REDUCTION OF VOLUNTARY ETHANOL INTAKE IN ETHANOL-PREFERRING sP RATS BY THE CANNABINOID ANTAGONIST SR-141716

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Abstract — The present study assessed the efficacy of the cannabinoid CB1 receptor antagonist, SR-141716, in reducing voluntary ethanol intake in selectively bred Sardinian alcohol-preferring (sP) rats. Ethanol (10%, v/v) and food were available in daily 4 h scheduled access periods; water was present 24 h/day. The acute administration of a 2.5 and a 5 mg/kg dose of SR-141716 selectively reduced ethanol intake, whereas a 10 mg/kg dose of SR-141716 reduced to a similar extent both ethanol and food intake. These results suggest that the cannabinoid CB1 receptor is involved in the mediation of the ethanol-reinforcing effects in sP rats.

INTRODUCTION

The recent availability of pharmacological tools, such as selective and potent agonists and antagonists has permitted the investigation of the function of the brain cannabinoid receptor. This receptor, normally activated by the endogenous ligand, anandamide, has been reported to modulate several effects, including the reinforcing ones, of Δ9-tetrahydrocannabinol (Δ9-THC), the active ingredient of marijuana and other synthetic agonists (Pertwee, 1995). Indeed, the selective cannabinoid CB1 receptor antagonist, SR-141716 (Rinaldi-Carmona et al., 1994), has been shown to block: (a) the self-administration of the cannabinoid agonist, WIN 55,212-2, in mice (Fratta et al., 1997); (b) the discriminative stimulus effects (of which the reinforcing effects constitute a part) of different cannabinoid agonists, such as Δ9-THC, CP 55,940, and WIN 55,212-2 in rats and monkeys (Wiley et al., 1995a, b; Péroy et al., 1996). Consistently, SR-141716 has been reported to affect some biochemical (Tanda et al., 1997) and electrophysiological (French, 1997; Gessa et al., 1997) events putatively related to cannabinoid reinforcement.

However, the existence of a common brain pathway mediating reinforcement of drugs of abuse has been proposed; this reward system primarily includes the brain dopamine, opioid, and GABA systems (Koob and Bloom, 1988). Were the brain cannabinoid CB1 receptor to constitute part of this neural substrate, its pharmacological manipulation might alter the reinforcing properties of addictive drugs and, in turn, their self-administration.

Selectively bred ethanol-preferring rodents constitute a powerful tool for investigating the neurobiological basis of ethanol reinforcement; indeed, these animals steadily self-administer pharmacologically relevant amounts of ethanol and show a clear preference for ethanol over water (Crawbe and Li, 1995). Thus, the present study was designed to assess the effect of SR-141716 on voluntary ethanol intake in Sardinian alcohol-preferring (sP) rats (Colombo, 1997).

MATERIALS AND METHODS

Experiment 1: effect of SR-141716 on voluntary ethanol intake in sP rats

Animals. Male sP rats, from the 39th generation and ~4 months old, were used. Rats were housed individually in standard plastic cages with wood
chip bedding. The animal facility was under an inverted 12 h:12 h light–dark cycle (lights on at 21:00), at a constant temperature of 22 ± 2°C and relative humidity of 60%. All rats used in the present study fulfilled the selection criteria adopted in this laboratory for sP rats (Colombo, 1997).

Procedure. Rats were offered two bottles containing ethanol (10% v/v, in tap water) and tap water, in daily 4 h drinking sessions (09:00 to 13:00). Food pellets (MIL Morini, San Polo d’Enza, RE, Italy) were available solely during the session; in contrast, water was available for the remaining 20 h. Rats were habituated to handling, i.p. injection, and frequent removal of bottles and food pellets.

After approximately 1 week of habituation to the experimental regimen, all rats showed stable ethanol and food intakes during the daily session. On the test day, rats were divided into four groups (n = 7) matched for ethanol, water, and food intakes during the three previous sessions. SR-141716 (donated by Sanofi Recherche, Montpellier, France) was suspended in 2 ml/kg saline with 0.1% Tween 80 and injected i.p. at doses of 0, 2.5, 5, and 10 mg/kg 20 min prior to the start of the session. On the test day, ethanol, water, and food intakes were monitored by weighing the bottles and food pellets at 60 min intervals from the start of the session.

Data analyses. Data on ethanol, water, and food intakes were expressed in g/kg, ml/kg, and g/kg, respectively, and analysed by a two-way (drug treatment; time interval) analysis of variance (ANOVA) with repeated measures on time intervals, followed by the Scheffe test for multiple comparisons.

Experiment 2: effect of SR-141716 on blood ethanol levels in Wistar rats

Animals. Male Wistar rats (Harlan Nossan, Correzzana, MI, Italy), weighing ~250 g, were used. After delivery to our animal facility, rats were housed individually under ambient conditions identical to those in Experiment 1 and left undisturbed for 7 days to adapt to the new housing conditions. Rat chow and tap water were always available.

Procedure. Rats were starved for 12 h prior to the start of the experiment. SR-141716 was prepared as described above and administered i.p. at doses of 0 (n = 9) and 5 (n = 9) mg/kg 20 min prior to the i.g. administration of 2 g/kg ethanol (15% w/v, in tap water).

Blood samples (50 μl) were collected from the tip of the tail of each rat at 0, 30, 60, 120 and 240 min post-ethanol administration. The procedure for gas-chromatographic determination of blood ethanol levels (BELs) was adapted from that previously described by Eriksson (1980). Briefly, blood samples were diluted 15-fold in distilled water containing n-propanol (9.3 mg/dl) as internal standard. Haemolysed samples were filtered (0.45 μm nylon filter) and injected (0.5 μl injection volume) into a gas chromatograph (Shimadzu GC 14 A, Shimadzu, Japan) equipped with a flame ionization detector and capillary fused silica column (Chrompack, The Netherlands; 25 m long and 0.53 mm inside diameter, packed with CP-WAX 52 CB). Temperatures of injector, column, and detector were 110, 50, and 130°C, respectively. Helium was used as the carrier gas (4 ml/min flow rate).

Data analyses. Data on BELs were expressed in mg/dl and analysed by a two-way (drug treatment; time interval) ANOVA with repeated measures on time intervals.

RESULTS

Experiment 1: effect of SR-141716 on voluntary ethanol intake in sP rats

Acute administration of SR-141716 produced a significant reduction of voluntary ethanol intake in sP rats \[ F_{\text{treatment}}(3;11) = 36.672, P < 0.0001; F_{\text{time}}(3;11) = 35.238, P < 0.0001; F_{\text{interaction}} (9;11) = 0.393, P > 0.05 \] (Fig. 1, top). The attenuating effect of SR-141716 on ethanol intake was already apparent after the first 1 h interval and persisted throughout the entire session. At the end of the 4 h access period, average ethanol intake was reduced by approximately 40, 35 and 50% in the 2.5, 5, and 10 mg/kg SR-141716-dosed rats, respectively, in comparison to vehicle-treated rats.

SR-141716 treatment slightly affected water intake \[ F_{\text{treatment}}(3;11) = 3.489, P < 0.05; F_{\text{time}}(3;11) = 9.767, P < 0.0001; F_{\text{interaction}} (9;11) = 0.603, P > 0.05 \], namely inducing: (a) a reduction at the highest dose (10 mg/kg) at the third and last observation time; (b) an increase at the lowest dose (2.5 mg/kg) at the end of the
Fig. 1. Effects of acute administration of the cannabinoid CB₁ receptor antagonist SR-141716 on ethanol, water, and food intake by Sardinian alcohol-preferring (sP) rats.

SR-141716 [0 (○), 2.5 (■), 5 (●), and 10 (□) mg/kg] was administered i.p. Ethanol (g/kg; top), water (ml/kg; centre), and food (g/kg; bottom) intakes were measured in sP rats having access to ethanol, water, and food in daily 4 h sessions (the first 4 h of the dark phase). Water was available during the remaining 20 h. Each point is the mean ± SEM (bars) of seven subjects. *P < 0.05 with respect to vehicle-treated rats (Scheffé test).

Finally, ANOVA revealed a significant effect of SR-141716 administration on food intake [F\text{treatment}(3;111) = 12.493, P < 0.0001; F\text{time}(3;111) = 38.624, P < 0.0001; F\text{interaction}(9;111) = 0.513, P > 0.05] (Fig. 1, bottom). However, at doses of 2.5 and 5 mg/kg, the reduction in food intake was confined to the first two time intervals; at the end of the session, average food intake in the 2.5 and 5 mg/kg SR-141716-dosed rats was virtually identical to that in vehicle-treated rats. In contrast, reduction of food intake exerted by 10 mg/kg SR-141716 lasted throughout the 4 h session.

Experiment 2: effect of SR-141716 on blood ethanol levels in Wistar rats

ANOVA failed to reveal any significant effect of 5 mg/kg SR-141716 on the pharmacokinetic profile of ethanol [F\text{treatment}(1;80) = 7.56, P > 0.05; F\text{time}(4;80) = 3.65, P < 0.01; F\text{interaction}(4;80) = 4.02, P > 0.05] (Table 1).

DISCUSSION

The results of Experiment 1: (a) demonstrate that acute administration of the selective cannabinoid CB₁ receptor antagonist, SR-141716, reduced voluntary ethanol intake in selectively bred, ethanol-preferring sP rats; (b) confirm and extend to a second ethanol-preferring rodent line the results of a recent study by Arnone et al. (1997) reporting a significant decrease of voluntary ethanol intake in C57BL/6 mice; (c) strengthen the hypothesis that the cannabinoid CB₁ receptor plays a role in the mediation of ethanol reinforcing properties (Arnone et al., 1997).

Besides its attenuating effect on ethanol intake, SR-141716 also reduced food consumption. This result is consistent with previous data from this laboratory, demonstrating an anorectic effect of SR-141716 in both Wistar and genetically obese Zucker rats (G. Colombo et al., in preparation). However, the results of the present study indicate that ethanol and food intakes were differentially affected by SR-141716 administration, at least within a certain dose range; SR-141716 being more effective on ethanol intake than on food intake. Indeed, the effects of the 2.5 and 5 mg/kg doses of SR-141716 on food intake were limited to
Table 1. Effect of acute administration of the cannabinoid CB₁ receptor antagonist SR-141716 on blood ethanol concentrations in Wistar rats

<table>
<thead>
<tr>
<th>SR-141716 (mg/kg)</th>
<th>Time interval (min) from ethanol administration</th>
<th>Blood ethanol concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>5</td>
<td>0 ± 0</td>
<td>95.0 ± 7.0</td>
</tr>
</tbody>
</table>

Ethanol (2 g/kg, i.g.) was administered 20 min after SR-141716 or vehicle i.p. injection. Blood samples were collected at different time intervals after ethanol administration. Each point is the mean ± SEM of nine subjects.

the first 2 h of the session, whereas the mean amount of food consumed at the end of the session was virtually identical to that in vehicle-treated rats. In contrast, ethanol intake was significantly lower in 2.5 and 5 mg/kg SR-141716-treated, than vehicle-treated, rats at each observation time as well as at the end of the session. A lack of selectivity was observed with the highest SR-141716 dose tested (10 mg/kg), which reduced to a similar extent both ethanol and food intakes.

The higher selectivity of SR-141716 on ethanol intake than on food intake appears to be dependent on the temporal availability of food. In the present study, SR-141716 differentially affected ethanol and food intakes in sP rats having food available solely during the daily drinking session; in contrast, in a previous experiment from this laboratory with no food restrictions, 2.5 and 5 mg/kg SR-141716 induced a parallel reduction of both ethanol and food intake (unpublished data). Thus, the temporally limited availability of food and, subsequently, its higher appetitive value allowed the disclosure of the selective effect of SR-141716 on ethanol intake.

Water intake was slightly modified by the administration of 2.5 and 5 mg/kg SR-141716, resulting in a modest increase only at the end of the drinking session in the lowest SR-141716 dose-treated group. The lack of a robust increase in water intake compensating for the reduced consumption of ethanol solution (a condition usually considered essential in animal studies where voluntary ethanol intake is pharmacologically manipulated) was likely to be due to the experimental procedure employed in the present study. Ethanol intake in ethanol-prefering rats is motivated by the search for specific pharmacological effects of ethanol and persists until these effects are perceived (Colombo, 1997). Under the limited access paradigm (as used in this study), ethanol-prefering rats tend to concentrate the ethanol drinking episodes temporally because of the restricted availability, and to consume a volume of ethanol solution that is usually greater than that needed for simply satisfying fluid requirements (Colombo, 1997). Thus, the reduction of ethanol intake observed in the present study after SR-141716 administration might not have affected the rats’ fluid needs but concerned the extra volume consumed by sP rats for the hedonic properties of ethanol.

Finally, the results of Experiment 2 demonstrate that the SR-141716-induced reduction of ethanol intake was not the result of any effect on ethanol pharmacokinetics. Indeed, although determined in unselected Wistar rats and not in sP rats, no difference in BELs was observed between SR-141716- and vehicle-treated rats.

In conclusion, the results of the present study and those recently reported by Arnone et al. (1997): (a) suggest the involvement of the cannabinoid CB₁ receptor in the neuronal circuitry mediating the reinforcing effects of ethanol in ethanol-preferring sP rats and C57BL/6 mice; (b) open promising avenues for future research in the field of the neurobiology of alcohol and of potentially effective drugs in the treatment of alcoholism.

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