

Cannabinoid CB₁ antagonist SR 141716A attenuates reinstatement of heroin self-administration in heroin-abstinent rats

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Received 7 September 2004; received in revised form 30 December 2004; accepted 26 January 2005

Abstract

Rats with a previous history of heroin self-administration were studied to assess interactions occurring between cannabinoids and opioids in an animal model of reinstatement of heroin-seeking behaviour. Rats were trained to self-administer heroin and after a long-term extinction were primed with one of the following non-contingent non-reinforced drug administrations: saline (or vehicle), heroin, synthetic cannabinoid CB₁ receptor agonists (WIN 55,212-2 or CP 55,940), opioid antagonist (naloxone) or CB₁ antagonist (SR 141716A), alone or in combination. After primings, lever-pressing activity was recorded and compared to those observed during previous phases of training and extinction. Results of this study showed that (i) priming injections of heroin (0.1 mg/kg) as well as CB₁ agonists WIN 55,212-2 (0.15 or 0.30 mg/kg) and CP 55,940 (0.05 or 0.1 mg/kg) completely restore heroin-seeking behaviour; (ii) primings of naloxone (1 mg/kg) and SR 141716A (0.3 mg/kg) had no effect when administered alone; (iii) heroin-induced reinstatement was fully prevented by pre-treatment with either naloxone or SR 141716A; (iv) pre-treatment with SR 141716A significantly reduced WIN 55,212-2 and CP 55,940 priming effects. These results suggest that cannabinoid CB₁ receptors play an important role in the mechanisms underlying relapse to heroin-seeking and depict CB₁ antagonists as possible therapeutic agents for use in the prevention of relapse to heroin abuse.

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Keywords: SR 141716A; Cannabinoid; Opioid; Relapse; Reinstatement of heroin-seeking; Self administration

1. Introduction

High rates of relapse represent a major clinical problem in detoxification from heroin addiction. Even after prolonged periods of withdrawal, several factors, such as re-exposure to drug or drug-associated cues (Ludwig and Wikler, 1974; Jaffe et al., 1989; O'Brien et al.,

1992) or social and stress factors (Krueger, 1981; Sinha, 2001) can trigger craving and heroin-seeking. At the present time there is no known pharmacological therapy capable of reliably preventing relapse to heroin. In animal studies, relapse is better defined as reinstatement of a behaviour that had previously been reinforced by a drug, i.e. reacquisition of responding after a drug-free period with consequent extinction of drug-associated behaviour.

The most commonly used animal models of reinstatement of extinguished drug-seeking behaviour are

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based on various drug self-administration (SA) or drug conditioned place preference (CPP) schedules (for a recent review see Shaham et al., 2003). These extinction/reinstatement models have been shown to possess good predictive validity since conditions that reliably reinstate drug-seeking behaviour in laboratory animals, such as re-exposure to the drug, drug-associated cues and stress are similar to those reported to induce drug craving and relapse in humans.

Mounting evidence has suggested that the endocannabinoid system plays an important role in the expression of opioid rewarding and addictive effects (Fattore et al., 2004, 2005) and a cross-sensitization between opioids and cannabinoids has been clearly demonstrated (Cadoni et al., 2001; Lamarque et al., 2001; Rubino et al., 2001).

Cannabinoid agents suppress naloxone-precipitated withdrawal in morphine-dependent rats (Hine et al., 1975; Vela et al., 1995) while the anandamide transport inhibitor *N*-(4-hydroxyphenyl)-arachidonyl-ethanolamide (AM404) attenuates spontaneous opiate withdrawal in mice (Del Arco et al., 2002). Moreover, the cannabinoid CB₁ receptor knock-out mice fail both to develop CPP to morphine (Castañé et al., 2003) as well as to self-administer morphine (Ledent et al., 1999) but not other drugs of abuse (Fattore et al., 2000; Cossu et al., 2001). On the other hand, cannabinoid rewarding effects are modulated by the opioid system, as the opioid antagonist naloxone blocks cannabinoid CPP in rats (Braida et al., 2001a), reduces cannabinoid intracerebral SA in rats (Braida et al., 2001b) and antagonizes cannabinoid SA in drug-naïve mice (Fratta et al., 1999; Fattore et al., 2002), while naltrexone decreases spontaneous intake of Δ^9 -tetrahydrocannabinol (THC) in squirrel monkeys (Justinova et al., 2004). In accordance with the above findings, Δ^9 -THC fails to induce CPP in mice lacking the μ opioid receptor (Ghozland et al., 2002).

Similarly, the CB₁ receptor antagonist SR 141716A has been reported to prevent heroin SA in trained rats (Navarro et al., 2001), to block the acquisition and expression of morphine CPP (Chaperon et al., 1998; Mas-Nieto et al., 2001; Navarro et al., 2001), to partially revert heroin intracerebral SA in rats (Braida et al., 2001b) and to reduce morphine intravenous SA in drug-naïve mice (Fratta et al., 1999; Navarro et al., 2001; Fattore et al., 2002). Earlier studies from our own and other laboratories revealed a cross-talk between the endogenous cannabinoid and opioid systems extending to the central mechanisms regulating relapse to drug-seeking (De Vries et al., 2003; Fattore et al., 2003; Spano et al., 2004).

The aim of the present study was therefore to further evaluate the hypothesis that reinstatement of heroin-seeking behaviour after extinction could be elicited or prevented by cannabinergic agents.

2. Methods

2.1. Animals

Male Lister Hooded rats (Harlan Nossan, Italy) weighing 260–280 g at the beginning of the experiments were used ($n=62$). Animals were housed 4/cage, handled daily for approximately 10 min for at least 6 days after arrival and maintained at a temperature of 21 ± 1 °C under a reversed 12:12 h light/dark cycle (lights on 07:00 h) with food and water freely available. Seven days after arrival, animals were surgically implanted with an intravenous silicon catheter in the right jugular vein, as previously described (Fattore et al., 2001). After surgery, rats were individually housed and maintained on a restricted diet of 20 g Purina laboratory chow per day, given after each daily testing session, to maintain body weight and growth rates.

All experiments were carried out during the dark phase of the cycle, between 09:00 and 14:00 h 7 days/week, and performed in strict accordance with both the Guide for the Care and Use of Laboratory Animals (NIH) and the E.C. regulations for animal use in research (86/609/EEC).

2.2. Drugs

For intravenous self-administration training, heroin was dissolved in heparinized (1%) saline solution at a dose of 0.03 mg/inf. For priming tests, heroin was dissolved in 0.9% sterile saline solution at a dose of 0.1 mg/kg and administered intravenously (i.v.) immediately before starting the session in a volume of 100 μ l followed by 0.2 ml saline solution to flush the drug solution through the catheter. Naloxone was dissolved in saline and injected 10 min before starting the session (volume of injection: 0.5 ml/kg i.p.).

WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-1-naphthalenyl-methanone mesylate) and CP 55,940 ((-)-*cis*-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol) were freshly dissolved in one drop of Tween-80, diluted in saline solution and administered i.p. in a volume of 0.5 ml/kg 10 min before starting the session. Heroin, naloxone and the CB₁ receptor agonists were purchased from Sigma, Italy.

SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) (Sanofi, Montpellier, France) was administered 20 min before starting the session (volume of injection: 0.5 ml/kg i.p.). As control studies, one group was primed with saline (i.v.) and an additional one with the same vehicle of cannabinergic agents (i.p.).

2.3. Experimental apparatus

Experiments took place in ten operant chambers (29.5×32.5×23.5 cm, Med Associates, USA), each located within a sound- and light-attenuating cubicle and equipped with a ventilation fan. The front panel contained two retractable levers, each 4 cm wide, positioned 12 cm apart and 8 cm from the grid, and extending 1.5 cm into the box. A white stimulus light was placed above each lever and a red house light was located on the opposite wall. Intravenous infusion of heroin was delivered by a software-operated infusion pump (Med Associates) through a counterbalanced single-channel swivel and an extra length of plastic tubing enclosed in a metal spring connecting the swivel to the catheter fitting on the animal's back.

Depression of one lever, defined as *active*, resulted in: (i) extinction of the house light and the illumination of the stimulus light above the active lever, which remained on for 20 s, (ii) retraction of both levers and (iii) activation of the infusion pump for 5.83 s delivering 0.1 ml intravenous infusion of 0.03 mg heroin solution. On completion of the 20 s time-out (TO) period, the levers were re-extended into the chamber, the stimulus light went out and the house light was switched on. Depressions on the other lever, defined as *inactive*, had no programmed consequences but were always recorded, thus providing an index of basal activity levels. The assignment of the active (drug-paired) and the inactive (not drug-paired) levers was counterbalanced between rats and remained constant for each subject throughout all experiments.

2.4. Surgery

Under deep anaesthesia with Equithesin (0.97 g pentobarbital, 2.1 g Mg sulphate, 4.25 g chloral hydrate, 42.8 ml propylene glycol, 11.5 ml ethanol 90%; 0.5 ml/kg i.p.), animals were implanted with silastic catheters inserted into the right external jugular vein as previously described (Fattore et al., 2001). After surgery, each animal recovered for six days with food and water freely available and received a daily subcutaneous administration of 0.1 ml Baytrill (Bayer). Free passage of 0.1 ml of heparinized saline solution (30 U/ml 0.9% sterile saline) through the catheter was checked after each session throughout all experiments. All antibiotics and anaesthetics were purchased as sterile solutions from local distributors.

2.5. Experimental procedure

The 'between-session' extinction/reinstatement model used in the present study was the same as described

previously (Fattore et al., 2003). Briefly, following recovery from surgery, animals were kept under food-restriction conditions and allowed 2 h daily access to heroin (0.03 mg/inf) under a continuous (FR1) schedule of reinforcement. Throughout training, no limit was fixed for heroin intake (i.e. rats earned as many heroin infusions as active lever presses) and in no case were priming injections given by the experimenter at the beginning of the session in an attempt to facilitate acquisition of SA behaviour. The daily sessions continued until the total number of heroin infusions per session stabilized to within $\pm 10\%$ for 7 consecutive days: subsequently, the extinction condition was imposed over 21 consecutive days. On days 16 and 19 of extinction, rats received an i.p. injection of saline (2 ml) to habituate them to subsequent drug priming administrations.

At the end of the extinction period (day 22), each animal received injections of either saline (i.v.) or cannabinoid vehicle (i.p.) or one of the following drug primings: heroin (0.1 mg/kg i.v.), WIN 55,212-2 (0.15 and 0.3 mg/kg i.p.), CP 55,940 (0.05 and 0.1 mg/kg i.p.), SR 141716A (0.3 mg/kg i.p.), naloxone (1.0 mg/kg i.p.), alone or in combination.

Treatments were assigned on the basis of a Latin square design whereby at least three extinction sessions separated two consecutive testing sessions to allow for assessment of carryover effects. Each animal was tested once with one drug and once with saline or vehicle in a counterbalanced manner.

Catheter life permitting, the same animals were tested with a different drug; however, no more than three drug sessions/animal were conducted. The order of presentation of different test drugs, where applicable, was varied between animals and each treatment group included at least seven animals. Animals receiving multiple tests were equally distributed across the different test conditions.

2.6. Statistical analyses

The number of responses on both the active and inactive levers as well as motor activity occurring during each reinstatement test session were evaluated. All measures were analysed with two-way repeated-measures analysis of variance (ANOVA) with drug primings as the between-group factor and the experimental phases (training:extinction:priming) as repeated within-subject measure. One-way ANOVA was used when comparing the three experimental phases within each treatment group. Differences among individual means were analysed using Newman–Keuls and Bonferroni post hoc tests following one-way and two-way ANOVA, respectively. Significance level was set at $P < 0.05$.

3. Results

3.1. Antagonism by naloxone and SR 141716A of heroin-induced reinstatement of heroin-seeking

As shown in Fig. 1, saline priming did not modify the mean number of responses on the active lever with respect to the previous extinction baseline. In contrast, priming injection of heroin (0.1 mg/kg i.p.) reinstated a mean number of responses on the active lever significantly higher with respect to both the extinction ($P < 0.001$) and the training ($P < 0.01$) baseline values ($F_{2,12} = 321.8$). Priming injections of naloxone (1 mg/kg) did not reinstate heroin-seeking behaviour; however, naloxone completely prevented heroin-induced reinstatement of heroin-seeking behaviour. It is noteworthy that none of the doses of naloxone tested in pilot experiments (ranging from 0.1 up to 2 mg/kg) was able to affect operant behaviour, even when using a different route of administration (i.e. subcutaneously, data not shown).

Similarly, priming injections of the cannabinoid CB_1 receptor antagonist SR 141716A were tested at different doses (0.1–1.0 mg/kg i.p.), none of which significantly modified the mean number of responses on the active lever with respect to extinction baseline. However, SR 141716A (0.3 mg/kg) was able to completely prevent the reinstatement of heroin-seeking induced by heroin primings. ANOVA revealed a drug \times phase significant interaction ($F_{8,90} = 41.12$, $P < 0.0001$), with an overall significant effect of phases ($F_{2,90} = 591.98$) as well as drug treatments ($F_{4,90} = 49.81$).

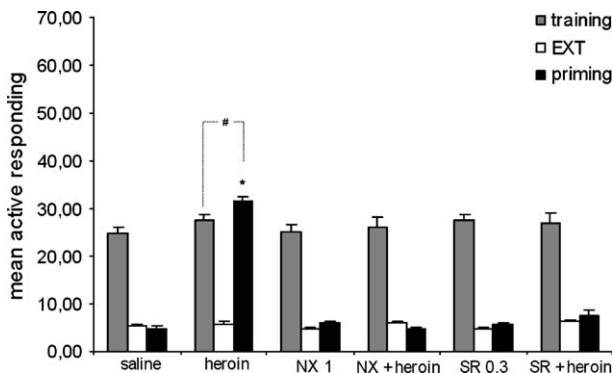


Fig. 1. Antagonism by naloxone (NX) and SR 141716A (SR) of the heroin-induced reinstatement of heroin-seeking behaviour following prolonged abstinence. Each bar represents the mean \pm SEM of active lever-presses over the last three days of heroin SA (training), over the last five consecutive sessions of extinction (EXT) and during the reinstatement test sessions (priming). Doses are expressed as mg/kg. * $P < 0.001$ vs. corresponding EXT and # $P < 0.01$ vs. respective training; ANOVA followed by post hoc test ($n = 7$).

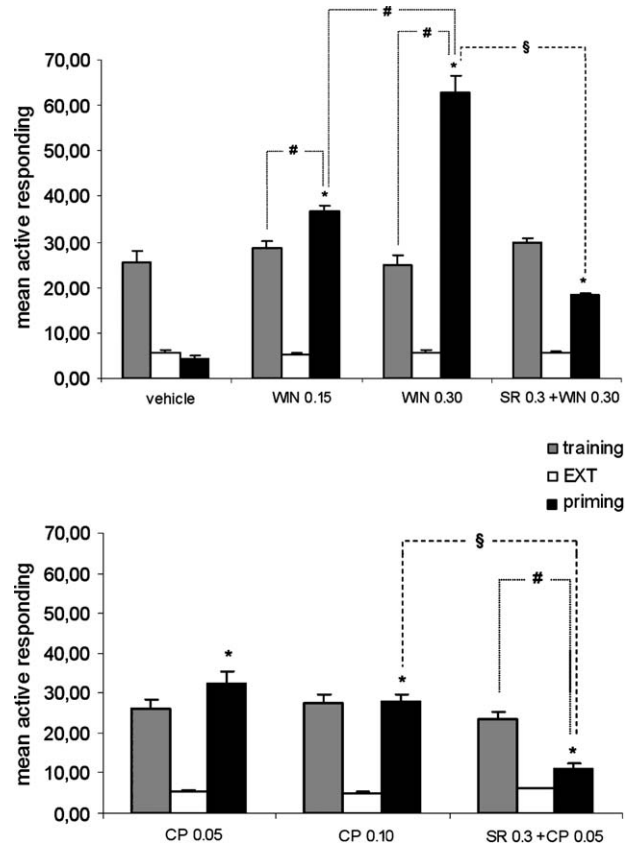


Fig. 2. Antagonism by SR 141716A of the reinstatement of heroin-seeking behaviour induced by WIN 55,212-2 (top) and CP 55,940 (bottom). Each bar represents the mean \pm SEM of active lever-presses over the last three days of heroin SA (training), over the last five consecutive sessions of extinction (EXT) and during the reinstatement test sessions (priming). Doses are expressed as mg/kg. * $P < 0.001$ vs. corresponding EXT and # $P < 0.001$ vs. respective training; ANOVA followed by post hoc test ($n = 7-8$).

3.2. Antagonism by SR 141716A of the CB_1 receptor agonists-induced reinstatement of heroin-seeking

Fig. 2 shows the effect of i.p. priming injection with the synthetic cannabinoid CB_1 receptor agonists WIN 55,212-2 (top) and CP 55,940 (bottom) on the reinstatement of heroin-seeking behaviour as well as antagonism of the above compounds by means of blockade of the CB_1 receptor by SR 141716A. The top panel shows that an acute priming with the vehicle of cannabinoid did not change the mean number of responses on the active lever with respect to previous extinction baseline. On the contrary, priming injection of WIN 55,212-2 at a dose of 0.15 mg/kg reinstated a mean number of responses on the active lever significantly higher ($P < 0.001$) compared with both the extinction and training baselines ($F_{2,12} = 195.8$). WIN 55,212-2 priming dose of 0.3 mg/kg produced a more intense effect, the mean number of responses on the active lever being much higher (+153%) with respect to the corresponding training baseline ($F_{2,12} = 175.8$, $P < 0.001$). Planned

comparisons showed a dose-dependent effect of WIN 55,212-2 primings, the reinstated levels of responding by the two cannabinoid doses being statistically different ($F_{2,36} = 37.98$, $P < 0.0001$).

SR 141716A (0.3 mg/kg) was able to significantly attenuate ($P < 0.001$), but not fully prevent, the reinstated responding for heroin induced by both doses of WIN 55,212-2 primings, a residual effect of the cannabinoid priming being still significantly present following drug primings ($F_{2,12} = 334.1$, $P < 0.001$).

The bottom panel of Fig. 2 illustrates how, similarly to WIN 55,212-2, priming injections of CP 55,940 also produced a significant effect on the reinstatement of heroin-seeking behaviour, the mean number of responses on the active lever being similar to the training baseline but significantly higher ($P < 0.001$) with respect to extinction baseline. No significant differences were found between the two doses (0.05 and 0.1 mg/kg) of CP 55,940 tested ($F_{2,36} = 0.95$, not significant).

Again, SR 141716A (0.3 mg/kg) pre-treatment was able to significantly attenuate the reinstatement of CP 55,940-induced heroin-seeking behaviour by decreasing active responding (-51.81%) with respect to the corresponding training baseline ($F_{2,12} = 56.85$, $P < 0.001$).

3.3. Response records of responding following heroin, WIN 55,212-2 and heroin + SR 141716A primings

Fig. 3 shows some examples of the response patterns of individual rats during heroin self-administration, extinction and following drug priming injections. In particular,

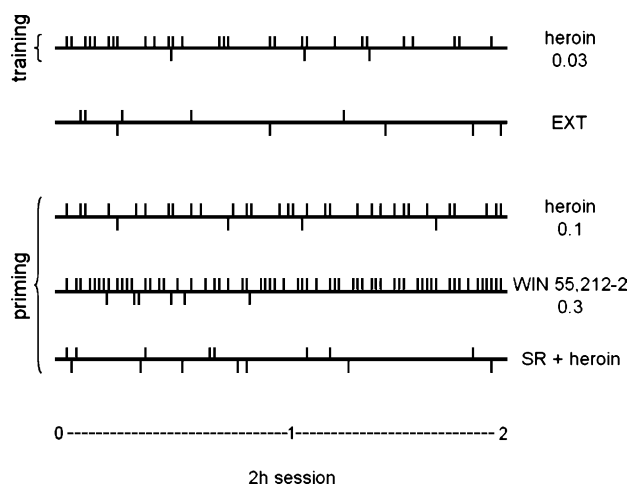


Fig. 3. Individual event records from representative rats during training, extinction and reinstatement test sessions. The vertical deflections above the horizontal line mark the exact time of each response on the active lever, while each deflection below the line represents a response on the inactive lever, over the 2h experimental session. *Training* refers to the last day of heroin self-administration before extinction, *EXT* refers to the last day of extinction before priming tests, while *primings* refer to the reinstatement test sessions following injection of heroin, WIN 55,212-2 or heroin in combination with SR 141716A. Doses are expressed as mg/kg.

the first record refers to the pre-extinction training phase (i.e. last day of heroin self-administration), during which the mean activity on the active lever was rated at 26.66 ± 2.25 , while during extinction (second record, day before priming test) the mean active responding was 5.57 ± 1.25 . Subsequently, the individual response patterns displayed during the different reinstatement test sessions following heroin, WIN 55,212-2 or heroin + SR 141716A primings are presented.

It can be noted that during heroin self-administration (i.e. training), rats exhibited within-session response patterns typically characterized by bursts of 2–3 lever-presses that occurred mainly during the first half of the session. As extinction developed (EXT), animals markedly decreased rate of responding and made very few lever-presses separated by longer inter-response intervals. Importantly, following both heroin and cannabinoid primings, animals display similar evenly spaced responding, regardless of the total number of responses made within the 2 h session. As can be observed in the last record, pre-treatment with SR 141716A drastically reduced the heroin priming effect by gradually lengthening the mean interval between two consecutive responses, so that responding was very slow and temporally dispersed.

However, absence of differences from previous baselines in the mean number of inactive lever presses during the reinstatement test sessions indicates that rats maintained a good discrimination between the active and inactive levers (data not shown), thus dispelling all doubts as to a possible non-specific effect (i.e. generalization) of drug priming injections.

4. Discussion

Results of the present investigation confirm that non-contingent acute priming injection of heroin reinstates drug-seeking behaviour in rats with a previous history of heroin self-administration, even following a long period of abstinence and consequent extinction of self-administration behaviour (De Wit and Stewart, 1983). Heroin priming effects were antagonized by naloxone, suggesting a specific involvement of opioid receptors. In line with previous observations (De Vries et al., 2003; Fattore et al., 2003), the present study demonstrates a clear involvement of the cannabinoid system in the neurobiological mechanisms controlling reinstatement of heroin-seeking behaviour in rats. Indeed, non-contingent priming injections of synthetic cannabinoid CB₁ receptor agonists WIN 55,212-2 and CP 55,940 promptly reinstate heroin-seeking behaviour, at a level even higher than heroin priming.

The effect of WIN 55,212-2 primings appears to be dose-related, with a dose of 0.3 mg/kg inducing a significantly greater effect than the lower dose of 0.15 mg/kg. However, the observation that WIN

55,212-2 resumes heroin-seeking behaviour at a level higher than those reinstated by the same heroin or another CB₁ receptor agonist (CP 55,940) was rather unexpected. At the present time it is somewhat arduous to explain such a finding, but it should be possible to speculate that WIN 55,212-2 might activate additional circuitries synergistic to those activated by heroin and/or CP 55,212-2. Follow-up studies are currently in progress in our laboratory to clarify the specific role of cannabinoid and opioid receptors in reinstating extinguished heroin-seeking behaviour.

Notably, heroin as well as WIN 55,212-2 and CP 55,940 primings significantly increased responding on the previously heroin-paired (active) lever only, and not on the non-drug-paired (inactive) lever, thus indicating the retention of a good discrimination between the two levers. Moreover, at all doses tested cannabinoids did not affect spontaneous motor activity in treated animals (data not shown), thus ensuring the absence of any aspecific effect on responding.

The present results support the hypothesis that in addition to opioid receptors, the cannabinoid CB₁ receptor may also be strictly involved in relapsing mechanisms to heroin. It is therefore reasonable to argue that CB₁ receptors play a permissive role in the expression of heroin priming effects, as these effects are completely antagonized by the CB₁ receptor antagonist SR 141716A at doses devoid of any intrinsic activity in this model. However, the possibility that a single animal model, although largely validated, may not capture the entire experience of human relapse should not be disregarded, the neurobiological, social and genetic factors contributing in various ways to relapse in humans being too numerous and complex.

Recently, increasing evidence has been reported to support the face validity, and likely the predictive validity, of the extinction/reinstatement animal model, at least as a medication screen (Epstein and Preston, 2003; Katz and Higgins, 2003; Littleton, 2003; Shaham et al., 2003). Undoubtedly, this model was successful in individuating brain areas involved in the reinstatement of drug-seeking behaviour and in determining the neuronal events that mediate such phenomena (Stewart, 2000; Kalivas and McFarland, 2003).

Since molecular mechanisms underlying reinstatement of drug-seeking behaviour are still unknown, it is somewhat premature to hypothesize which mechanisms are involved in the observed interactions between cannabinoids and opioids. Under appropriate experimental conditions (i.e. drug doses, routes of drug administration), reinstatement of heroin-seeking can also be obtained by dopaminergic agents such as cocaine or amphetamine (De Wit and Stewart, 1983; De Vries et al., 1998; for a review see Shalev et al., 2002). The present findings might suggest that the interaction between cannabinoids and opioids could in some way

involve the dopaminergic system as a possible functional link. Such a role for the dopaminergic system has already been suggested by different authors with regard to the interactions between cannabinoids and opioids in the reciprocal mechanisms of reward (Tanda et al., 1997; Mascia et al., 1999). It should, however, be borne in mind that negative results have also been reported with regard to a possible interaction between these two endogenous systems in modulating the dopaminergic transmission in reward-relevant areas (French, 1997; Caillé and Parsons, 2003).

A most interesting study by De Vries et al. (1998) shows that reinstatement of heroin-seeking is associated with the expression of behavioural sensitization and therefore primings with drugs inducing cross-sensitization with heroin, such as amphetamine, are able to trigger reinstatement of heroin-seeking. In line with this finding, a cross-sensitization between cannabinoids and opioids has been clearly demonstrated (Cadoni et al., 2001; Rubino et al., 2001, 2003; Lamarque et al., 2001). On the other hand, heroin itself does not reinstate cocaine-seeking and does not induce locomotor sensitization in rats with previous history of cocaine self-administration.

Independently from the possible underlying mechanisms, our findings might have an important clinical impact. In fact, assuming that the animal model of reinstatement of drug-seeking behaviour could be reasonably predictive for relapse to drug-craving and drug-seeking in humans, the present results might suggest that SR 141716A or other CB₁ receptor antagonists could be useful as drug therapies for use in preventing relapse to heroin. Furthermore, since SR 141716A also attenuates reinstatement of cocaine-seeking behaviour induced by priming injections of cocaine in rats with a previous history of cocaine self-administration (De Vries et al., 2001), it can be hypothesized that a block of CB₁ receptors could have a more general role in the mechanisms of relapse to different drugs of abuse. This assertion is further strengthened by a very recent study showing that SR 141716A also blocks methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behaviour (Anggadiredja et al., 2004).

Acknowledgements

This study was supported by funds from the Italian Ministry of University and Scientific Research (MIUR) and Centre of Excellence on 'Neurobiology of Dependence'.

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