Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 81 (2005) 369 – 380

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Review

Endocannabinoid system and alcohol addiction: Pharmacological studies

Giancarlo Colombo[a,*], Salvatore Serra[b], Giovanni Vacca[b], Mauro A.M. Carai[a,b], Gian Luigi Gessa[a,b]

[a]*C.N.R. Institute of Neuroscience, Viale Diaz, 182 I-09126 Cagliari (CA), Italy*
[b]*Bernard B. Brodie Department of Neuroscience, University of Cagliari, I-09126 Cagliari, Italy*

Received 2 July 2004; received in revised form 15 December 2004; accepted 17 January 2005

Abstract

The present paper describes the results of recent pharmacological studies implicating the cannabinoid CB_1 receptor in the neural circuitry regulating alcohol consumption and motivation to consume alcohol. Cannabinoid CB_1 receptor agonists have been found to specifically stimulate alcohol intake and alcohol's motivational properties in rats. Conversely, the cannabinoid CB_1 receptor antagonist, SR 141716, has been reported to specifically suppress acquisition and maintenance of alcohol drinking behavior, relapse-like drinking and alcohol's motivational properties in rats. More recent data indicate that opioid receptor antagonists a) blocked the stimulatory effect of cannabinoids on alcohol intake, and b) synergistically potentiated the suppressing effect of SR 141716 on alcohol intake and alcohol's motivational properties. Consistently, SR 141716 blocked the stimulatory effect of morphine on alcohol intake. These results suggest a) the existence of a functional link between the cannabinoid and opioid receptor systems in the control of alcohol intake and motivation to consume alcohol, and b) that novel and potentially effective therapeutic strategies for alcoholism may come from the combination of cannabinoid and opioid receptor antagonists.

Keywords: Cannabinoid CB_1 receptor; WIN 55,212-2; CP 55,940; SR 141716 (rimonabant); Opioid receptor; Naloxone/Naltrexone; Alcohol intake; Alcohol's motivational properties; Sardinian alcohol-preferring (sP) rats

Contents

1. Introduction

The results of recent pharmacological studies suggest the involvement of the brain cannabinoid CB_1 receptor in the neural circuitry regulating alcohol consumption and moti-

* Corresponding author. Tel.: +39 070 302227; fax: +39 070 302076.
E-mail address: colomb@unica.it (G. Colombo).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.01.022

vation to consume alcohol in different rodent models of excessive alcohol consumption. These results invariably suggest that cannabinoid receptor agonists and antagonists stimulate and suppress, respectively, alcohol intake, alcohol self-administration and alcohol's motivational properties.

The present paper is intended to briefly review these data. Particular attention will be dedicated to the recently collected data suggesting the existence of an interaction between the cannabinoid and opioid receptor systems in the control of alcohol seeking and drinking behavior.

2. Stimulating effect of cannabinoid CB_1 receptor agonists on alcohol drinking and seeking behaviors

Recent work from this laboratory (Colombo et al., 2002) demonstrated that the acute administration of the cannabinoid receptor agonists WIN 55,212-2 and CP 55,940 markedly stimulated voluntary alcohol intake in Sardinian alcohol-preferring (sP) rats, one of the few rat lines selectively bred worldwide for high alcohol preference and consumption. When exposed to the standard, homecage 2-bottle free choice between an alcohol solution (10% v/v, in water) and water with unlimited access for 24 h/day, sP rats usually consume approximately 6 g alcohol per kg body weight over the 24 h and 0.7–0.8 g/kg alcohol during the first hour of the dark phase of the light/dark cycle (e.g.: Serra et al., 2001; Colombo et al., 2002). Voluntary alcohol intake in sP rats has been found to give rise to blood alcohol levels in the 50–70 mg% range (Colombo et al., 2002), and to produce specific psychopharmacological effects, including amelioration of anxiety-related behaviors (Colombo et al., 1995) and motor stimulation (Colombo et al., 1998b).

The study (Colombo et al., 2002) testing the effect of WIN 55,212-2 and CP 55,940 on alcohol intake used alcohol-experienced sP rats, that is rats in which the consumption of pharmacologically relevant doses of alcohol was already established before administration of the cannabinoid receptor agonists; these rats have been proposed to model the "active drinking" phase of human alcoholism. Rats were individually housed and exposed to the 2-bottle free choice regimen for 8 consecutive weeks. Food pellets were continuously available. WIN 55,212-2 (0.5, 1 and 2 mg/kg, i.p.) and CP 55,940 (3, 10 and 30 μg/kg, i.p.) were acutely injected 20 min before lights off. Alcohol, water and food intake was recorded 1 h after lights off.

Both drugs produced a dose-dependent increase in alcohol intake. At the doses of 2 mg/kg WIN 55,212-2 and 30 μg/kg CP 55,940, alcohol intake was increased by 70–90% in comparison to baseline levels (vehicle-treated rats). The selectivity of the stimulating effect of WIN 55,212-2 and CP 55,940 on alcohol intake was demonstrated by the lack of significant effect on water and food intake as well as by the ineffectiveness of both drugs to alter sucrose intake in a separate group of sP rats given a sucrose solution (1.5–15% v/v, in water) and water under the 2-bottle free choice.

The increase in alcohol intake induced by 2 mg/kg WIN 55,212-2 and 30 μg/kg CP 55,940 was associated to a marked increase in blood alcohol levels, suggesting that activation of the cannabinoid CB_1 receptor likely shifted upward the hypothesized hedonic set-point that control alcohol drinking behavior in sP rats. The observed increase in blood alcohol levels led to exclude that rats increased their alcohol intake to compensate for a reduced alcohol absorption secondary to cannabinoid-induced inhibition of gastric emptying.

Pretreatment with the cannabinoid CB_1 receptor antagonist, SR 141716 (rimonabant), given at a dose (0.3 mg/kg, i.p.) that did not alter alcohol intake per se, resulted in the complete blockade of the stimulating effect of 2 mg/kg WIN 55,212-2 and 30 μg/kg CP 55,940 on alcohol intake (Fig. 1, panels A and B), suggesting that the effect of WIN 55,212-2 and CP 55,940 was indeed secondary to activation of the cannabinoid CB_1 receptor.

In agreement with the above results, Gallate and coworkers (1999) demonstrated that the acute administration of CP 55,940 increased the motivation to consume alcohol in Wistar rats. In this study, rats were initially trained to repeatedly lick on a tube spout to obtain the delivery of a drop of beer (containing 4.5% v/v alcohol). Once this behavior was established, the effect of CP 55,940 (10–50 μg/kg, i.p.) on the break-point for beer was determined. Specifically, on the test session the number of licks to be emitted for each beer delivery was progressively increased. Break-point for beer was defined as the number of licks at which responding on the tube spout ceased. Break-point represented a reliable index of motivation to consume beer. The results of the study by Gallate et al. (1999) demonstrated that CP 55,940 dose-dependently increased the break-point for beer; at the dose of 30 μg/kg, break-point for beer was increased by 35–40% with respect to vehicle-dosed rats. The stimulating effect of CP 55,940 on break-point for beer was completely prevented by the acute treatment with SR 141716 (1.5 mg/kg, i.p.).

At variance with the results of the study with sP rats (see above), where WIN 55,212-2 and CP 55,940 increased alcohol intake without altering food and sucrose consumption, the study by Gallate and colleagues (1999) reported a concomitant increase in the break-point for a sucrose solution (8.6%, w/v) or a beer-like beverage (containing <0.5% v/v alcohol), posing some question on the specificity of CP 55,940 action. A possible explanation for this discrepancy may reside, beside the differences in the methodological procedures and behaviors under investigation (ingestive vs seeking behaviors), in the rat strains used in the two studies. Accordingly, selectively bred alcohol-preferring sP rats might possess, when compared to unselected Wistar rats, a greater sensitivity to the stimulating effect of cannabinoids on different aspects of alcohol drinking behavior.

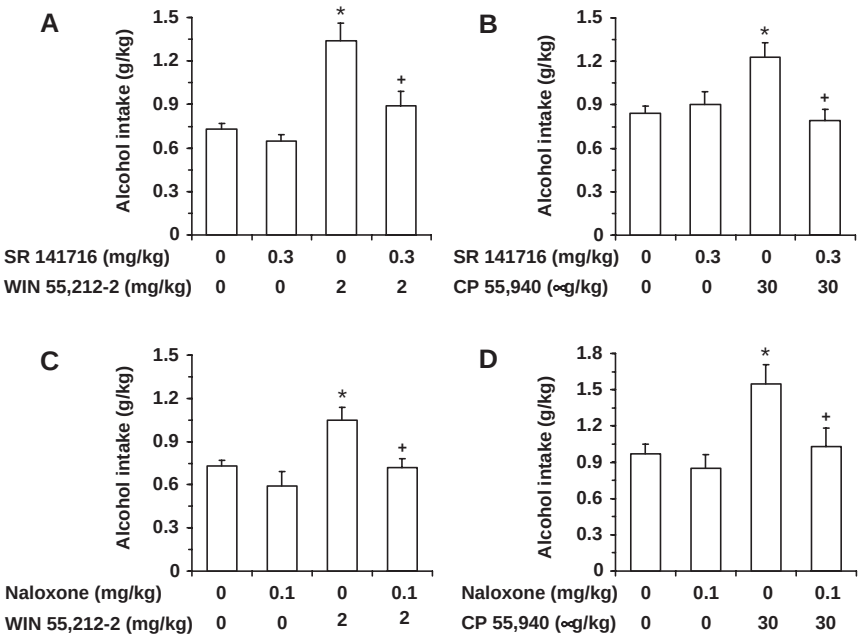

Fig. 1. Suppressing effect of the cannabinoid CB_1 receptor antagonist, SR 141716 (panels A and B), and opioid receptor antagonist, naloxone (panels C and D), on the stimulatory effect of the cannabinoid receptor agonists, WIN 55,212-2 (panels A and C) and CP 55,940 (panels B and D), on alcohol intake in Sardinian alcohol-preferring (sP) rats. Alcohol (10% v/v) was offered under the standard, homecage 2-bottle free choice regimen with water and unlimited access for 24 h/day. Food pellets were always available. Alcohol intake was recorded 60 min after lights off. SR 141716 (0 and 0.3 mg/kg, i.p.) and naloxone (0 and 0.1 mg/kg, i.p.) were injected 10 min before administration of WIN 55,212-2 (0 and 2 mg/kg, i.p.) and CP 55,940 (0 and 30 μg/kg, i.p.). WIN 55,212-2 and CP 55,940 were administered 20 min before lights off. Each bar is the mean ± SEM of $n = 16$ rats. *$P < 0.05$ with respect to 0 mg/kg SR 141716 plus 0 mg/kg WIN 55-212,2-treated rats in panel A, 0 mg/kg SR 141716 plus 0 μg/kg CP 55,940-treated rats in panel B, 0 mg/kg naloxone plus 0 mg/kg WIN 55-212,2-treated rats in panel C, and 0 mg/kg naloxone plus 0 μg/kg CP 55,940-treated rats in panel D (Newman–Keuls test); +$P < 0.05$ with respect to 0 mg/kg SR 141716 plus 2 mg/kg WIN 55-212,2-treated rats in panel A, 0 mg/kg SR 141716 plus 30 μg/kg CP 55,940-treated rats in panel B, 0 mg/kg naloxone plus 2 mg/kg WIN 55-212,2-treated rats in panel C, and 0 mg/kg naloxone plus 30 μg/kg CP 55,940-treated rats in panel D (Newman–Keuls test). Reprinted with permission from Springer-Verlag, in Colombo et al., Psychopharmacology 2002; 159:181–7.

As a final observation, consistent with the above results, the acute administration of the endocannabinoid, anandamide (1 mg/kg, s.c.), stimulated by approximately 45% the 24 h/day intake of alcohol in alcohol-consuming C57BL/6J mice tested under the standard, 2-bottle free choice paradigm (Wang et al., 2003). As predictable, this effect was blocked by pretreatment with 3 mg/kg SR 141716 (i.p.) (Wang et al., 2003).

3. Interaction of the cannabinoid and opioid receptor systems in the control of alcohol drinking and seeking behaviors

Several research data suggest the existence of functional links between the mechanisms of action of opioids and cannabinoids (see Manzanares et al., 1999); this interaction apparently extends also to the control of addictive behaviors. For example, the opioid receptor antagonist, naloxone, has been found to block the self-administration of CP 55,940 and WIN 55,212-2 (Braida et al., 2001b; Navarro et al., 2001), as well as CP 55,940-induced conditioned place preference (Braida et al., 2001a) in rats; opioid receptor antagonists prevented the Δ^9-tetrahydrocannabinol-stimulated release of dopamine in the rat nucleus accumbens (Chen et al., 1990; Tanda et al., 1997), an event thought to be associated with the reinforcing properties of drugs of abuse; cross-sensitization between cannabinoids (namely, Δ^9-tetrahydrocannabinol and WIN 55,212-2) and morphine has been observed in rats (Cadoni et al., 2001); finally, Δ^9-tetrahydrocannabinol-induced conditioned place preference was absent in opioid μ receptor knockout mice (Ghozland et al., 2002).

Conversely, pretreatment with SR 141716 blocked the development of conditioned place preference to morphine (Chaperon et al., 1998; Mas-Nieto et al., 2001; Navarro et al., 2001), reduced heroin and morphine self-administration (Braida et al., 2001b; Navarro et al., 2001; De Vries et al., 2003) and reinstatement of heroin self-administration induced by heroin itself or a heroin-associated cue (De Vries et al., 2003; Fattore et al., 2003) in rodents; different cannabinoid receptor agonists have been found to reinstate heroin seeking behavior (De Vries et al., 2003; Fattore et al., 2003) and stimulate morphine self-administration (Norwood et al., 2003) in rats; in CB_1 receptor knockout mice, the reinforcing properties of morphine were weak (as evidenced by a reduced number of self-administration responses in knockout than wild-type mice) (Ledent et al., 1999; Cossu et al., 2001), and morphine failed to produce conditioned place preference (Martin et al., 2000; see however Rice et al.,

2002), induce behavioral sensitization (Martin et al., 2000) and stimulate dopamine release in the nucleus accumbens (Mascia et al., 1999).

On the wake of these results, this laboratory investigated the effect of naloxone pretreatment on the promoting effect of WIN 55,212-2 and CP 55,940 on alcohol intake in alcohol-experienced sP rats. Naloxone was administered acutely, 10 min before the injection of WIN 55,212-2 and CP 55,940, at a dose (0.1 mg/kg, i.p.) that did not alter alcohol intake when given alone. Similarly to the effect of SR 141716, pretreatment with naloxone completely antagonized the increasing effect of 2 mg/kg WIN 55,212-2 and 30 μg/kg CP 55,940 on alcohol intake (Fig. 1, panels C and D), suggesting the presence of an opioid component in the cannabinoid-mediated control of alcohol intake. Accordingly, naloxone (2.5 mg/kg, i.p.) also prevented CP 55,940-induced increase in break-point for beer in Wistar rats (Gallate et al., 1999).

A large body of experimental evidence indicates that administration of low to moderate doses of morphine (e.g., 1–2.5 mg/kg, s.c.) stimulates voluntary alcohol intake in rats tested under multiple experimental procedures (e.g.: Hubbell et al., 1993; Hodge et al., 1995), extending to facilitation of alcohol intake the similarity in pharmacological profiles of opioids and cannabinoids. As an example, the acute administration of 1 mg/kg morphine (s.c.), 20 min before lights off, specifically stimulated—by 50–100% over baseline-alcohol intake in sP rats during the first hour of the dark phase (Vacca et al., 2002a). This increase in alcohol intake resulted in a proportional increase in blood alcohol levels.

Antagonism experiments (Vacca et al., 2002a) found that morphine-induced stimulation of voluntary alcohol intake in sP rats was prevented by both naloxone and SR 141716. Indeed, pretreatment with either naloxone (0.1 mg/kg, i.p.) and SR 141716 (0.3 mg/kg, i.p.), 10 min before injection of morphine (1 mg/kg, s.c.), resulted in a complete blockade of the increasing effect of morphine on alcohol intake during the first hour of the dark phase of the light/dark cycle (Fig. 2).

Taken together, the results of these antagonism experiments with SR 141716 and naloxone suggest that the cannabinoid CB_1 and opioid receptors involved in the promoting effect of cannabinoids and morphine on alcohol intake likely coexist in the same neuronal circuitry, and that their concomitant activation is needed for the stimulating effect to occur. Accordingly, the fact that blockade of one or the other receptor prevents the response suggests 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.

4. Involvement of the $GABA_B$ and 5-HT_3 receptor systems in the stimulatory effect of WIN 55,212-2 and morphine on alcohol intake

Subsequently, we investigated whether other receptor systems are involved in the mediation of the stimulating effect of cannabinoids and morphine on alcohol intake in sP rats. Specifically, we tested the effect of pretreatment with the $GABA_B$ receptor agonist, baclofen (Colombo et al., 2004b), and the 5-HT_3 receptor antagonist, tropisetron (known also as ICS 205-930), on WIN 55,212-2- and morphine-stimulation of alcohol intake in sP rats. These experiments were conducted using the procedure previously used in the other antagonism experiments. Specifically, sP rats were continuously exposed to the 2-bottle, "alcohol vs water" free choice with unlimited access. Baclofen (0.5 and 1 mg/kg, i.p.) and tropisetron (5.6 and 10 mg/kg, i.p.) were administered acutely, 10 min before WIN 55,212-2 or morphine administration. Baclofen and tropisetron doses were chosen as to be ineffective on alcohol intake in sP rats

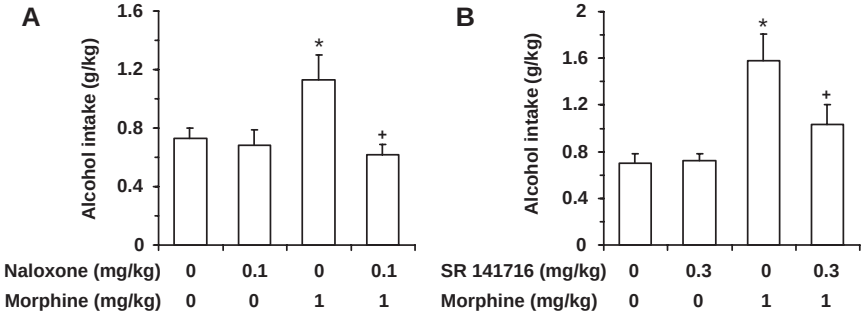

Fig. 2. Suppressing effect of the opioid receptor antagonist, naloxone (panel A), and cannabinoid CB_1 receptor antagonist, SR 141716 (panel B), on the stimulatory effect of morphine on alcohol intake in Sardinian alcohol-preferring (sP) rats. Alcohol (10% v/v) was offered under the standard, homecage 2-bottle free choice regimen with water and unlimited access for 24 h/day. Food pellets were always available. Alcohol intake was recorded 60 min after lights off. Naloxone (0 and 0.1 mg/kg, i.p.) and SR 141716 (0 and 0.3 mg/kg, i.p.) were injected 10 min before administration of morphine (0 and 1 mg/kg, s.c.). Morphine was administered 20 min before lights off. Each bar is the mean±SEM of $n=8$ rats. *$P<0.05$ with respect to 0 mg/kg naloxone plus 0 mg/kg morphine-treated rats in panel A, and 0 mg/kg SR 141716 plus 0 mg/kg morphine-treated rats in panel B (Newman–Keuls test); +$P<0.05$ with respect to 0 mg/kg naloxone plus 1 mg/kg morphine-treated rats in panel A, and 0 mg/kg SR 141716 plus 1 mg/kg morphine-treated rats in panel B (Newman–Keuls test). Reprinted with permission from Elsevier Publ., in Vacca et al., Eur J Pharmacol 2002;445:55–9.

when given alone. WIN 55,212-2 (2 mg/kg, i.p.) and morphine (1 mg/kg, s.c.) were injected acutely, 20 min before lights off. Alcohol intake was recorded 60 min after lights off.

Baclofen pretreatment produced a significant, dose-dependent blockade of the stimulatory effect of WIN 55,212-2 and morphine on alcohol intake (Colombo et al., 2004b). The dose of 1 mg/kg baclofen completely abolished the increase in alcohol intake produced by WIN 55,212-2 and morphine. Water and food intake was not significantly modified by drug treatment.

Similarly, pretreatment with tropisetron dose-dependently antagonized the stimulatory effect of WIN 55,212-2 [1-way ANOVA: $F(5,66)=5.20$, $P<0.0005$] (Fig. 3, panel A) and morphine [1-way ANOVA: $F(5,65)=3.88$, $P<0.005$] (Fig. 3, panel B) on alcohol intake. When given at the dose of 10 mg/kg, tropisetron completely abolished WIN 55,212-2- and morphine-induced increase in alcohol intake. Water and food intake was not significantly affected by drug treatment. In agreement with these results, Hodge and colleagues (1995) reported that tropisetron (10–17 mg/kg, i.p.) reduced the stimulating effect of morphine (1.5 mg/kg, s.c.) on alcohol intake in Long Evans rats given alcohol and water under the homecage 2-bottle free choice regimen.

These results suggest the involvement of the $GABA_B$ and 5-HT_3 receptors in the neural substrate mediating the stimulating effect of cannabinoids and morphine on alcohol consumption. We hypothesize that this substrate also includes the mesolimbic dopamine system. Indeed, low to moderate doses of cannabinoids and morphine have been found to stimulate the electrical activity of mesolimbic dopamine neurons (Gysling and Wang, 1983; Latimer et al., 1987; French et al., 1997; Wu and French, 2000), and dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988; Chen et al., 1990; Pontieri et al., 1995; Tanda et al., 1997), the terminal area of mesolimbic dopamine neurons arising in the ventral tegmental area. The latter actions are shared also by alcohol: low to moderate doses of alcohol indeed stimulate the activity of the mesolimbic dopamine system (Gessa et al., 1985; Imperato and Di Chiara, 1986; Brodie et al., 1990; Weiss et al., 1993; for review, see Weiss and Porrino, 2002).

It can be proposed that WIN 55,212-2- and morphine-induced stimulation of the mesolimbic dopamine system functioned as a "primer" on alcohol intake, mimicking the effect of alcohol on dopamine release (Ulm et al., 1995; Colombo et al., 2002). In other words, the stimulating effect of morphine and WIN 55,212-2 on dopamine release in the nucleus accumbens may have triggered alcohol intake to further stimulate the system. Conversely, $GABA_B$ and 5-HT_3 receptors, located on mesolimbic dopamine neurons or afferent neurons controlling the former (Bowery et al., 1987; Kilpatrick et al., 1987), would offset—when activated and blocked, respectively—this stimulation and the extra-intake of alcohol induced by WIN 55,212-2 and morphine. The observation that baclofen and tropisetron suppressed the increase in extracellular dopamine concentration induced by morphine (Carboni et al., 1989; Imperato and Angelucci, 1989; Pei et al., 1993; Fadda et al., 2003) and alcohol (Carboni et al., 1989; Wozniak et al., 1990; Campbell and McBride, 1995; Colombo et al., 2004a) in the rat nucleus accumbens strengthens this hypothesis.

5. Reducing effect of cannabinoid CB_1 receptor antagonists on alcohol drinking and seeking behaviors

Together with the above-mentioned studies with the cannabinoid receptor agonists, the results of different pharmacological investigations with the cannabinoid CB_1 receptor antagonist, SR 141716, have supported the hypothesis that the brain cannabinoid CB_1 receptor system plays a role in the control of alcohol preference and

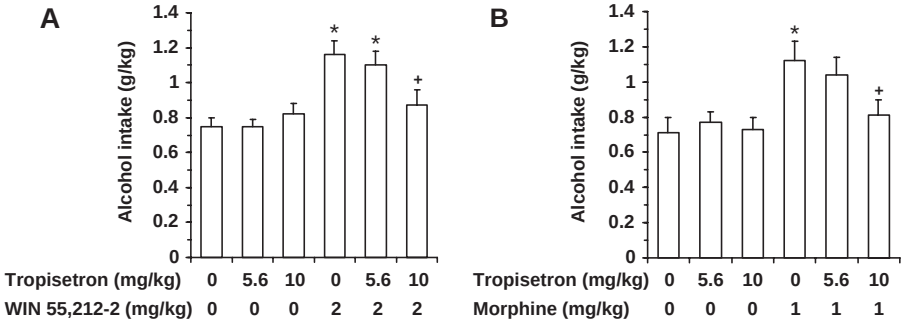

Fig. 3. Suppressing effect of the 5-HT_3 receptor antagonist, tropisetron (ICS 205-930), on the stimulatory effect of the cannabinoid receptor agonist, WIN 55,212-2 (panel A), and morphine (panel B) on alcohol intake in Sardinian alcohol-preferring (sP) rats. Alcohol (10% v/v) was offered under the standard, homecage 2-bottle free choice regimen with water and unlimited access for 24 h/day. Food pellets were always available. Alcohol intake was recorded 60 min after lights off. Tropisetron (0, 5.6 and 10 mg/kg, i.p.) was injected 10 min before administration of WIN 55,212-2 (0 and 2 mg/kg, i.p.) and morphine (0 and 1 mg/kg, s.c.). WIN 55,212-2 and morphine were administered 20 min before lights off. Each bar is the mean ± SEM of $n=11-12$ rats. *$P<0.05$ with respect to 0 mg/kg tropisetron plus 0 mg/kg WIN 55,212-2-treated rats in panel A, and 0 mg/kg tropisetron plus 0 mg/kg morphine-treated rats in panel B (Newman–Keuls test); +$P<0.05$ with respect to 0 mg/kg tropisetron plus 2 mg/kg WIN 55,212-2-treated rats in panel A, and 0 mg/kg tropisetron plus 1 mg/kg morphine-treated rats in panel B (Newman–Keuls test).

consumption and the mediation of alcohol reinforcing and motivational properties.

The repeated administration of SR 141716 has been recently found to completely prevent the acquisition of alcohol drinking behavior in alcohol-naive sP rats, that is rats which had never consumed alcohol before the start of the experiment. In this experiment (Serra et al., 2001), SR 141716 (0, 0.3, 1 and 3 mg/kg, i.p.) was administered twice a day for 10 consecutive days. Alcohol (10% v/v, in water) and water were offered under the 2-bottle free choice regimen immediately after the first injection of SR 141716. Food pellets were always available. Alcohol, water and food intake was monitored once a day immediately before lights off.

In vehicle-treated rats, mean alcohol intake was higher than 4 g/kg/day from the first day of exposure to the "alcohol vs water" free choice and rose to approximately 6 g/kg within 7 days, indicative of a rapid disclosure and experience of the psychopharmacological effects of alcohol that sustain alcohol drinking behavior in sP rats. In contrast, treatment with SR 141716 resulted in a dose-dependent suppression of daily alcohol intake throughout the 10-day treatment period; accordingly, in the rat group treated with 3 mg/kg SR 141716, mean daily alcohol intake was steadily lower than 1 g/kg. Importantly, in terms of the specificity of the drug action on alcohol intake, a compensatory increase in daily water intake left total fluid intake virtually unchanged. Also food intake was not affected by treatment with SR 141716.

Genetic inactivation of CB_1 receptors apparently reproduced the effects of the repeated pharmacological blockade of these receptors on acquisition of alcohol drinking behavior. Indeed, CB_1 receptor knockout mice tested under the 2-bottle free choice regimen displayed a significantly slower rate of acquisition of alcohol drinking behavior and lower levels of alcohol preference and consumption in comparison to wild-type mice (Hungund et al., 2003; Poncelet et al., 2003; Wang et al., 2003; Naassila et al., 2003; see however Racz et al., 2003).

Administration of SR 141716 has been found to reduce alcohol intake and preference also in alcohol-experienced sP rats. In this study (Colombo et al., 1998a), rats were offered alcohol (10% v/v, in water) and water under the 2-bottle free choice regimen with limited access for 4 h/day. Water was available also during the remaining 20 h. In order to limit the anorectic effect of SR 141716 (see Harrold and Williams, 2003), and therefore to possibly unravel the specificity of the drug action on alcohol intake, availability of food pellets was restricted to the 4 h of the drinking session, resulting in an enhancement of the appetitive value of food. SR 141716 (0, 2.5, 5 and 10 mg/kg, i.p.) was administered acutely, 20 min before the start of the drinking session (which coincided with lights off).

Administration of SR 141716 induced a significant reduction in voluntary alcohol intake, by approximately 40–50% in comparison to vehicle-dosed rats. Water intake was significantly reduced only at the dose of 10 mg/kg SR 141716, while the dose of 2.5 mg/kg induced a significant increase in water intake, that compensated the reduction in alcohol consumption. Food intake was significantly reduced only by the dose of 10 mg/kg SR 141716. These results indicate that doses of SR 141716 in the range of 2.5–5 mg/kg specifically reduced alcohol intake in alcohol-experienced sP rats.

More recently, this laboratory investigated the effect of SR 141716 on "alcohol deprivation effect", that is the transient increase in alcohol intake which occurs in several animal species after a period of abstinence from alcohol. Interestingly, this phenomenon has been proposed to model the loss of control over alcohol and the episodes of alcohol relapse of human alcoholics (see Boening et al., 2001; McBride et al., 2002). sP rats appear to constitute a proper model for this investigation, since they have been found to display a pronounced "alcohol deprivation effect" during the first hour of re-access to alcohol after a period of alcohol abstinence (Agabio et al., 2000; Serra et al., 2003).

In this experiment (Serra et al., 2002), sP rats had been initially offered the free choice between alcohol (10% v/v, in water) and water with unlimited access for 8 consecutive weeks. Subsequently, rats were divided into 2 groups: one group was deprived of alcohol for 15 consecutive days, during which water was the sole fluid available (alcohol-deprived rats), while the second group continued to have unlimited access to alcohol and water (alcohol-nondeprived rats). At the end of the deprivation phase, rats of both groups were acutely injected with SR 141716 (0, 0.3, 1 and 3 mg/kg, i.p.). Alcohol was re-presented at lights off and its consumption was recorded 60 min later.

Alcohol intake was approximately two times higher in vehicle-treated alcohol-deprived rats than -nondeprived rats, indicative of the development of a robust "alcohol deprivation effect". Interestingly, all doses of SR 141716 resulted in a virtually complete suppression of the extra-intake of alcohol produced by the deprivation. No dose of SR 141716 affected water and food intake, indicating the selectivity of the reducing effect of SR 141716 on alcohol intake.

These results suggest that the CB_1 receptor may be part of the neural substrate mediating "alcohol deprivation effect" in sP rats. Further, because of the predictive validity of alcohol deprivation effect as an experimental model of alcohol relapse, the results of the present study suggest that SR 141716 might possess some efficacy in preventing relapses in human alcoholics.

The above results have been recently replicated in selectively bred Indiana alcohol-preferring (P) rats; indeed, treatment with SR 141716 (0.3–2 mg/kg, i.p.) suppressed acquisition and maintenance of alcohol drinking behavior as well as alcohol deprivation effect in P rats exposed to the 2-bottle "alcohol vs water" choice regimen (Bell et al., 2004).

The previous experiments, performed with the 2-bottle free choice paradigm, focused on the effect of SR 141716

on some *consummatory* aspects of alcohol ingestive behavior in sP rats. Recently, this laboratory investigated whether SR 141716 could affect, in this rat line, also the *appetitive*, or *motivational*, properties of alcohol. Specifically, we evaluated the effect of SR 141716 on the extinction responding for alcohol, defined as the maximal amount of "work" that a rat trained to lever-press for alcohol is willing to perform to obtain alcohol. Extinction responding has been proposed to represent an index of the appetitive strength of alcohol (Samson et al., 2001; Samson et al., 2003). Recent work has shown that sP rats trained to lever-press for alcohol displayed high values of extinction responding for alcohol (Vacca et al., 2002b), confirming that alcohol has strong motivational capacities in sP rats, and indicating the suitability of this rat line for the planned study.

In the study testing SR 141716 (Colombo et al., 2004c), sP rats were initially trained to lever-press for oral alcohol (15% v/v, in water) in standard operant chambers under a fixed ratio 4 (FR4) schedule. All rats rapidly displayed a stable lever-pressing behavior, resulting in a mean alcohol intake of approximately 0.6 g/kg during the 30-min session and blood alcohol levels in the range of 40–50 mg%. In order to assess the specificity of the SR 141716 effect on extinction responding for alcohol, a separate group of sP rats was trained to lever-press for a sucrose solution (3% w/v, in water) on an FR4 schedule. Extinction responding for alcohol or sucrose was defined as the maximal number of lever responses reached by each rat in the absence of alcohol or sucrose reinforcement, respectively; specifically, during extinction sessions, rats were exposed to the operant chamber for 30 min but lever-pressing did not result in any alcohol or sucrose presentation. On test sessions, SR 141716 (0, 0.3, 1 and 3 mg/kg, i.p.) was injected, 20 min before the start of the session, to all rats of both groups under a Latin-square design.

Pretreatment with SR 141716 resulted in a dose-dependent suppression of extinction responding for alcohol. Extinction responding for alcohol in the rat groups treated with 0.3, 1 and 3 mg/kg SR 141716 was approximately 30%, 60% and 90% lower, respectively, than that recorded in vehicle-treated rats. In the 3 mg/kg SR 141716-dosed group, 4/7 rats completely avoided to perform any press on the lever. In contrast, pretreatment with SR 141716 failed to significantly alter extinction responding for sucrose.

These results (a) implicate the CB_1 receptor in the neural system mediating alcohol's motivational properties in sP rats, and (b) suggest that the reducing effect of SR 141716 on the consummatory aspects of alcohol drinking behavior in sP rats (see above) is likely secondary to a reduction in the motivation to consume alcohol.

The above results on the reducing effects of SR 141716 on alcohol consumption and alcohol's motivational properties in sP rats have found a large consensus among the results of other studies using different methodological procedures. Specifically, SR 141716 has been found to reduce (a) alcohol intake and preference in C57BL/6 mice (Arnone et al., 1997), congenic B6.Cb4i5-β/13C/Vad and B6.Cb4i5-β14/Vad mice (Hungund et al., 2002), and Wistar rats (Lallemand et al., 2001) tested under the homecage 2-bottle free choice regimen, and (b) oral alcohol self-administration in unselected rats tested under operant procedures (Freedland et al., 2001; Hungund et al., 2002). Further, SR 141716 has been found to decrease the probability of response requirement completion for access to alcohol (a procedure for measuring the appetitive strength of alcohol comparable to the above-mentioned extinction responding procedure) in unselected rats trained to orally self-administer alcohol under an operant procedure (Gallate and McGregor, 1999; Freedland et al., 2001).

Interestingly, the ability of SR 141716 to suppress acquisition and maintenance of alcohol drinking behavior and relapse-like drinking in sP rats has been recently extended to the newly synthesized cannabinoid CB_1 receptor antagonist, SR 147778 (Rinaldi-Carmona et al., 2004), which produced a reduction in alcohol intake in sP rats comparable to that observed with SR 141716 (Gessa et al., 2005). Accordingly, SR 147778 has been reported to reduce alcohol intake also in mice tested under the 2-bottle free choice regimen (Rinaldi-Carmona et al., 2004).

With regard to the possible mechanism of action by which cannabinoid CB_1 receptor antagonists exert their reducing effect on alcohol consumption and alcohol's motivational properties, recent microdialysis experiments demonstrated that doses of SR 141716 comparable to those that reduce alcohol intake suppressed alcohol-stimulated dopamine release in the nucleus accumbens of anaesthetized, freely moving rats and mice (Cohen et al., 2002; Hungund et al., 2003); further, alcohol-induced stimulation of dopamine release in the nucleus accumbens was completely absent in CB_1 receptor knockout mice (Hungund et al., 2003). As mentioned above, 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). Cannabinoid CB_1 receptors have been reported to modulate the activity of mesolimbic dopamine neurons, likely via an inhibitory action (a) on GABA interneurons located in the VTA and controlling mesolimbic dopamine neurons or, alternatively, (b) on excitatory glutamatergic neurons controlling long-loop GABA-containing neurons projecting from the nucleus accumbens to the ventral tegmental area (see Schlicker and Kathmann, 2001). The results of the microdialysis studies by Cohen et al. (2002) and Hungund et al. (2003) led to hypothesize that SR 141716 may remove the inhibitory cannabinoidergic tone on the GABA interneurons, resulting in the observed suppression of alcohol-stimulated dopamine release and, in turn, dopamine-mediated alcohol-reinforced and -motivated behaviors.

Changes in alcohol intake following pharmacological manipulations may be secondary to changes in the rewarding or aversive attributes of alcohol. In other words, a given

drug might decrease alcohol consumption because it renders alcohol less rewarding or, conversely, more aversive. Accordingly, it may be hypothesized that SR 141716 treatment reduces alcohol intake rendering alcohol aversive. This hypothesis has been recently tested evaluating whether the combination of SR 141716 with a dose of alcohol comparable to those usually consumed by sP rats in each drinking binge would have been associated with aversive properties in sP rats. To this aim, we used the conditioned taste aversion (CTA) procedure, i.e. a behavioral technique that assesses the aversive properties of psychoactive drugs. CTA procedures are based on the association—made by the rat—of the psychopharmacological effects of the tested drug with the flavor of a specific tastant, such as saccharin; specifically, on a daily basis rats are exposed to a saccharin solution for a given period of time (usually, 20–30 min); immediately after the removal of the bottle containing the saccharin solution, rats are injected with the tested drug. Therefore the rats tend to associate, in a cause–effect relationship, the psychopharmacological effects of the drug to the previous ingestion of the saccharin solution. After a period of conditioning of proper length, avoidance by the rat of the saccharin solution is inferred to indicate that the drug produced central aversive effects. Previous experiments demonstrated that alcohol, when given alone and up to the dose of 1 g/kg (i.p.), produced a modest, if any, CTA in sP rats (Brunetti et al., 2002).

Male sP rats, 75-days-old at the start of the study, were used. Rats were individually housed and had food always available. Rats were initially habituated to consume water in daily 20-min sessions for 6 consecutive days (Preconditioning phase). The drinking session coincided with the first 20 min of the dark phase of the light/dark cycle. A second, empty bottle was also presented [in order to avoid any bias at the time of the Postconditioning phase (see below)]. Water intake was monitored by bottle weighing immediately after the end of the session. The Conditioning phase started the day after the end of the Preconditioning phase. Each conditioning session consisted in exposure for 20 min/day to a bottle containing a saccharin solution (0.1% w/v, in water) and a second, empty bottle; within 1 min of bottle removal, rats were injected with SR 141716 (0, 1 and 3 mg/kg, i.p.; suspended in 1 ml/kg saline with 0.1% Tween 80). Twenty minutes after the injection of SR 141716, rats were treated with 0 and 0.5 g/kg alcohol (20% w/v, in saline; i.p.). The alcohol dose was chosen on the basis of previous results indicating that it gives rise to blood alcohol levels comparable to those observed in sP rats after a single episode of alcohol drinking and it did not elicit any CTA in sP rats when given alone (Brunetti et al., 2002). After injections, rats were returned to their homecage and then left undisturbed. Five "saccharin–SR 141716 plus alcohol" pairings were given, one every other day. On intervening days, a bottle containing water alone and a second, empty bottle were presented for 20 min (development of conditioned aversion to saccharin is associated with a progressive decrease in the intake of saccharin solution; access to water on alternate days would prevent possible dehydration of the rat). No injection followed the water session. The Postconditioning phase started the day after completion of the Conditioning phase. Rats were offered the free choice between 2 bottles containing the saccharin solution (0.1% w/v) and water, respectively, in daily 20-min sessions and in the absence of any injection. Postconditioning sessions were repeated for 7 consecutive days. Fluid consumption was monitored immediately after the end of the drinking session.

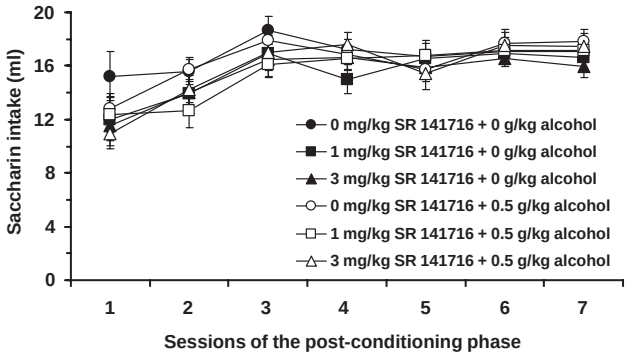

Fig. 4. Lack of development of conditioned taste aversion to the combination of the cannabinoid CB_1 receptor antagonist, SR 141716, and alcohol in Sardinian alcohol-preferring (sP) rats. On 5 of the 10 daily sessions of the Conditioning phase, treatment with SR 141716 (0, 1 and 3 mg/kg, i.p.) plus alcohol (0 and 0.5 g/kg, i.p.) was paired with saccharin solution (which was the only fluid available). On intervening days, a bottle containing water alone and a second, empty bottle were presented; no injection followed the water session. During the 7 sessions of the Postconditioning phase, rats were offered a free choice between saccharin solution and water. Data refer to intake of saccharin solution during the daily 20-min/sessions of the Postconditioning phase. Each point is the mean ± S.E.M. of $n = 8$.

Two-way (drug treatment; day) ANOVA did not reveal any difference in saccharin intake [$F_{\text{drug treatment}}(5,252) = 0.50$, $P > 0.05$] among rat groups during the Postconditioning phase (Fig. 4), indicating that the combination of SR 141716 (injected at doses that have been reported to effectively reduce alcohol intake in sP rats) and alcohol (injected at a dose which gives rise to blood alcohol levels similar to those deriving from voluntary alcohol intake) did not result in the development of CTA in sP rats. These results suggest that SR 141716-induced reduction of alcohol intake in sP rats is probably not secondary to an increase in the aversive properties of alcohol.

6. Synergistic potentiation by the combination of cannabinoid and opioid receptor antagonists of the reducing effect on alcohol drinking and seeking behaviors

More recently, this laboratory investigated the effect of the combination of SR 141716 plus an opioid receptor antagonist on alcohol intake in sP rats. Different lines of

evidence indicate the ability of opioid receptor antagonists to a) decrease alcohol intake and alcohol-motivated behaviors in laboratory animals, and b) reduce alcohol consumption and prevent alcohol relapses in human alcoholics (see Modesto-Lowe and Van Kirk, 2002; Anton and Swift, 2003). Similarly to the mechanism of action proposed for the cannabinoid CB_1 receptor antagonists, reduction in alcohol intake and alcohol-motivated behaviors by opioid receptor antagonists is thought to be secondary to an inhibition of alcohol-stimulated dopamine function in the mesolimbic dopamine pathway (Acquas et al., 1993; Benjamin et al., 1993; Gonzales and Weiss, 1998). On the basis of these results, we predicted that the combination of SR 141716 and an opioid receptor antagonist would have resulted in an additive or even synergistic reducing effect on voluntary alcohol intake in sP rats.

The first experiment evaluated the effect of the combination of SR 141716 plus naltrexone on the acquisition of alcohol drinking behavior. This experiment used male sP rats, 75-days-old and alcohol-naive at the start of the study. Rats were divided into groups of $n=8$, matched for body weight. On Day 1, rats were treated with SR 141716 (0 and 0.1 mg/kg, i.p.; suspended in 1 ml/kg saline with 0.1% Tween 80) and naltrexone (0 and 0.5 mg/kg, i.p.; dissolved in 1 ml/kg saline) 30 min before alcohol presentation. Drug doses were chosen on the basis of the results of preliminary experiments in order to not affect acquisition of alcohol drinking behavior in sP rats when given alone. Alcohol (10% v/v, in water) was presented at the start of the dark phase, under the homecage 2-bottle free choice with water and unlimited access for 24 h/day. Food pellets were always available. Drugs were administered once a day (30 min before lights off) for 10 consecutive days. Bottles were refilled every day with fresh solution and their left–right positions interchanged daily. Daily alcohol, water and food intake was monitored every day immediately before lights off. Recording of daily alcohol, water and food intake was performed for an additional 3 days after termination of treatment (post-treatment phase).

Statistical analysis [2-way (drug treatment; day) ANOVA with repeated measures on the drug treatment factor] revealed a significant effect of drug treatment on alcohol intake during the treatment phase [$F(3;243)=3.01$, $P<0.05$]. Daily alcohol intake in the control rat group (0 mg/kg SR 141716 plus 0 mg/kg naltrexone) ranged between 4 and 5 g/kg over the first days of exposure to the "alcohol vs water" free choice, indicative of a rapid disclosure and acquisition of alcohol drinking behavior (Fig. 5, panel A). Daily alcohol intake in the rat groups treated with either SR 141716 or naltrexone alone (0.1 mg/kg SR 141716 plus 0 mg/kg naltrexone, or 0 mg/kg SR 141716 plus 0.5 mg/kg naltrexone, respectively) did not significantly differ from that recorded in the control rat group (Fig. 5, panel A). Conversely, daily alcohol intake in the combination rat group (0.1 mg/kg SR 141716 plus 0.5 mg/kg naltrexone) was significantly lower, with respect to that of the control

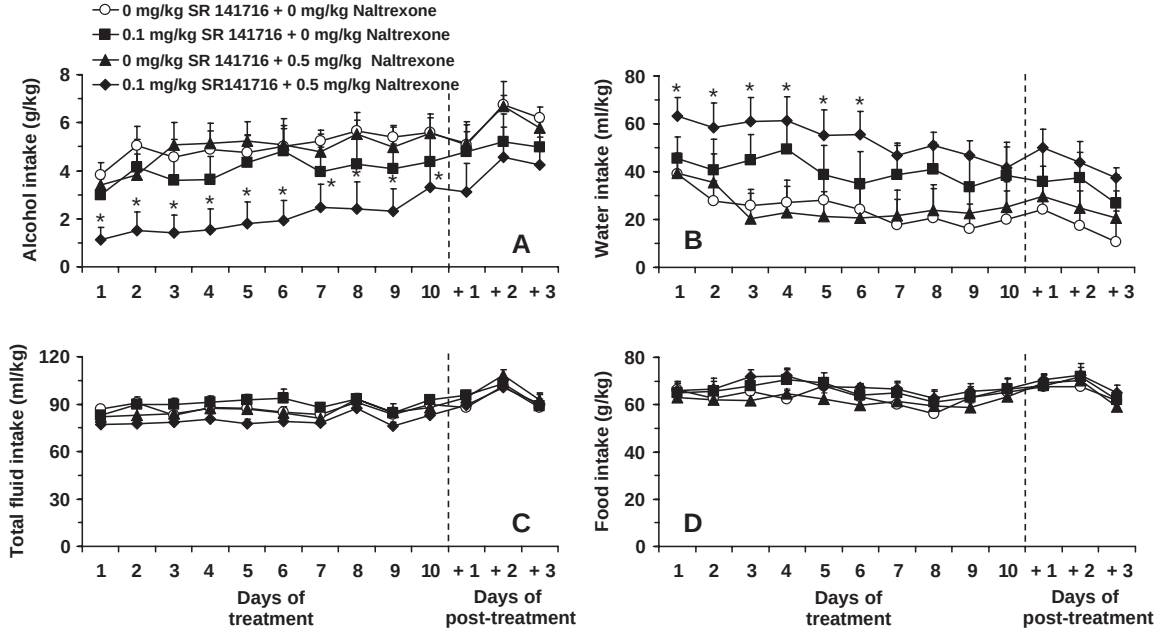

Fig. 5. Suppressing effect of the repeated administration of the combination of the cannabinoid CB_1 receptor antagonist, SR 141716, plus the opioid receptor antagonist, naltrexone, on the acquisition of alcohol drinking behavior in alcohol-naive, Sardinian alcohol-preferring (sP) rats. Alcohol (10% v/v) was offered under the standard, homecage 2-bottle free choice regimen with water and unlimited access for 24 h/day. Alcohol presentation started immediately after the first drug treatment. Food pellets were always available. Alcohol intake was recorded once a day immediately before lights off. SR 141716 (0 and 0.1 mg/kg, i.p.) and naltrexone (0 and 0.5 mg/kg, i.p.) were injected once a day (30 min before lights off) for 10 consecutive days. The dashed line indicates the completion of the 10-day treatment period and the start of the 3-day post-treatment period. Each point is the mean ± SEM of $n=8$ rats. *$P<0.05$ with respect to 0 mg/kg SR 141716 plus 0 mg/kg naltrexone-treated rats (Newman–Keuls test).

rats, on each of the 10 days of treatment (Fig. 5, panel A). Reduction in daily alcohol intake in the combination rat group was associated with a compensatory increase in water intake [$F(3;243)=4.07$, $P<0.05$] (Fig. 5, panel B), so that daily total fluid intake remained unchanged [$F(3;243)=2.06$, $P>0.05$] (Fig. 5, panel C). Food intake was not significantly affected by drug treatment [$F(3;243)=0.48$, $P>0.05$] (Fig. 5, panel D), indicating the specificity of the combination effect on alcohol intake. Finally, once treatment was completed, alcohol intake in the combination rat group returned to control values within a few days (Fig. 5). The results of the present experiment indicate that the combination of SR 141716 and naltrexone exerted a synergistic action in suppressing acquisition of alcohol drinking behavior in sP rats.

Subsequently, this laboratory investigated the effect of the combination on SR 141716 and naloxone on the maintenance of alcohol drinking behavior. Specifically, this experiment used alcohol-experienced sP rats given alcohol (10% v/v, in water) and water under the 2-bottle free choice regimen, with unlimited access for 24 h/day, for 8 consecutive weeks before drug treatment. On the test day, rats were divided into groups on $n=16$, matched for body weight as well as alcohol and water intake over the 3 days preceding the start of the experiment. SR 141716 (0 and 0.3 mg/kg, i.p.; suspended in 1 ml/kg saline with 0.1% Tween 80) and naloxone (0 and 0.1 mg/kg, i.p.; dissolved in 1 ml/kg saline) were administered acutely 15–20 min before lights off. Drug doses were chosen on the basis of the results of preliminary experiments in order to not affect alcohol intake in alcohol-experienced sP rats when given alone. Alcohol, water and food intake was monitored 60 min after lights off.

One-way ANOVA revealed a significant effect of drug treatment on alcohol intake [$F(3;60)=4.26$, $P<0.01$]. Alcohol intake in the control rat group (0 mg/kg SR 141716 plus 0 mg/kg naloxone) averaged 0.64 g/kg (Fig. 6). Neither naloxone nor SR 141716 affected alcohol intake when given alone; indeed, alcohol intake in 0.3 mg/kg SR 141716 plus 0 mg/kg naloxone rat group and in 0 mg/kg SR 141716 plus 0.1 mg/kg naloxone rat group averaged 0.70 and 0.63 g/kg, respectively (Fig. 6). Conversely, the rat group treated with the combination of 0.3 mg/kg SR 141716 plus 0.1 mg/kg naloxone consumed 0.42 g/kg alcohol, which was significantly lower than the intake recorded in each other rat group (Fig. 6). Drug treatment did not significantly affect food intake [$F(3;60)=1.51$, $P>0.05$].

Again, in close agreement with the present data, Gallate and colleagues (2004) recently reported that the combination of doses of SR 141716 and either naloxone or naltrexone comparable to those tested in our studies synergistically reduced the break-point for beer and beer consumption in Wistar rats.

The possible generalization to human alcoholics of the results of the combination experiments would suggest a novel therapeutic strategy, characterized by a higher efficacy and likely fewer and less pronounced side-effects than each single drug therapy.

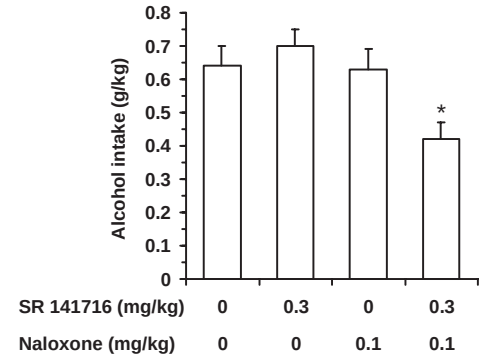

Fig. 6. Reducing effect of the acute administration of the combination of the cannabinoid CB_1 receptor antagonist, SR 141716, plus the opioid receptor antagonist, naloxone, on alcohol intake in alcohol-experienced, Sardinian alcohol-preferring (sP) rats. Alcohol (10% v/v) was offered under the standard, homecage 2-bottle free choice regimen with water and unlimited access for 24 h/day. Food pellets were always available. Alcohol intake was recorded 60 min after lights off. SR 141716 (0 and 0.3 mg/kg, i.p.) and naloxone (0 and 0.1 mg/kg, i.p.) were injected 15-20 min before lights off. Each point is the mean ± SEM of $n=16$ rats. *$P<0.05$ with respect to 0 mg/kg SR 141716 plus 0 mg/kg naloxone-treated rats (Newman–Keuls test).

References

Acquas E, Meloni M, Di Chiara G. Blockade of delta-opioid receptors in the nucleus accumbens prevents ethanol-induced stimulation of dopamine release. Eur J Pharmacol 1993;230:239–41.

Agabio R, Carai MAM, Lobina C, Pani M, Reali R, Vacca G, et al. Development of short-lasting alcohol deprivation effect (ADE) in Sardinian alcohol-preferring rats. Alcohol 2000;21:59–62.

Anton RF, Swift RM. Current pharmacotherapies of alcoholism: a US perspective. Am J Addict 2003;12:S53–68.

Arnone M, Maruani J, Chaperon F, Thiébot ME, Poncelet M, Soubrié P, et al. Selective inhibition of sucrose and alcohol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology 1997;132:104–6.

Bell RL, Rodd ZA, Sable HJK, McQueen VK, Davids MR, Murphy JM, et al. Cannabinoid CB1 antagonist reduces alcohol intake during acquisition, maintenance, and relapse in inbred alcohol preferring (IP) rats. Abstract viewer/It planner. Washington DC: Society for Neuroscience; 2004. Program No. 489.6.

Benjamin D, Grant ER, Pohorecky LA. Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. Brain Res 1993;621:137–40.

Boening JAL, Lesch OM, Spanagel R, Wolffgramm J, Narita M, Sinclair D, et al. Pharmacological relapse prevention in alcohol dependence: from animal models to clinical trials. Alcohol Clin Exp Res 2001; 25:S127–31.

Bowery NG, Hudson AL, Price GW. $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Neuroscience 1987;20: 365–83.

Braida D, Pozzi M, Cavallini R, Sala M. Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. Neuroscience 2001a;104:923–6.

Braida D, Pozzi M, Parolaro D, Sala M. Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. Eur J Pharmacol 2001b;413:227–34.

Brodie MS, Shefner SA, Dunwiddie TV. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res 1990;508:65–9.

Brunetti G, Carai MAM, Lobina C, Melis S, Serra S, Vacca G, et al. Differences in ethanol-induced conditioned taste aversion in Sardinian alcohol-preferring and Sardinian alcohol-nonpreferring rats. Alcohol 2002;26:167–72.

Cadoni C, Pisanu A, Solinas M, Acquas E, Di Chiara G. Behavioural sensitization after repeated exposure to Δ^9-tetrahydrocannabinol and cross-sensitization with morphine. Psychopharmacology 2001;158: 259–66.

Campbell AD, McBride WJ. Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens. Pharmacol Biochem Behav 1995;51:835–42.

Carboni E, Acquas E, Frau R, Di Chiara G. Differential inhibitory effects of a 5-HT_3 antagonist on drug-induced stimulation of dopamine release. Eur J Pharmacol 1989;164:515–9.

Chaperon F, Soubrié P, Puech AJ, Thiébot MH. Involvement of the central cannabinoid (CB_1) receptors in the establishment of place conditioning in rats. Psychopharmacology 1998;135:324–32.

Chen JP, Paredes W, Li J, Smith D, Lowinson J, Gardner EL. Δ^9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious freely-moving rats as measured by intracerebral microanalysis. Psychopharmacology 1990;102:156–62.

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrié P. SR 141716, a central cannabinoid (CB_1) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol 2002;13:451–63.

Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, Fadda F, et al. Sardinian alcohol-preferring rats: a genetic animal model of anxiety. Physiol Behav 1995;57:1181–5.

Colombo G, Agabio R, Fà M, Guano L, Lobina C, Loche A, et al. Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR 141716. Alcohol Alcohol 1998a;33: 126–30.

Colombo G, Agabio R, Lobina C, Reali R, Vacca G, Gessa GL. Stimulation of locomotor activity by voluntarily consumed ethanol in Sardinian alcohol-preferring rats. Eur J Pharmacol 1998b;357:109–13.

Colombo G, Serra S, Brunetti G, Gomez R, Melis S, Vacca G, et al. Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. Psychopharmacology 2002;159: 181–7.

Colombo G, Addolorato G, Agabio R, Carai MAM, Pibiri F, Serra S, et al. Role of $GABA_B$ receptor in alcohol dependence: reducing effect of baclofen on alcohol intake and alcohol motivational properties in rats and amelioration of alcohol withdrawal syndrome and alcohol craving in human alcoholics. Neurotox Res 2004a;6:403–14.

Colombo G, Serra S, Vacca G, Gessa GL, Carai MAM. Suppression by baclofen of the stimulation of alcohol intake induced by morphine and WIN 55,212-2 in alcohol-preferring rats. Eur J Pharmacol 2004b;492: 189–93.

Colombo G, Vacca G, Serra S, Carai MAM, Gessa GL. Suppressing effect of the cannabinoid CB_1 receptor antagonist, SR 141716, on alcohol's motivational properties in alcohol-preferring rats. Eur J Pharmacol 2004c;498:119–23.

Cossu G, Ledent C, Fattore L, Imperato A, Böhme GA, Parmentier M, et al. Cannabinoid CB_1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. Behav Brain Res 2001;118: 61–5.

De Vries TJ, Homberg JR, Binnekade R, Raasø H, Schoffelmeer ANM. Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology 2003;168:164–9.

Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 1988;85:5274–8.

Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse 2003;50:1–6.

Fattore L, Spano MS, Cossu G, Deiana S, Fratta W. Cannabinoid mechanism in reinstatement of heroin seeking after a long period of abstinence in rats. Eur J Neurosci 2003;17:1723–6.

Freedland CS, Sharpe AL, Samson HH, Porrino LJ. Effects of SR 1417161A on alcohol and sucrose self-administration. Alcohol Clin Exp Res 2001;25:277–82.

French ED, Dillon K, Wu X. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. NeuroReport 1997;8: 649–52.

Gallate JE, McGregor IS. The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. Psychopharmacology 1999;142: 302–8.

Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB_1 receptor agonist. Eur J Pharmacol 1999;370:233–40.

Gallate JE, Mallet PE, McGregor IS. Combined low dose treatment with opioid and cannabinoid receptor antagonists synergistically reduces the motivation to consume alcohol in rats. Psychopharmacology 2004; 173:210–6.

Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res 1985;348:201–3.

Gessa GL, Serra S, Vacca G, Carai MAM, Colombo G. Suppressing effect of the cannabinoid CB_1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. Alcohol Alcohol 2005;40:46–53.

Ghozland S, Matthes HWD, Simonin F, Filliol D, Kieffer BL, Maldonado R. Motivational effects of cannabinoids are mediated by μ-opioid and κ-opioid receptors. J Neurosci 2002;1146–54.

Gonzales RA, Weiss F. Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. J Neurosci 1998;18:10663–71.

Gysling K, Wang RY. Morphine-induced activation of A10 dopamine neurons in the rat. Brain Res 1983;277:119–27.

Harrold JA, Williams G. The cannabinoid system: a role in both the homeostatic and hedonic control of eating? Br J Nutr 2003;90: 729–34.

Hodge CW, Niehus JS, Samson HH. Morphine induced changes in ethanol- and water-intake are attenuated by the 5-$HT_{3/4}$ antagonist tropisetron (ICS 205-930). Psychopharmacology 1995;119:186–92.

Hubbell CL, Mankes RF, Reid LD. A small dose of morphine leads rats to drink more alcohol and achieve higher blood alcohol concentrations. Alcohol Clin Exp Res 1993;17:1040–3.

Hungund BL, Basavarajappa BS, Vadasz C, Kunos G, Rodriguez de Fonseca F, Colombo G, et al. Ethanol, endocannabinoids, and the cannabinoidergic signaling system. Alcohol Clin Exp Res 2002;26: 565–74.

Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C. Cannabinoid CB1 receptor knockout exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. J Neurochem 2003;84:698–704.

Imperato A, Angelucci L. 5-HT_3 receptors control dopamine release in the nucleus accumbens of freely moving rats. Neurosci Lett 1989;101: 214–7.

Imperato A, Di Chiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 1986;239:219–28.

Kilpatrick GJ, Jones BJ, Tyers MB. Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 1987; 330:746–8.

Lallemand F, Soubrié PH, De Witte PH. Effects of CB_1 cannabinoid receptor blockade on ethanol preference after chronic ethanol administration. Alcohol Clin Exp Res 2001;25:1317–23.

Latimer LG, Duffy P, Kalivas PW. Mu opioid receptor involvement in enkephalin activation of dopamine neurons in the ventral tegmental area. J Pharmacol Exp Ther 1987;241:328–37.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. Science 1999;283:401–4.

Manzanares J, Corchero J, Romero J, Fernández-Ruiz JJ, Ramos JA, Fuentes JA. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol Sci 1999;20:287–94.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. Eur J Neurosci 2000;12:4038–46.

Mascia MS, Obinu MC, Ledent C, Parmentier M, Böhme GA, Imperato A, et al. Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB_1 receptor knockout mice. Eur J Pharmacol 1999;383:R1–2.

Mas-Nieto M, Pommier B, Tzavara ET, Caneparo A, Da Nascimento S, Le Fur G, et al. Reduction of opioid dependence by the CB_1 antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction. Br J Pharmacol 2001;132:1809–16.

McBride WJ, Le AD, Noronha A. Central nervous system mechanisms in alcohol relapse. Alcohol Clin Exp Res 2002;26:280–6.

Modesto-Lowe V, Van Kirk J. Clinical uses of naltrexone: a review of the evidence. Exp Clin Psychopharmacol 2002;10:213–27.

Naassila M, Pierrefiche O, Ledent C, Daoust M. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. Neuropharmacology 2003;46:243–53.

Navarro M, Carrera MRA, Fratta W, Valverde O, Cossu G, Fattore L, et al. Functional interaction between opioid and cannabinoid systems in drug self-administration. J Neurosci 2001;21:5344–50.

Norwood CS, Cornish JL, Mallet PE, McGregor IS. Pre-exposure to the cannabinoid receptor agonist CP 55,940 enhances morphine behavioral sensitization and alters morphine self-administration in Lewis rats. Eur J Pharmacol 2003;465:105–14.

Pei Q, Zetterström T, Leslie RA, Grahame-Smith DG. 5-HT_3 receptor antagonists inhibit morphine-induced stimulation of mesolimbic dopamine release and function in the rat. Eur J Pharmacol 1993;230:63–8.

Poncelet M, Maruani J, Calassi R, Soubrié P. Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. Neurosci Lett 2003;343:216–8.

Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci U S A 1995;92:12304–8.

Racz I, Bilkei-Gorzo A, Toth ZE, Michel K, Palkovits M, Zimmer A. A critical role for the cannabinoid CB_1 receptors in alcohol dependence and stress-stimulated ethanol drinking. J Neurosci 2003;23:2453–8.

Rice OV, Gordon N, Gifford AN. Conditioned place preference to morphine in cannabinoid CB1 receptor knockout mice. Brain Res 2002;945:135–8.

Rinaldi-Carmona M, Barth F, Congy C, Martinez S, Oustric D, Perio A, et al. SR147778, a new potent and selective antagonist of the CB1 cannabinoid receptor Biochemical and pharmacological characterization. J Pharmacol Exp Ther 2004;310:905–14.

Samson HH, Chappell A, Czachowski C, Sharpe A. Measuring ethanol-seeking behavior: the effect of using repeated extinction trials. Alcohol 2001;24:205–9.

Samson HH, Czachowski CL, Chappell A, Legg B. Measuring the appetite strength of ethanol: use of an extinction trial procedure. Alcohol 2003;31:77–86.

Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci 2001;22:565–72.

Serra S, Carai MAM, Brunetti G, Gomez R, Melis S, Vacca G, et al. The cannabinoid receptor antagonist SR 141716 prevents acquisition of drinking behaviour in alcohol-preferring rats. Eur J Pharmacol 2001;430:369–71.

Serra S, Brunetti G, Pani M, Vacca G, Carai MAM, Gessa GL, et al. Blockade by the cannabinoid CB_1 receptor antagonist, SR 141716, of alcohol deprivation effect in alcohol-preferring rats. Eur J Pharmacol 2002;443:95–7.

Serra S, Brunetti G, Vacca G, Lobina C, Carai MAM, Gessa GL, et al. Stable preference for high ethanol concentrations after alcohol deprivation in Sardinian alcohol-preferring (sP) rats. Alcohol 2003;29:101–8.

Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. Science 1997;276:2048–50.

Ulm RR, Volpicelli JR, Volpicelli LA. Opiates and alcohol self-administration in animals. J Clin Psychiatry 1995;56(Suppl. 7):5–14.

Vacca G, Serra S, Brunetti G, Carai MAM, Gessa GL, Colombo G. Boosting effect of morphine on alcohol drinking is suppressed not only by naloxone but also by the cannabinoid CB_1 receptor antagonist, SR 141716. Eur J Pharmacol 2002a;445:55–9.

Vacca G, Serra S, Brunetti G, Carai MAM, Samson HH, Gessa GL, et al. Operant self-administration of ethanol in Sardinian alcohol-preferring rats. Alcohol Clin Exp Res 2002b;26:1678–85.

Wang L, Liu J, Harvey-White J, Zimmer A, Kunos G. Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. Proc Natl Acad Sci U S A 2003;100:1393–8.

Weiss F, Porrino LJ. Behavioral neurobiology of alcohol addiction: recent advances and challenges. J Neurosci 2002;22:3332–7.

Weiss F, Lorang MT, Bloom FE, Koob GF. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 1993;267:250–8.

Wozniak KM, Pert A, Linnoila M. Antagonism of 5-HT_3 receptors attenuates the effects of ethanol on extracellular dopamine. Eur J Pharmacol 1990;187:287–9.

Wu X, French ED. Effects of chronic Δ^9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. Neuropharmacology 2000;39:391–8.