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ORIGINAL INVESTIGATION

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Behavioural sensitization after repeated exposure to Δ^9-tetrahydrocannabinol and cross-sensitization with morphine

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Abstract *Rationale:* Repeated exposure to several drugs of abuse has been reported to induce behavioural sensitization. So far no evidence has been provided that such a phenomenon also applies to cannabinoids. *Objectives:* In this study we investigated if repeated exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC) induces behavioural sensitization. In addition we tested the possibility of cross-sensitization between Δ^9-THC and morphine. *Methods:* Male Sprague-Dawley rats were administered for 3 days, twice daily, with increasing doses of Δ^9-tetrahydrocannabinol (2, 4 and 8 mg/kg i.p.) or increasing doses of morphine (10, 20 and 40 mg/kg s.c.) or vehicle. After a washout of 14 days the animals were challenged with Δ^9-THC (75 and 150 µg/kg i.v.), with a synthetic cannabinoid agonist WIN55212–2 (75 and 150 µg/kg i.v.) or with morphine (0.5 mg/kg i.v.), through a catheter inserted into the left femoral vein 24 h before, and the behaviour recorded. *Results:* Rats previously administered with Δ^9-THC showed a greater behavioural activation compared to controls in response to challenge with Δ^9-THC (150 µg/kg i.v.) and to challenge with morphine (0.5 mg/kg i.v.). Similar to that observed after repeated opiates, this behavioural sensitization was characterized by stereotyped activity. Animals administered with a schedule of morphine that induces behavioural sensitization to morphine also showed a behavioural sensitization to challenge with cannabinoids (Δ^9-THC and WIN55212–2, 75 and 150 µg/kg i.v.). The effect of the challenge with Δ^9-THC was prevented by the administration of the CB1 antagonist SR141716A (1 mg/kg i.p.), 40 min beforehand. *Conclusions:* The results of the present study demonstrate that repeated exposure to Δ^9-THC induces behavioural sensitization not only to cannabinoids but also to opiates. This cross-sensitization was symmetrical since rats behaviourally sensitized to morphine were also sensitized to cannabinoids. These observations further support the evidence of an interaction between the opioid and the cannabinoid system and might provide a neurobiological basis for a relationship between cannabis use and opiate abuse.

Keywords Behavioural sensitization · Cross-sensitization · Δ^9-Tetrahydrocannabinol · WIN55212–2 · SR141716A · Morphine

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Introduction

Cannabis is the most widely abused drug in western countries. Its psychoactive component, Δ^9-tetrahydrocannabinol (Δ^9-THC), produces a variety of effects in humans as well as in animals that are mediated by specific receptors in the brain (CB1) (Matsuda et al. 1990; Devane et al. 1992). Recent studies revealed an important functional relationship between the endogenous cannabinoid and opioid system in mediating the pharmacological and behavioural actions produced by these agents including their reinforcing effects (see Ambrosio et al. 1999 for review). Interest in cannabis/opiate interactions derives mainly from the possibility that cannabis use may lead or predispose to opiate abuse (Clayton and Voss 1981; O'Donnel and Clayton 1982; Kandel 1984). Both cannabinoids and opiates stimulate mesolimbic dopamine (DA) transmission through an activation of μ_1 opioid receptors (Tanda et al. 1997). Moreover in transgenic mice lacking the cannabinoid CB1 receptor the reinforcing properties of morphine and the severity of the withdrawal syndrome are greatly reduced (Ledent et al. 1999). On the other hand, maternal exposure to Δ^9-THC has been reported to facilitate morphine self-administration and to increase the expression of the µ opioid receptors in several cortical and limbic areas of the offspring (Vela et al. 1998). Further evidence for an interaction between the cannabinoid and opioid system derives from studies showing that naloxone, the opiate antagonist, precipitates a withdrawal syndrome in rats

exposed, during the perinatal period, to cannabinoids (Vela et al. 1995).

Behavioural sensitization after repeated exposure to drugs of abuse has been proposed to play a role in drug addiction (Robinson and Berridge 1993; Stewart and Badiani 1993; De Vries et al. 1998; Kalivas et al. 1998; Vezina et al. 1999; Lorrain et al. 2000). Behavioural sensitization has been reported to develop to most drugs of abuse such as opiates, psychostimulants, nicotine and phencyclidine. To the best of our knowledge behavioural sensitization has not yet been reported after repeated cannabinoid exposure (Arnold et al. 1998). This, as well as the possibility that cannabinoids produce their effects on DA transmission through an indirect action on μ_1 opioid receptors, prompted us to test the hypothesis of a cross-sensitization between morphine and cannabinoids. To this aim we have utilized the schedule of repeated treatment with morphine previously shown by us to induce opiate sensitization (Cadoni and Di Chiara 1999). We utilized a similar schedule with Δ^9-THC to induce behavioural sensitization to Δ^9-THC.

Materials and methods

Animals

Male Sprague-Dawley rats (Charles River, Calco, Italy) of 125–150 g at the beginning of the treatment were housed in groups of three per cage, with food and water ad libitum, under an artificial 12-h light-dark cycle and standard conditions of temperature and humidity. After 3–4 days of habituation to the animal room, rats were administered according to the group treatment. One group received for 3 days, twice a day, increasing doses of Δ^9-THC (2, 4 and 8 mg/kg i.p.). A second group received an equivalent volume of vehicle (3 ml/kg i.p.) according to the same schedule. A third group was injected with increasing doses of morphine hydrochloride (10, 20 and 40 mg/kg s.c.) twice daily for 3 days. A fourth group received an equivalent volume of saline (2 ml/kg s.c.). Rats were taken from their home cages, injected and returned immediately to the cage.

All animal experimentations have been conducted in accordance with the "Principles of Laboratory Animal Care" (NIH publication number 85–23, revised 1985) and with the guidelines for care and use of experimental animals of the European Union (86/609/EEC; D.L.: 27.01.1992, N°116).

Surgery

The morning of the 14th day after the last injection the rats were anaesthetized with 300 mg/kg i.p. of chloral hydrate (Carlo Erba, Italy). The left femoral vein was exposed and a polyethylene catheter was inserted in the vein and tunnelled subcutaneously to exit at the nape of the neck according to the method of Crane and Porrino (1989).

Behavioural testing

After surgery the animals were transferred to another room and each placed in an hemispherical bowl. The following day the animals, habituated to the novel environment, were tested by a challenge with the drug. During testing each animal was videotaped and then the behavioural response to the drug analysed by an observer unaware of the treatment the animal received. As previously described (Cadoni and Di Chiara 1999, 2000; Cadoni et al. 2000) two behavioural categories were distinguished: a *non-stereotyped activity* consisting of forward locomotion with sniffing around and a *stereotyped activity* consisting of repetitive gnawing, sniffing downward and licking confined and apparently purposeless. The percentage of time spent by the rat performing each behavioural category was recorded at 10-min intervals for 40–60 min following drug challenge. Furthermore, the percentage of time spent by the animal in each behavioural item (locomotion, sniffing, gnawing, etc.) during the total period of observation (40 or 60 min) was also recorded.

Drugs

Δ^9-THC, kindly supplied by the National Institute on Drug Abuse (NIDA, Research Triangle Park, N.C., USA) and WIN55212–2 (RBI Chemicals, Amersham, Milan, Italy) were suspended in 0.3% Tween 80 in saline and administered i.p. (3 ml/kg) or i.v. (1 ml/kg). Morphine hydrochloride (Sigma Chemical, St. Louis, Mo., USA) was dissolved in saline and injected in a volume of 2 ml/kg body weight s.c. or i.v. (1 ml/kg). SR171416A, kindly supplied by Sanofi Research (Montpellier, France) was suspended in 0.3% Tween 80 in saline and administered i.p. (3 ml/kg).

Statistics

The results were expressed as mean (± SEM) of time spent performing each behavioural item. Differences in behavioural scores were assessed by one-way ANOVA or two-way ANOVA for repeated measure with drug treatment as independent variable. When a significant F value was found, post hoc analysis was performed by Tukey's test.

Results

Sensitization to Δ^9-THC and to morphine after repeated exposure to Δ^9-THC

Repeated exposure to Δ^9-THC induced behavioural sensitization to the same cannabinoid administered 14 days after the last Δ^9-THC administration (Fig. 1). While 150 µg/kg of Δ^9-THC injected i.v. produced a short-lasting non-stereotyped behavioural activation (exploratory behaviour with locomotion and sniffing around) the same dose injected to rats pretreated with the cannabinoid induced a greater locomotor response associated with a more intense sniffing around [total locomotion $F(1,7)=93.33$, $P<0.0001$, Tukey's post hoc $P<0.001$; total sniffing around $F(1,7)13.95$, $P<0.01$, Tukey's post hoc $P<0.01$], both in terms of degree and duration (Fig. 1A, B) [*non-stereotyped activity*: $F_{group}(1,7)=13.62$, $P<0.01$; $F_{time}(3,21)=50.73$, $P<0.0001$; $F_{time\times group}(3,21)=10.23$, $P<0.001$] and a stereotyped activity [*stereotyped activity*: $F_{group}(1,7)=12.86$, $P<0.01$; $F_{time}(3,21)=8.34$, $P<0.001$; $F_{time\times group}(3,21)=8.34$, $P<0.001$] otherwise absent in controls (Fig. 1A, B). This stereotyped activity resembled that elicited by morphine in rats sensitized to opiates since it consisted mainly of repetitive gnawing and confined sniffing directed at the cage floor [total gnawing $F(1,7)=15.07$, $P<0.01$, Tukey's post hoc $P<0.01$; total confined sniffing $F(1,7)=70.67$, $P<0.0001$, Tukey's post hoc $P<0.001$]. Pretreatment with Δ^9-THC induced sensitization also to the effects of morphine

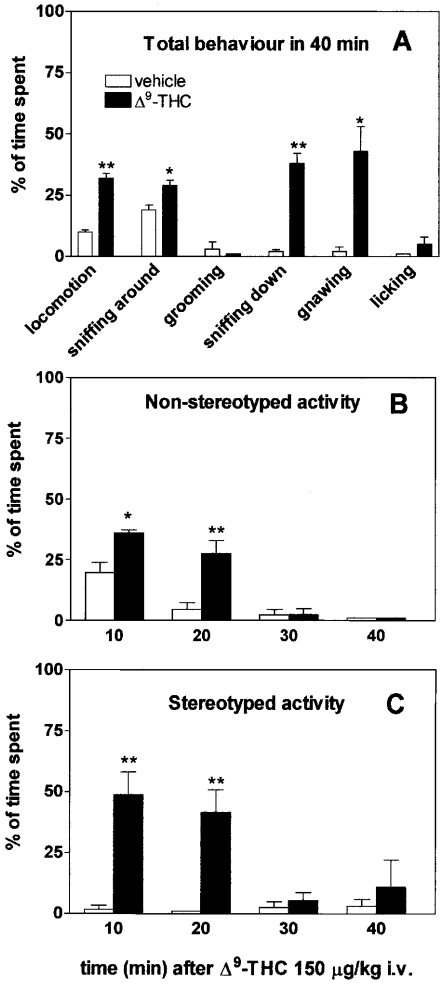

Fig. 1A–C Effect of a challenge with Δ^9-tetrahydrocannabinol (Δ^9-THC; 150 μg/kg i.v.) in rats repeatedly exposed to vehicle (*unfilled bars*) or to Δ^9-THC (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=5) of the percentage of time spent performing each behavioural item. * P<0.01 and ** P<0.001 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural categories is not 100, the difference has to be due to sedation or no activity

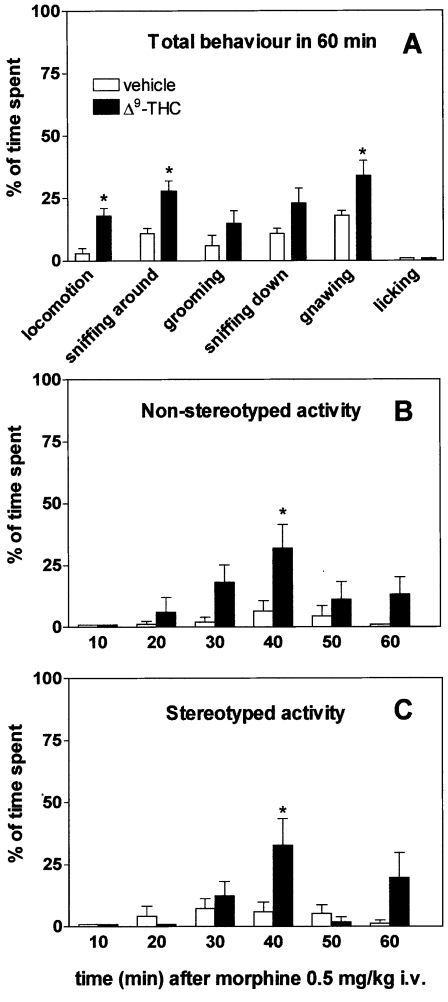

Fig. 2A–C Effect of a challenge with morphine (0.5 mg/kg i.v.) in control rats (*unfilled bars*) and in rats sensitized to Δ^9-THC (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=6) of the percentage of time spent performing each behavioural item. * P<0.05 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural categories is not 100, the difference has to be due to sedation or no activity

(0.5 mg/kg i.v.) as shown in Fig. 2 [*non-stereotyped activity*: $F_{group}(1,10)=8.31$, $P<0.05$; $F_{time}(5,50)=3.72$, $P<0.01$; $F_{time\times group}(5,50)=1.84$, n.s.; *stereotyped activity*: $F_{group}(1,10)=3.47$, $P<0.05$; $F_{time}(5,50)=4.56$, $P<0.01$; $F_{time\times group}(3,50)=3.57$, $P<0.01$]. Post hoc test showed a significant difference between groups at 40 min ($P<0.05$ Tukey's test). The analysis of each behaviour (Fig. 2A) revealed a significant difference between groups in total locomotion [$F(1,10)=6.67$, $P<0.05$, post hoc $P<0.05$], total sniffing around [$F(1,10)=8.07$, $P<0.05$, post hoc $P<0.05$] and total gnawing [$F(1,10)=5.40$, $P<0.05$, post hoc$P<0.05$].

Effects of cannabinoids in rats sensitized to morphine

As shown in Fig. 3 administration of 75 μg/kg i.v of Δ^9-THC induced in control rats only a short-lasting behavioural activation characterized mainly by forward locomotion and sniffing around. The same dose of Δ^9-THC injected to animals sensitized to morphine induced a stereotyped behavioural activation with gnawing and confined sniffing [*non-stereotyped activity*: $F_{group}(1,6)=4.82$, n.s.; $F_{time}(3,18)=28.42$, $P<0.0001$; $F_{time\times group}(3,18)=3.11$, $P<0.05$; *stereotyped activity*: $F_{group}(1,6)=70.90$, $P<0.0001$; $F_{time}(3,18)=14.50$, $P<0.0001$; $F_{time\times group}(3,18)=12.32$, $P<0.001$]. Tukey's post hoc test revealed a significant difference between groups at 10 and 20 min ($P<0.05$).

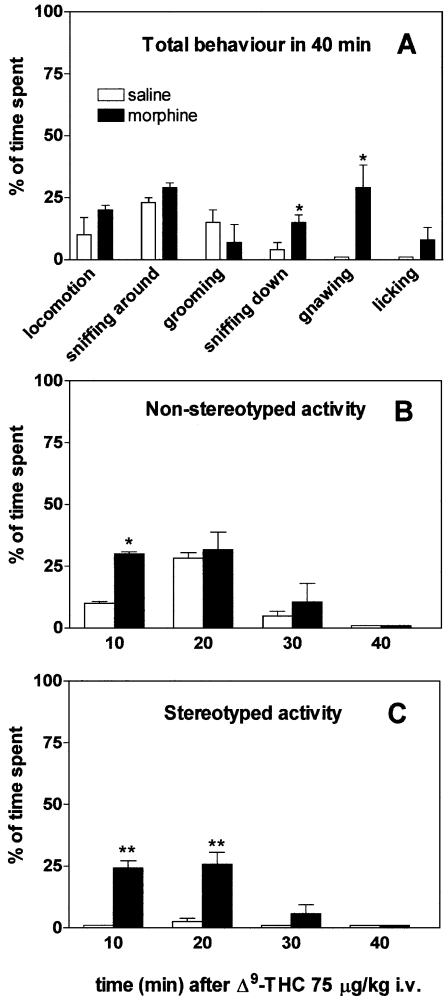

Fig. 3A–C Effect of a challenge with Δ^9-THC (75 μg/kg i.v.) in controls (*unfilled bars*) and in rats sensitized to morphine (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=4) of the percentage of time spent performing each behavioural item. * P<0.05 and ** P<0.001 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural categories is not 100, the difference has to be due to sedation or no activity

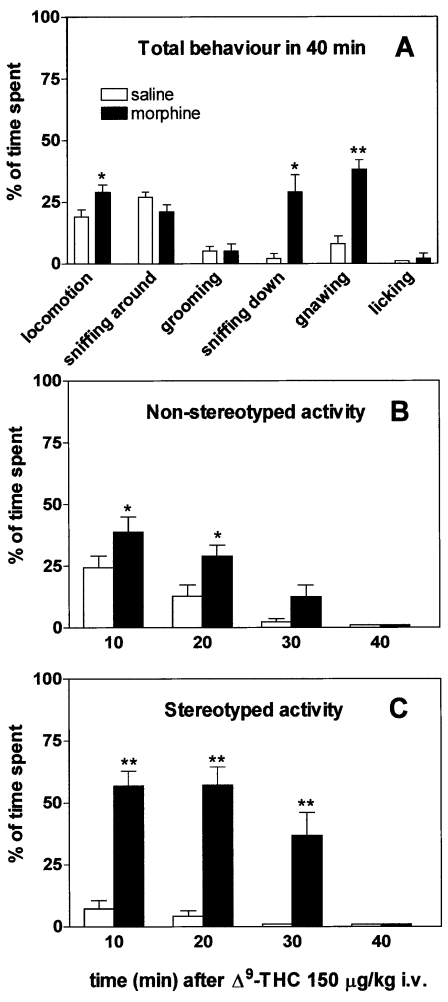

Fig. 4A–C Effect of a challenge with Δ^9-THC (150 μg/kg i.v.) in controls (*unfilled bars*) and in rats sensitized to morphine (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=7) of the percentage of time spent performing each behavioural item. * P<0.05 and ** P<0.001 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural categories is not 100, the difference has to be due to sedation or no activity

The analysis of each behaviour (Fig. 3A) revealed a significant difference between groups only in sniffing downward [F(1,6)=5.67, P<0.05, post hoc P<0.05] and gnawing [F(1,6)=9.80, P<0.05, post hoc P<0.05]. Challenge with 150 μg/kg of Δ^9-THC (Fig. 4A, B) induced a greater locomotor activation [total locomotion F(1,12)=12.47, P<0.01, post hoc P<0.01; *non-stereotyped activity*: F_{group}(1,12)=7.26, P<0.05; F_{time}(3,36)= 42.24, P<0.0001; $F_{time\times group}$(3,36)=2.80, P<0.05] and stereotyped activity (Fig. 4A, C) in morphine-pretreated rats as compared to controls [*stereotyped activity*: F_{group}(1,12)=58.63, P<0.0001; F_{time}(3,36)=25.07, P<0.0001; $F_{time\times group}$(3,36)=16.72, P<0.0001; total sniffing downward F(1,12)=14.56, P<0.01, post hoc P<0.05; total gnawing F(1,12)=46.66, P<0.0001, post hoc P<0.001]. Post hoc analysis of the data of non-stereotyped and stereotyped activity revealed a significant difference between groups at 10, 20 and 30 min (P<0.05; Fig. 4B, C). Similar results were obtained with the synthetic cannabinoid agonist WIN55212–2 (Figs. 5, 6) administered at the same doses [75 μg/kg: *non-stereotyped activity*: F_{group}(1,10)=18.32, P<0.01; F_{time}(3,30)=25.51, P<0.0001; $F_{time\times group}$(3,30)=5.81, P<0.01; *stereotyped activity*: F_{group}(1,10)=32.66, P<0.001; F_{time}(3,30)=11.93, P<0.0001; $F_{time\times group}$(3,30)=8.31, P<0.001; 150 μg/kg: *non-stereotyped activity*: F_{group}(1,6)=9.28, P<0.05; F_{time}(3,18)=5.83, P<0.01; $F_{time\times group}$(3,18)=0.44, n.s.; *stereotyped activity*: F_{group}(1,6)=27.70, P<0.01; F_{time}(3,18)=

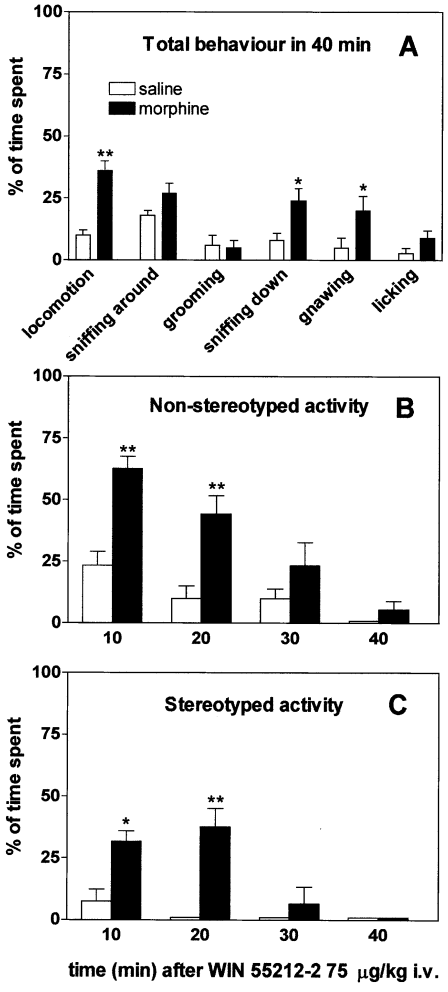

Fig. 5A–C Effect of a challenge with WIN55212–2 (75 μg/kg i.v.) in controls (*unfilled bars*) and in rats sensitized to morphine (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=6) of the percentage of time spent performing each behavioural item. * P<0.05 and ** P<0.001 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural categories is not 100, the difference has to be due to sedation or no activity

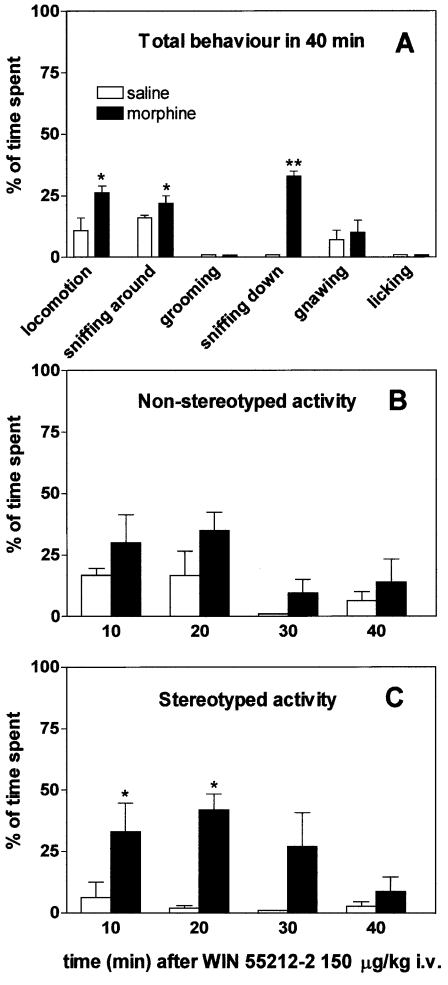

Fig. 6A–C Effect of a challenge with WIN55212–2 (150 μg/kg i.v.) in controls (*unfilled bars*) and in rats sensitized to morphine (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=4) of the percentage of time spent performing each behavioural item. * P<0.05 and ** P<0.001 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural categories is not 100, the difference has to be due to sedation or no activity

1.82, n.s.; $F_{time\times group}(3,18)$=1.67, n.s.]. The analysis of each behaviour after challenge with 75 μg/kg WIN55212–2 (Fig. 5A) revealed a significant difference between groups in total locomotion [$F(1,10)$=23.70, P<0.001, Tukey's test P<0.001], total sniffing downward [$F(1,10)$=5.93, P<0.05, Tukey's test P<0.05] and total gnawing [$F(1,10)$=5.27, P<0.05, Tukey's test P<0.05]. Analysis of the results obtained after 150 μg/kg WIN55212–2 showed a significant difference in the sensitized group compared to controls in total locomotion [$F(1,6)$=21.05, P<0.01, post hoc P<0.01], total sniffing around [$F(1,6)$=5.90, P<0.05, post hoc P<0.05] and total stereotyped sniffing [$F(1,6)$=205.11, P<0.0001, post hoc P<0.001] (Fig. 6A).

The effect of Δ^9-THC was prevented by pretreatment with the cannabinoid antagonist SR141716A (1 mg/kg i.p.) given 40 min in advance [*non-stereotyped activity*: $F_{group}(1,6)$=319.81, P<0.0001; $F_{time}(3,18)$= 23.76, P<0.0001; $F_{time\times group}(3,18)$=14.58, P<0.001; *stereotyped activity*: $F_{group}(1,6)$=223.91, P<0.0001; $F_{time}(3,18)$=16.44, P<0.0001; $F_{time\times group}(3,18)$=15.17, P<0.0001] (Fig. 7B, C). In Fig. 7A are shown the total activities for each group. Significant differences between groups were obtained for total locomotion [$F(1,6)$=98.67, P<0.0001, post hoc P<0.001], total sniffing around [$F(1,6)$=6.92, P<0.05, post hoc P<0.05], total sniffing downward [$F(1,6)$=20.51, P<0.01, post hoc P<0.01] and total gnawing [$F(1,6)$=82.56, P<0.001,

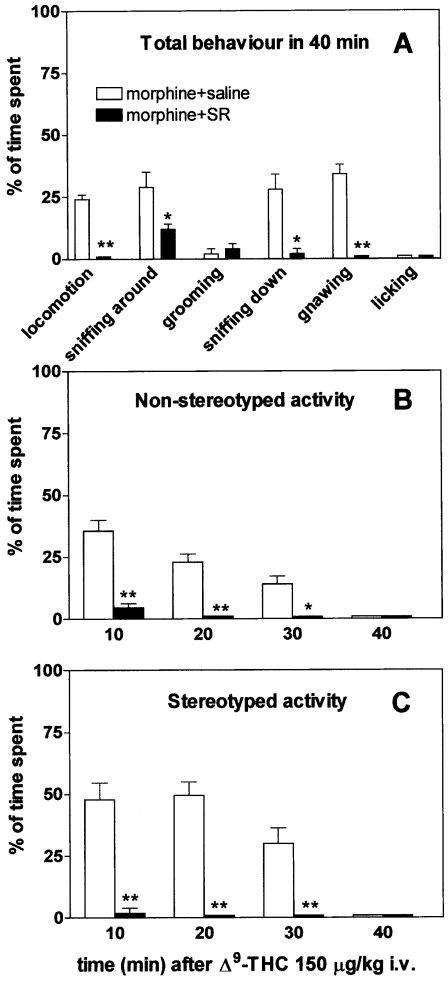

Fig. 7A–C Effect of Δ^9-THC (150 μg/kg i.v.) in rats sensitized to morphine and pretreated, 40 min before Δ^9-THC challenge, with vehicle (*unfilled bars*) or with SR141716A 1 mg/kg i.p. (*filled bars*). **A** Percentage of time spent performing each behavioural item during the total period of observation. In **B** and **C** two or more behavioural items, shown in **A**, are grouped and scored every 10 min. Results are expressed as mean ± SEM (n=4) of the percentage of time spent performing each behavioural item. * P<0.05 and ** P<0.001 by Tukey's test versus the corresponding control value. In **B** and **C** the time points at which the sum of the percentage of time spent in the different behavioural items is not 100, the difference has to be due to sedation or no activity

post hoc P<0.001]. SR141716A by itself did not induce behavioural activation (data not shown).

Discussion

The findings of the present study are twofold. The first finding is that repeated exposure to Δ^9-THC induces behavioural sensitization to the same drug. This adds Δ^9-THC to the list of drugs of abuse capable of inducing behavioural sensitization. The second finding is that cross-sensitization takes place between Δ^9-THC and morphine. This cross-sensitization is context-independent since it was induced in the home cage and was expressed in a different environment (hemispherical bowls).

The present observations contrast with the report of Arnold et al. (1998) who failed to observe sensitization after repeated treatment with the synthetic cannabinoid agonist CP 55,940. In addition to differences in the strain of rats utilized in that study (Lewis instead of Sprague-Dawley) there are further differences in the doses and schedule of administration of the cannabinoid. Thus, assuming a potency ratio of 30 between CP 55,940 and Δ^9-THC (Gold et al. 1992; Wiley et al. 1995), the doses of CP 55,940 utilized by Arnold et al. (1998) correspond to 0.3, 0.75 and 1.5 mg/kg of Δ^9-THC and were given every 2nd day for 2 weeks. In the present study we utilized doses of Δ^9-THC five to seven times larger than those of CP 55,940 employed by Arnold et al. (1998) and we administered them twice a day for 3 days. Therefore, in comparison with that of Arnold et al. (1998), the regimen of drug administration utilized by us involved exposure to higher doses of the cannabinoid for a shorter time period.

Behavioural sensitization might result from adaptive changes related to those that result in tolerance (reverse tolerance). Thus, while acute cannabinoids in large doses reduce motor activity, repeated exposure results in tolerance to their motor depressant effects (see Chaperon and Thiébot 1999 for review). Indeed, downregulation of CB1 receptors develops after chronic exposure to Δ^9-THC or CP 55,940 and the extent of this effect is different in different brain areas (Oviedo et al. 1993; Rodriguez de Fonseca et al. 1994; Romero et al. 1997). Thus, while CB1 receptor binding markedly decreased in the lateral caudate putamen, substantia nigra and septum no significant reduction was observed in the nucleus accumbens (NAc) and basolateral amygdala (Romero et al. 1997, 1998). To date, however, the relationship of these changes to the sensitization observed here is unknown.

The mechanism of sensitization to Δ^9-THC and of the cross-sensitization with morphine might involve the ability of these drugs to release DA in the NAc, a property common to many drugs that induce sensitization (Kalivas and Stewart 1991). On the other hand, release of DA by Δ^9-THC has been suggested to occur through an activation of an endogenous opioid system acting through μ_1 opioid receptors located in the ventral tegmental area (Tanda et al. 1997). Therefore, both DA and opioid mechanisms might be involved in the mechanism of sensitization to cannabinoids and to morphine. Release of DA by cannabinoids has also been suggested to involve a depression of glutamate release on GABAergic neurons of the NAc which tonically inhibit dopaminergic neurons (Robbe et al. 2001). However, the role of opioid receptors in this mechanism and its role in the sensitization reported here is unknown.

In view of the cross-sensitization between morphine and cannabinoids a more likely explanation of sensitization is one that takes into account the common role of opioid receptors in the mechanism of DA release by

Δ^9-THC and by morphine (Tanda et al. 1997). DA and opioid mechanisms, however, might be differentially involved in the mechanism of the induction and of the expression of sensitization to cannabinoids.

A primary role of DA in the expression rather than in the induction of sensitization seems indicated by the observation that blockade of DA D1 receptors impairs the expression but not the induction of sensitization to morphine (Kalivas 1985; Vezina and Stewart 1989; Jeziorski and White 1995). On the other hand, sensitization to the locomotor effects of morphine is abolished while sensitization to cocaine motor stimulant effects is preserved in CB1 knockout mice (Martin et al. 2000). These observations are consistent with the idea of an involvement of opioid and CB1 receptors in the induction of sensitization to opiates and cannabinoids and with a distinction between the mechanism of induction of sensitization to opiates and that to psychostimulants. The substrate for the interaction between morphine and cannabinoids reported in the present and other studies might be the ability of cannabinoids to release endogenous opioids. Evidence for this mechanism has been reported by Valverde et al. (2001) who have recently shown that Δ^9-THC releases endogenous enkephalins in the NAc and facilitates their antinociceptive and antidepressant-like effects. Further studies on the relative effects of DA and opioid antagonists in the induction and expression of behavioural sensitization to cannabinoids are needed to clarify this issue.

Chronic Δ^9-THC treatment has been reported to induce sensitization to the psychomotor effects of amphetamine in rats (Gorriti et al. 1999). In this study, however, sensitization to Δ^9-THC was not tested and it is unclear if the adaptive changes induced by Δ^9-THC in the study by Gorriti et al. (1999) have any relationship with those induced in the present study. Thus, sensitization to amphetamine in the study by Gorriti et al. (1999) was tested as early as 30 min or 24 h after the last injection of Δ^9-THC at a time when acute abstinence to Δ^9-THC takes place. In our study instead, sensitization to morphine was tested 14 days after Δ^9-THC treatment, an interval correspondent to that utilized by us for testing sensitization to morphine (Cadoni and Di Chiara 1999). It is notable, on the other hand, that in our studies, in contrast to the observation of cross-sensitization between morphine and Δ^9-THC, no cross-sensitization between amphetamine and morphine was obtained (Cadoni and Di Chiara 1999). It is possible therefore that the mechanism of the sensitization to amphetamine induced by Δ^9-THC in the study by Gorriti et al. (1999) is different from that of the sensitization to Δ^9-THC and morphine obtained in the present study. Given the high colocalization of the DA and CB1 receptors in neurons of the basal ganglia and of the limbic cortex (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1993) and of the recently reported interactions between the cannabinoid and dopaminergic systems (Giuffrida et al. 1999; Beltramo et al. 2000) an interaction between CB1 and DA D1 receptors might be the basis of the sensitization to amphetamine induced by Δ^9-THC in the conditions of Gorriti et al. (1999). An alternative mechanism suggested by these authors is the involvement of the pituitary adrenal axis. In fact CB1 cannabinoid agonists are potent activators of the pituitary adrenal axis (Kubena et al. 1971; Rodriguez de Fonseca et al. 1991). Each one of these mechanisms, although applicable to the results of Gorriti et al. (1999), is unlikely to be applicable to our observations given the dissociation between opioid/cannabinoids sensitization and psychostimulants sensitization under our conditions.

This study provides further evidence for the notion of the existence of strong homologies between cannabinoids and opiates. Given the role assigned to behavioural sensitization in current theories of drug addiction (Robinson and Berridge 1993; Stewart and Badiani 1993; Di Chiara 1995), the demonstration of cross-sensitization between Δ^9-THC and morphine provides a neurobiological substrate for the postulated role of cannabis use in the vulnerability to opiate abuse (Clayton and Voss 1981; O'Donnel and Clayton 1982; Kandel 1984).

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