted to date, mainly focused on the pharmacological manipulation of the alcohol deprivation effect in rats and mice, have suggested the possible involvement of glutamate (Salimov and Salimova, 1993; Hölter et al., 1996, 1997, 2000a; Spanagel et al., 1996; Heyser et al., 1998) and opioid (Cowen et al., 1999; Hölter and Spanagel, 1999; Hölter et al., 2000b) receptor systems. The results of the present study suggest that the cannabinoid CB_1 receptor may be part of this substrate.

In conclusion, the results of the present study, demonstrating the ability of SR 141716 to suppress the alcohol deprivation effect in sP rats, add a further piece of evidence to the preclinical characterization of the reducing effect of SR 141716 on alcohol intake and support the hypothesis that blockade of the cannabinoid CB_1 receptor may constitute a novel strategy for the pharmacotherapy of alcoholism.

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