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# Boosting effect of morphine on alcohol drinking is suppressed not only by naloxone but also by the cannabinoid $CB_1$ receptor antagonist, SR 141716

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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 the ability of low and high doses of morphine to, respectively, augment and suppress voluntary alcohol intake in selectively bred Sardinian alcohol-preferring rats. Acute administration of a low dose of morphine (1 mg/kg, s.c.) produced a specific and marked increase in alcohol intake, which correlated with an increase in blood alcohol levels and was prevented by either SR 141716 (0.3 mg/kg, i.p.) or naloxone (0.1 mg/kg, i.p.). A higher dose (10 mg/kg, s.c.) of morphine reduced both alcohol and food intakes and produced sedation and hypomotility. The suppressant effect of morphine on alcohol intake was blocked by naloxone (0.1 mg/kg, i.p.) but not by SR 141716 (0.3 mg/kg, i.p.). These results are in agreement with those showing the ability of SR 141716 to antagonize the appetitive and positive reinforcing properties of morphine and add further support to the hypothesis of the existence of a functional link between the action of opioids and of cannabinoids. 

*Keywords:* Morphine; Naloxone; Cannabinoid; SR 141716; Alcohol intake; Sardinian alcohol-preferring (sP) rat

## 1. Introduction

It has been repeatedly reported that the acute and chronic administration of low doses of morphine (1–2.5 mg/kg, s.c.; 1–10 μg, i.c.v.) stimulates alcohol intake in rats as tested using various procedures (e.g.: Reid and Hunter, 1984; Hubbell et al., 1986; Czirr et al., 1987; Hubbell et al., 1987; Reid et al., 1987; Linseman and Harding, 1990; Wild and Reid, 1990; Nichols et al., 1991; Reid et al., 1991; Hubbell et al., 1993; Hodge et al., 1995). It has been suggested that alcohol activates opioid receptors, by increasing β-endorphin release and/or the affinity of opioid receptors for endogenous opioids (see Ulm et al., 1995; Herz, 1997) and that morphine functions as a "primer" for the additional opioid stimulation derived from the increased alcohol intake (see Ulm et al., 1995). On the other hand, higher doses of morphine (17–60 mg/kg, s.c. or i.p.) have been found to suppress alcohol intake in rats (Sinclair et al., 1973; Sinclair, 1974; Reid et al., 1987; Hodge et al., 1995), the likely result of the replacement of the reinforcing properties of alcohol by those of morphine (see Ulm et al., 1995).

Similarly to morphine, the cannabinoid receptor agonists, WIN 55,212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl] -1-naphthalenylmethanone] and CP 55,940 [(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol], have been found to enhance alcohol drinking (Colombo et al., 2002) and to promote the motivation to consume alcohol (Gallate et al., 1999) in rats, extending to stimulation of alcohol intake the similarity in pharmacological profiles of opioids and cannabinoids (see Manzanares et al., 1999). Interestingly, the stimulating effect of WIN 55,212-2 and CP 55,940 on alcohol intake and motivation to consume alcohol was completely abolished by

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pretreatment with the opioid receptor antagonist, naloxone (Gallate et al., 1999; Colombo et al., 2002), adding further support to the hypothesis of the existence of functional links between the action of opioids and that of cannabinoids (see Manzanares et al., 1999).

In order to further examine the interaction of opioids and cannabinoids in the control of alcohol seeking behavior, we investigated whether the cannabinoid $CB_1$ receptor antagonist, SR 141716 (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide), was able to block the changes produced by morphine in voluntary alcohol intake in rats. The present study was done with the Sardinian alcohol-preferring (sP) rats, one of the few lines of rats selectively bred for high alcohol preference and consumption.

## 2. Methods

### 2.1. Animals

Male sP rats, from the 49th generation and approximately 5 months old, were used. The rats were from a population of sP rats which had undergone caesarian delivery at Charles River (Lyon, France) for production of Specific Pathogen Free individuals. The rats were individually housed in standard plastic cages with wood-chip bedding. The animal facility had an inverted 12:12 h light–dark cycle (lights on at 23:00), at a constant temperature of $22 \pm 2$ °C and relative humidity of approximately 60%.

Animal care and experimental procedures employed in the present study were approved by the Ethical Committee of the University of Cagliari.

### 2.2. Procedures of the alcohol intake tests

The rats were continuously offered two bottles containing alcohol (10% v/v, in tap water) and tap water, respectively. The 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. The rats were habituated to handling and intraperitoneal and subcutaneous injection. After approximately 1 week of habituation to the experimental regimen, all rats showed stable daily intakes of alcohol, water and food.

The dose–response for the effect of morphine on alcohol intake was first determined. To this aim, the rats were divided into five groups ($n = 8$) matched for daily alcohol, water and food intakes during the 3 preceding days. Morphine (sulfate; Salars, Como, CO, Italy) was dissolved in 1 ml/kg saline and injected s.c. at the doses of 0, 0.3, 1, 3 and 10 mg/kg 20 min before lights off. Alcohol, water and food intakes were monitored by weighing the bottles and food pellets (0.1 g accuracy) 60 min after lights off. Data for alcohol, water and food intakes were expressed in g/kg, ml/kg and g/kg, respectively, and analyzed by one-way analysis of variance (ANOVA), followed by the Newman–Keuls test for multiple comparisons.

In blockade tests, the rats were divided into groups ($n = 8$) matched for daily alcohol, water and food intakes during the 3 preceding days, and given SR 141716 or naloxone 10 min prior to administration of morphine. SR 141716 (Sanofi-Synthelabo, Montpellier, France) was suspended in 1 ml/kg saline with 0.1% Tween 80 and injected intraperitoneally at the doses of 0 and 0.3 mg/kg. Naloxone (Salars) was dissolved in 2 ml/kg saline and injected intraperitoneally at the doses of 0 and 0.1 mg/kg. Morphine was prepared as previously specified and injected, in two separate tests, at the doses of 0 and 1 mg/kg or 0 and 10 mg/kg 20 min before lights off. Alcohol, water and food intakes were monitored 60 min after lights off and analyzed by one-way ANOVA, followed by the Newman–Keuls test for multiple comparisons.

### 2.3. Procedure for testing blood alcohol levels

A separate experiment assessed the blood alcohol levels deriving from the stimulating effect of 1 mg/kg morphine on voluntary alcohol intake. The rats were offered unlimited access to alcohol (10%, v/v) and water under the two-bottle free-choice regimen described above for a period of time comparable to that of rats used for the alcohol intake studies. On the test day, the rats were divided into two groups ($n = 7–8$), matched for daily alcohol, water and food intakes during the 3 preceding days, and injected subcutaneously with saline (1 ml/kg) or morphine (1 mg/kg in 1 ml/kg saline) 20 min before lights off. Food pellets and alcohol bottles were removed 12 and 3 h before the test, respectively. Alcohol bottles were re-exposed at lights off; food pellets were re-presented after termination of the experiment. Blood samples (50 μl) were collected from the tip of the tail of each rat 60 min after lights off. At that time, alcohol intake was also recorded (intake of the first hour of the dark phase).

Blood samples were analyzed by means of an enzymatic system [AM1 Analyser (Analox Instruments, London, UK)] based on measurement of oxygen consumption in the alcohol–acetaldehyde reaction. Data on alcohol intake and blood alcohol levels were expressed in g/kg and mg/dl, respectively, and analyzed by the Mann–Whitney test.

## 3. Results

Morphine modified voluntary alcohol intake in a dose-dependent manner [$F_{\text{treatment}}(4;35) = 7.33$, $P < 0.001$] (Fig. 1, left panel). Alcohol intake was increased by approximately 70% and decreased by approximately 60% by 1 and 10 mg/kg morphine, respectively, in comparison to that of

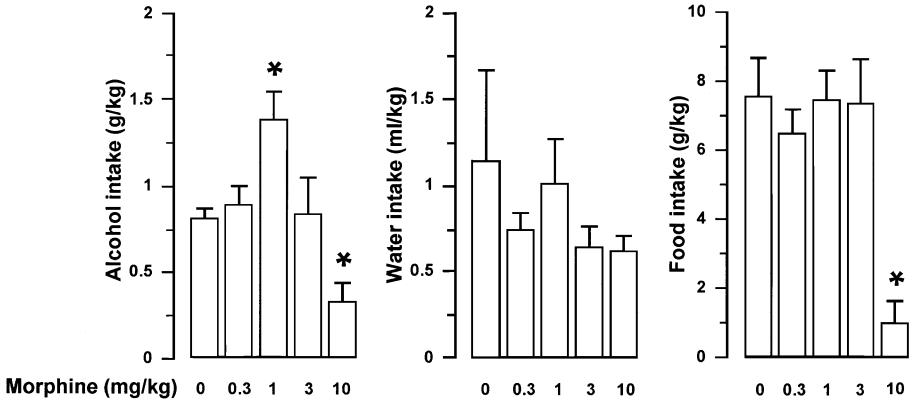


Fig. 1. Effect of the acute administration of morphine on alcohol (g/kg; left panel), water (ml/kg; center panel) and food (g/kg; right panel) intake in Sardinian alcohol-preferring (sP) rats. Alcohol (10%, v/v) and water were offered under the two-bottle, free-choice regimen with unlimited access for 24 h per day. Food pellets were always available. Alcohol, water and food intake was monitored 60 min after lights off. Morphine (0, 0.3, 1, 3 and 10 mg/kg, s.c.) was administered 20 min before lights off. Each bar is the mean ± S.E.M. for $n = 8$ rats. *: $P < 0.05$ with respect to saline-treated rats (Newman–Keuls test).

saline-treated rats. Treatment with morphine failed to significantly affect water intake [$F_{\text{treatment}}(4;35) = 0.73$, $P > 0.05$] (Fig. 1, center panel), while food intake was significantly reduced only by the 10-mg/kg dose of morphine [$F_{\text{treatment}}(4;35) = 8.77$, $P < 0.0001$] (Fig. 1, right panel). As revealed in a separate experiment using an open-field arena, 10 mg/kg morphine also inhibited, by approximately 80%, the spontaneous locomotor activity in sP rats (data not shown).

In a separate experiment, we evaluated whether the effect of 1 mg/kg morphine on alcohol intake was correlated with increased blood alcohol levels. Morphine stimulated alcohol intake by approximately 40% (Fig. 2), when compared to that of saline-dosed rats, and increased blood alcohol levels by about 50% with respect to those in saline-treated rats.

Pretreatment with SR 141716 (0.3 mg/kg), which did not affect alcohol intake per se, suppressed the stimulating effect of 1 mg/kg morphine on alcohol intake [$F(3;28) = 7.28$, $P < 0.001$] (Fig. 3, left panel); in contrast, as revealed by the post hoc analysis, SR 141716 was ineffective to prevent the inhibitory effect of 10 mg/kg morphine on alcohol intake [$F(3;28) = 45.57$, $P < 0.0001$] (Fig. 3, right panel). SR 141716 also failed to prevent the suppressant effect of 10 mg/kg morphine on food intake (data not shown). On the other hand, a dose of 0.1 mg/kg naloxone, which failed to alter alcohol intake when given alone, antagonized both the increasing effect of 1 mg/kg morphine [$F(3;28) = 4.29$,

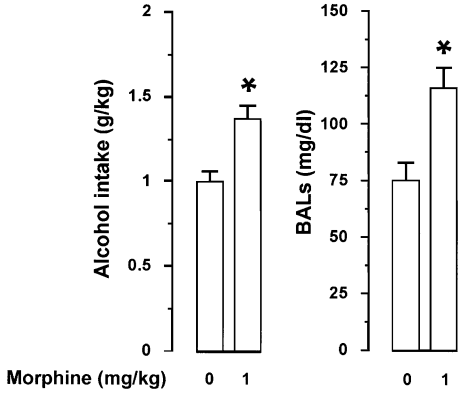


Fig. 2. Effect of the acute administration of 1 mg/kg morphine (s.c.) on voluntary alcohol intake (g/kg) (left panel) and the resulting blood alcohol levels (BALs; mg/dl) (right panel) in Sardinian alcohol-preferring (sP) rats. Alcohol (10%, v/v) was offered under the two-bottle, free-choice regimen with water and unlimited access for 24 h per day. Alcohol intake was monitored 60 min after lights off. At that time, blood samples were collected from the tip of the tail of each rat. Saline and morphine were administered 20 min before lights off. Each bar is the mean ± S.E.M. for $n = 7–8$ rats. *: $P < 0.05$ with respect to vehicle-treated rats (Mann–Whitney test).

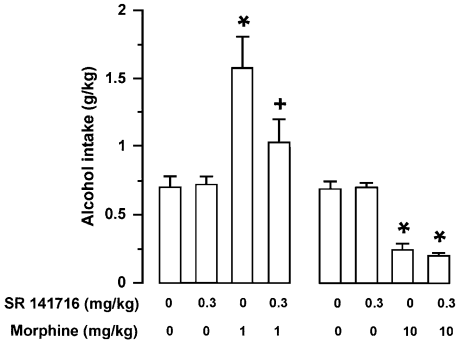


Fig. 3. Effect of the cannabinoid $CB_1$ receptor antagonist, SR 141716, on the stimulating (left panel) and suppressing (right panel) effect of 1 and 10 mg/kg morphine (s.c.), respectively, on alcohol intake (g/kg) in Sardinian alcohol-preferring (sP) rats. Alcohol (10%, v/v) was offered under the two-bottle, free-choice regimen with water and unlimited access for 24 h per day. Food pellets were always available. Alcohol intake was monitored 60 min after lights off. SR 141716 (0.3 mg/kg, i.p.) was injected 10 min before morphine administration. Morphine was administered 20 min before lights off. Each bar is the mean ± S.E.M. for $n = 8$ rats. * $P < 0.05$ with respect to 0 mg/kg SR 141716 plus 0 mg/kg morphine-treated rats (Newman–Keuls test); +: $P < 0.05$ with respect to 0 mg/kg SR 141716 plus 1 mg/kg morphine-treated rats (Newman–Keuls test).

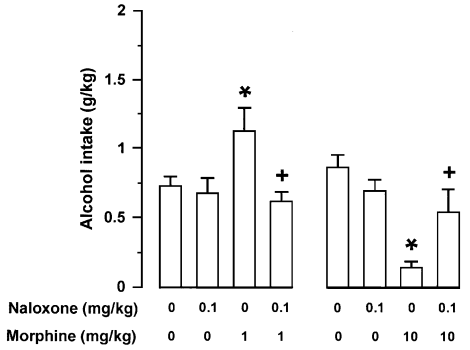


Fig. 4. Effect of the opioid receptor antagonist, naloxone, on the stimulating (left panel) and suppressing (right panel) effect of 1 and 10 mg/kg morphine (s.c.), respectively, on alcohol intake (g/kg) in Sardinian alcohol-preferring (sP) rats. Alcohol (10%, v/v) was offered under the two-bottle, free-choice regimen with water and unlimited access for 24 h per day. Food pellets were always available. Alcohol intake was monitored 60 min after lights off. Naloxone (0.1 mg/kg, i.p.) was injected 10 min before morphine administration. Morphine was administered 20 min before lights off. Each bar is the mean ± S.E.M. for $n=8$ rats. *: $P<0.05$ with respect to 0 mg/kg naloxone plus 0 mg/kg morphine-treated rats (Newman–Keuls test); +: $P<0.05$ with respect to 0 mg/kg naloxone plus 1 mg/kg morphine-treated rats in the left panel and 0 mg/kg naloxone plus 10 mg/kg morphine-treated rats in the right panel (Newman–Keuls test).

$P<0.05$] (Fig. 4, left panel) and the inhibitory effect of 10 mg/kg morphine [$F(3;28)=8.89$, $P<0.001$] (Fig. 4, right panel) on alcohol intake.

## 4. Discussion

The effect of morphine on alcohol intake now found confirms previous observations (see Introduction for references) showing that low doses of the drug stimulate, while high doses suppress, the intake of alcohol in rats. The low dose of 1 mg/kg morphine specifically stimulated alcohol intake, being totally ineffective on food consumption. Further, morphine produced an increase in blood alcohol levels proportional to the increase in alcohol intake, which excludes the possibility that rats increased their intake of alcohol to compensate for a reduced alcohol absorption secondary to morphine-induced inhibition of gastric emptying (Hubbell et al., 1993).

A morphine-induced boost in alcohol drinking might be interpreted as an increased appetite for alcohol, similarly to the increased appetition for alcohol observed after a period of alcohol abstinence [the so-called "alcohol deprivation effect" (e.g.: Agabio et al., 2000)]. Both conditions are blocked by the opioid receptor antagonist, naloxone (alcohol deprivation effect: Hölter and Spanagel, 1999; morphine-induced stimulation: present study) and by the cannabinoid $CB_1$ receptor antagonist, SR 141716 (alcohol deprivation effect: Serra et al., 2002; morphine-induced stimulation: present study), suggesting that they are mediated by the same neuronal mechanism.

The finding that alcohol drinking can be stimulated interchangeably by either morphine or cannabinoids and is suppressed by either naloxone or SR 141716 may suggest the hypothesis that $CB_1$ and opioid receptors involved in this effect coexist in the same neuron, and that their concomitant activation is needed for the boosting effect to occur. Accordingly, the fact that blockade of one or the other receptor prevents the response indicates that the morphine effect is permitted by the concomitant activation of $CB_1$ receptors by endogenous cannabinoids and, vice versa, opioid receptor activation by endogenous opioids is needed for the cannabinoid response.

The mutually permissive role of cannabinoid and opioid receptors does not seem to be restricted to the boosting effect on alcohol drinking since SR 141716 has been shown to antagonize the appetitive and positive reinforcing properties of opioid receptor activation, including morphine and heroin self-administration (Braida et al., 2001b; Navarro et al., 2001) and acquisition of conditioned place preference to morphine and heroin (Chaperon et al., 1998; Mas-Nieto et al., 2001; Navarro et al., 2001) in rats and mice. Conversely, naloxone has been reported to antagonize the self-administration of the cannabinoid receptor agonists, CP 55,940 (Braida et al., 2001b), WIN 55,212-2 (Navarro et al., 2001) and HU 210 [(6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol] (Navarro et al., 2001), as well as the conditioned place preference induced by CP 55,940 (Braida et al., 2001a) in rodents.

The inhibition of alcohol intake produced by 10 mg/kg morphine might reflect the replacement of the reinforcing properties of alcohol by those of morphine (see Ulm et al., 1995). It should be noted, however, that the decrease in alcohol intake produced by 10 mg/kg morphine was accompanied by a similar reduction in food intake as well as by marked hypomotility, as observed in a separate test in the open-field arena, which might be incompatible with normal rates of drinking and eating. This observation makes sedation the most likely explanation of the reducing effect of 10 mg/kg morphine on alcohol intake. Unlike the stimulatory effect, the morphine-induced inhibition of alcohol intake was antagonized by naloxone but not by SR 141716, suggesting that different neuronal mechanisms may underlie the two phenomena. The inability of SR 141716 to antagonize the reducing effect of the high dose of morphine is consistent with the recently reported failure of 1 mg/kg SR 141716 (i.e., a dose threefold higher than that used in the present study) to prevent the sedation and motor impairment produced by 10 mg/kg morphine in rats (Brodkin and Moerschbaecher, 1997). Testing higher doses of SR 141716, which could theoretically attenuate the suppressing effects of morphine, would, however, have been of limited interest within the frame of the present work, because they would themselves have reduced alcohol intake (Arnone et al., 1997; Colombo et al., 1998; Gallate and McGregor, 1999; Freedland et al., 2001; Lallemand et al., 2001; Serra et al., 2001).

In conclusion, the results of the present study demonstrate the ability of SR 141716 to antagonize the stimulating effect of a low dose of morphine on alcohol intake in alcohol-preferring sP rats, and add a further piece of evidence to the hypothesised cross-talk between opioid and cannabinoid brain systems.

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