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European Journal of Pharmacology 395 (2000) 1–7

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Changes in rat brain energetic metabolism after exposure to anandamide or Δ^9-tetrahydrocannabinol

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Received 28 October 1999; received in revised form 21 February 2000; accepted 25 February 2000

Abstract

The objective of this study was to investigate whether single and repeated administration of the cannabinoids anandamide or Δ^9-tetrahydrocannabinol affected brain energetic metabolism. Single administration of either anandamide (20 mg/kg) or Δ^9-tetrahydrocannabinol (10 mg/kg) in rats induced a behaviour typical with cannabinoids. An increase in both brain mitochondria oxidative phosphorylation and cerebral lipoperoxidation was shown ex vivo. The cannabinoid CB_1 receptor-specific antagonist, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716A; 3 mg/kg), reversed the anandamide-induced metabolic effects. Prolonged exposure to anandamide (20 mg/kg, 16 days) induced behavioural tolerance and the disappearance of the increased mitochondria oxygen uptake and lipoperoxidation. Repeated Δ^9-tetrahydrocannabinol injection (10 mg/kg, twice daily, 4.5 days) reduced brain metabolism and uncoupled respiration from oxidative phosphorylation. The present findings showed that both anandamide and Δ^9-tetrahydrocannabinol enhanced the energetic brain metabolism, probably via the cannabinoid CB_1 receptor; the anandamide-tolerant brain of rats showed tolerance to the drug for metabolic effects, while the brain of Δ^9-tetrahydrocannabinol-tolerant rats showed metabolic signs of neuronal damage, i.e. low energy production.

Keywords: Cannabinoid; Behavioural tolerance; Brain; Energetic metabolism; Cannabinoid CB_1 receptor-specific antagonist

1. Introduction

Natural and synthetic cannabinoids exert a large spectrum of effects — alterations in cognition and memory, euphoria, sedation, analgesia, anti-convulsing, anti-inflammation and attenuation of both intraocular pressure of glaucoma and emesis (Abood and Martin, 1992). Some evidence suggests that Δ^9-tetrahydrocannabinol — the main psychoactive component of marijuana — as well as other cannabinoids, exert pharmacological effects via interactions with lipid constituents of biological membranes (Hillard et al., 1985). However, it is currently well established that cannabinoids exert most of their effects through receptor-coupled mechanisms. To date, two subtypes of cannabinoid receptors have been characterised and cloned in mammalian tissues: the neuronal cannabinoid CB_1 receptor (Matsuda et al., 1990), which is mainly distributed in the nervous system, and the cannabinoid CB_2 receptor, (Munro et al., 1993) which is mainly present in cells of the immune system. Both receptors are members of the G protein-coupled receptor family (Howlett, 1995). Many derivatives of long-chain fatty acids have been identified as endogenous ligands of cannabinoid receptors (Mechoulam et al., 1998).

Marijuana is the most widely used illicit drug of abuse, but its acute and chronic effects on brain metabolic functions are not clearly understood. Most studies evaluating the effects of single drug-abused administration on human brain metabolism have demonstrated decreases after cocaine (London et al., 1990b), heroin (London et al., 1990a), amphetamines (Wolkin et al., 1987), alcohol (DeWit et al., 1990; Volkow et al., 1990) and benzodiazepines (DeWit et al., 1991; Volkow et al., 1995). Volkow et al. (1991) have assessed the metabolic effects of acute Δ^9-tetrahydrocannabinol in normal subjects in various brain regions and have reported an increase only in cerebellum metabolism. Physiologically relevant doses of Δ^9-tetrahydrocannabinol have been shown to produce, in vitro, a dose-dependent increase in the rate of glucose oxidation to CO_2 of C6 glioma cells (Sanchez et al., 1997) and of rat astrocytes (Sanchez et al.,

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00170-9

1998). Furthermore, Δ^9-tetrahydrocannabinol (0.2 mg/kg, intravenous (i.v.)) has been shown to stimulate glucose uptake by rat brain cortical and limbic areas in vivo (Margulies and Hammer, 1991). The first aim of the present study was to evaluate the oxidative phosphorylation of brain mitochondria from rats treated acutely with Δ^9-tetrahydrocannabinol and with anandamide, a putative endogenous ligand for the cannabinoid receptor (Devane et al., 1992), administered at doses that induce a profile of behavioural effects typically elicited by cannabinoids (Compton et al., 1993). The second purpose was to study the brain energetic metabolism of rats treated chronically, so that they were tolerant to the two cannabinoids.

2. Materials and methods

2.1. Animals and cannabinoid treatment

Male Sprague–Dawley rats (Charles River, Italy) weighing 140–160 g at the beginning of the experiment were used. All animals were adapted to the home cage for 1 week before the start of the experiment. The rats were held in a temperature- and light-controlled environment on a 12/12 h light/dark cycle, with free access to food and water until they were killed. All the procedures were carried out in accordance with the Italian State regulations governing the care and treatment of laboratory animals (Ministry of Health permit number: 36/1994-A). The rats received intraperitoneal (i.p.) administration of Δ^9-tetrahydrocannabinol (10 mg/kg), anandamide (20 mg/kg) or their respective vehicles. Two different treatments were used. For acute treatment, the animals received a single i.p. injection of cannabinoid or its vehicle and their behaviour was tested. For chronic treatment, the animals received a twice daily injection of Δ^9-tetrahydrocannabinol for 4.5 days or a daily i.p. injection of anandamide for 16 days, according to Fride's report (Fride, 1995) of tolerance development following the chronic exposure of mice to anandamide (20 mg/kg, i.p.). Behaviour was tested on the 1st and 16th days of anandamide treatment and on the 1st and 5th days of Δ^9-tetrahydrocannabinol treatment. For the study of antagonism, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716A; 3 mg/kg) or its vehicle was i.p. administered 30 min before the injection of anandamide or its vehicle, so that there were four treatment groups: SR141716A vehicle plus anandamide vehicle; SR141716A vehicle plus anandamide; SR141716A plus anandamide vehicle; SR141716A plus anandamide.

2.2. Behavioural testing

Rats were adapted to the evaluation room for 1 h before treatment. Tail-flick response and body temperature were evaluated 30 min after anandamide and 60 min after Δ^9-tetrahydrocannabinol; and locomotor activity 20–25 min after anandamide and 50–55 min after Δ^9-tetrahydrocannabinol; ring immobility 25–30 min after anandamide and 55–60 min after Δ^9-tetrahydrocannabinol. Antinociception was assessed in the tail-flick test (D'Amour and Smith, 1941) and was quantified in terms of response latency (s); core (rectal) temperature was measured before treatment (baseline) and was measured again after drug treatment, with a rectal thermistor probe inserted 5 cm into the anus and connected to a digital monitor (Ellab, Roedovre, Denmark). Spontaneous locomotor activity was measured by placing rats in an open field and recording the number of crossings in a 5-min period with an activity meter (Animex, LKB, USA). Ring immobility was determined with a modification of the ring immobility test (Pertwee, 1972); rats were placed on a horizontal ring and the immobility time (s) was recorded for 5 min.

The rats were decapitated and brains were rapidly removed, weighed, homogenised and used for both measure-

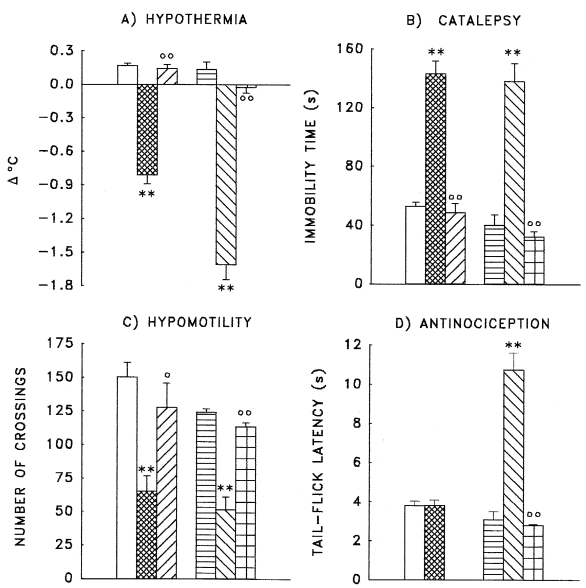

Fig. 1. Effects of acute (box filled with diagonally crossed lines) and 16-day (box filled with wavy, right-leaning diagonal lines) administration of anandamide (20 mg/kg, i.p.) or of acute (box filled with wavy, left-leaning diagonal lines) and 4.5-day (box filled with crossed lines) twice daily administration of Δ^9-tetrahydrocannabinol (10 mg/kg, i.p.) on behaviour testing. Body temperature is expressed as change in rectal temperature (Δ°C) before and after administration (A). Ring immobility (s) was assessed on a horizontal wire ring, 12 cm in diameter, attached to a stand at a height of 38 cm (B). Spontaneous locomotor activity is expressed as the number of crossovers, using an Animex activity meter (40×25 cm divided into six magnetic solenoids of equal size on two lines) (C). Analgesia is expressed as tail-flick response latency (s) (D). Bar heights represent the means ± S.E.M. for 8–10 rats. Statistical significance of differences was obtained by ANOVA followed by Tukey's test (** $P \leq 0.01$, as compared with vehicle; ° $P \leq 0.01$, ° $P \leq 0.05$ as compared with the 1st day of treatment). The vehicle value for anandamide (□) is the mean of the 1st and 16th days; the vehicle value for Δ^9-tetrahydrocannabinol (box filled with horizontal lines) of the 1st and 5th days of treatment because these values were not significantly different.

ment of lipid peroxide level and preparation of the mitochondria fraction 30 min after the injection of anandamide or 60 min after Δ^9-tetrahydrocannabinol, because at these times, all behavioural effects of the two cannabinoids were at their maximum.

2.3. Mitochondria

The brain mitochondria were prepared according to the method of Davis et al. (1974). Mitochondria respiratory rates were determined polarographically using a Clark-type oxygen electrode at 35°C according to the method described by Chance and Williams (1956); the respiratory medium was as described by Davis et al. (1974), with 13 mM glutamate and 6.5 mM succinate as respiratory substrates. Mitochondria protein concentration was measured through the method of Lowry et al. (1951) with bovine serum albumin as standard.

2.4. Lipid peroxide assay

This assay was used to determine malondialdehyde level in a brain homogenate prepared with a Teflon Potter homogenizer in a ratio of 1 g wet tissue to 9 ml 50 mM potassium phosphate buffer pH 7.4 + 0.1 mM EDTA. The lipid peroxide level was estimated spectrophotometrically at 532 nm by the thiobarbituric acid test of Ohkawa et al. (1979), employing 0.156/μM/cm as the extinction coefficient, and was expressed in terms of nmol malondialdehyde/g wet weight.

2.5. Chemicals

All chemicals were purchased from normal commercial sources and were of the highest purity available. Anandamide and Δ^9-tetrahydrocannabinol, in ethanol, were kindly supplied by NIDA (Research Triangle Institute, Rockville, MD, USA). After drying off the ethanol under a stream of nitrogen gas, the Δ^9-tetrahydrocannabinol residue

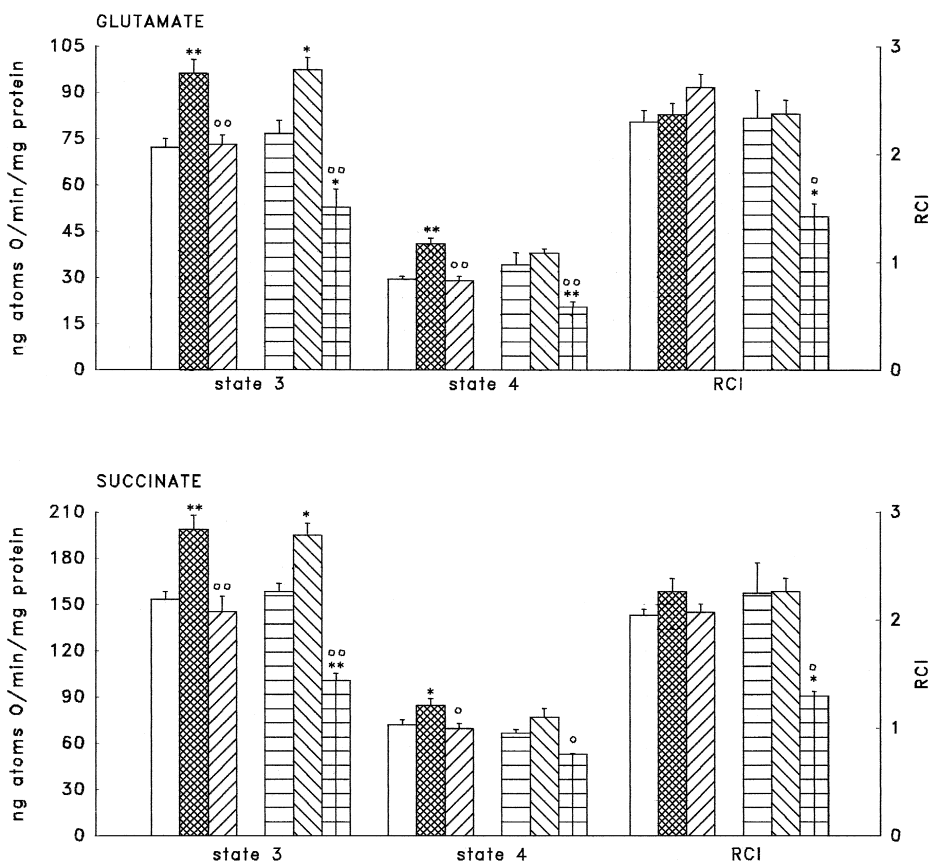

Fig. 2. Effects of acute (box filled with diagonally crossed lines) and 16-day (box filled with wavy, right-leaning diagonal lines) administration of anandamide (20 mg/kg, i.p.) or of acute (box filled with wavy, left-leaning diagonal lines) and 4.5-day (box filled with crossed lines) twice daily administration of Δ^9-tetrahydrocannabinol (10 mg/kg, i.p.) on rat brain mitochondria. Brain mitochondria were prepared and assayed for respiratory rate in the presence of substrate alone (state 4) (glutamate or succinate) as well as for ADP-stimulated (state 3) respiratory rate and for RCI. Bar heights represent the means ± S.E.M. of the brain mitochondria results from 8–10 rats. Statistical analysis was done by ANOVA followed by Tukey's test ($^{**}P \leq 0.01$, $^{*}P \leq 0.05$ as compared with anandamide vehicle (□) or Δ^9-tetrahydrocannabinol vehicle (box filled with horizontal lines); $^{\circ}P \leq 0.01$; $^{\circ}P \leq 0.05$ as compared with the acute treatment).

was emulsified in Cremophor, ethanol and saline (1:1:18), and the anandamide residue in Tween 80 and saline (1:24). SR141716A was a generous donation of Sanofi Recherche (Montpellier, France). It was emulsified in Tween 80, dimethylsulfoxide and distilled water (1:2:7). Drugs and vehicles were administered i.p. at a volume of 1 ml/kg body weight.

2.6. Analysis of data

The data are expressed as mean values ± S.E.M. Statistical comparisons of the means were performed with a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. In all statistical comparisons, a P value ≤ 0.05 was considered significant.

3. Results

3.1. Behavioural tolerance

The development of behavioural tolerance to either Δ^9-tetrahydrocannabinol or anandamide is shown in Fig. 1. A single injection of Δ^9-tetrahydrocannabinol (10 mg/kg, i.p.) decreased body temperature by 1.6°C; the hypothermia disappeared after 4.5 days of Δ^9-tetrahydrocannabinol treatment, indicating tolerance. The Δ^9-tetrahydrocannabinol single injection increased immobility time on a ring by about 245%, indicating a strong cataleptic effect, and reduced spontaneous locomotor activity by about 60%; the effects on immobility and locomotion disappeared after nine doses of Δ^9-tetrahydrocannabinol, indicating the development of tolerance to the cataleptic effects and to the motor disturbance induced by the cannabinoid. Another significant behavioural effect — strong antinociception — was observed at this Δ^9-tetrahydrocannabinol dose. Tolerance also developed to the antinociceptive effect after 5 days of Δ^9-tetrahydrocannabinol treatment. The latency (s) of the tail-flick response of Δ^9-tetrahydrocannabinol-once-treated rats was increased by about 250%; the antinociceptive effect disappeared after nine doses of Δ^9-tetrahydrocannabinol. As shown recently (Costa et al., 1999), anandamide induced in rats a significant increase in ring immobility (171%) and a significant decrease in locomotor activity (57%) and body temperature (about 1°C); no analgesic effect was induced by this dose of anandamide. Full tolerance developed to these behavioural effects after 16 days of anandamide treatment, hypothermia disappeared early, after two injections (Costa et al., 2000).

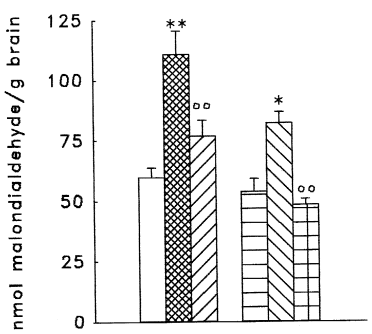

Fig. 3. Effects of acute (box filled with diagonally crossed lines) and 16-day (box filled with wavy, right-leaning diagonal lines) administration of anandamide (20 mg/kg, i.p.) and of acute (box filled with wavy, left-leaning diagonal lines) and 4.5-day (box filled with crossed lines) twice daily administration of Δ^9-tetrahydrocannabinol (10 mg/kg, i.p.) on the cerebral lipoperoxide level. Brain homogenate was prepared and assayed for the content of malondialdehyde. Bar heights represent the means ± S.E.M. of the experimental results from 8–10 rats. Statistical analysis was done by ANOVA followed by Tukey's test ($^{**}P \leq 0.01$, $^{*}P \leq 0.05$ as compared with anandamide vehicle (□) or Δ^9-tetrahydrocannabinol vehicle (box filled with horizontal lines); $^{\circ}P \leq 0.01$ as compared with acute treatment).

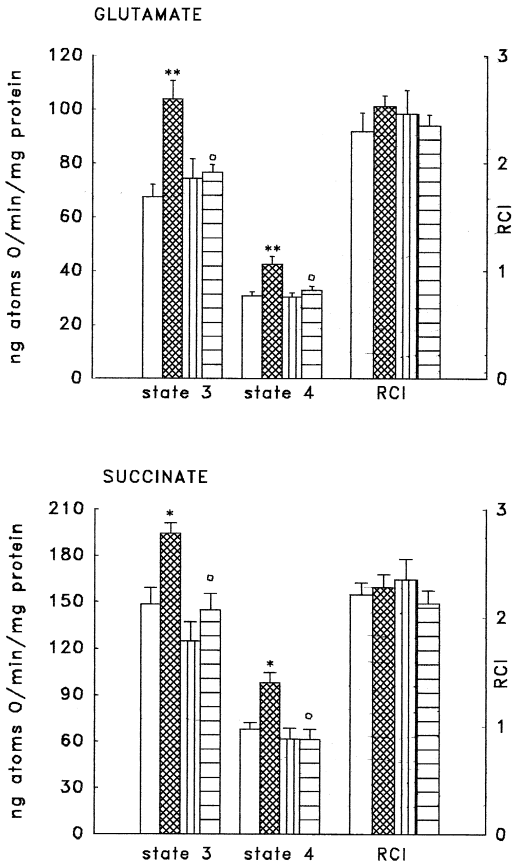

Fig. 4. Effects of the cannabinoid CB_1 receptor-specific antagonist, SR141716A (3 mg/kg, i.p.), on brain mitochondria oxidative phosphorylation increase induced by acute administration of anandamide (20 mg/kg, i.p.) in rats. Bar heights represent the means ± S.E.M. of the experimental results from 4–5 rats per group. The treatment groups were: vehicles (□), SR141716A plus vehicle (box filled with vertical lines), vehicle plus anandamide (box filled with diagonally crossed lines) and SR141716A plus anandamde (box filled with horizontal lines). Statistical analysis was done by ANOVA followed by Tukey's test ($^{**}P \leq 0.01$, $^{*}P \leq 0.05$ as compared with vehicle; $^{\circ}P \leq 0.05$ as compared with acute anandamide).

3.2. Effects on brain mitochondria

The effects of the acute and prolonged Δ^9-tetrahydrocannabinol or anandamide treatment on the brain mitochondria respiratory rates in states 3 and 4 and on the respiratory control index (RCI) in the presence of two oxidative substrates (glutamate and succinate) are shown in Fig. 2. After a single Δ^9-tetrahydrocannabinol administration, the brain mitochondria oxygen uptake in state 3 was significantly increased with both glutamate and succinate, by approximately 25%, while the uptake in state 4 was unaffected; the increase in oxygen consumption was not accompanied by a change in coupling between oxidation and oxidative phosphorylation. The RCI of Δ^9-tetrahydrocannabinol-treated rats had the same values as those for vehicle-treated rats. Repeated i.p. administration of Δ^9-tetrahydrocannabinol decreased the brain mitochondria oxygen consumption of state 3 with two oxidative substrates by approximately 30%, the oxygen uptake of state 4 by 40% in glutamate and by about 20% in succinate and uncoupled oxidative phosphorylation; the RCI was decreased by 40% in glutamate and in succinate. Also, after a single anandamide administration, the brain mitochondria oxygen consumption in glutamate and succinate was increased significantly by approximately 30% in both states 3 and 4 without changes in RCI. After anandamide chronic treatment, the increase in brain mitochondria oxygen consumption disappeared and the parameter of coupling, RCI, was not modified.

3.3. Cerebral lipoperoxidative effects

After a single dose of anandamide or Δ^9-tetrahydrocannabinol, there was a significant increase in cerebral peroxide level of 85% and 52%, respectively, that disappeared after repeated doses of cannabinoids, showing the development of tolerance (Fig. 3).

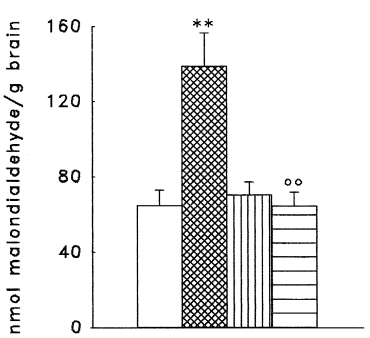

Fig. 5. Effects of the cannabinoid CB_1 receptor-specific antagonist, SR141716A (3 mg/kg, i.p.), on brain lipoperoxide increase induced by acute administration of anandamide (20 mg/kg, i.p.) in rats. Bar heights represent the means ± S.E.M. of the experimental results from 4–5 rats per group. The treatment groups were: vehicles (□), SR141716A plus vehicle (box filled with vertical lines), vehicle plus anandamide (box filled with diagonally crossed lines) and SR141716A plus anandamide (box filled with horizontal lines). Statistical analysis was done by ANOVA followed by Tukey's test (** $P \leq 0.01$ as compared with vehicle; ° $P \leq 0.01$ as compared with acute anandamide).

3.4. Antagonism of biochemical effects

We previously showed that the cannabinoid CB_1 receptor-specific antagonist, SR141716A, reversed the anandamide-induced behavioural effects (Costa et al., 1999). In the present experiments, SR141716A reversed both the increase in brain oxidative metabolism (Fig. 4) and the lipoperoxidative effect (Fig. 5). The brain mitochondria oxidative phosphorylation and the lipoperoxide level of brains from anandamide-treated rats and pre-treated with the specific antagonist were not different from those of vehicle-treated rats. SR141716A itself did not affects these parameters.

4. Discussion

We had shown that a single i.p. administration of 0.4 mg/kg of the synthetic bicyclic cannabinoid, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)cyclo hexanol) (CP-55,940), inducing in rats antinociception, hypothermia, hindlimb splaying and hypomotility increased rat brain mitochondria respiration but did not modify the ADP/O (adenosine 5′-diphosphate/oxygen) ratio (Costa et al., 1996). In the bioassay we used, known as the ''tetrad model'' (Compton et al., 1993), anandamide at the dose employed had no antinociceptive effects. Although some authors reported a short duration (5 min) and rapid onset (1 min) of antinociception (Stein et al., 1996), in our experiment, a dose of 20 mg/kg anandamide (dose selected in the light of the findings of Fride and Mechoulam, 1993, who showed that in mice, this i.p. dose produced maximal effects in pharmacological tests) did not induce analgesia, even at 1 and 5 min. Pharmacological and biochemical data suggest that anandamide acts as a partial agonist (Kulkarni et al., 1996). Moreover, Smith et al. (1994a) obtained minimal antinociception in mice with the i.p. route, and it appeared that anandamide had a maximum antinociceptive effect when it was injected i.v. or i.t. Finally, Smith et al. (1994b) showed that the antinociceptive effects of the cannabinoids are mediated through mechanisms distinct from those responsible for other behavioural effects.

The findings of the present study showed that both anandamide and Δ^9-tetrahydrocannabinol, administered at doses inducing a typical behavioural pattern (Compton et al., 1993) in rats, produced an analogous increase in brain energetic metabolism without modification of the coupling between mitochondria oxidation and oxidative phosphorylation. This anandamide-induced metabolic effect was specific for brain tissue; states 3 and 4 oxygen uptake in glutamate and succinate of mitochondria from liver of the

same rats was unaffected by the treatment (data not shown). To study whether this anandamide-induced metabolic effect was mediated by the cannabinoid CB_1 receptor, the rats were pre-treated with the cannabinoid CB_1 receptor-specific antagonist, SR141716A, at a dose producing reversal of typical behaviour (Costa et al., 1999). This pre-treatment also antagonised the increase in brain mitochondria oxidative phosphorylation, thus showing a receptor-mediated effect. It is known that there are effects that do not appear to be related to an action at the cannabinoid receptor but to the disturbance of the physical properties of biological and model membranes (Bloom et al., 1997). The cerebral lipoperoxide level was increased after treatment with either cannabinoid. This increase seemed to favour this non-specific mechanism, but the reversal by SR141716A pre-treatment of this anandamide-produced increase indicated cannabinoid CB_1 receptor mediation. Therefore, we conclude that the increase in lipoperoxide level was caused by the increase in brain mitochondria oxygen uptake via the cannabinoid CB_1 receptor. Further studies are needed to clarify whether the increase in both brain energy metabolism and lipoperoxidation induced by Δ^9-tetrahydrocannabinol and by other cannabinoids is also cannabinoid CB_1 receptor-mediated.

Our previous study showed that the development of behavioural tolerance to CP-55,940 appeared together with the disappearance of the increase in mitochondria oxygen consumption induced by a single administration (Costa et al., 1996). In line with this previous report, the present findings demonstrated that also the behavioural tolerance induced by prolonged treatment with anandamide again occurred together with the disappearance of the increase in brain oxygen uptake and in brain lipoperoxidation induced by a single anandamide injection. Different results were obtained with brain mitochondria from Δ^9-tetrahydrocannabinol-tolerant rats. Both the decrease in oxygen uptake and the uncoupling of oxidation and oxidative phosphorylation indicated a low ATP production, little disposable energy and consequent neuronal damage, subsequent to the Δ^9-tetrahydrocannabinol prolonged treatment. Whether chronic marijuana use leads to cerebral dysfunction and brain damage is still debatable (Stefanis et al., 1976). Various and conflicting results have long been reported about the effects of single and repeated Δ^9-tetrahydrocannabinol administration on brain metabolism and functions. Volkow et al. (1991) reported that marijuana is the only drug of abuse that does not produce a reduction in absolute metabolism but an increase in cerebellum metabolism after a single acute administration in normal subjects and that marijuana abusers have a lower relative cerebellum metabolism than do normal subjects (Volkow et al., 1996). In the light of our present findings and of what Volkow et al. (1996) showed for human brain, we hypothesise that the increase in brain energetic metabolism after a single Δ^9-tetrahydrocannabinol administration and its decrease after a prolonged treatment could reflect changes in cannabinoid receptors attributable to repeated Δ^9-tetrahydrocannabinol doses (Rodriguez de Fonseca et al., 1994). Because other authors could not find a change in cannabinoid receptor density, by using Δ^9-tetrahydrocannabinol (Abood et al., 1993), we plan to confirm this hypothesis by measuring receptor density and by studying the coupling between cannabinoid receptor and second messenger system in rats treated with Δ^9-tetrahydrocannabinol under our experimental conditions. It has been shown that Δ^9-tetrahydrocannabinol (0.2 mg/kg, i.v.) administration in rats stimulated 2-deoxyglucose uptake by the brain cortex and limbic system, regions characterised by a high density of cannabinoid receptors (Margulies and Hammer, 1991) and that in vitro Δ^9-tetrahydrocannabinol produced a dose-dependent increase in the rate of glucose oxidation to CO_2 and glucose incorporation into phospholipid and glicogen by C6 glioma cells and by rat astrocytes through a cannabinoid receptor-mediated process (Sanchez et al., 1997, 1998).

In summary, the response of brain energetic metabolism to acute exposure to the endocannabinoid agonist, anandamide, and to the main psychoactive component of marijuana is similar. Behavioural and brain metabolic tolerance develops after chronic exposure to anandamide, while after chronic exposure to the exogenous agonist, in this case Δ^9-tetrahydrocannabinol, the brain energetic metabolism was inhibited and oxidation was uncoupled from ATP production, indicating neuronal damage after prolonged exposure to Δ^9-tetrahydrocannabinol.

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