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Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system

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Abstract

The effect of CP 55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclo-hesanol}, heroin and etonitazene on intracerebroventricular (i.c.v.) self-administration in a free-choice procedure was evaluated in rats. Animals were trained in 1-h daily sessions with a continuous reinforcement schedule to press two active levers to obtain the vehicle of each drug. Then, when a stable baseline was reached, each drug could be self-administered by pressing the lever found to be less preferred during training, while the vehicle came from the other. The number of bar pressings associated with the delivery of increasing unit doses of CP 55,940 (0.1, 0.2, 0.4, 0.8, 1.6 μg/2 μl/infusion), heroin (0.125, 0.25, 0.5, 1, 2 μg/2 μl/infusion) or etonitazene (0.1–0.2–0.5–1 μg/ 2 μl/infusion) and with the delivery of the corresponding vehicle was fitted by symmetrical parabolas. The mean drug intake was linearly related to the log of self-administered drugs. Pretreatment with SR141716A [*N*-piperidino-5-(4-chlorophenyl)1-(2,4-dichloro-phenyl)-4-methylpyrazole-3-carboxamide] (0.5 mg/kg) or naloxone HCl (2 mg/kg/i.p.) 15 min before each daily session reduced the self-administration of both CP 55,940 and heroin. The combination of CP 55,940 with heroin or etonitazene reduced the number of drug-associated lever pressings compared to that obtained with the maximal reinforcing unit dose of each drug alone. These findings suggest there may be a strong interaction between opioids and the cannabinoid system.

Keywords: Cannabinoid; Opiate; Self-administration; Free-choice

1. Introduction

Evidence from animal studies suggests that natural cannabinoids are unable to produce rewarding effects, especially using electrical self-stimulation (Thorat and Bhargava, 1994) or self-administration models such as intravenous self-administration (Harris et al., 1974; Smith et al., 1994; Meng et al., 1998) or the drinking test (Corcoran and Amit, 1974), in rodents and primates. The failure to find these reinforcing properties has been attributed to the slow onset and long duration of the effects, or to aversive effects, which can mask the appetitive properties. However, some reports indicate a facilitation of brain stimulation reward (Gardner et al., 1988), sustained self-administration (Takahashi and Singer, 1979; Tanda et al., 2000) and conditioned place preference (Lepore et al., 1995) by Δ^9 tetrahydrocannabinol in rats.

Since the characterisation of the brain CB_1 cannabinoid receptor (Matsuda et al., 1990; Munro et al., 1993), the discovery of synthetic cannabinoids has led to a valuable tool for investigating the cannabinoid system. Sustained intravenous self-administration of WIN 55,212-2 {*R*(+)-(2,3-dihydro-5-methyl-3-[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphtalenyl)methanone mesylate} was seen in drug-naive mice in a concentration-dependent manner, according to a two-phase "bell-shaped" curve (Martellotta et al., 1998). CP 55,940 [(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol] caused place avoidance in the conditioned place preference paradigm (McGregor et al., 1996).

Opioids and cannabinoids appear to share a variety of pharmacological properties, such as hypothermia, sedation, hypotension and block of intestinal motility (Dewey, 1986), but the most important interactions are related to antinociception (Welch and Stevens, 1992; Smith et al., 1994) and, to a lesser extent, to drug reinforcement (Chen et al., 1990). The cannabinoids and heroin may activate mesolimbic dopaminergic neurotransmission in a similar way,

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probably through a common μ_1-opioid receptor mechanism (Tanda et al., 1997). Opioid receptor antagonists, at least partially, block some effects of Δ^9 tetrahydrocannabinol related to alterations in the reward substrates (Chen et al., 1990). By contrast, no reversal was found of the Δ^9 tetrahydrocannabinol-induced increased firing of dopaminergic neurons in the ventral tegmental area, an effect which, however, was blocked by a specific cannabinoid receptor antagonist, SR 141716A [*N*-piperidino-5-(4-chlorophenyl) 1-(2,4-dichloro-phenyl)-4-methylpyrazole-3-carboxamide] (French, 1997). The reinforcing properties of morphine and the severity of the withdrawal syndrome were strongly reduced in mutant mice lacking the cannabinoid CB_1 receptor, suggesting that this receptor is involved in the motivational properties of opiates (Ledent et al., 1999).

An increase in prodynorphin and proenkephalin gene expression in the spinal cord of the rat was observed after subchronic treatment with Δ^9 tetrahydrocannabinol (Corchero et al., 1997) further indicating probable interaction between the cannabinoid and opioid systems in this region.

The purpose of this study was to examine intracerebroventricular (i.c.v.) self-administration of the potent synthetic cannabinoid receptor agonist, CP 55,940, and two opiates, heroin and etonitazene, on operant responding in a free-choice situation. 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 side effects. Second, we also assessed whether the cannabinoid receptor antagonist SR 141716A and the opioid receptor antagonist naloxone reversed the effect of the cannabinoid and the opiates. Third, we studied the effects of the combination of cannabinoids and opioids in the same task.

2. Materials and methods

2.1. Animals

Fifty outbred male Wistar rats (Charles River, Calco, Como, Italy), weighing 350 ± 10 g, housed in single cages, under standard laboratory conditions with a 12 h light/dark cycle, were used. Food was given ad libitum, but water was allowed only for 1 h/session and then for 10 min afterwards throughout the experiment. All procedures were carried out in accordance with the Italian Government Decree No. 36/1994-A.

2.2. 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). Stereotaxic coordinates were: L = 1.5 mm, AP = 1.8 mm, V = 4 mm. The cannulas were attached to the rat's head with acrylic dental cement. Three micro-screws (1 mm × 1 mm) were inserted, one rostrally and two caudally from the bregma (AP 2 mm, L 0.5 mm) and fixed with the same cement to the pedestal.

When the animal was not being tested, a double-dummy cannula was inserted to seal the top of the guide cannula and to keep tissue out of the guide tubing. A dust cap was installed to hold the dummy cannula securely to the guide cannula. Each rat was allowed to recover for approximately one week. Cannula placement was verified by observation of the drinking response to an i.c.v. injection of angiotensin II (100 ng/rat; Dib and Duclaux, 1982). All rats were injected twice with angiotensin II: once 1 week before the first session and the second time, the day after the last session. Only rats that drank 5 ml or more in 30 min after injection on both occasions were included in data analysis. In any case, to ascertain the accuracy of the i.c.v. injections, the rats were injected by the same route at the end of the experiment 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.

2.3. 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 houselight, exhaust fan, a liquid swivel on the ceiling, two response levers 6.8 cm above the floor on the front wall and the right side wall, and two solenoid-activated dipper dispensers to the left of each lever. A response on either lever resulted in illumination of a cue light fitted in each dispenser, and 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 Basile assembler gathered, listed, and every 5 min, printed the total number of bar pressings and the total number of reinforced bar pressings for each lever.

2.4. Procedure

2.4.1. Training procedure

Rats were kept without water for 23 h before surgery, and were individually trained for 1 h daily to press both

active levers to obtain water as reinforcer for one 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. The double-dummy cannula was removed and the double-injection cannula was inserted into the double-guide cannulas for liquid delivery. Two microliters of sterile cerebrospinal fluid or cannabinoid vehicle, depending on the drug self-administration pattern (see Section 2.4.2) was obtained each time 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 two weeks.

2.4.2. 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 cerebrospinal fluid or cannabinoid vehicle (2 μl/infusion) and the non-preferred one with the drug. 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 self-administration of the different drugs during a 1-h daily session. During the testing procedure, water was delivered after each lever pressing. The experiment continued until a stable baseline on five consecutive days was again achieved (about 2 weeks).

2.5. Drugs and treatments

Three groups of five rats each were randomly assigned to the following treatments: CP 55.940 ((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol) (Tocris Kookson, Bristol, UK) dissolved in cannabinoid vehicle (cerebrospinal fluid: ethanol: cremophor, 18:1:1) and delivered at increasing unit doses (0.1, 0.2, 0.4, 0.8 and 1.6 μg/2 μl/infusion); heroin HCl (S.A.L.A.R.S., Como, Italy) dissolved in cerebrospinal fluid and delivered at unit doses of 0.125, 0.25, 0.5, 1 and 2 μg/2 μl/infusion); etonitazene HCl (Ciba-Geigy, Basel, Switzerland), dissolved in cerebrospinal fluid and delivered at increasing unit doses of 0.1, 0.2, 0.5 and 1 μg/2 μl/infusion). Within each treatment the unit dose was increased in a counterbalanced order and the higher dose was given only when the baseline response for the preceding unit dose was stable.

Two further groups (five animals each), which had reached five days of stable baseline with the unit dose of heroin or etonitazene that produced the maximal response to lever pressing, were allowed to self-administer the same drug in combination with the unit dose of CP 55,940 that produced maximal lever pressing. A third group, trained to self administer the unit dose of CP 55,940 that produced the maximal response to lever pressing, was allowed to self-administer the same drug in combination with the unit dose of heroin which produced maximal lever pressing. This last group was included in order to verify whether the combination interfered with self-administration (CP 55,940 and then combined with heroin, or heroin and then combined with CP 55,940). The infusions were warmed to 37°C by means of a water jacket around the bilateral injection cannula, in which water flowed heated to 37°C by a heating element. Sterile cerebrospinal fluid was prepared according to Silvia et al. (1994) as follows: 124 mM NaCl, 1 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 10 mM glucose at a concentration of 50 μM.

For the antagonism studies, two naive groups of 10 rats received an intraperitoneal (i.p). injection of naloxone HCl (S.A.L.A.R.S., Como, Italy) or SR 141716A [*N*-piperidino-5-(4-chlorophenyl)1-(2,4-dichlorophenyl)-4-methyl pyrazole-3-carboxamide] (Sanofi, Montpellier, France) vehicle 15 min before the daily session of self-administration of the unit dose that produced maximal lever pressing for CP 55,940 or heroin. When the baseline was stable for 5 days, the two groups were further divided, one subgroup receiving, 15 min before starting the daily self-administration session, SR 141716A dissolved in its vehicle (saline, ethanol, cremophor, 18:1:1) and administered i.p. at a dose of 0.5 mg/kg and the second subgroup being given naloxone HCl dissolved in saline at the same time, at a dose of 2 mg/kg i.p.

2.6. Data analysis

Due to 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.

The data for operant responding, expressed as means ± S.E.M. of the number of bar pressings delivering the drug or the vehicle i.c.v. in each daily 1-h session, were plotted against the log of the unit doses and calculated by the usual statistical analysis adapted to curvilinear regression, or evaluated by one-way analysis of variance (ANOVA) for multiple comparisons, followed by Tukey's test where appropriate. The mean total intake (μg) of the drugs during the last 5 days of stable baseline was plotted against the log of the self-administered unit doses and adapted to linear regression. All statistical analysis was done using the Prism version 3 software (Graph Pad).

3. Results

Operant responding during training of all rats trained to press both levers simultaneously did not change before and

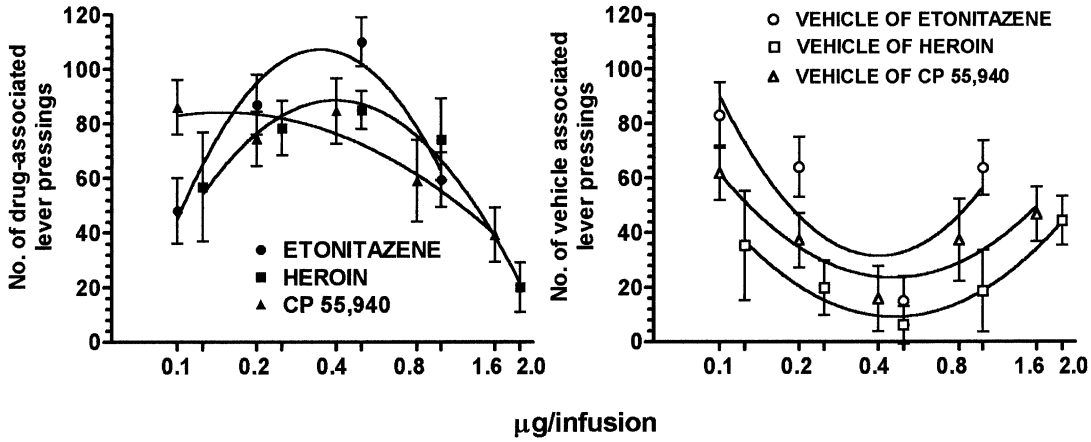

Fig. 1. Parabolic regression lines of free-choice operant responding, during a 1-h daily session, to the drug (left) or to cerebrospinal fluid or the cannabinoid vehicle (right) lever plotted against the log of self-administered unit doses. Each value is the mean (±S.E.M.) of the last five daily sessions obtained after 15–20 days of acquisition. Cerebrospinal fluid was the vehicle for heroin and etonitazene and the cannabinoid vehicle was the vehicle for CP 55,940. See text for unit doses used in each study.

after surgery (data not shown). 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 drugs (left panel) or vehicle (right panel) is shown in Fig. 1, where a statistically symmetrical parabola was obtained for each drug [$r^2(1,20) = 0.98$ for CP 55,940; $r^2(1,20) = 0.99$ for heroin; $r^2(1,16) = 0.97$ for etonitazene ($P < 0.0001$)] and for vehicle [$r^2(1,20) = 0.97$ for vehicle of CP 55,940; $r^2(1,20) = 0.99$ for vehicle of heroin; $r^2(1,16) = 0.85$ for vehicle of etonitazene ($P < 0.0001$)]. The maximal reinforcing concentrations were 0.4 μg/2 μl/ infusion for CP 55,940 and 0.5 μg/2 μl/infusion for heroin and etonitazene.

The mean daily intake of each drug (μg) was linearly related to the log of the self-administered unit doses: [$r^2(1,20) = 0.98$ for CP 55,940; $r^2(1,16) = 0.93$ for heroin; $r^2(1,16) = 0.99$ for etonitazene ($P < 0.0001$)] (Fig. 2). The maximal unit dose led to a reduction in intake only for heroin, so it was discarded from the linear regression calculation. The estimated ED_{50} (±confidence limits) (μg/2 μl/ infusion) values were 0.57 (±0.01) for etonitazene, 0.6 (±0.02) for heroin and 0.9 (±0.05) for CP 55,940.

I.c.v. self-administration of different drugs significantly changed operant responding [$F(13,56) = 14.01$ $P < 0.0001$] (Fig. 3). Post-hoc comparison indicated that the self-administration of CP 55,940 (0.4 μg/2 μl/ infusion) or heroin (0.5 μg/2 μl/ infusion) significantly increased the number of drug-associated and decreased the number of vehicle-associated lever pressings in comparison with vehicle ($P < 0.001$, Tukey's test). Pretreatment with SR 141716A or naloxone reduced the drug-associated lever pressings and increased vehicle-associated lever pressings in comparison with CP 55,940 ($P < 0.001$, Tukey's test) or heroin alone ($P < 0.01$, Tukey's test). In addition, SR 141716A was more active than naloxone in reversing the number of CP 55,940-associated lever pressings ($P < 0.05$, Tukey's test).

SR 141716A or naloxone per se did not affect the mean number of pressings of the levers delivering the appropriate vehicle, in comparison with that obtained during training (data not shown).

The combination of the maximal self-administered concentration of CP 55,940 with heroin or etonitazene delivered by pressing the same lever significantly affected operant responding [$F(13,56) = 23.40$, $P < 0.0001$] (Fig. 4). Post-hoc comparison indicated that the self-administration of CP 55,940 (0.4 μg/2 μl/ infusion), heroin (0.5 μg/2 μl/ infusion) or etonitazene (0.5 μg/2 μl/ infusion) significantly increased the number of drug-associated and decreased the number of vehicle-associated lever pressings in comparison with vehicle ($P < 0.001$, Tukey's test). There was a significant reduction in the number of

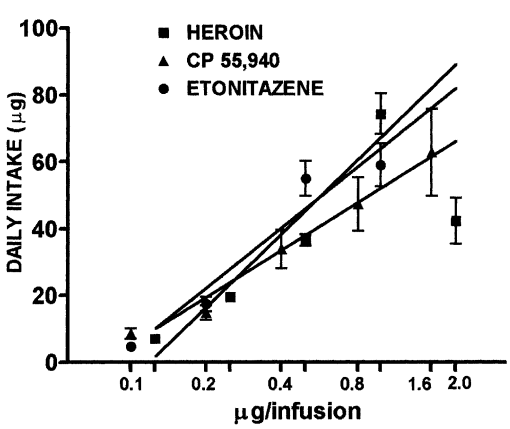

Fig. 2. Linear regression lines of daily intake (μg) of different drugs plotted against the log of self-administered unit doses. Each value is the mean (±S.E.M.) of the last five daily sessions obtained after 15–20 days of acquisition. See text for exact unit doses used in each study.

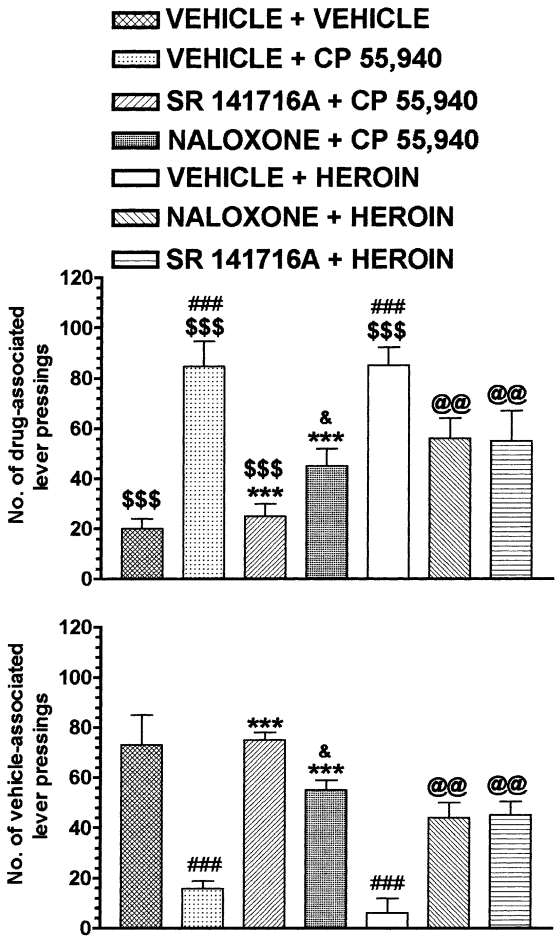

Fig. 3. Operant responding mean (±S.E.M), in a free-choice situation, to the drug (top) and to vehicle (bottom) lever pressing during the last five stable daily sessions of 15–20 days of acquisition. Vehicle, SR 141716A (0.5 mg/kg) or naloxone (2 mg/kg) was given i.p. 15 min before. Each drug-lever pressing delivered 0.4 μg/2 μl/infusion of CP 55,940 or 0.5 μg/2 μl/infusion of heroin. Cerebrospinal fluid was the vehicle for heroin and the cannabinoid vehicle (ethanol: cremophor: cerebrospinal fluid; 1:1:18) was the vehicle for CP 55,940. $\$\$\$P < 0.001$ vs. the corresponding vehicle-associated lever pressing; $\#\#\#P < 0.001$ vs. corresponding vehicle; $^{***}P < 0.001$ vs. CP 55,940 alone; $\&P < 0.05$ vs. SR 141716A + CP 55,940 group; $@@P < 0.01$ vs. heroin alone group (Tukey's test).

the drug combination-associated lever pressings compared with that for the drugs alone (CP 55,940 + heroin vs. CP 55,940 or heroin alone: $P < 0.05$; etonitazene + CP 55,940 vs. etonitazene alone: $P < 0.01$). Similarly, the number of vehicle-associated lever pressings was significantly increased in the groups of animals self-administering the above combinations (CP 55,940 + heroin vs. CP 55,940 alone: $P < 0.01$; heroin + CP 55,940 vs. heroin alone: $P < 0.05$; etonitazene + CP 55,940 vs. etonitazene alone: $P < 0.001$).

Fig. 5 shows a daily response pattern for four representative rats during the training and testing procedures under CP 55,940 self-administration. For the sake of brevity, only the last 10 days for each period of self-administration are shown. There was a progressive increase in the number of bar pressings associated with the less preferred lever when CP 55,940 was delivered at different concentrations (upper panel, left). The increase was greatest with a unit dose of 0.4 μg/2 μl/infusion. Higher concentrations produced a gradual decrease in the number of pressings delivering the cannabinoid and an increase of that delivering vehicle. The pattern of the response under daily pretreatment with SR 141716A (upper panel, right) or naloxone (bottom panel, left) was different from that with self-administration of CP 55,940 alone. There was an increase in the number of vehicle-associated lever press-

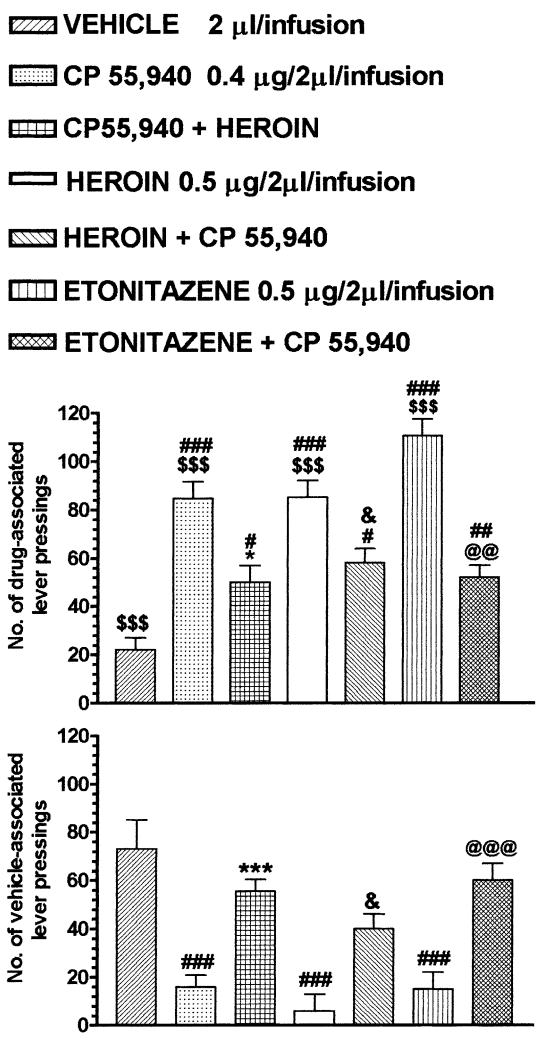

Fig. 4. Operant responding (mean ± S.E.M.), in a free-choice situation, to the drug (top) and to vehicle (bottom) lever pressing during the last five stable daily sessions of 15–20 days of acquisition. Each drug-lever pressing delivered 0.4 μg/2 μl/infusion of CP 55,940, 0.5 μg/ 2 μl/infusion of heroin or etonitazene, or both. Cerebrospinal fluid was the vehicle for heroin and the cannabinoid vehicle (ethanol:cremophor: cerebrospinal fluid; 1:1:18) was the vehicle for CP 55,940. $\$\$P < 0.001$ vs. the corresponding vehicle-associated lever pressing. $\#P < 0.05$, $\#\#\#P < 0.001$ vs. corresponding vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. CP 55,940 alone; $\&P < 0.05$ vs. heroin alone; $@@P < 0.01$, $@@@P < 0.001$ vs. etonitazene alone (Tukey's test).

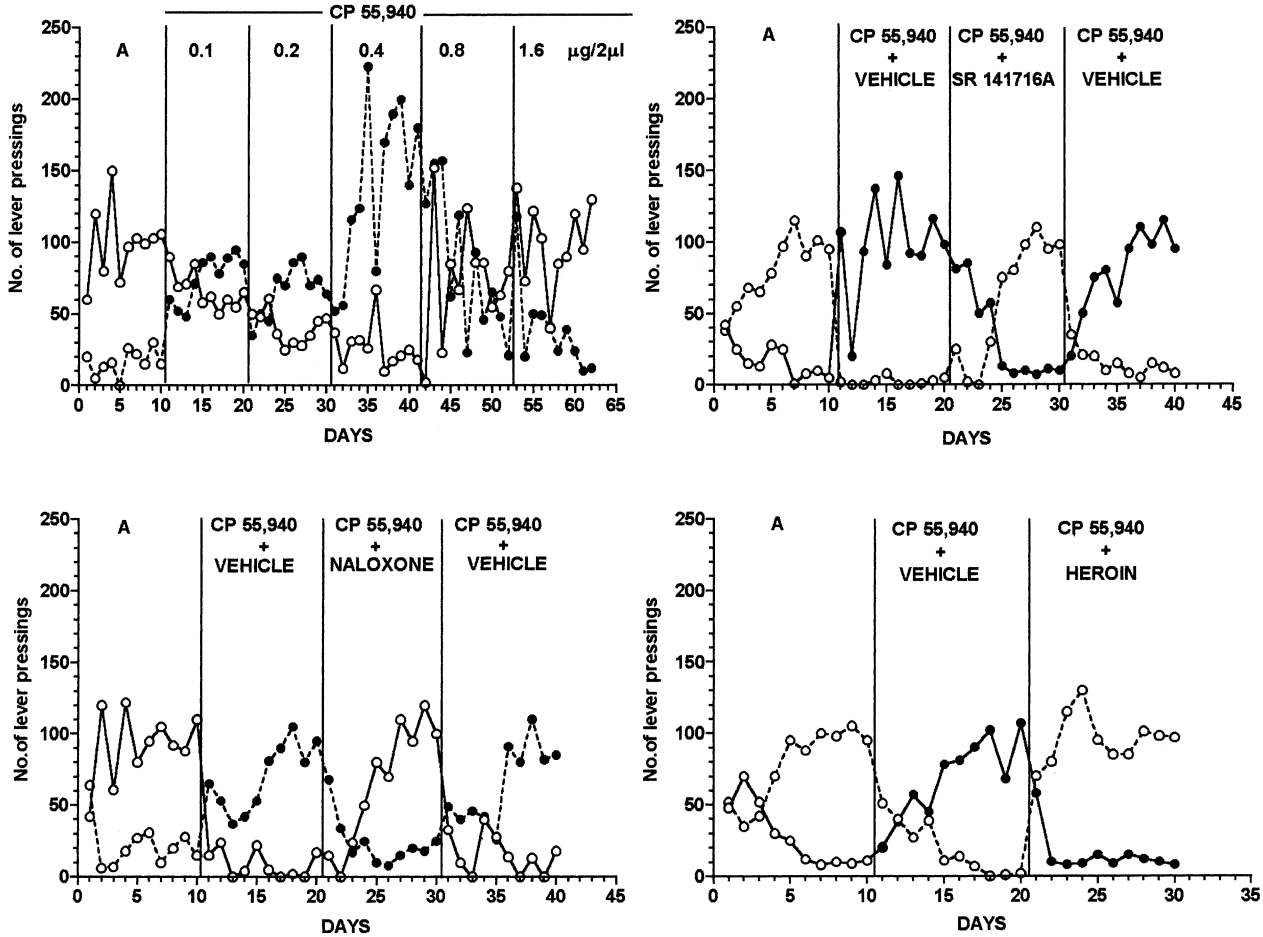

Fig. 5. Number of pressings, in a free-choice situation, on lever 1(—) and lever 2 (----) of four representative rats under a continuous reinforcement schedule during a 1-h daily training session (A) or testing. (○) = No. of daily pressings on the lever delivering i.c.v. cannabinoid vehicle. (●) = Number of lever pressings delivering increasing concentrations of CP 55,940 (upper panel, left); CP 55,940 (0.4 μg/2 μl/infusion) preceded 15 min by a daily i.p. injection of vehicle, SR 141716A (0.5 mg/kg) (upper panel, right) or naloxone (2 mg/kg) (bottom panel, left) CP 55,940 (0.4 μg/2 μl/infusion) alone or in combination with heroin (0.5 μg/2 μl/infusion) (bottom panel, right) during testing.

ings, with a loss of drug-associated lever pressings. Finally, the simultaneous self-administration of CP 55,940 and heroin (bottom panel, right) reversed the preference for the drug-associated lever.

4. Discussion

The first finding of this study is that CP 55,940 and heroin were able to sustain operant responding, in an i.c.v. drug self-administration free-choice procedure, as previously described for etonitazene (Braida et al., 1998). Opiates are widely self-administered using the classical i.v. route (Piazza and Le Moal, 1998) but the reinforcing properties of the cannabinoids are difficult to demonstrate in animals using self-administration models (Corcoran and Amit, 1974; Van Ree et al., 1978; Gardner and Lowinson, 1991). However, recent data (Tanda et al., 2000) indicate a persistent i.v. self-administration behavior with low doses of Δ^9 tetrahydrocannabinol comparable to doses in marijuana smoke inhaled by humans.

This is the first time that CP 55,940 has been shown to exhibit reinforcing properties, since it was aversive in the conditioned place preference paradigm when given peripherally (McGregor et al., 1996) at doses of 10 and 100 μg/kg. Several explanations can be put forward to explain these contradictory findings. First of all, the different route of administration (central or peripheral) might have induced a feeling of malaise due to the peripheral effects of the cannabinoid agonist, such as inhibition of intestinal motility as recently found by Izzo et al. (1999). Alternatively, the i.c.v. route might have unmasked the reinforcing effects of CP 55,940, because the compound avoided the paraventricular nucleus of the hypothalamus, central nucleus of the amygdala and lateral septum, all areas known to be involved in stress (Weindenfeld et al., 1994) and in the anxiogenic (Onaivi et al., 1995) responses of cannabinoids.

It cannot be excluded that some aversive substance might be created by the hepatic metabolism when the cannabinoid agonist is given peripherally, although until

now no active metabolite has been described for CP 55,940.

Tanda et al. (1997, 1999) reported that stimulation of central CB_1 cannabinoid receptors by natural and synthetic cannabinoid agonists leads to increased dopaminergic activity, measured by in vivo microdialysis, in mesolimbic areas, particularly in the shell of the nucleus accumbens. Given the extensive connections of the nucleus accumbens with the limbic brain areas involved in emotion, the activation of dopamine transmission by CP 55,940 may be involved in the motivational properties of the synthetic cannabinoid, since it was injected into the lateral ventricles, which are very close to the nucleus accumbens.

The pattern of self-administration with the three drugs indicates a biphasic effect, showing both positive and negative reinforcing effects depending on the unit dose used (Martellotta et al., 1998). Thus, under our experimental conditions, the positive reinforcing effect was highest at unit doses of 0.4 μg for CP 55,940 and 0.5 μg for heroin and etonitazene, while higher unit doses induced aversion due to other non-reinforcing (e.g. toxic) effects of each drug.

The total daily drug intake appears to be a linear function of the unit dose even if, at least for heroin, a biphasic effect was seen with the maximal unit dose (2 μg/2 μl/infusion). Higher unit doses of etonitazene and CP 55,940 might possibly lead to a reduction of daily intake too. Etonitazene was as potent as heroin based on the estimated ED_{50}, and CP 55,940 was the least potent. These different estimated values agree with the greater potency of heroin compared with natural and synthetic cannabinoids found by Tanda et al. (1997), who selectively obtained a 150% increase in extracellular dopamine concentrations—whose role in the motivational properties of many drugs of abuse is well known— in the shell of the nucleus accumbens.

In our opinion, drug intake seems to better reflect the appetitive properties of the drug more than the response frequency (mean number of bar pressings). Rats self-administering drugs at stable levels tend to adjust the dose during sessions by modifying the response frequency (Koob, 1993): the responding rate usually drops when the unit dose of the reinforcer is increased and vice versa. Thus, the descending part of the curve for the mean number of drug associated lever pressings does not mean that an aversive effect of a drug has appeared. Indeed, the mean drug intake seems to be a more precise indicator of the reinforcing efficacy of a drug.

SR 141716A and naloxone antagonised cannabinoid and heroin-induced self-administration, indicating that the rewarding effects are specifically mediated by cannabinoid CB_1 and opiate receptors, respectively. The selective reduction of i.c.v. self-administration of CP 55,940 in rats pretreated with the cannabinoid antagonist is in line with that observed in SR 141716A pretreated squirrel monkeys self-administering i.v. Δ^9 tetrahydrocannabinol (Tanda et al., 2000). The fact that naloxone also antagonised the CP 55,940-induced reinforcing effect is in line with other studies using Δ^9 tetrahydrocannabinol with in vivo microdialysis (Tanda et al., 1997) and brain self-stimulation (Gardner et al., 1988), in which the opioid receptor antagonist blocked reward substrates. Our findings on the antagonistic effect of SR 141716A on heroin-induced self-administration agree with those of Chaperon et al. (1998), who reported a block of morphine-induced conditioned place preference after pretreatment with the cannabinoid antagonist, in the same dose range as used by us, supporting the possibility of an interconnected role for cannabinoid and opioid receptors in brain regions mediating addictive behaviours.

The combined self-administration of CP 55,940 and heroin resulted in a dramatic decrease in operant responding on the drug-associated lever, suggesting a negative cannabinoid–opioid interaction in motivation. Studies similar to ours are lacking so far and data published in the past few years are related only to antinociception induced by combinations of cannabinoid and opiates, leading to an additive or synergistic effect (Welch and Stevens, 1992; Manzanares et al., 1999). We can exclude the possibility that non-specific effects were responsible for the lower mean number of bar pressings since no signs of sedation or motor impairment were observed. In fact, the mean number of pressings on the drug-associated lever was followed by an increase in those for the vehicle-associated lever. Similar findings were recently obtained by Fattore et al. (1999), who reported that i.v. pre-treatment with WIN 55,212-2 of rats self-administering cocaine reduced cocaine intake in a dose-dependent manner.

It is difficult to explain the mechanism by which the above combination leads to a loss of effect. One possible explanation comes from a study by Vaysse et al. (1987) in which treatment of rat cerebral membranes with Δ^9 tetrahydrocannabinol consistently reduced specific in vitro binding of [^{3}H]dihydromorphine (μ-opioid receptor agonist) in a dose-dependent fashion. More recently, Vasquez and Lewis (1999) reported that the human cannabinoid CB_1 receptor can sequester $G_{i/o}$-proteins from a common pool, preventing other $G_{i/o}$-coupled receptors (opioid) from transducing their biological signals. These molecular findings suggest that the simultaneous presence of cannabinoids and opiates might reduce the reinforcing potential of each drug.

In conclusion, the present study shows for the first time the reinforcing properties of CP 55,940 and heroin, using a method for i.c.v. self-administration in a free-choice procedure. Blockade of central cannabinoid CB_1 receptors completely reversed the reinforcing properties of the cannabinoid receptor agonist and partially reversed those of heroin. Blockade of opioid receptors partially reversed the reinforcing properties induced by heroin and CP 55,940. The combination of CP 55,940 and heroin or etonitazene completely reversed preference for the drug-associated lever.

There may be a loss of transducing biological signal due to the presence of cannabinoids and opioids. Further elucidation of the interconnected roles of cannabinoid and opioid receptors might help clarify how these compounds produce addiction.

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