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Δ^9-Tetrahydrocannabinol-induced conditioned place preference and intracerebroventricular self-administration in rats

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Abstract

On the basis of contradictory findings on the rewarding effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) in laboratory animals, the effect of the compound on conditioned place preference and intracerebroventricular (i.c.v.) self-administration in a free-choice procedure, using a wide range of doses (0.015–6 mg/kg for conditioned place preference test and 0.01–1 μg/2 μl/infusion for i.c.v. self-administration), was studied in Wistar rats. The present results showed that Δ^9-THC induced reward in both tests, but only at the lowest tested doses (0.075–0.75 mg/kg i.p. for conditioned place preference test and 0.01–0.02 μg/infusion for i.c.v. self-administration). This effect was fully antagonised by i.p. pretreatment with the cannabinoid CB_1 receptor antagonist, SR 141716A [*N*-piperidino-5-(4-chlorophenyl)1-(2,4-dichlorophenyl)-4 methyl pyrazole 3-carboxamide] (0.25–1 mg/kg), and the opiate receptor antagonist, naloxone (0.5–2 mg/kg), suggesting the involvement of both endocannabinoid and opioid systems. In conclusion, these findings demonstrate, for the first time, that low doses of Δ^9-THC can act as an effective reinforcer in Wistar rats providing a reliable animal model of human marijuana abuse.

Keywords: Δ^9-THC; Conditioned place preference; i.c.v. self-administration

1. Introduction

Although cannabinoids have clear addictive potential at the human level, knowledge of the abuse liability of cannabis and of the reinforcing/dependence-producing effects of its psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), is still controversial. Animal studies indicate that Δ^9-THC and other synthetic cannabinoid receptor agonists can induce both rewarding and aversive effects, using several paradigms (Gardner, 2002; Maldonado, 2002; Tanda and Goldberg, 2003). Among them, conditioned place preference and intravenous (i.v.) self-administration are the most currently used procedures to provide an indication of drug-related motivational effects in animals.

In the conditioned place preference paradigm, Δ^9-THC administration has been shown to produce place aversion or no effect in rats (Parker and Gillies, 1995; Sanudo-Peña et al., 1997; Mallet and Beninger, 1998; Cheer et al., 2000; Robinson and Berridge, 2003) and mice (Hutchenson et al., 1998). On the other hand, some of the same authors reported that Δ^9-THC produced place preference both in rats (Lepore et al., 1995) and mice (Valjient and Maldonado, 2000; Valjient et al., 2002; Castañe et al., 2003), using the same test but with a procedural modification (time between dosing and number of pairings) designed to minimize or eliminate possible anhedonic or dysphoric effects.

Past attempts to demonstrate intravenous self-administration of Δ^9-THC were relatively unsuccessful (Kaymakcalan, 1972, 1973; Pickens et al., 1973; Harris et al., 1974; Leite and Carlini, 1974; Carney et al., 1977; Van Ree et al., 1978; Mansbach et al., 1994), both in rats and monkeys. Recently, persistent intravenous self-administration of Δ^9-THC, rapidly extinguished by administering the cannabinoid CB_1 receptor antagonist [*N*-piperidino-5-(4-chlorophenyl)1-(2,4-dichlorophenyl)-4 methyl pyrazole 3-carboxamide] (SR 141716A), was first demonstrated by Tanda et al. (2000) and confirmed

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by Justinova et al. (2003) in squirrel monkeys using a dose range lower than that previously used in other Δ^9-THC self-administration studies and comparable with that contained in a single puff of a marijuana cigarette (Agurell et al., 1986).

Until recently, the cannabinoid literature was marked by failure to demonstrate reliable Δ^9-THC self-administration in rodents. Only in one early series of studies (Takahashi and Singer, 1979, 1980) was Δ^9-THC self-administration behavior above placebo level found in food-deprived rats, but it immediately disappeared when either food deprivation or the automatic food presentation was discontinued.

The present study was designed to investigate the reinforcing properties of Δ^9-THC in the rat, using a range of doses lower that those, until now, employed in literature. For this purpose, we have first evaluated the possibility of revealing rewarding effects of Δ^9-THC in the classical conditioned place preference task. In a second set of experiments, we sought to examine whether Δ^9-THC was self-administered through an intracerebroventricular (i.c.v.) route. This method (Braida et al., 1998) presents advantages, such as a durable preparation, the possibility of simultaneous choice between the addicting drug and vehicle, and the avoidance of peripheral effects. Finally, the involvement of both cannabinoid CB_1 and opioid receptors on Δ^9-THC-induced rewarding effects was investigated peripherally, pretreating rats with SR 141716A or naloxone in both tests.

2. Materials and methods

2.1. Conditioned place preference

2.1.1. Animals

Male Wistar rats (Charles River, Calco, Como, Italy) weighing 200–300 g were housed in individual cages in a climatically controlled colony room under a 12-h light–dark cycle (lights on 8 a.m.). Food and water were continuously available, and each animal was handled daily through the first week. Experimental testing began 7 days after this acclimatisation period.

All procedures were carried out in accordance with the Italian Government Decree No. 94/2000-A.

2.1.2. Apparatus

Conditioned place preference was tested in a rectangular Plexiglass shuttle box (80×25×36 cm), as described elsewhere (Sala et al., 1995) with slight modifications. Briefly, the apparatus was divided into two equal-sized compartments separated by a guillotine door. One compartment was white with a stainless steel mesh floor and the other was black with a Plexiglass floor. The visual and tactile cues were balanced such that no evident preference was exhibited prior to conditioning.

2.1.3. Measurement of conditioned place preference

Conditioned place preference consisted of three phases: preconditioning, conditioning, and postconditioning.

2.1.3.1. Preconditioning. On Days 1–2, the rats were allowed to explore the two compartments of the shuttle box for 15 min each day. To check for any initial unconditioned preference for either of the two sites, the time spent by each animal in the two large compartments on the third day was recorded.

2.1.3.2. Conditioning. Conditioning sessions (four for Δ^9-THC, four for vehicle) were conducted once daily in the morning (9 a.m.) for 8 days. Ten min after the i.p. injection of Δ^9-THC (0.015–6 mg/kg), the animals were placed in the conditioned compartment for 30 min, with the door closed. On alternate days, animals receiving cannabinoid vehicle (cremophor, ethanol, and saline, 1:1:18) were placed in the opposite compartment for 30 min. For the antagonism studies, rats received an i.p. injection of SR 141716A HCl (0.25–1 mg/kg) or naloxone HCl (0.5–2 mg/kg) or the appropriate vehicle (cannabinoid vehicle or saline) 20 min before the maximal reinforcing dose of Δ^9-THC or cannabinoid vehicle and were placed in the conditioned compartment for 30 min. On alternate days, animals receiving double injection of appropriate vehicle were placed in the opposite compartment for 30 min. Drug–texture pairings were always counterbalanced.

2.1.3.3. Postconditioning. On the test day, neither drug nor vehicle was injected. Each rat was placed at the intersection of the two compartments, with access to both sides, and the time spent in each of the two compartments was measured over a 15-min period as an indicator of reinforcing properties.

2.2. Intracerebroventricular self-administration

2.2.1. Surgical procedure

Animals were anesthetized with chloral hydrate (450 mg/kg i.p.) and implanted with i.c.v. double-guide stainless-steel cannulas (22 gauge), anchored to a pedestal as described elsewhere (Braida et al., 1998).

Each rat was allowed to recover for approximately 1 week. To ascertain the accuracy of the i.c.v. injections, at the end of the experiments, the rats were injected by the same route with 10 μl of a saturated solution of Evans blue (Merck) and killed immediately; mascroscopic examination of the brain confirmed that only the area around the lateral ventricles was stained.

2.2.2. Apparatus

An operant chamber (Coulbourn Instruments, England) was housed in a sound-attenuating cubicle. The chamber was equipped, as previously described (Braida et al., 1998), with a house light, an exhaust fan, a liquid swivel on the ceiling, two response levers 6.8 cm above the floor on the

front and the right side walls, and two solenoid-activated dipper dispensers to the left of each lever. A response on either lever resulted in the illumination of a cue light fitted in each dispenser and the delivery of 0.1 ml of water over a period of 8 s.

During the daily experimental session, a bilateral injection cannula (28 gauge) was placed inside the double-guide cannula. The distal ends of the injection cannula were connected to two Silastic flexible coiled spring tubes, which, in turn, were connected to a flow-through swivel. The swivel was connected by tubing to two infusion pumps (Mod.A-99, Razel), for drug delivery, outside the sound-attenuated cubicle. The perfusion tubes easily rotated the liquid swivel. Each infusion delivered a volume of 2 μl/8 s. If the rat pressed the lever twice within the 8-s period, the event was recorded as not reinforced.

The chamber was connected to a Basilink Data Acquisition System (Ugo Basile, Comerio, Varese, Italy), which controlled reinforcement schedules. A microprocessor assembler (Ugo Basile) gathered, listed, and, every 5 min, printed the total number of bar pressings and the total number of reinforced bar pressings for each lever.

2.2.3. Procedure

2.2.3.1. Training procedure. Before surgery, rats previously deprived of water for 23 h were individually trained for 1 h daily to press both active levers to obtain water as reinforcer for 1 week, in a continuous reinforcement schedule. One week after surgery, single rats were again placed in the operant chamber, in the same continuous reinforcement schedule. Two microliters of sterile vehicle (cremophor, ethanol, and cerebrospinal fluid, 1:1:18) was obtained each time the rats pressed either lever. During the training procedure, water was delivered after each lever pressing. This procedure was repeated daily for 1 h until baselines were judged to be stable (5 days at least). Lever pressing was usually acquired within four/five sessions, and a stable pattern of responding developed within 2 weeks.

2.2.3.2. Testing procedure. The drug sessions were carried out on the basis of individual preference for one of the levers, the preferred one always being associated with the vehicle (2 μl/infusion) and the nonpreferred one with Δ^9-THC (0.01–1 μg/ 2 μl). Then, each rat, already checked during training for its preference for one of the two levers, was evaluated in a continuous reinforcement schedule for operant responding after the self-administration of the different concentrations of Δ^9-THC during a 1-h daily session. Each unit dose was given in a counterbalanced order and only when the baseline response for the preceding unit dose was stable.

For the antagonism studies, further groups of rats received an i.p. injection of SR 141716A HCl (0.5 mg/kg) or naloxone HCl (2 mg/kg) or the appropriate vehicle (cannabinoid vehicle or saline) 20 min before each daily session, during which the maximally self-administered unit dose of Δ^9-THC was available.

2.3. Drugs

The following drugs were used: Δ^9-THC (kindly supplied by GW Pharma, Salisbury, England), in a range of doses from 0.015 to 6 mg/kg for the conditioned place preference test and from 0.01 to 1 μg/2 μl/infusion for i.c.v. self-administration; SR 141716A HCl [*N*-piperidino-5-(4-chlorophenyl)1-(2,4-dichlorophenyl)-4 methyl pyrazole 3-carboxamide] (0.25-1 mg/kg) dissolved in the cannabinoid vehicle, kindly supplied by Synthelabo-Sanofi Recherche, Montpellier, France; or naloxone HCl (0.5–2 mg/kg; S.A.L.A.R.S., Como, Italy) dissolved in saline. The doses of the drugs were calculated as salt.

2.4. Statistical analysis

All the data were expressed as mean±S.E.M. and were analysed by one-way analysis of variance (ANOVA) for multiple comparisons, followed by Tukey's test where appropriate. Due to the individual animal's sensitivity, different numbers of sessions (from 15 to 20) were needed to reach a stable baseline of lever pressing (no more than about 15% difference across the sessions) with each drug unit dose. Thus, statistical analyses involved only the last 5 days of stable baseline. During the last 5 days of stable baseline, the mean total daily intake (μg) of Δ^9-THC was plotted against the log of the self-administered unit doses and was adapted to linear regression. The accepted level of significance was $P<0.05$. All statistical analyses were done using software Prism, version 4 (GraphPad, USA).

3. Results

3.1. Conditioned place preference

Fig. 1 shows the effect of different doses of Δ^9-THC (0.015, 0.075, 0.15, 0.37, 0.75, 0.75, 1.00, 3.00, and 6.00 mg/kg) on the conditioned place preference test. Significant treatment effect was found between subjects when comparing the time during the pre- and postconditioning period in the drug-paired compartment [$F(17,126)=171.20$, $P<0.0001$, ANOVA]. Thus, post hoc analysis revealed that Δ^9-THC produced a significant increase in the time spent in the drug-paired compartment on the postconditioning day, only between 0.075 and 0.75 mg/kg, when compared with that in the preconditioning period. Vehicle group exhibited no significant difference in the time spent in the drug-paired side. Starting from 1 to 3 mg/kg, Δ^9-THC had no effect, while the highest dose induced place aversion.

For the antagonism studies (Fig. 2), significant treatment effect was found between subjects when comparing the time in the drug-paired compartment during pre-

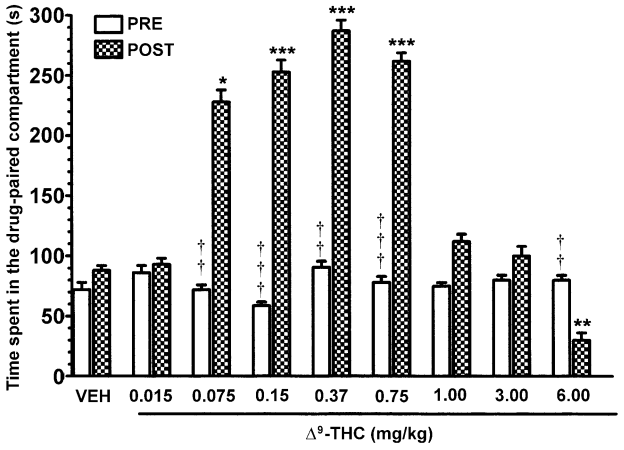

Fig. 1. Effect of increasing i.p. doses of Δ^9-THC on conditioned place preference evaluated as the time (mean±S.E.M.) spent in the drug-paired compartment before and after conditioning on the test day, during which neither drug nor vehicle was injected. N=8 rats for each group. VEH=vehicle. *P<0.05, **P<0.01, and ***P<0.001 as compared with vehicle group during postconditioning; $^{\dagger\dagger}P$<0.01 and $^{\dagger\dagger\dagger}P$<0.001 as compared with corresponding postconditioning (Tukey's test).

and post conditioning periods in rats given SR 141716 [F(15,112)=61.35, P<0.0001, ANOVA] or naloxone before each drug pairing [F(15,112)=96.02, P<0.0001, ANOVA]. Post hoc analysis showed that neither SR 141716 nor naloxone, administered alone, at all the tested doses, affected the time spent in the drug-paired side between the pre- and postconditioning periods. However, when given in combination with the maximally effective dose of Δ^9-THC (0.75 mg/kg), the two antagonists progressively reduced the time spent in the drug-paired compartment during postconditioning, in comparison with Δ^9-THC alone. The highest dose of both CB_1 cannabinoid (1 mg/kg) and opioid (2 mg/kg) receptor antagonists fully reversed Δ^9-THC-induced conditioned place preference.

3.2. i.c.v. self-administration

Operant responding during the training of all rats trained to press both levers simultaneously did not change before and after surgery (data not shown). The intake of water, delivered after each lever pressing, did not change during the training or testing procedure. Food intake and body weight were not modified throughout the experiment (data not shown).

The mean number of pressings on the lever delivering vehicle or increasing concentrations of Δ^9-THC is shown in Fig. 3. The i.c.v. self-administration of different unit doses of the cannabinoid receptor agonist significantly changed operant responding [F(11,84)=10.40, P<0.0001)]. Post hoc comparison indicated that self-administration of Δ^9-THC (0.01 and 0.02 μg/2 μl) significantly increased the mean number of drug-associated lever pressings in comparison with vehicle. A significant reduction in the number of vehicle-associated lever pressings was observed only for Δ^9-THC at the unit dose of 0.02 μg/2 μl. The highest concentrations (0.05, 0.5, and 1 μg/2 μl) produced a gradual reduction in the number of drug-associated lever pressings and a concomitant increase in the vehicle-associated lever pressings. In addition, a unit dose of 1 μg/2 μl resulted in a suppression of responding below vehicle level. The mean daily intake of Δ^9-THC was linearly related to the log of the self-administered unit doses (R^2 value=0.99, P<0.001) between a range of 0.01–0.5 μg/2 μl (data not shown). The estimated ED_{50} (±confidence limits; μg/2 μl/infusion) was 0.1 (±0.003).The highest unit dose produced a significant decrease of mean intake.

Fig. 4 shows the mean number of lever pressings under Δ^9-THC or vehicle self-administration in combination with SR 141716 (0.5 mg/kg) or naloxone (2 mg/kg). The mean number of lever pressings significantly changed between groups [F(11,84)=11.25, P<0.0001, ANOVA]. Post hoc comparison indicated that pretreatment with SR 141716 or naloxone per se did not affect the mean number of pressings on the levers delivering vehicle, in comparison with that

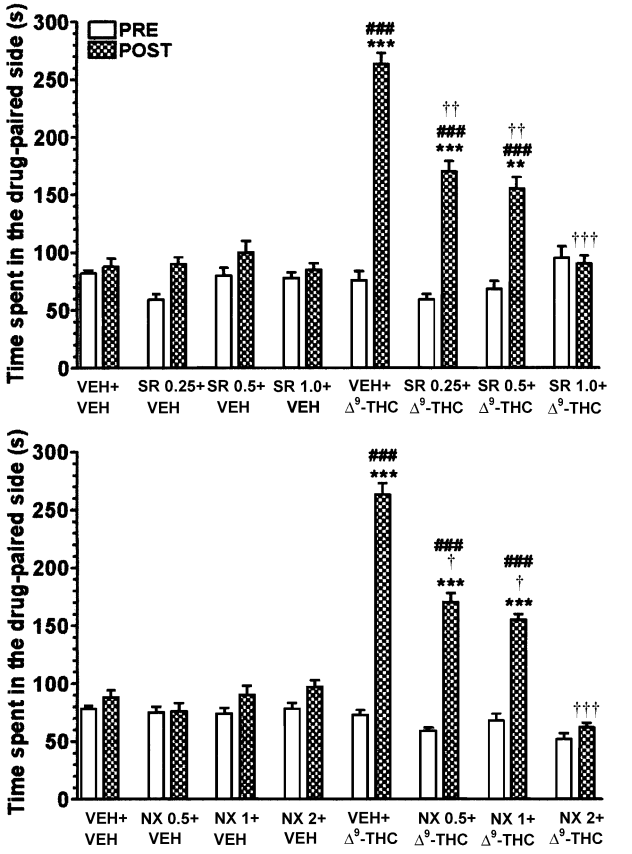

Fig. 2. Effect of increasing i.p. doses of SR 141716 (SR) (top) or naloxone (NX) (bottom) on Δ^9-THC-induced (0.75 mg/kg i.p.) conditioned place preference. Data were evaluated as time (mean±S.E.M.) spent in the drug-paired compartment before and after conditioning on the test day, during which neither drug nor vehicle was injected. Doses are expressed as mg/kg i.p. N=8 rats for each group. VEH=vehicle. ***P<0.001 as compared with corresponding preconditioning; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, and $^{\dagger\dagger\dagger}P$<0.001 as compared with VEH+Δ^9-THC postconditioning; $^{\#\#\#}P$<0.001 as compared with VEH group during postconditioning (Tukey's test).

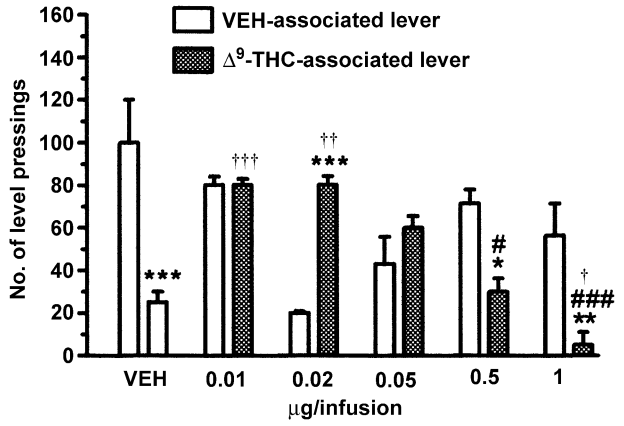

Fig. 3. Effect of increasing concentrations of Δ^9-THC on mean (±S.E.M.) number of lever pressings, evaluated the last five daily sessions after 15–20 days of acquisition. Two microliters of sterile vehicle (cremophor, ethanol, and cerebrospinal fluid, 1:1:18) was delivered i.c.v. by pressing the lever found preferred during training. Δ^9-THC was delivered i.c.v. by pressing the lever found nonpreferred during training. N=8 rats for each group. *P<0.05, **P<0.01, and ***P<0.001 as compared with the corresponding vehicle-associated lever; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, and $^{\dagger\dagger\dagger}P$<0.001 as compared with the vehicle group, same lever; $^{\#}P$<0.05 and $^{\#\#\#}P$<0.001 as compared with 0.01 and 0.02 of Δ^9-THC-associated lever (Tukey's test), respectively.

obtained during training. Peripheral pretreatment with both antagonists significantly decreased the number of Δ^9-THC-associated lever pressings in comparison with Δ^9-THC alone.

4. Discussion

The present work shows that Δ^9-THC, in a range of doses between 0.075 and 0.75, clearly produced conditioned place preference, as indicated by the increase in time spent in the drug-paired compartment, while higher doses produced no effect or aversion.

This is the first time that the reinforcing effects of Δ^9-THC have been obtained in Wistar rats using a classical conditioned place preference protocol. In the same strain of rats and using a similar range of doses, no conditioned place preference was previously obtained by Mallet and Beninger (1998). Different methodological details and experimental design could account for this discrepancy. A conditioned place preference was seen in Long Evans rats given 2–4 mg/kg of Δ^9 THC, using a different number of drug pairings (Lepore et al., 1995). It should be noted that differences in rat strain and in the number of pairings might explain the different range of Δ^9-THC doses that we found rewarding. In the same study, when the schedule of daily injections was changed, allowing a longer wash-out time period between drug injections (i.e., vehicle, day off, Δ^9-THC, day off, vehicle, day off, Δ^9-THC, etc.), Δ^9-THC produced a conditioned place preference at a low, 1 mg/kg, dose but produced place aversion at higher, 2 and 4 mg/kg, doses. More recently, Δ^9-THC-induced conditioned place preference was obtained only when mice received a previous priming injection and were exposed to the drug-paired conditioning chamber for a longer period of time (45 min instead of 15–30 min; Valjient and Maldonado, 2000; Valjient et al., 2002). The same dose was found to produce aversive effects using a standard schedule of injection. As suggested by Valjient and Maldonado (2000), the low doses of Δ^9-THC employed in our experiments could have minimized the possible dysphoric consequences considered to be responsible for aversion. In addition, in our experiments, Δ^9-THC produced aversive effects at a dose (6 mg/kg) within a range previously shown to have similar effects in rats (Sanudo-Peña et al., 1997; Cheer et al., 2000).

The conditioned place preference rewarding findings are corroborated by those obtained with self-administration task. Δ^9-THC (0.01–0.05 μg/infusion) sustained an i.c.v. self-administration behavior in rats without a history of exposure to other drugs at concentrations comparable with those in marijuana smoke inhaled by humans (Agurell et al., 1986). Recent findings (Justinova et al., 2003) confirmed a persistent intravenous self-administration of Δ^9-THC in squirrel monkeys without a history of exposure to other drugs. Δ^9-THC i.v. self-administration was also obtained in monkeys with a cocaine experience (Tanda et al., 2000) and in rats diet restricted (Takahashi and Singer, 1979, 1980).

The obtained biphasic effect for the number of bar pressings (increase with 0.01–0.05 μg per infusion and decrease with 0.5–1 μg per infusion) indicated that rats tended to adjust the dose during sessions by modifying the response frequency (Koob, 1993). A similar pattern was also observed for other drugs of abuse and for the cannabinoid synthetic agonist ((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol) (CP 55,940; Braida et al., 2001), using the

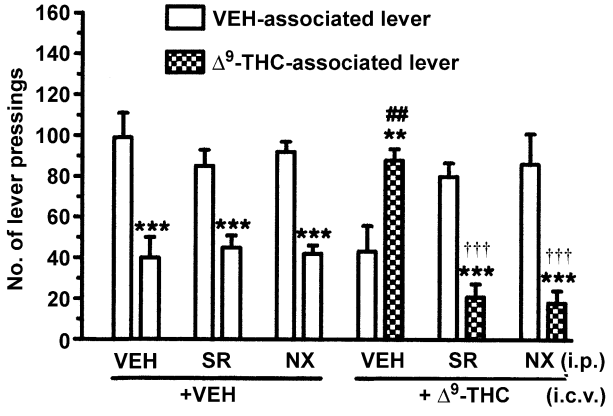

Fig. 4. Effect of i.p. pretreatment with vehicle (VEH), SR 141716 (SR; 0.5 mg/kg), or naloxone (NX; 2 mg/kg) on operant responding under vehicle or Δ^9-THC i.c.v. self-administration. Results are mean (±S.E.M.) of the last five daily sessions after 15–20 days of acquisition. N=8 rats for each group. **P<0.01 and ***P<0.001 as compared with the corresponding vehicle-associated lever; $^{\dagger\dagger\dagger}P$<0.001 as compared with VEH+Δ^9-THC-associated lever; $^{\#\#}P$<0.01 as compared with all groups, same lever (Tukey's test).

same test. In addition, the number of self-injections per session decreased markedly with the highest unit dose below vehicle levels. The observed bar pressing decrease on both levers may be the result of Δ^9-THC- impaired operant behavior or aversive/anxiogenic reactions, as previously reported (Carriero et al., 1998; Onaivi et al., 1990).

Biphasic effects of cannabinoids have been previously found, including motor activity, aggressive behavior, catalepsy, defecation (Sulcova et al., 1998), and anxiety (Rodriguez de Fonseca et al., 1996). A possible mechanism, at least suggested for anandamide, has been proposed: Low doses involve a G_s protein as opposite to a G_i protein, which is activated by higher doses (Fride, 1995; Gilman, 1984). A similar mechanism may be proposed to explain Δ^9-THC-induced reinforcing/aversive effects.

The reinforcing effect of Δ^9-THC demonstrated in both conditioned place preference and i.c.v. self-administration tasks was significantly blocked by pretreatment with SR 141716 and naloxone, suggesting that the rewarding effects are specifically mediated by both cannabinoid CB_1 and opioid receptors. The possibility that nonspecific effects were responsible for the observed antagonism in both tasks can be excluded since the antagonists per se had no effect on reward. At least for the conditioned place preference paradigm, the obtained antagonism agrees with that found for the synthetic cannabinoid receptor agonists, [2,3-dihydro-5-methyl-3-[(4-merpholino) methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone (WIN 55212-2) and CP 55,940 (Chaperon et al., 1998; Braida et al., 2001).

The selective reduction shown in SR 141716 pretreated rats self-administering i.c.v Δ^9-THC is in line with that observed in squirrel monkeys self-administering i.v. Δ^9-THC (Tanda et al., 2000).

Naloxone also antagonized Δ^9-THC-reinforcing effects, and this is in agreement with other studies using in vivo microdialysis (Tanda et al., 1997) and brain stimulation (Gardner et al., 1988). The observed SR 141716 antagonism agrees with that obtained in rats self-administering i.c.v. CP 55,940 (Braida et al., 2001) and i.v. WIN 55212-2 (Fattore et al., 2001).

The positive reinforcing effects of opioids are notably absent in cannabinoid CB_1 receptor knockout mice, and the self-administration of opioids in intact rats and mice is decreased by SR 141716 (Ledent et al., 1999; Navarro et al., 2001).

These effects may reflect a joint action of opioids and cannabinoids in the mesolimbic dopamine system. Both Δ^9-THC and morphine excite neurons in the ventral tegmental area, leading to increased dopamine release in the nucleus accumbens (Melis et al., 2000; Tanda et al., 1997). It is also worthy to note that both μ-opioid and cannabinoid CB_1 receptors are coexpressed in limbic forebrain structures, such as nucleus accumbens, septum, dorsal striatum, central amygdala, hippocampus, and medial habenula (Navarro et al., 1998). In addition, both μ-opioid and cannabinoid CB_1 receptors are members of the G-protein-coupled family of receptors and modulate similar transduction system.

In conclusion, the present findings demonstrate that low doses of Δ^9-THC can induce conditioned place preference, and it is possible to maintain an i.c.v. self-administration behavior in naive Wistar rats. Thus, a reliable animal model of human marijuana abuse can be suitable for the study of the abuse liability of cannabinoids.

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