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Short communication

Dependence of mesolimbic dopamine transmission on Δ^9-tetrahydrocannabinol

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Abstract

Rats were administered daily for 8 days with increasing doses (2–12 mg/kg/day) of Δ^9-tetrahydrocannabinol (Δ^9-THC) and than challenged with different doses of SR141716A, an antagonist of cannabinoid receptors. SR141716A dose dependently reduced dialysate dopamine (DA) in the nucleus accumbens shell and precipitated a physical withdrawal syndrome. No such effects were obtained after administration of SR141716A to saline controls. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the active principle of *Cannabis*, resembles heroin in the property of stimulating dopamine (DA) transmission in the nucleus accumbens shell and in being antagonized in this action by naloxone, the prototypical opiate antagonist (Tanda et al., 1997). Moreover, after chronic cannabinoid exposure, the synthetic cannabinoid receptor antagonist SR141716A (Rinaldi-Carmona et al., 1994) precipitates a physical abstinence syndrome and elicits changes in brain corticotropin releasing factor that resemble those associated to opiate withdrawal (De Fonseca et al., 1997). We now provide evidence that in rats, a relatively mild and short-lasting schedule of exposure to Δ^9-THC induces a state of dependence of in vivo DA transmission in the nucleus accumbens shell as estimated by microdialysis.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Calco, Como, Italy) of 250 g were housed for at least a week, before use, in group of six under standard conditions of temperature and humidity, in an artificial light–dark cycle (light on 8:00 a.m., off 8:00 p.m.). They had free access to food and water. All animal experimentation was approved by the local committee, in accordance with European Economic Community guidelines for care and use of experimental animals.

2.2. Experimental procedure

Thirty rats were divided into two groups. The first group ($n = 15$) was administered intraperitoneally twice a day ($\sim$8:00 a.m. and $\sim$8:00 p.m.) with Δ^9-THC, for a total daily dose of 2 mg/kg on the 1st day, 4 mg/kg on the 2nd day, 8 mg/kg on the 3rd day and 12 mg/kg from the 4th to the 8th day. The second group of rats ($n = 15$) was administered intraperitoneally twice a day (8:00 a.m. and 8:00 p.m.) with saline for 8 days. On the 8th day, 3 h after the first daily dose of Δ^9-THC or saline, the rats were implanted with microdialysis probes in the nucleus accumbens shell in order to estimate changes in extracellular DA as an index of in vivo DA transmission. Dialysate DA was monitored the next day, about 12 h after the last dose of Δ^9-THC and about 24 h after probe implant. Saline controls were run in parallel.

2.3. Probe preparation

Concentric dialysis probes were prepared with AN 69 fibers (Hospal Dasco, Italy) as described by Tanda and Di Chiara (1998).

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2.4. Surgery

Rats were anaesthetized with ketamine (100 mg/kg i.p.) and placed in a stereotaxic apparatus. The skull was exposed and a small hole drilled to expose the dura. Each rat was implanted with one dialysis probe, aimed at the nucleus accumbens shell according to the rat brain atlas Paxinos and Watson (1987) (uncorrected coordinates: A = +2.0, L = 1.1, V = 8.0; Anterior, A; Lateral, L; Vertical, V).

2.5. Microdialysis experiments

Experiments were performed about 24 h after probe implant on freely moving rats. Ringer's solution (147 mM NaCl, 2.2 mM $CaCl_2$ and 4 mM KCl) was pumped through the dialysis probes at a constant rate of 1 μl/min. Collection of samples started after 30 min. After stabilization of dialysate DA (i.e., after about 3 h), rats were administered with SR141716A or saline.

2.6. Analytical procedure

Dialysate samples (10 μl) were taken every 10 min and injected without purification into a high-performance liquid chromatography apparatus equipped with a reverse-phase column (LC-18 DB, 15 cm, particle size 5 μm; Supelco) and a coulometric detector (ESA, Coulochem II, Bedford, MA) to quantify DA. The oxidation and reduction electrodes of the analytical cell (5014A, ESA, Bedford, MA) were set at +130 mV and −175 mV, respectively. The mobile phase, containing 50 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 0.5 mM *n*-octyl sulfate, and 18% (v/v) methanol (pH adjusted to 5.5 with Na_2HPO_4), was pumped with an LKB 2150 pump at 1.0 ml/min. Assay sensitivity for DA was femtomoles per sample.

2.7. Histology

At the end of the experiment, rats were transcardially perfused with 100 ml saline and 500 ml of 4% formaldehyde, 1% calcium acetate, and 100 mM NaCl solution. Probes were removed and brains were cut on a vibratome in serial coronal slices oriented according to the atlas (Fig. 1, Paxinos and Watson, 1987). Sections were processed for Luxol fast Cresyl violet stain and for calbindin immunohistochemistry in order to identify the location of the probes in relation to the shell and core subdivisions of the nucleus accumbens.

2.8. Behaviour

The behaviour of rats was videotaped and the number of wet dog shakes (WDS) was recorded during the whole experimental sessions in agreement with Aceto et al. (1995) and Tsou et al. (1995).

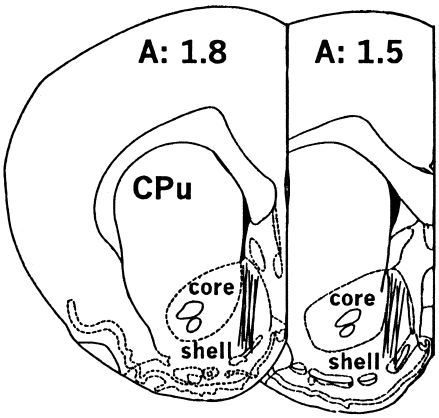

Fig. 1. Brain sections, redrawn from Paxinos and Watson (1987), represent the track corresponding to the dialysing surface of the probes (dialysing portion within the nucleus accumbens shell). On each section, the anterior coordinate (measured from bregma) is indicated. Abbreviations: A, anterior coordinate; CPu, caudate putamen.

2.9. Drugs

Δ^9-THC (Makor Chemicals, Jerusalem, Israel) and SR141716A (kindly donated by Dr. P. Soubrié, Sanofi, Paris, France) were suspended in 0.3%. Tween-80 in saline and administered intraperitoneally (3 ml/kg). Calbindin antibodies were kindly provided by Dr. C. Gerfen.

2.10. Statistics

One-way or two-way analysis of variance for repeated measures over time was applied to the data obtained from behavioural observation or to the data obtained from serial assays of DA normalized as percentage of basal DA values of each group. Basal DA values were the means of three consecutive samples differing no more than 10%. Results from treatments showing overall changes were subjected to post hoc. Tukey's test with significance at $P < 0.05$.

3. Results

3.1. Effect of repeated Δ^9-THC administration on basal DA output in the nucleus accumbens shell

Basal values of DA in the nucleus accumbens shell (expressed as femtomoles × 10 min sample ± S.E.M.) were not significantly different in Δ^9-THC-treated (57.69 ± 4.20, $n = 15$) as compared to saline controls (50.80 ± 2.82, $n = 15$) (chronic Δ^9-THC vs. chronic saline: $F(1.28) = 1.801$, $P = 0.209$).

3.2. Behavioural effects of acute challenge with SR141716A on chronic Δ^9-THC-treated rats

In agreement with previous reports (Aceto et al., 1995; Tsou et al., 1995), administration of the cannabinoid recep-

tor antagonist SR141716A (1 and 5 mg/kg i.p.) elicited in Δ^9-THC-treated rats a physical abstinence syndrome characterized by grooming, WDS, and hunched-back posture. Administration of SR141716A to saline-treated rats elicited frequent grooming, but only rare WDS. Administration of saline to rats chronically exposed to Δ^9-THC resulted only in rare WDS. Therefore, in agreement with Aceto et al. (1995) and Tsou et al. (1995), we evaluated the intensity of the syndrome induced by SR141716A exclusively on the basis of the number of WDS observed. Two-way ANOVA showed a significant effect of exposure to Δ^9-THC on the incidence of WDS (chronic Δ^9-THC + SR141716A 1 mg/kg vs. chronic saline + SR141716A 1 mg/kg: $F(1.68) = 7.200$, $P < 0.001$; chronic Δ^9-THC + SR141716A 5 mg/kg vs. chronic saline + SR141716A 5 mg/kg: $F(1.68) = 520.200$, $P < 0.001$) and of dose of SR141716A ($F(2.102) = 212.167$, $P < 0.001$) and a significant treatment × time interaction for both doses of SR141716A (1 mg/kg: $F(6.56) = 7.250$, $P < 0.001$; 5 mg/kg: $F(6.56) = 40.583$, $P < 0.001$). Post hoc test showed an increase of WDS after SR141716A in a relation to the exposure to Δ^9-THC and to the dose of SR141716A.

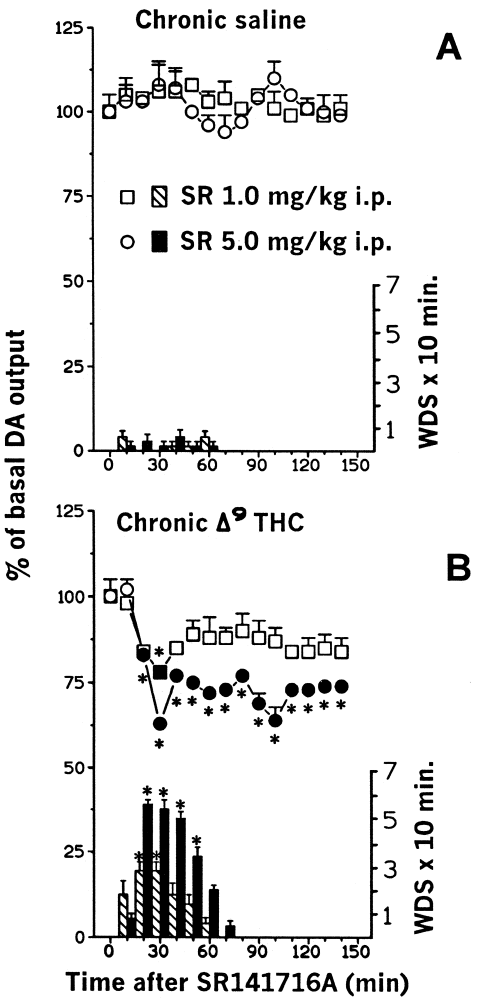

Fig. 2. (A) Time course of the effects of the administration of SR141716A on DA output in dialysates from nucleus accumbens shell and on WDS of rats chronically injected with saline (control group). DA in dialysates is expressed as percentage of basal values (mean ± S.E.M.) (1 mg/kg i.p., squares; 5 mg/kg i.p., circles). Bars represent the number of WDS (mean ± S.E.M.) observed every 10 min after the administration of SR141716A (1 mg/kg i.p., diagonal bars; 5 mg/kg i.p., solid bars). (B) Time course of the effects of the administration of SR141716A on DA output in dialysates from nucleus accumbens shell and on WDS of rats chronically treated with Δ^9-THC. DA in dialysates is expressed as percentage of basal values (mean ± S.E.M. (1 mg/kg i.p., squares; 5 mg/kg i.p., circles)). Bars represent the number of WDS (mean ± S.E.M.) observed every 10 min, after the administration of SR141716A (1 mg/kg i.p., diagonal bars; 5 mg/kg i.p., solid bars). Filled symbols: $P < 0.05$ compared with basal values. Asterisk: $P < 0.05$ compared with the corresponding time point of chronic saline-injected rats challenged with SR141716A.

3.3. Effect of SR141716A on mesolimbic DA transmission in chronic Δ^9-THC-treated rats

As shown in Fig. 2, dialysate DA rapidly decreased in the Δ^9-THC-treated group upon administration of 1.0 and 5.0 mg/kg of SR141716A. No changes were observed after administration of both doses of SR141716A to saline controls or of saline to Δ^9-THC-treated rats. Two-way ANOVA showed a significant effect of Δ^9-THC treatment (chronic Δ^9-THC + SR141716A, 1 mg/kg vs. chronic saline + SR141716A, 1 mg/kg: $F(1.128) = 90.413$, $P < 0.001$; chronic Δ^9-THC + SR141716A, 5 mg/kg vs. chronic saline + SR141716A 5 mg/kg: $F(1.128 = 631.767$, $P < 0.001$). After the dose of 1 mg/kg of SR141716A, significant reduction of DA was observed only at 30 min, while after 5 mg/kg, dialysate DA decreased up to 150 min post-SR141716A, when physical symptoms had subsided. Two-way ANOVA revealed a significant effect of dose (chronic Δ^9-THC + SR141716A, 1 mg/kg vs. chronic Δ^9-THC + SR141716A, 5 mg/kg: $F(1.128) = 102.112$, $P < 0.001$).

4. Discussion

The present results indicate that in the rat, a relatively mild and short-lasting schedule of exposure to Δ^9-THC induces a state of dependence of DA transmission in the nucleus accumbens shell. The induction of a dependence state to Δ^9-THC is shown by the acute and dose-related fall of extracellular DA in the nucleus accumbens shell associated to physical withdrawal signs upon challenge with the cannabinoid receptor antagonist SR141716A. After the present study was submitted for publication and in agreement with it. Diana et al. (1998) reported that abstinence from Δ^9-THC precipitated by SR141716A reduces the activity of presumed DA neurons recorded extracellularly in the ventral tegmentum of paralyzed rats. Given the

Δ^9-THC property of stimulating mesolimbic DA transmission acutely (French et al., 1997; Tanda et al., 1997), one can hypothesize that repeated exposure to Δ^9-THC results in a progressive substitution by Δ^9-THC of endogenous tonic excitatory inputs to DA neurons projecting to the nucleus accumbens shell. Under these conditions, homeostasis of DA transmission in the nucleus accumbens becomes dependent upon *Cannabis* self-administration. Induction of dependence in DA neurons, however, is not exclusive of *Cannabis* exposure. Thus, a dependence state is induced in DA neurons also by repeated exposure to opiates, psychostimulants and ethanol (see Di Chiara, 1995 for review). This adaptive change might contribute, together with other mechanisms, to drug addiction (Koob and Le Moal, 1997). Therefore, the present evidence strengthens and extends the conclusion of previous studies (De Fonseca et al., 1997; Tanda et al., 1997) that Δ^9-THC, the active principle of *Cannabis*, shares with other drugs of abuse those neurochemical properties that are regarded as the biological substrate of drug addiction.

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