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Chronic (−)-Δ^9-tetrahydrocannabinol treatment induces sensitization to the psychomotor effects of amphetamine in rats

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Abstract

Clinical and basic research studies have linked cannabinoid consumption to the onset of psychosis, specially schizophrenia. In the present study we have evaluated the effects of the natural psychoactive constituent of *Cannabis* (−)-Δ^9-tetrahydrocannabinol on the acute actions of the psychostimulant, D-amphetamine, on behaviour displayed by male rats on a hole-board, a proposed animal model of amphetamine-induced psychosis. Cannabinoid–amphetamine interactions were studied (1) 30 min after acute injection of (−)-Δ^9-tetrahydrocannabinol (0.1 or 6.4 mg/kg, i.p.); (2) 30 min after the last injection of 14-daily treatment with (−)-Δ^9-tetrahydrocannabinol (0.1 or 6.4 mg/kg) and 3) 24 h after the last injection of 14-daily treatment with (−)-Δ^9-tetrahydrocannabinol (6.4 mg/kg). Acute cannabinoid exposure antagonized the amphetamine-induced dose–dependent increase in locomotion, exploration and the decrease in inactivity. Chronic treatment with (−)-Δ^9-tetrahydrocannabinol resulted in tolerance to this antagonistic effect on locomotion and inactivity but not on exploration, and potentiated amphetamine-induced stereotypies. Lastly, 24 h of withdrawal after 14 days of cannabinoid treatment resulted in sensitization to the effects of D-amphetamine on locomotion, exploration and stereotypies. Since (−)-Δ^9-tetrahydrocannabinol is a cannabinoid CB_1 receptor agonist, densely present in limbic and basal ganglia circuits, and since amphetamine enhances monoaminergic inputs (i.e., dopamine, serotonin) in these brain areas, the present data support the hypothesis of a role for the cannabinoid CB_1 receptor as a regulatory mechanism of monoaminergic neuron-mediated psychomotor activation. These findings may be relevant for the understanding of both cannabinoid-monoamines interactions and *Cannabis*-associated psychosis.

Keywords: Cannabinoid; Amphetamine; Behavior; Locomotion; Sensitization; Dopamine; Psychosis

1. Introduction

Cannabis sativa derivatives are the most widely used illicit drugs (Gardner and Lowinson, 1991; Abood and Martin, 1992), and their acute and chronic consumption has been associated with both an increased risk for the onset of psychotic syndromes (Andreasson et al., 1987; Nuñez-Dominguez and Gurpegui-Fernández de Legaria, 1997) and with a decrease of the therapeutic effectiveness of neuroleptics (Knudsen and Vilmar, 1984). Since dopaminergic neurons have been proposed to contribute significantly to the psychopathology of schizophrenia (Crow, 1980; Ashcroft et al., 1981), the above described clinical features associated with acute and chronic *Cannabis* exposure link the recently described endogenous cannabinoid system to brain dopaminergic neurotransmission. The endogenous cannabinoid system includes the cannabinoid CB_1 receptor (Devane et al., 1988) and the endogenous ligand, anandamide (Devane et al., 1992). Brain cannabinoid receptors are distributed in the mammalian brain at higher levels than any other known G-protein-coupled receptor (Herkenham et al., 1990; Matsuda et al., 1990; Mailleux and Vanderhaeghen, 1992). They are expressed in areas of the central nervous system that contribute to the control of movement (caudate-putamen, globus pallidum, entopeduncular nucleus, substantia nigra and cerebellum), memory and cognition (hippocampal for-

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mation, cingulate cortex), processing of emotions and motivational responses (amygdalar complex, nucleus accumbens, olfactory cortex), pain perception (central gray matter, dorsal horn of spinal medulla), and neuroendocrine integration (paraventricular, arcuate, supraoptic and ventromedial hypothalamic nuclei) (Matsuda et al., 1990; Mailleux and Vanderhaeghen, 1992). Although cannabinoid CB_1 receptors appear to be absent in brain dopaminergic neurons (Herkenham et al., 1990, 1991), they colocalize with dopamine receptors in neurons of the basal ganglia and limbic cortex (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1993), providing a mechanism by which dopamine and cannabinoids might interact in psychotic processes. A possible interaction between dopamine receptors and cannabinoid CB_1 receptors has neurobiological support from the similar structure of both receptor systems. These receptors belong to the family of G-protein coupled receptors for neurotransmitters (Matsuda et al., 1990; Howlet, 1995). Both types of receptors are coupled to the same transduction systems, including the control of cAMP synthesis, and the regulation of Ca^{2+} and K^+ channels (Howlet, 1995). Experimental approaches to cannabinoid–dopamine interactions have recently provided evidence for the link between the two systems that might be relevant for the understanding of dopamine-related diseases such as stress, addiction and psychosis (Rodriguez de Fonseca et al., 1992, 1994a,b, 1997; Navarro et al., 1993a,b, 1997; Emrich et al., 1997). Additionally, it is noteworthy that acute and cannabinoid exposure is associated with both dysfunctions of motor behaviours (Moss et al., 1981; Navarro et al., 1993b; Romero et al., 1995, 1996; Rodríguez de Fonseca et al., 1997) and alterations in the activity of nigrostriatal (Ng Cheong Ton et al., 1988; Rodriguez de Fonseca et al., 1992; Cadogan et al., 1997) and mesolimbic dopaminergic neurons (Bowers and Hoffman, 1986; Chen et al., 1990; Navarro et al., 1993a).

The fact that cannabinoid exposure activates the pituitary–adrenal axis, leading to an increase in circulating levels of glucorticoids (Kubena et al., 1971; Rodríguez de Fonseca et al., 1991, 1995; Weidenfeld et al., 1993), is also potentially relevant for the understanding of cannabinoid-induced psychosis, since these steroid hormones play a key role in (1) the activation of mesocorticolimbic neurons, which, as stated above, contribute significantly to the positive symptoms of schizophrenia (Crow, 1980; Ashcroft et al., 1981) and (2) the sensitization of motor behaviors to psychostimulants, a rat model of psychosis (Piazza et al., 1989, 1996; Kalivas and Stewart, 1991; Coryell and Tsunag, 1992; Piazza and Le Moal, 1996). Together, these data support the existence of multiple neurobiological factors converging in brain dopaminergic systems, underlying the facilitatory role of *Cannabis* exposure in the initiation and relapse of psychosis. However, there is a lack of studies addressing dopamine–cannabinoid interactions in animal models of psychosis. Early studies described the existence of an acute antagonistic interaction between amphetamine and (−)-Δ^9-tetrahydrocannabinol on locomotor and stereotyped activity (Hattendorf et al., 1977; Pryor et al., 1978; Moss et al., 1984), but they were limited and did not include chronic (−)-Δ^9-tetrahydrocannabinol treatments and withdrawal groups. Makanjoula and et al. described in 1977 a simple test using a hole-board on which to study the effects of psychostimulants on behavioral organization. The hole-board has been used extensively in the evaluation of amphetamine effects (Piazza et al., 1989, 1996; Palomo, 1994; Makanjoula et al., 1977). Since amphetamine can produce in humans a toxic syndrome indistinguishable from paranoid psychosis (Snyder, 1973; Robinson and Becker, 1986), the evaluation of the effects of this psychostimulant in laboratory animals has been proposed as a model for schizophrenia studies (Makanjoula et al., 1977; Palomo, 1994). We now present data obtained with this model on the interactions between cannabinoid exposure and amphetamine, which might help to set a framework within which the potential role of cannabinoid exposure in the onset of psychosis as a dopamine-related disease could be evaluated.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Panlab, Barcelona) weighing 300 ± 35 g at the start of the experiment were housed 2 per cage, and were maintained in a temperature- and light-controlled environment on a 12-h light/dark reversed cycle (lights on at 8:00 P.M.) with free access to food and water. The animals were used only once for behavioral studies. All procedures were carried out according to European Communities directive 86/609/EEC regulating animal research.

2.2. Drugs

(−)-Δ^9-tetrahydrocannabinol and D-amphetamine were obtained through Sigma (St. Louis, MO, USA). Commercial (−)-Δ^9-tetrahydrocannabinol was obtained as an ethanol solution. Immediately before use, the alcohol was evaporated under a nitrogen flow, and the residue was emulsified in saline/propylene–glycol/Tween 80 (90:5:5 v/v) as vehicle, as previously described (Rodriguez de Fonseca et al., 1994a). Amphetamine was prepared in sterile 0.9% saline. Both drugs were made up daily to the appropriate concentrations to be administered intraperitoneally in a volume of 0.1 ml/100 g b.wt.

2.3. Behavioural studies

The study was performed with a hole-board apparatus, similar to that described by Makanjoula et al. (1977). It consisted of a 0.5 × 0.5 m square open field. Evenly

spaced on the floor were 25 holes arranged in five parallel rows of five holes each. Four black lines painted on the floor divided the apparatus into 25 equal square sectors. The field was surrounded by vertical opaque walls of 40 cm height. The field was illuminated with halogen ceiling light, adjusted to yield 350 lux at the center of the field. The test was initiated by placing the rat in the center of the field. The rat was observed in four consecutive intervals of 4 min each, every 20 min. The following behavioral acts were scored by trained observers blind to experimental conditions: *Locomotion* was recorded as the number of lines crossed by the animal; *Exploration* was recorder as the sum of hole dippings, rearings or sniffing activities; *Inactivity* was defined as the time spent by the animals in absolute quietness and *Stereotypies* were repetitive behavioral acts, counted without considering the initial one. According to this model, a theoretical sequence of 5 dippings in hole 1, followed by a dip in hole 2, a rearing, and two dips in hole 6 will be scored as 4 units of exploration (three dips + a rearing) and 5 units of stereotyped behavior (5 repetitive dippings).

2.4. Data analysis

Data recorded (from each 4-min interval, or cumulative scores of the 80-min-test) were evaluated by multifactorial analysis of variance. The data from first interval (0–4 min) were discarded from the analysis to avoid the effects of novelty. When a significant F value was found, post hoc analyses (Newman–Keuls) were performed for assessing specific group comparisons. Calculations were performed using the Bio-Medical Data Analyses Package (BMDP statistical package).

2.5. Experimental designs

2.5.1. Experiment 1: (−)-Δ^9-Tetrahydrocannabinol and amphetamine dose–response studies in male rats

In a first experiment, a full dose–response study on the effects of either D-amphetamine and (−)-Δ^9-tetrahydrocannabinol on the hole-board was studied. Dose-response to amphetamine was studied using 47 male rats which

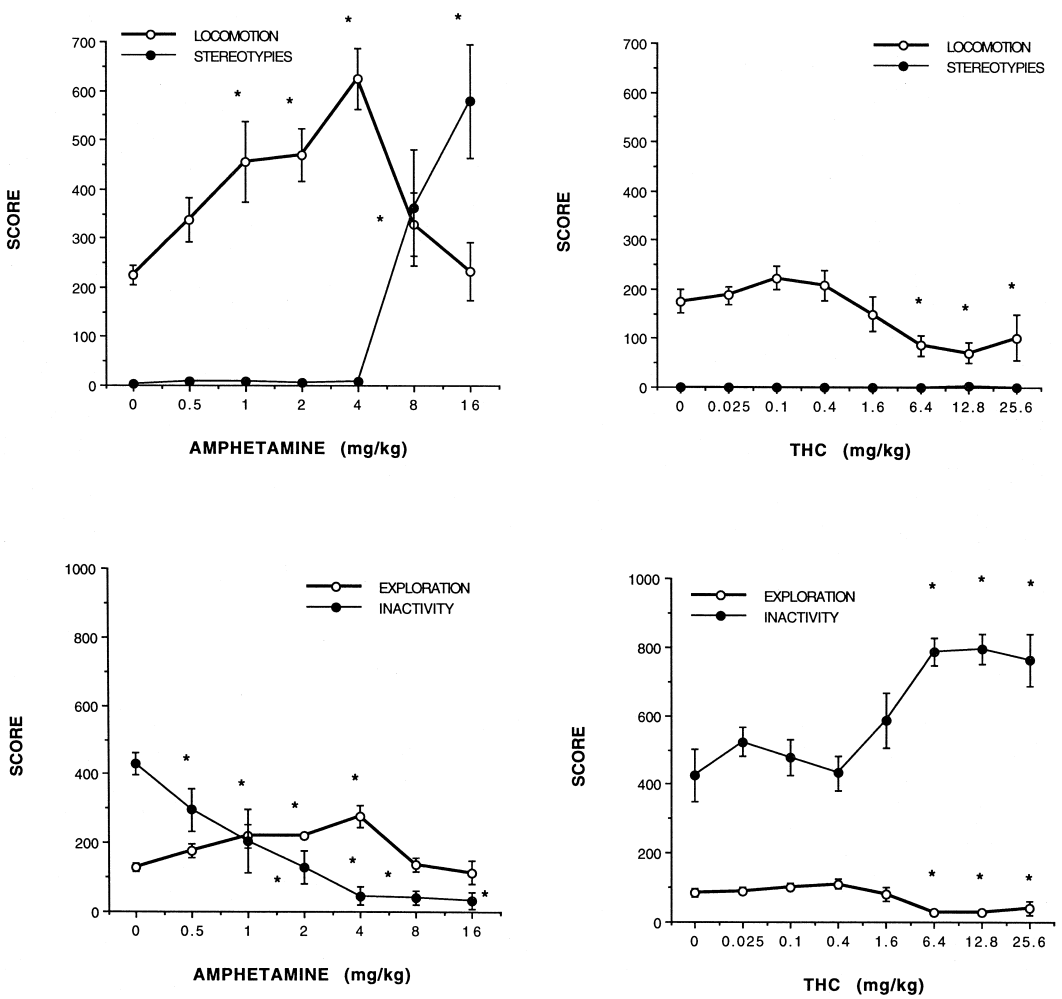

Fig. 1. Dose-response for D-amphetamine (left graphs) or for (−)-Δ^9-tetrahydrocannabinol (right graphs) on the hole-board. Upper panels display horizontal locomotion and stereotypies, while lower panels depict exploration and inactivity scores. Data are means ± S.E.M. of cumulated values measured in 7–9 animals per group. $* P < 0.05$, Newman–Keuls, vs. vehicle-treated animals.

received single i.p. injections of amphetamine (0, 0.5, 1, 2, 4, 8 and 16 mg/kg). The dose response for (−)-Δ^9-tetrahydrocannabinol was studied in 62 male rats that received single i.p. injections of (−)-Δ^9-tetrahydrocannabinol (0, 0.025, 0.1 0.4, 1.6, 6.4, 12.8 and 25.6 mg/kg). The animals were placed in the apparatus immediately after the injection of the drugs.

2.5.2. Experiment 2: Effect of acute administration of (−)-δ^9-tetrahydrocannabinol on acute amphetamine dose–response

The interaction between acute (−)-Δ^9-tetrahydrocannabinol exposure and amphetamine was studied in 115 male rats. The animals were divided into three groups. The first ($n = 38$) received an i.p injection of vehicle (Tween 80:propylenglycol:saline), the second group ($n = 39$) received (−)-Δ^9-tetrahydrocannabinol 0.1 mg/kg i.p., and the third one ($n = 38$) received (−)-Δ^9-tetrahydrocannabinol 6.4 mg/kg i.p. 30 min after the injection, the different groups were divided into 5 subgroups of 7–8 animals each, that received various doses of amphetamine (0, 1, 2, 4 and 8 mg/kg, i.p.). After the injection of amphetamine, each animal was placed on the hole-board as described above. (−)-Δ^9-tetrahydrocannabinol and amphetamine doses were selected on the basis of the dose–response study of experiment 1.

2.5.3. Experiment 3: Effect of chronic administration of (−)-δ^9-tetrahydrocannabinol on acute amphetamine (4 mg / kg) effects

A total of 47 rats was divided into three groups which received daily injections of vehicle ($n = 15$), (−)-Δ^9-tetrahydrocannabinol 0.1 mg/kg ($n = 16$) or (−)-Δ^9-tetrahydrocannabinol 6.4 mg/kg ($n = 16$), for 14 days. Thirty minutes after the last injection, the animals were divided into two subgroups that received either saline or amphetamine, 4 mg/kg. Immediately after the injection, the animals were placed on the hole-board and studied as described above.

2.5.4. Experiment 4: Amphetamine dose-response 24 h after last (−)-δ^9-tetrahydrocannabinol administration (Fig. 4)

A total of 60 rats was divided in two groups that received daily injections of vehicle ($n = 30$), or (−)-Δ^9-te-

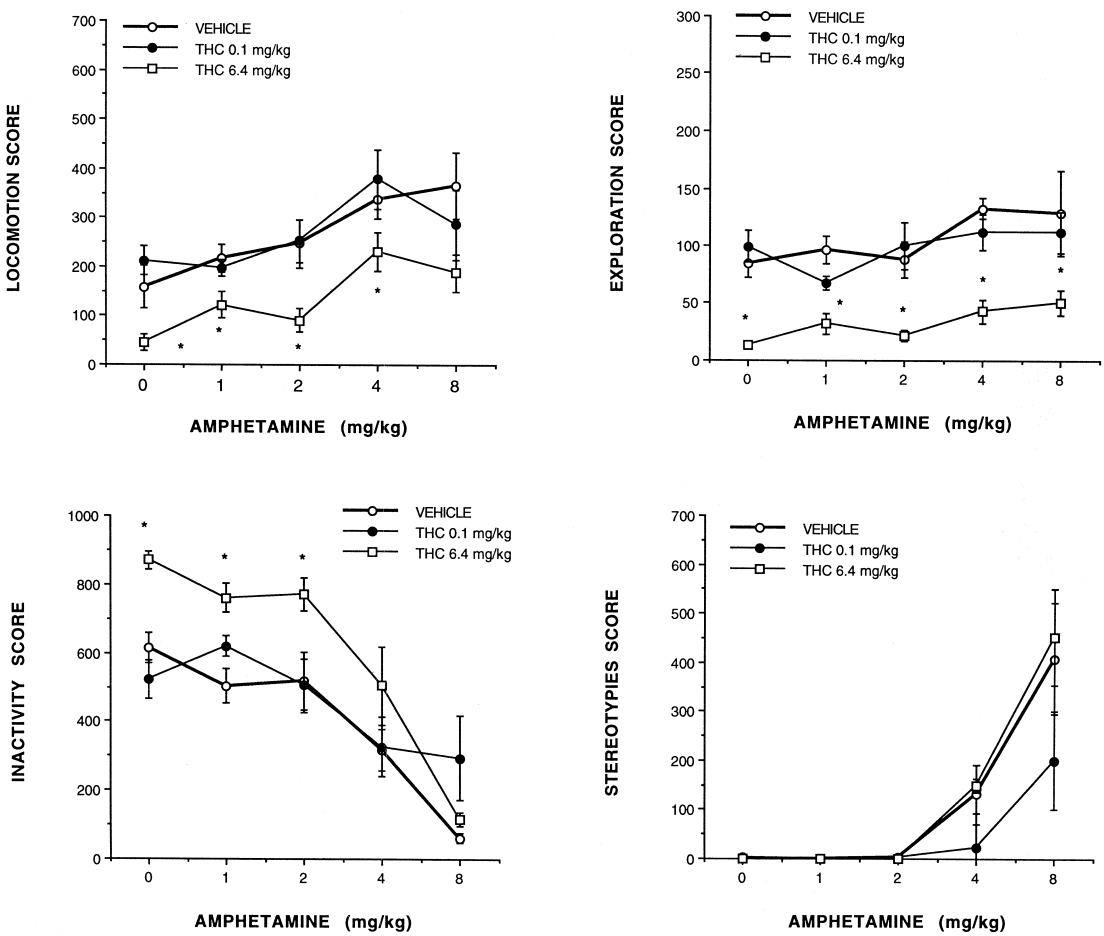

Fig. 2. Effects of 30-min acute pretreatment with vehicle or (−)-Δ^9-tetrahydrocannabinol (0.1 and 6.4 mg/kg, i.p.) on the acute dose-response for D-amphetamine on the hole-board. Data are means ± S.E.M. of cumulated values measured in 7–9 animals per group. * $P < 0.05$, Newman–Keuls, vs. vehicle-treated animals.

trahydrocannabinol, 6.4 mg/kg ($n = 30$), for 14 days. Twenty-four hours after the last injection, the animals were divided into five subgroups ($n = 6$) which received amphetamine (0, 1, 2, 4 or 8 mg/kg, i.p.). Immediately after the injection, the animals were placed on the hole-board and studied as described above.

3. Results

3.1. Experiment 1: (−)-Δ^9-Tetrahydrocannabinol and amphetamine dose-response studies in male rats (Fig. 1)

The cumulative scores for the 4-min study intervals (periods 2nd to 5th) are depicted in Fig. 1. Acute exposure to amphetamine produced a characteristic bell-shaped curve for locomotor activity, which peaked at the 4 mg/kg dose, decreasing thereafter (dose-effect, $F(6,37) = 6.05$, $P < 0.005$). A similar effect was observed for exploration (dose-effect, $F(6,34) = 5.1$, $P < 0.005$). Inactivity decreased in a dose-dependent fashion (dose-effect, $F(6,37) = 6.05$, $P < 0.005$), whereas stereotypies started at the 4 mg/kg dose (dose-effect, $F(6,37) = 13.83$, $P < 0.0001$). In contrast, acute (−)-Δ^9-tetrahydrocannabinol exposure produced a dose-dependent decrease in locomotion (dose-effect, $F(7,50) = 3.7$, $P < 0.0005$) and exploration (dose-effect, $F(7,50) = 3.2$, $P < 0.005$), which started at the 6.4 mg/kg dose, while it increased the time spent in immobility (dose-effect, $F(7,50) = 5.9$, $P < 0.0001$) with a similar dose profile. Stereotyped activity was not affected by (−)-Δ^9-tetrahydrocannabinol administration ($F(7,50) = 0.8$, $P = 0.76$, n.s.).

3.2. Experiment 2: Effect of acute administration of (−)-δ^9-tetrahydrocannabinol on acute amphetamine dose–response (Fig. 2)

Cumulative scores for the four 20-min study intervals are depicted in Fig. 2. Acute pretreatment with (−)-Δ^9-tetrahydrocannabinol 6.4 mg/kg flattened the dose-response curve for amphetamine for locomotion, (pretreatment × treatment interaction effect, $F(14,99) = 5.1$, $P < 0.0001$), eliminating the bell-shaped profile. This antagonistic effect was not observed after amphetamine, 8 mg/kg. (−)-Δ^9-

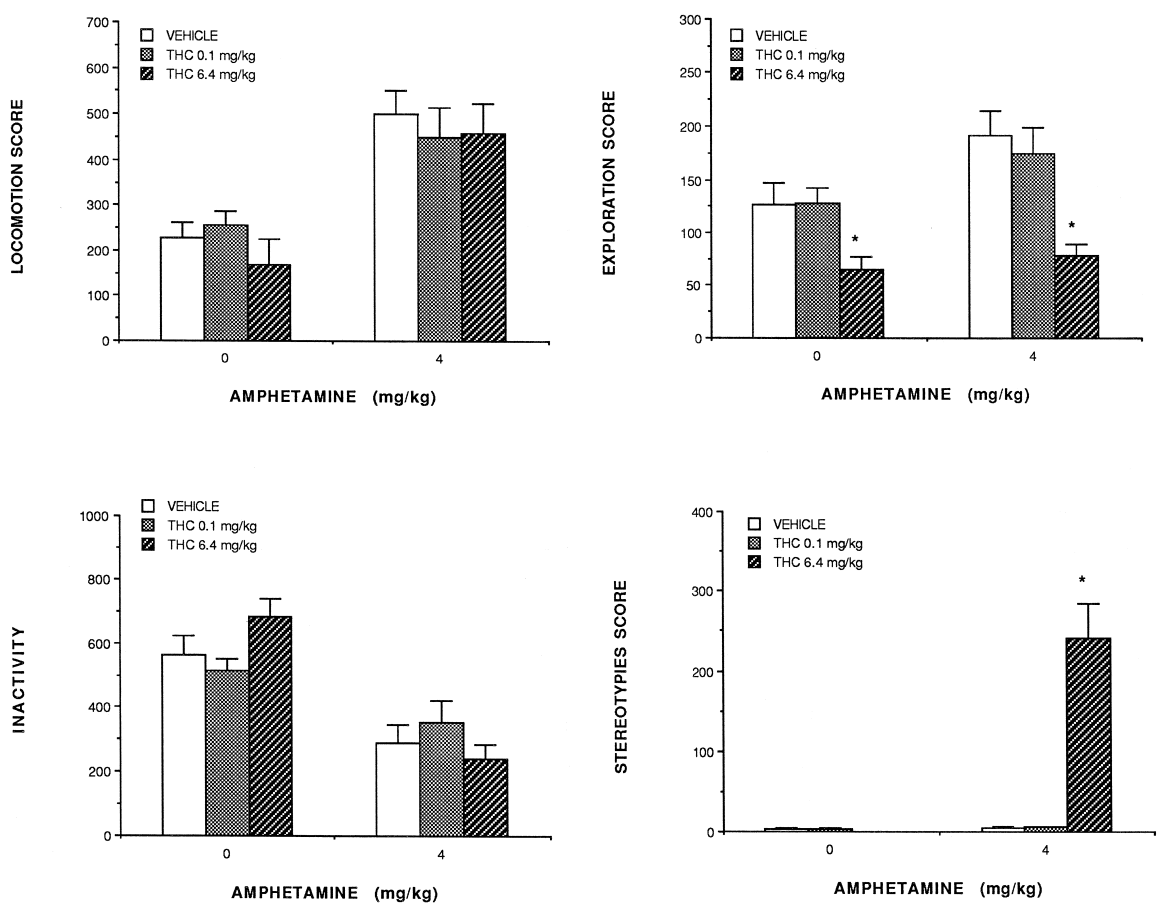

Fig. 3. Effects of chronic (14 days) pretreatment with vehicle or (−)-Δ^9-tetrahydrocannabinol (0.1 and 6.4 mg/kg, i.p.) on the acute effects of D-amphetamine (4 mg/kg) on the hole-board. Amphetamine was injected 30 min after the last (−)-Δ^9-tetrahydrocannabinol injection. Data are means ± S.E.M. of cumulated values measured in 7–9 animals per group. * $P < 0.05$, Newman–Keuls, vs. vehicle-treated animals of the same group.

Tetrahydrocannabinol 0.1 mg/kg did not affect the cumulative locomotor score induced by amphetamine. (−)-Δ^9-Tetrahydrocannabinol, 6.4 mg/kg, pretreatment antagonized the increased exploration induced by all doses of amphetamine (pretreatment × treatment interaction effect, $F(14,99) = 5.9$, $P < 0.0001$), and only attenuated the increased activity found after amphetamine, 1 and 2 mg/kg (pretreatment × treatment interaction effect, $F(14,99) = 10.9$, $P < 0.0001$). Amphetamine-induced stereotyped activity was only affected by (−)-Δ^9-tetrahydrocannabinol 0.1 mg/kg, pretreatment during the second half of the test (pretreatment × treatment interaction effect, $F(74,481) = 3.17$, $P < 0.0001$), but the cumulative scores were not changed after any of the (−)-Δ^9-tetrahydrocannabinol doses.

3.3. Experiment 3: Effect of chronic administration of (−)-δ^9-tetrahydrocannabinol on acute amphetamine (4 mg / kg) effects (Fig. 3)

Cumulative scores for the four 20-min study intervals are depicted in Fig. 3. Chronic pretreatment with (−)-Δ^9-tetrahydrocannabinol 0.1 or 6.4 mg/kg resulted in tolerance to the attenuating effects on amphetamine-induced locomotion in the different time intervals studied (pretreatment × treatment × time interaction effect, $F(23,162) = 1.2$, $P = 0.29$, n.s.), although the attenuating effects on cumulative exploration scores persisted (pretreatment × treatment interaction effect, $F(5,41) = 7.5$, $P < 0.0001$). Total inactivity was also not affected in spite of the chronic (−)-Δ^9-tetrahydrocannabinol treatment (pretreatment × treatment × time interaction effect, $F(23,162) = 0.6$, $P = 0.71$, n.s.), while stereotyped activity was clearly increased as a result of chronic (−)-Δ^9-tetrahydrocannabinol treatment (pretreatment × treatment × time interaction effect, $F(23,162) = 4.86$, $P < 0.00001$).

3.4. Experiment 4: Amphetamine dose–response 24 h after last (−)-δ^9-tetrahydrocannabinol administration (Fig. 4)

Cumulative scores for the four 20-min study intervals are depicted in Fig. 4. Chronic pretreatment with (−)-Δ^9-tetrahydrocannabinol 6.4 mg/kg resulted in sensitization

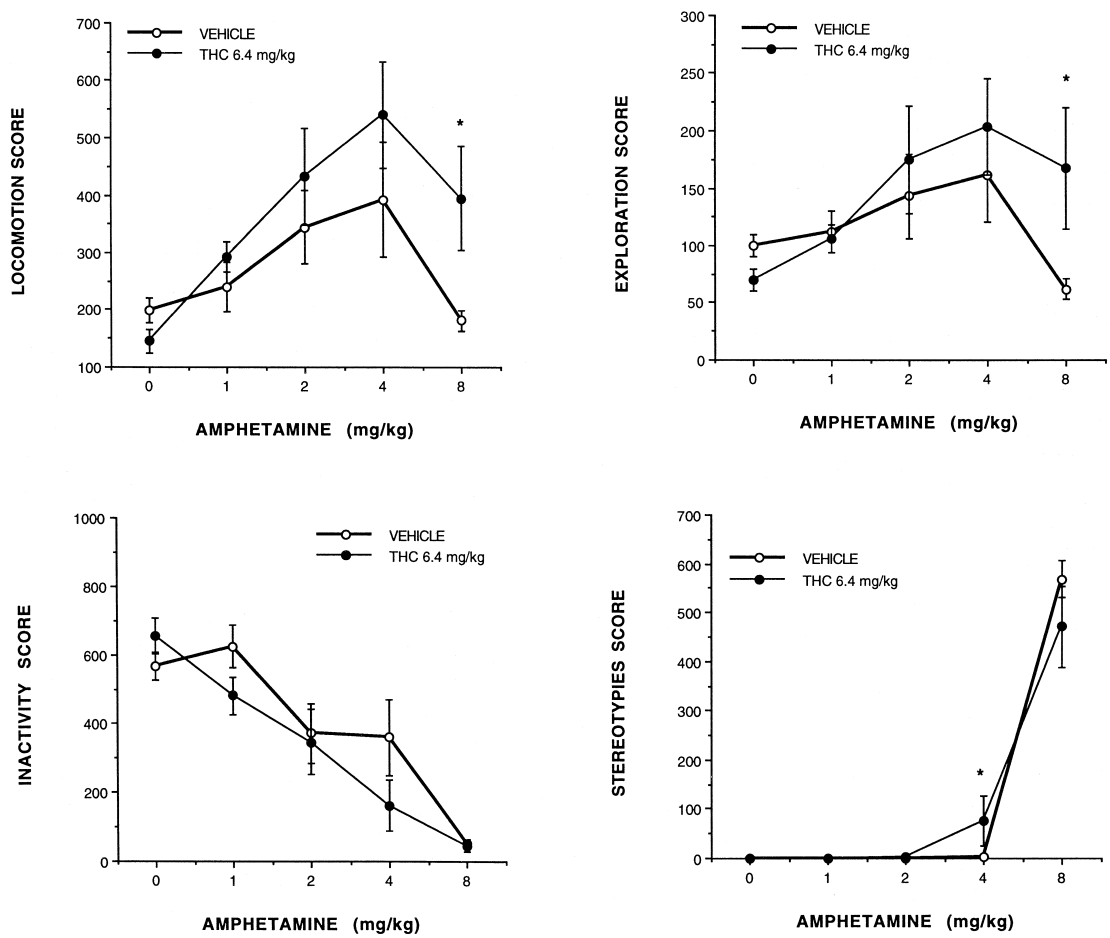

Fig. 4. Effects of chronic (14 days) pretreatment with vehicle or (−)-Δ^9-tetrahydrocannabinol (6.4 mg/kg, i.p.) on the acute dose-response to D-amphetamine on the hole board. Amphetamine was injected 24 h after the last (−)-Δ^9-tetrahydrocannabinol injection (withdrawal group). Data are means ± S.E.M. of cumulative values measured in 7–9 animals per group. * $P < 0.05$, Newman–Keuls, vs. vehicle-treated animals of the same group.

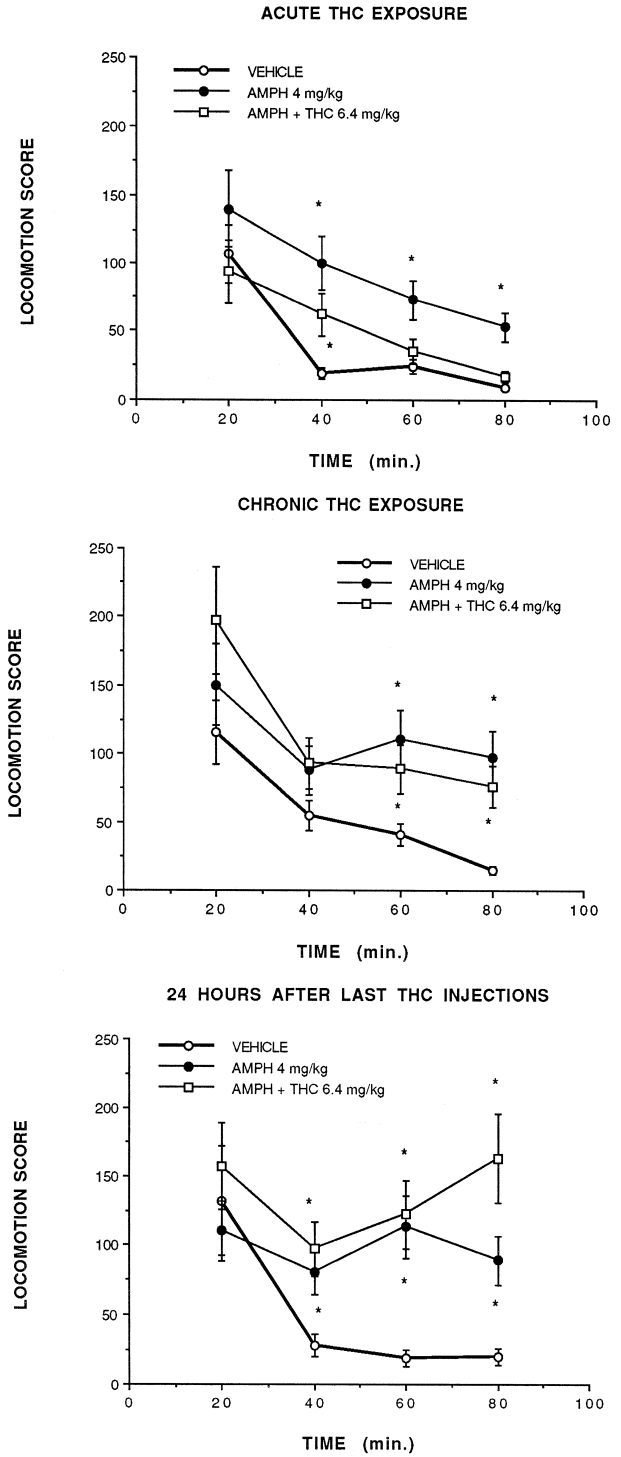

Fig. 5. Time course of the acute effects of amphetamine (4 mg/kg) in animals pretreated with (−)-Δ^9-tetrahydrocannabinol (acute, chronic, or withdrawal groups) pretreatment with vehicle or (−)-Δ^9-tetrahydrocannabinol (6.4 mg/kg, i.p.) on the locomotion scored on the hole board. Data are means ± S.E.M. of line crossings scored during 4 consecutive intervals of 20 min each measured in 7–9 animals per group. $* P < 0.05$, Newman–Keuls, vs. vehicle-treated animals.

to the stimulatory effect of amphetamine on locomotion ($F(9,50) = 3.76$, $P < 0.005$), exploration ($F(9,50) = 2.15$, $P < 0.05$), inactivity ($F(9,50) = 10.3$, $P < 0.0001$, n.s.), and stereotypies ($F(9,50) = 42.7$, $P < 0.0001$).

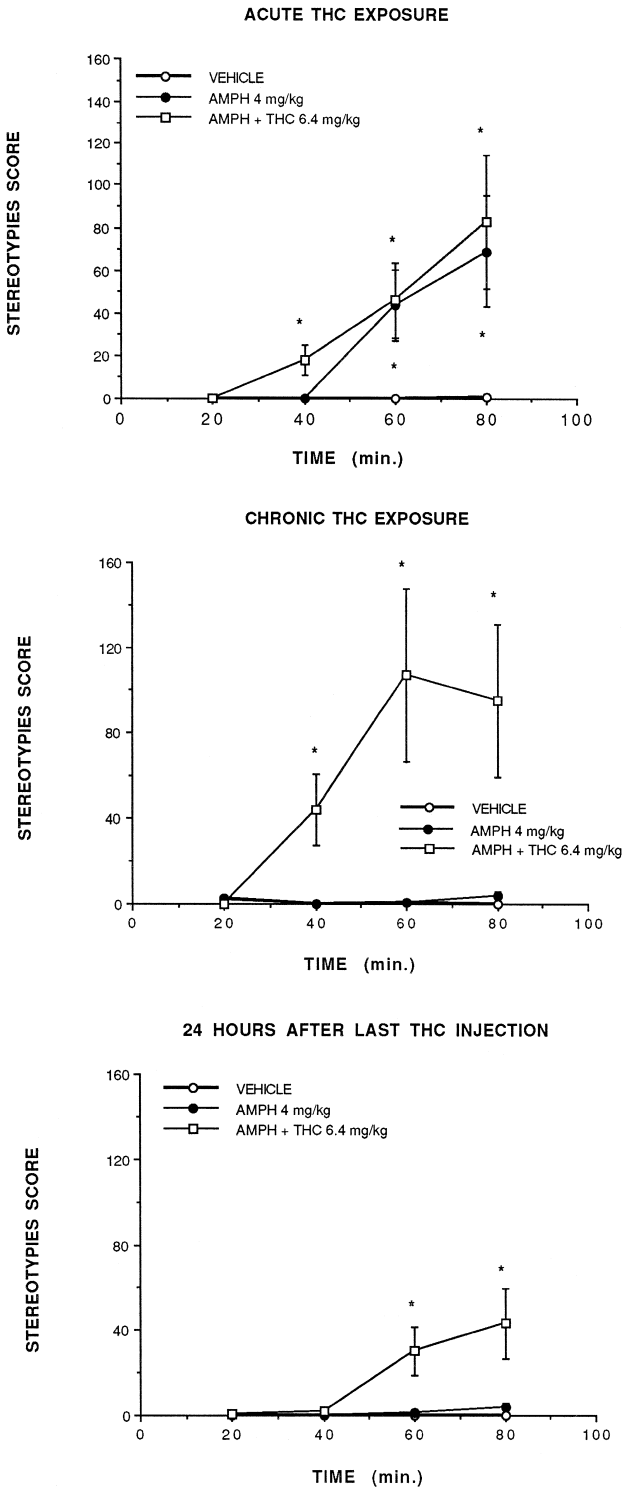

Fig. 6. Time course of the acute effects of amphetamine (4 mg/kg) in animals pretreated with (−)-Δ^9-tetrahydrocannabinol (acute, chronic, or withdrawal groups) pretreatment with vehicle or (−)-Δ^9-tetrahydrocannabinol (6.4 mg/kg, i.p.) on the stereotypies scored on the hole board. Data are means ± S.E.M. of stereotypies scored during 4 consecutive intervals of 20 min each measured in 7–9 animals per group. $* P < 0.05$, Newman–Keuls, vs. vehicle-treated animals.

3.5. Time course of the effects of amphetamine in animals receiving acute or chronic (−)-δ⁹-tetrahydrocannabinol, or 24 h after last (−)-δ⁹-tetrahydrocannabinol administration (Figs. 5 and 6)

The temporal profile of the effects of the interaction between (−)-Δ⁹-tetrahydrocannabinol and amphetamine, 4 mg/kg, in experiments 2, 3 and 4 is depicted in Fig. 5 (locomotion) and Fig. 6 (stereotypies). As can be seen (open square symbol for (−)-Δ⁹-tetrahydrocannabinol + amphetamine group), acute treatment with (−)-Δ⁹-tetrahydrocannabinol attenuated the time course and intensity of amphetamine effects, chronic (−)-Δ⁹-tetrahydrocannabinol resulted in tolerance to this effect, while giving amphetamine 24 h after last chronic (−)-Δ⁹-tetrahydrocannabinol injection reversed the acute effects of the cannabinoid, indicating the appearance of sensitization. Similar results were obtained for exploration and inactivity (data not shown).

4. Discussion

The present results revealed that acute (−)-Δ⁹-tetrahydrocannabinol exposure attenuated the psychomotor activation induced by amphetamine, while chronic cannabinoid administration resulted in the development of tolerance to these effects, facilitating the induction of stereotyped activity. For this later situation, we described the existence of down-regulation of cannabinoid CB_1 sites (Rodriguez de Fonseca et al., 1994a). A similar effect was found with the G-proteins needed for its signalling pathways (Rubino et al., 1997). Both findings provide neurochemical support for the (−)-Δ⁹-tetrahydrocannabinol-induced tolerance. Finally, when the animals were allowed 24 h of withdrawal after the last (−)-Δ⁹-tetrahydrocannabinol injection in a 14 day-lasting schedule of treatment, sensitization to the acute actions of amphetamine could be observed. These results support the existence of interactions between the endogenous cannabinoid system and monoaminergic neurons (mainly dopamine) in the elicitation of the behavioural effects of psychostimulants. The results also extend early findings regarding an amphetamine–(−)-Δ⁹-tetrahydrocannabinol interaction in rodents (Moss et al., 1984; Hattendorf et al., 1977; Pryor et al., 1978), supporting a mutual antagonism of the effects of both drugs on psychomotor activation. Amphetamine can block dopamine, noradrenaline and serotonin uptake, or promote the reverse transport of dopamine in the terminal fields of ascending monoaminergic neurons (Sulzer et al., 1993), including the striatum, the nucleus accumbens, and prefrontal and limbic cortices, resulting in significant psychomotor activation and a positive reinforcing process (Kalivas and Stewart, 1991; Sorg and Kalivas, 1993; Darracq et al., 1998). The increase in amphetamine-induced dopaminergic output (and in that of serotonin and noradrenaline) can be counteracted by the activation of cannabinoid CB_1 receptors that not only co-exist in dopaminergic receptors-containing neurons (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992), but are actively regulated through the stimulation of dopamine receptors (Mailleux and Vanderhaeghen, 1993; Rodríguez de Fonseca et al., 1994b; Glass et al., 1997). This counter-regulatory mechanism will act as a brake to the increased monoaminergic input, leading to flattening of the dose–response curve for amphetamine in animals acutely pretreated with (−)-Δ⁹-tetrahydrocannabinol. Chronic stimulation of cannabinoid CB_1 receptors with (−)-Δ⁹-tetrahydrocannabinol will desensitize this brake, increasing the response to amphetamine, as reflected in the sensitized behavioural responses observed in experiments 3 and 4. Although the withdrawal effect was only studied 24 h after the last (−)-Δ⁹-tetrahydrocannabinol injection, further research is needed to fully evaluate the intensity and persistence of the behavioural sensitization during early and late cannabinoid withdrawal stages.

In view of reports that there is a dose threshold for amphetamine to elicit psychomotor activation when there is an organized pattern of behavior, and that high doses of amphetamine disorganize the behavior of rats, inducing stereotypies (Makanjoula et al., 1977), the present results support the hypothesis of a role for the endogenous cannabinoid system as a physiological regulatory mechanism for psychomotor activation mediated by monoaminergic neurons. This hypothesis might be relevant in the context of the dopaminergic hypothesis of positive symptoms in schizophrenia (Crow, 1980). If the dopamine (and probably serotonin) message exceeds certain limits, the behavior will appear disorganized and purposeless. This hypothesis has been called *the constricted dopamine tolerance hypothesis of schizophrenia* (Ashcroft et al., 1981; reviewed in Palomo, 1994). In schizophrenia, the limits of tolerance are constricted, with the positive symptoms appearing as the clinical manifestation. In the amphetamine model proposed as a useful tool for studying the dopaminergic hypothesis of schizophrenia (Crow, 1980; Ashcroft et al., 1981), an amphetamine-induced increase in the presence of dopamine (and that of noradrenaline and serotonin) may result in the activation of postsynaptic dopamine receptors, finally resulting in an increased behavioral output. Pharmacological manipulation of physiological regulatory mechanisms, like that proposed in the present study, might alter the sensitivity to amphetamine, inducing either attenuation or sensitization of the response to this psychostimulant. In fact, if this is a correct model, this hypothesis might explain the relapse in positive symptomatology, and the decrease in neuroleptic efficacy observed in schizophrenic patients after marijuana exposure (Knudsen and Vilmar, 1984; Andreasson et al., 1987). In this regard, we have recently demonstrated that acute treatment with a cannabinoid CB_1 receptor antagonist facilitates the psychomotor activation by high doses of the dopamine D_2

receptor agonist quinpirole (Rodríguez de Fonseca et al., submitted), a finding similar to that described in the present study.

However, there are alternative mechanisms that can contribute to the sensitized response to amphetamine observed after chronic exposure to (−)-Δ^9-tetrahydrocannabinol. Natural and synthetic cannabinoid CB_1 receptor agonists are potent activators of the pituitary adrenal axis (Kubena et al., 1971; Weidenfeld et al., 1993; Rodríguez de Fonseca et al., 1991, 1995), promoting the release of glucocorticoids. Alterations in the secretion of these steroid hormones have been proposed as crucial factors in the development of behavioural sensitization to psychostimulants (Kalivas and Stewart, 1991; Maccari et al., 1991; Sorg and Kalivas, 1993; Piazza et al., 1989, 1996; Piazza and Le Moal, 1996; Koob and Le Moal, 1997). Chronic (−)-Δ^9-tetrahydrocannabinol administration might result in persistent high plasma levels of glucocorticoids, which might result in sensitization. The recently described cannabinoid-induced neuroadaptions in the endogenous corticotropin-releasing factor containing neurons could contribute to this effect (Rodríguez de Fonseca et al., 1997). This neuropeptide contributes to the sensitization processes induced by amphetamine and cocaine (Richter et al., 1995; Koob and Le Moal, 1997). Additional cannabinoid-induced alterations in dopamine autoreceptors, which are also affected during the development of amphetamine sensitization (Wolf et al., 1993) cannot be excluded and also need to be examined.

In summary, the present study showed that acute and chronic (−)-Δ^9-tetrahydrocannabinol exposure induced opposite effects in the sensitivity to acute amphetamine challenges, supporting a role for the endogenous cannabinoid system in the establishment of monoaminergic alterations associated with the positive symptoms of schizophrenia.

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