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Characterization of the acute endocrine actions of (−)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210), a potent synthetic cannabinoid in rats

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Abstract

In the present study we have characterized the effects of the acute administration of the synthetic cannabinoid (−)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210, 4, 20 and 100 μg/kg), on the secretion of prolactin, growth hormone, luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone and corticosterone in adult male rats. HU-210 administration resulted in a dose-dependent inhibition of plasma growth hormone, follicle-stimulating hormone and luteinizing hormone 60 min after the acute intraperitoneal injection, starting at 20 μg/kg. Plasma adrenocorticotropic hormone and corticosterone levels revealed a dose-dependent activation of the pituitary–adrenal axis after acute exposure to HU-210. Plasma prolactin levels reflected a biphasic action of HU-210: the 4 μg/kg dose resulted in high prolactin levels and the 20 and 100 μg/kg doses induced a decrease in the levels of this hormone. The time course of the endocrine effects of HU-210 was examined using the 20 μg/kg dose and was found to parallel the onset of the immobility and hypothermic effects of this cannabinoid. HU-210 (20 μg/kg) was also found to block the hormonal surges of luteinizing hormone, follicle-stimulating hormone and prolactin occurring during the afternoon of the proestrus phase in adult female rats. This dose induced activation of tubero-infundibular dopaminergic neurons, as reflected by the decrease in hypothalamic contents of dopamine in both males and females in the afternoon of the proestrus phase. The actions of HU-210 during early postnatal development revealed a delayed maturation of the endocrine response to HU-210, with respect to the behavioral effects. The findings of the present study reveal that HU-210 induces a set of endocrine alterations closely related to those described for natural cannabinoids such as Δ^9-tetrahydrocannabinol but at doses 50–200 times lower than those required for Δ^9-tetrahydrocannabinol. © 1998 Elsevier Science B.V.

Keywords: Cannabinoid; Pituitary hormone; Corticosterone; Dopamine; Hypothalamus; Estrous cycle

1. Introduction

The acute administration of drugs acting on the recently characterized brain cannabinoid receptor, CB_1 (Devane et al., 1988; Herkenham et al., 1990; Matsuda et al., 1990; Howlett et al., 1991), produces a characteristic set of endocrine effects (for review see Murphy et al., 1990a). Acute exposure to Δ^9-tetrahydrocannabinol, the main psychoactive constituent of marijuana, results in a decrease in the release of prolactin, gonadotropins and growth hormone and an increase in both adrenocorticotropic hormone and corticosterone secretion in male rodents (Kubena et al., 1971; Kramer and Ben-David, 1978; Steger et al., 1983). The acute administration of Δ^9-tetrahydrocannabinol during the proestrus phase of the estrous cycle also blocks the afternoon hormonal surges of prolactin and the gonadotropins (Ayalon et al., 1977; Murphy et al., 1990a). Some of these effects also appear after the administration of anandamide (Weidenfeld et al., 1993; Romero et al., 1994), the proposed endogenous ligand for brain cannabinoid receptors (Devane et al., 1992). It has been proposed that the endocrine effects of Δ^9-tetrahydrocannabinol are mediated through alterations in the hypothalamic mechanisms controlling pituitary hormone synthesis and release

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(Hughes et al., 1981; Rettori et al., 1988; Murphy et al., 1990b). Additionally, cannabinoid CB_1 receptors have been reported in several hypothalamic nuclei such as the ventromedial, paraventricular and arcuate nuclei, as well as in brain areas regulating hypothalamic activity, such as the central amygdala (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992). These findings suggest that cannabinoids act on the hypothalamus to elicit the above-described endocrine effects. However, Δ^9-tetrahydrocannabinol and most natural cannabinoids induce a broad range of non-specific effects. These include alterations in membrane fluidity and Na^+ membrane conductance, interactions with sex steroids and glucocorticoids, etc. (Dewey, 1986; Eldridge and Landfield, 1990; Murphy et al., 1991; Bonnin et al., 1992). Moreover, anandamide might also act through mechanisms unrelated to the cannabinoid CB_1 receptor (Venance et al., 1995), hindering the analysis of the neuroendocrine mechanisms underlying the endocrine actions of the cannabinoids.

In order to establish a role for cannabinoid CB_1 receptors in the neuroendocrine control of anterior pituitary function, the synthesis of highly potent cannabinoid analogs could be a useful tool for avoiding the non-CB_1-mediated effects of natural cannabinoids. One such synthetic cannabinoid, (−)-11-hydroxy-Δ8-tetrahydrocannabinol-dimethylheptyl (HU-210), has been found to display 100–500 times greater activity than Δ^9-tetrahydrocannabinol in various pharmacological tests, including the induction of hypothermia, analgesia, or the inhibition of adenylate cyclase (Little et al., 1989; Howlett et al., 1990; Ovadia et al., 1995). HU-210 binds with high affinity to cannabinoid CB_1 receptors (Howlett et al., 1990) and has been used as a tool for characterizing the anatomical distribution of cannabinoid CB_1 receptors in the brain (Thomas et al., 1992). However, only one preliminary study on the effects of HU-210 on the endocrine system has been carried out (Rodríguez de Fonseca et al., 1995).

The purpose of this work was to characterize: (a) the dose-dependent effects of HU-210 on several endocrine parameters in male rats; (b) the time profile of the endocrine effects of HU-210; (c) the effects of HU-210 on the hormonal surges appearing in the afternoon of the proestrus phase in female rats and (d) the effects of HU-210 on hormone secretion at different stages of postnatal development. The study was carried out using Wistar rats and the following were tested: (a) plasma prolactin, growth hormone, luteinizing hormone, follicle-stimulating hormone, adrenocorticotropin and corticosterone levels and (b) the medial basal hypothalamus contents of dopamine, L-3,4-dihydroxyphenylacetic acid (DOPAC, the main dopamine intraneuronal metabolite) and noradrenaline. Additionally, selected variables, classically described as altered after cannabinoid treatment, were evaluated after treatment with HU-210 (Rodríguez de Fonseca et al., 1994b; Ovadia et al., 1995). These include hypothermia, locomotor activity, immobility or catalepsy.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Panlab, Barcelona) weighing 300 ± 35 g at the start of the experiment were housed 3 per cage and were maintained in a temperature and light-controlled environment on a 12 h light/dark cycle (lights on at 8.00 a.m.) with free access to food and water. Female virgin Wistar rats weighing 220 ± 30 g were housed 4 per cage under similar conditions. Daily vaginal smears were taken between 10.00–12.00 h and only those animals exhibiting three or more consistent 4-day cycles were used in this study. The animals were allowed a period of at least 2-weeks to become acclimatized to the animal room before they were subjected to the different research protocols. Litters from our colony of Wistar rats were used for the developmental studies. Pups were weaned on day 24 of postnatal age and housed 4–6 per cage. All procedures were carried out according to the European Communities directive 86/609/EEC regulating animal research.

2.2. Drugs

(−)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210) was synthesized at the Hebrew University at Jerusalem, Israel. The drug was dissolved in saline/propylene-glycol/Tween 80 (90:5:5 v/v) as vehicle and made up daily to the appropriate concentrations to be administered intraperitoneally in a volume of 0.1 ml/100 g b.w.

2.3. Behavioral studies, cannulae implantation and rectal temperature

The intensity of catalepsy was determined by means of the bar test, measuring the length of time a rat remained immobile with both forelegs over a 10 cm high bar and both hindlegs on the bench. Animals were tested at selected times (0, 30, 60 or 120 min after the injection of the drug), for a maximum period of 180 s. Tests were ended when the animals placed both forepaws on the bench and the time elapsed was taken as the descent latency (Rodríguez de Fonseca et al., 1994b). For the developmental studies, the bar was placed progressively higher, to achieve a 45° angle between the body of the animal and the bench. Locomotor activity was evaluated using 40 × 25 cm photocell cages. Adult animals were adapted for 8 h to the cages 24 h before the testing sessions. The animals were studied for 2 h and the number of two consecutive beam interruptions (crossovers) was automatically recorded every 10 min. One group of six animals was implanted under Equithesin anesthesia with indwelling atrial cannulae, inserted via the right external jugular vein. Cannulae were filled with heparinized saline (10 U/ml) to maintain

patency (Tyrey, 1986). The animals were allowed at least 48 h for recovery, before experimental procedures were begun. For developmental studies immobility was monitored by noting the time spent by the animal in absolute quietness in an observation box to which the animal had been previously habituated (Rodríguez de Fonseca et al., 1994b). The test length was 5 min. Rectal temperature was obtained with a digital thermometer and measured several times 0, 30, 60 or 120 min after drug injection.

2.4. Sampling

300 μl of blood was withdrawn from the jugular vein with the help of a heparinized syringe at −20, 0, 30, 60 and 120 min before and after HU-210 injection. 60 min after the last sampling, these animals, as well as those from the other experimental groups, were killed by rapid decapitation using a guillotine. The animals were previously familiarized with the handling procedure. Trunk blood was collected in tubes containing 400 μl of 6% EDTA and centrifuged at 2500 × *g* at 4°C. Plasma was stored frozen at −20°C until assayed for hormonal determinations. The brain of each animal was quickly removed and frozen at −70°C. On the day of analysis, the medial basal hypothalamus was dissected out as previously described (Rodríguez de Fonseca et al., 1995) and used for neurochemical determinations. This area consisted of a

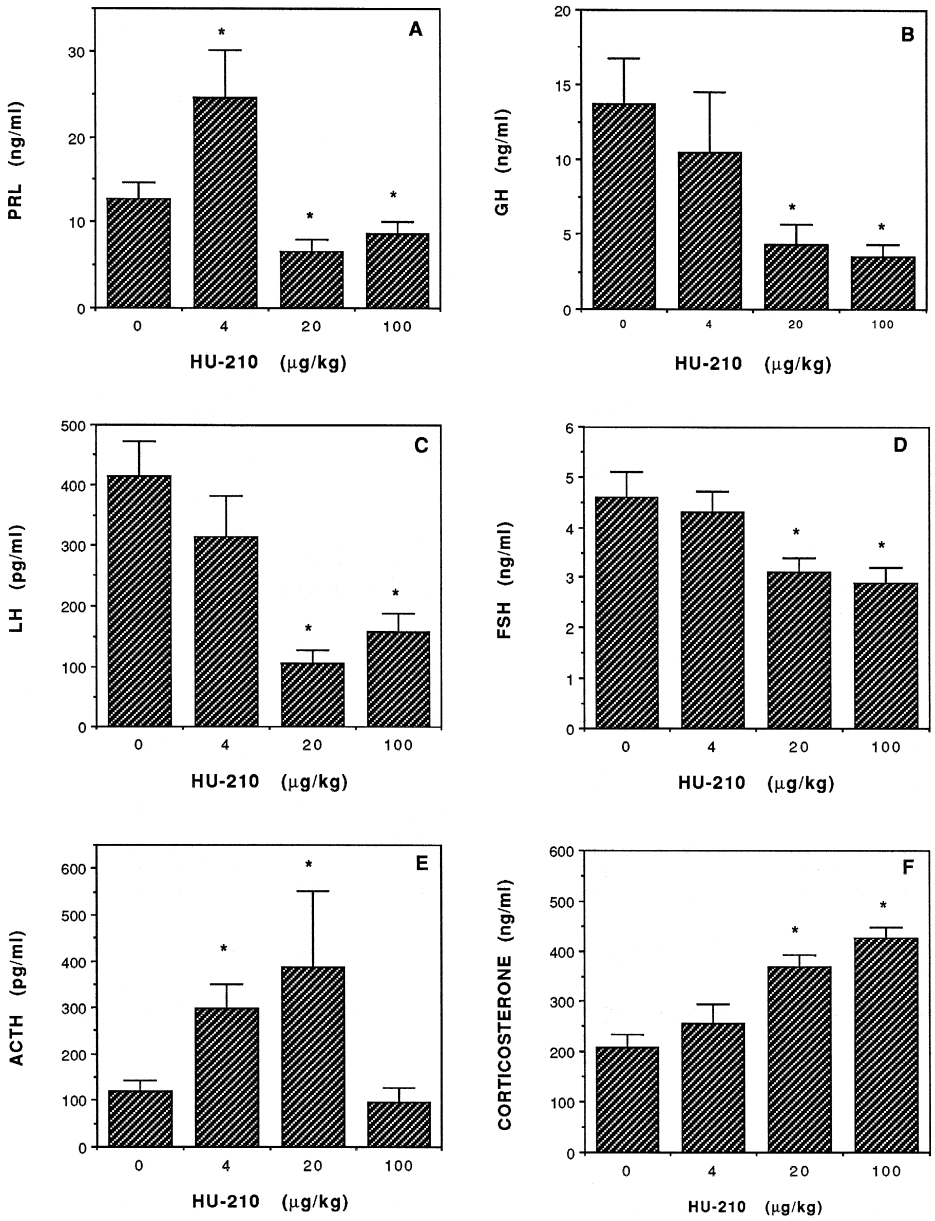

Fig. 1. Endocrine effects of the highly potent cannabinoid receptor agonist HU-210 (0, 4, 20 or 100 μg/kg), in male rats, 60 min after its acute i.p. administration. Values are means ± S.E.M. of plasma prolactin (A), growth hormone (GH) (B), luteinizing hormone (LH) (C), follicle-stimulating hormone (FSH) (D), adrenocorticotropic hormone (ACTH) (E) or corticosterone (F) levels measured in at least 12 animals per group. * $P < 0.05$, Newman–Keuls, versus vehicle-treated animals.

tissue block 2.5 mm deep, extending from just caudal to the optic chiasm to the rostral margin of the mammillary bodies and lateral to the hypothalamic sulci.

2.5. Hormonal determinations

Plasma prolactin was measured by a specific double-antibody radioimmunoassay system (RIA) using materials kindly provided by the National Hormone and Pituitary Program (NIH, Bethesda, MD). Values are expressed in ng/ml of reference preparation r-prolactin-RP3. The intra-assay coefficient of variation was 3.3% and sensitivity was 0.025 ng/ml. Plasma growth hormone was determined by a specific RIA using reagents provided by NIH. Results are expressed as ng/ml of r-growth hormone-RP3. The sensitivity of the assay was 0.5 ng/ml and the intra-assay coefficient of variation was 6%. Plasma luteinizing hormone levels were measured by a specific double-antibody RIA system, using materials kindly supplied by the NIH. The data were expressed as pg/ml of reference preparation r-luteinizing hormone-RP3. The intra-assay coefficient of variation was 4.5% and sensitivity was 0.02 ng/ml. Plasma follicle-stimulating hormone was measured by a specific double-antibody RIA system, using materials provided by the NIH. Plasma follicle-stimulating hormone levels were expressed as ng/ml of reference preparation r-follicle-stimulating hormone-RP2. The intra-assay coefficient of variation was 10% and sensitivity 3.7 ng/ml. Details of the methods have been previously published (Esquifino et al., 1995; Rodríguez de Fonseca et al., 1995, 1996). Plasma adrenocorticotropic hormone levels were measured using a commercial kit (CIS-Biointernational, Gif-Sur-Yvette, France). The intra-assay coefficient of variation was 5% and sensitivity was 10 pg/ml. Plasma corticosterone levels were measured with a RIA system, using a specific antibody from Bio Clin (Cardiff, England) with hydroxyapatite for separating the free and bound phases. This RIA system yields basal values of corticosterone of 175 ± 25 ng/ml in undisturbed adult male animals and 500 ± 70 ng/ml in stressed animals (Gamallo et al., 1986; Rodríguez de Fonseca et al., 1996). The variability of the method was 15.3% and the detection limit was 62 pg/ml.

All samples were run in duplicate in the same assay for each hormone to avoid interassay variations.

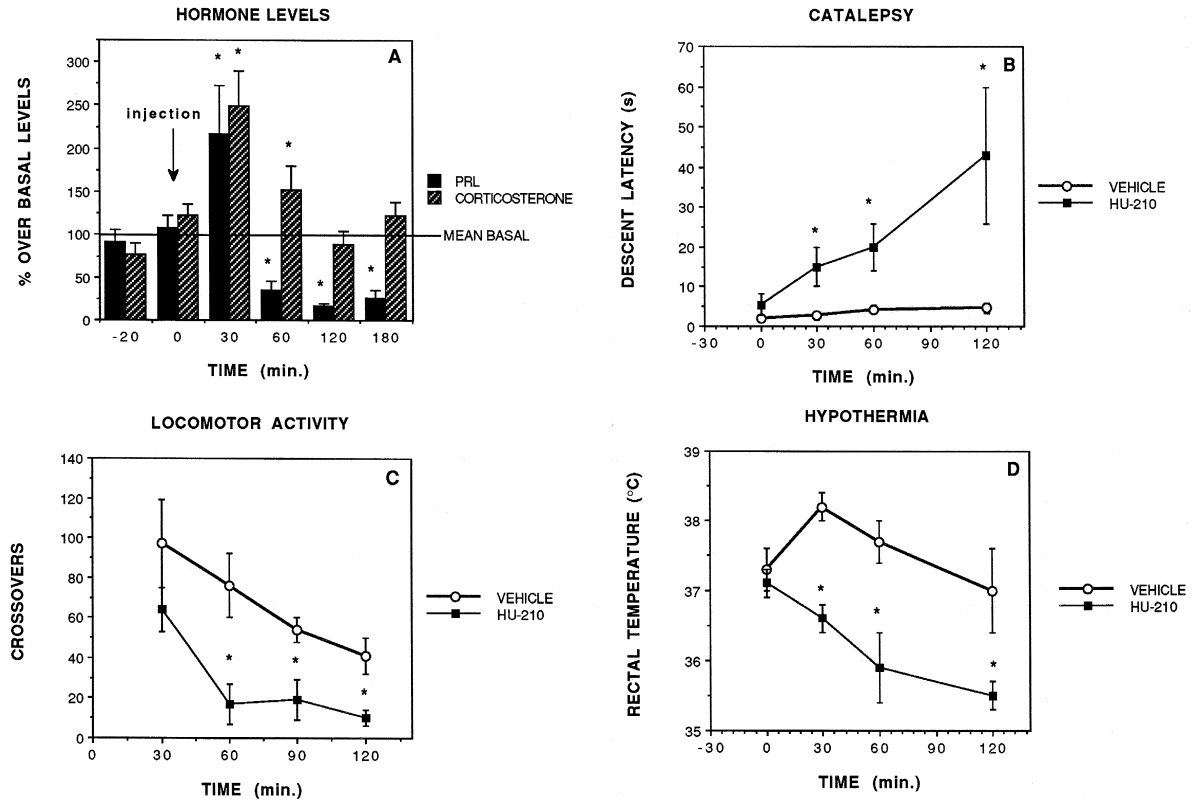

Fig. 2. (A) Time-course of the effects of acute administration of HU-210 (20 μg/kg) on plasma corticosterone and prolactin levels. Values are means $\pm$ S.E.M. of the % over basal levels measured in 6 animals bearing jugular cannulae. $^{*}P < 0.05$, Newman–Keuls, versus first fraction. (B) Time-course of the effects of acute administration of either vehicle or HU-210 (20 μg/kg) on catalepsy measured in the bar test. (C) Time-course of the effects of acute administration of either vehicle or HU-210 (20 μg/kg) on locomotor activity measured in photocell cages. (D) Time-course of the effects of acute administration of either vehicle or HU-210 (20 μg/kg) on rectal temperature. Values of (B), (C) and (D) are means $\pm$ S.E.M. for 8–12 animals per group. $^{*}P < 0.05$, Newman–Keuls, versus vehicle-treated animals.

2.6. Neurochemical determinations

Dopamine, noradrenaline and DOPAC contents were analyzed using high performance liquid chromatography with electrochemical detection. Hypothalami were weighed and homogenized in 400 μl of ice-cold 0.4 M $HClO_4$, containing 0.1% $Na_2S_2O_5$ and centrifuged at $12\,000 \times g$ for 20 min at 5°C. A 25 μl aliquot of the clear supernatant was injected into the chromatography system. The mobile phase consisted of 0.1 M Na_2HPO_4, 0.1 M citric acid, 1.8 mM octanesulfonic acid and 6% v/v methanol, adjusted to pH 4. Elution was performed at a constant rate of 1 ml/min and the working electrode potential was set at 0.80 V. The column was a stainless steel reverse-phase column (Nova-Pak®, C-18, 4 μm, 0.39×15 cm) and the precolumn was a Guard-Pak® C-18. Chromatography was performed at room temperature. Values are expressed as ng/mg tissue weight. Details of the method have been published previously (Rodríguez de Fonseca et al., 1995).

2.7. Data analysis

The data were evaluated by multifactorial analysis of variance. 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).

3. Results

3.1. Experiment 1: Dose–response in male rats (Fig. 1)

Adult male rats received an acute injection of HU-210 (4, 20 or 100 μg/kg, i.p.) and plasma samples were obtained 60 min after the injection. Plasma prolactin levels were affected after acute exposure to HU-210 in a biphasic fashion ($F(3, 68) = 9.7$, $P < 0.0001$), the 4 μg/kg dose stimulating and the 20 and 100 μg/kg resulting in a decrease in the levels of this hormone (Fig. 1A). The administration of HU-210 resulted in a dose-dependent reduction of plasma growth hormone ($F(3, 54) = 3.51$, $P < 0.03$), plasma luteinizing hormone ($F(3, 54) = 7.3$, $P < 0.0005$) and plasma follicle-stimulating hormone ($F(3, 58) = 3.98$, $P < 0.02$) levels, starting at the 20 μg/kg dose (Fig. 1B, C and D). Plasma adrenocorticotropic hormone concentrations (Fig. 1E) were found to be increased in the 4 and 20 μg/kg groups, but unaffected after the

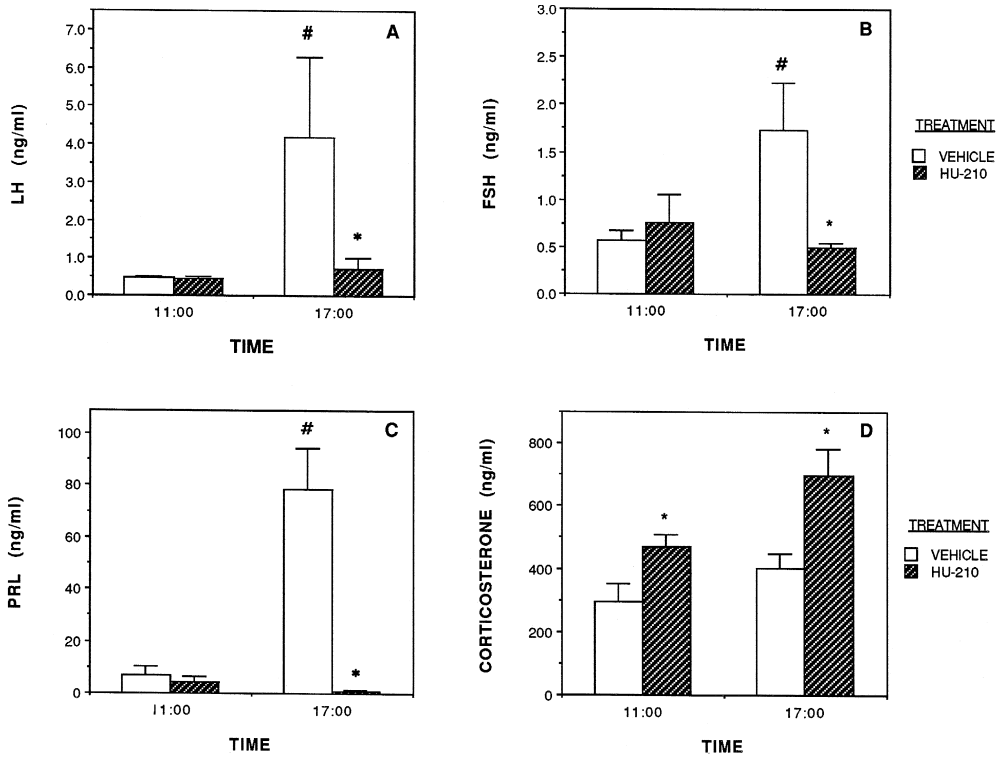

Fig. 3. Acute HU-210 (20 μg/kg) blocks the afternoon hormonal surges in female rats in the proestrus phase of the estrous cycle. Values are means ± S.E.M. of plasma luteinizing hormone (A), follicle-stimulating hormone (B), prolactin (C) and corticosterone (D) levels measured in 8–9 animals per group. $^{\#}P < 0.05$, Newman–Keuls, afternoon (17.00 p.m.) versus morning time (11.00 a.m.). $^{*}P < 0.05$, Newman–Keuls, HU-210 versus vehicle-treated animals.

100 μg/kg dose ($F(3, 48) = 3.96$, $P < 0.02$). Finally, plasma corticosterone increased in a dose-dependent manner ($F(3, 68) = 8.1$, $P < 0.0001$) after acute HU-210, starting at the 20 μg/kg dose (Fig. 1F).

3.2. Experiment 2: Time profile of the effects of HU-210 in male rats (Fig. 2)

Animals implanted with jugular cannulae received a single dose of HU-210 (20 μg/kg). Blood was withdrawn from the jugular vein 20 and 0 min prior to injection, (basal levels) and 30, 60 and 120 min after the injection of the cannabinoid. The animals were killed 180 minutes after HU-210 injection. Plasma samples obtained were used for the evaluation of corticosterone and prolactin, selected on the basis of their constant response to cannabimimetics and their opposite nature (Murphy et al., 1990a; Rodríguez de Fonseca et al., 1991b, 1995). Mean basal levels of both hormones were obtained as the mean value of the first 2 fractions, and were used for normalization (percentage of change over mean basal value). A parallel group of animals was used for evaluating catalepsy, locomotor activity and rectal temperature. Acute exposure to HU-210 (20 μg/kg) resulted in a biphasic profile of changes in plasma prolactin levels ($F(5, 25) = 9.6$, $P < 0.0001$, ANOVA for repeated measures, Fig. 2A). Plasma prolactin levels rose 30 min after the injection of HU-210, but were depressed thereafter. Plasma corticosterone levels were elevated 30 and 60 min after acute administration of the cannabinoid ($F(5, 25) = 8.2$, $P < 0.0001$). Acute administration of HU-210 resulted in a time-dependent increase in catalepsy (time × treatment interaction, $F(3, 48) = 3.1$, $P < 0.04$, Fig. 2B), which started at 30 min and peaked at 120 min after the injection. Locomotor activity was also depressed, starting 60 min after the injection ($F(3, 48) = 9.1$, $P < 0.001$, Fig. 2C). Rectal temperature decreased by as much as 1.5°C 120 min after the injection of the cannabinoid and significant effects were observed 30 min after the injection ($F(3, 33) = 6.4$, $P < 0.002$, Fig. 2D).

3.3. Experiment 3: Effects of HU-210 on proestrus hormonal surges in female rats (Fig. 3)

Female rats in the proestrus phase of the estrous cycle were injected in the morning (11.00 a.m.) or the afternoon (17.00 p.m.) with either vehicle or HU-210 (20 μg/kg). The animals were killed 60 min after administration of the

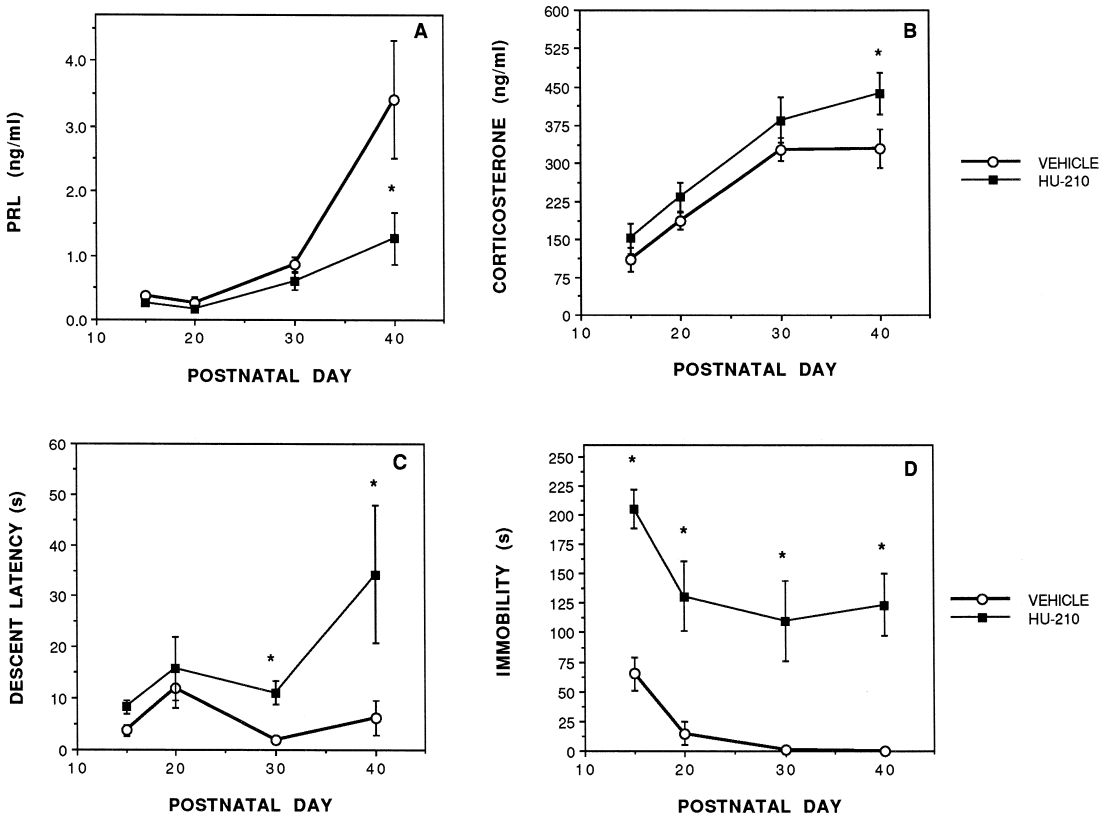

Fig. 4. Effects of acute HU-210 (20 μg/kg) on plasma prolactin (A) and corticosterone levels (B), catalepsy measured in the bar test (C) and immobility measured in observational boxes (D) 60 min after its i.p. administration at different stages of the postnatal development. Values are means ± S.E.M.; 10–13 animals per group. * $P < 0.05$, Newman–Keuls, versus vehicle-treated animals.

cannabinoid and plasma samples obtained. Hormonal surges of luteinizing hormone ($F(1, 27) = 4.6$, $P < 0.05$, Fig. 3A), follicle-stimulating hormone ($F(1, 31) = 2.7$, $P < 0.05$, Fig. 3B) and prolactin ($F(1, 31) = 17.6$, $P < 0.0002$, Fig. 3C) were observed during the afternoon of the proestrus phase. Acute exposure to HU-210 did not alter the plasma levels of these hormones during the morning of proestrus phase, but did block the surges of luteinizing hormone (time × treatment interaction $F(1, 27) = 4.4$, $P < 0.05$, Fig. 3A), follicle-stimulating hormone (time × treatment interaction $F(1, 31) = 5$, $P < 0.04$, Fig. 3B) and prolactin (time × treatment interaction $F(1, 27) = 20.9$, $P < 0.0001$, Fig. 3C). Plasma corticosterone levels rose equally after HU-210 administration in the morning and the afternoon times ($F(1, 29) = 18.9$, $P < 0.0003$, Fig. 3D).

3.4. Experiment 4: Developmental effects of HU-210 (Fig. 4)

Plasma prolactin and corticosterone levels as well as immobility and catalepsy were monitored at 15, 20, 30 and 40 days of postnatal life in both male and female pups 60 min after they had an acute dose of either vehicle or HU-210 (20 μg/kg). The data for both sexes were pooled since they showed a similar developmental profile and a similar response to HU-210 (data not shown). Plasma prolactin levels (Fig. 4A) rose with postnatal age (time effect, $F(3, 91) = 23.7$, $P < 0.0001$). Acute exposure to HU-210 only lowered plasma prolactin levels at 40 days of postnatal age (time × treatment interaction, $F(3, 91) = 6.5$, $P < 0.01$). Plasma corticosterone levels increased progressively during postnatal ageing (time effect, $F(3, 92) = 28.4$ $P < 0.0001$, Fig. 4B). Acute HU-210 administration increased plasma corticosterone levels at 40 days of postnatal age ($F(1, 92) = 6.2$, $P < 0.02$). Acute HU-210 injection resulted in prolonged immobility times (Fig. 4D) on all days tested ($F(1, 81) = 37.0$, $P < 0.0001$) but only increased the catalepsy score (Fig. 4C) starting at 30 days of postnatal age ($F(1, 81) = 9.1$, $P < 0.005$).

3.5. Effects of HU-210 on hypothalamic catecholamines (Table 1)

Hypothalamic contents of noradrenaline, dopamine and DOPAC, and DOPAC/dopamine ratio were analyzed in the male and female animals of experiments 1 and 3, at 60 min after the injection of HU-210 (20 μg/kg). In the male rat, acute exposure to the synthetic cannabinoid resulted in a decrease in hypothalamic dopamine ($F(1, 24) = 5.9$, $P < 0.03$) and an increase in the DOPAC/dopamine ratio ($F(1, 24) = 5.3$, $P < 0.04$). Neither hypothalamic noradrenaline ($F(1, 24) = 2.6$, $P = 0.12$, n.s.) nor DOPAC contents ($F(1, 24) = 1.9$ $P = 0.2$, n.s.) were affected as a result of acute HU-210 treatment. In female animals in the proestrus phase there was only a significant effect of HU-210 on hypothalamic dopamine contents, which were reduced in the afternoon group (time × treatment interaction $F(1, 30) = 9.3$, $P < 0.005$). Noradrenaline, DOPAC and the DOPAC/dopamine ratio remained unaltered after acute treatment with HU-210 in female rats.

Table 1
Hypothalamic contents of noradrenaline (NA), dopamine (DA), L-3,4-dihydroxyphenylacetic acid (DOPAC) and DOPAC/DA ratio in male and female rats 60 min after the injection of a single dose of HU-210 (20 μg/kg) or vehicle

	NA (ng/mg)	DA (ng/mg)	DOPAC (ng/mg)	DOPAC/DA
Males				
+ Vehicle	2.8 ± 0.3	0.56 ± 0.06	0.21 ± 0.04	0.46 ± 0.1
+ HU-210	2.2 ± 0.2	0.38 ± 0.04[a]	0.24 ± 0.05	0.90 ± 0.16[a]
Females 11.00 a.m.				
+ Vehicle	3.4 ± 0.4	0.16 ± 0.02	0.18 ± 0.03	1.10 ± 0.2
+ HU-210	3.2 ± 0.3	0.36 ± 0.1	0.18 ± 0.04	1.10 ± 0.4
Females 17.00 p.m.				
+ Vehicle	4.3 ± 0.5	0.53 ± 0.14[b]	0.22 ± 0.06	0.70 ± 0.2
+ HU-210	3.7 ± 0.4	0.20 ± 0.03[a]	0.24 ± 0.11	0.63 ± 0.2

Female rats were studied in the proestrus phase of the estrus cycle, and were injected either in the morning (11.00 a.m.) or the afternoon (17.00 p.m.).
Values are means ± SEM for 9–14 animals per group.
[a] Mean value is significantly different from vehicle value, one-way analysis of variance, $P < 0.05$.
[b] Different from morning vehicle value (11.00 a.m.), one-way analysis of variance, $P < 0.05$.

4. Discussion

The present results clearly indicated that acute administration of HU-210 results in profound alterations in the rat endocrine system. Most of the effects were markedly dose-dependent and resembled those described for natural cannabinoids such as Δ^9-tetrahydrocannabinol (Kubena et al., 1971; Ayalon et al., 1977; Murphy et al., 1990a,b; Bonnin et al., 1993), or the endogenous CB_1 receptor ligand anandamide (Weidenfeld et al., 1993; Romero et al., 1994). HU-210 was found to be effective starting at the 20 μg/kg dose i.p., a dose 250 times greater than an equipotent oral dose of Δ^9-tetrahydrocannabinol (Rodríguez de Fonseca et al., 1991b; Fernández-Ruiz et al., 1992); 100 times greater than that described for i.p. Δ^9-tetrahydrocannabinol (Murphy et al., 1990a,b) and 50 times greater than that for i.v. Δ^9-tetrahydrocannabinol (Rettori et al., 1988). These findings correspond to the previously described efficacy of HU-210 to elicit cannabinoid-like behavioral alterations (Little et al., 1989; Rodríguez de Fonseca et al., 1994b, 1996, 1997) and correlate with its extreme affinity for CB_1 cannabinoid receptors (Howlett et al., 1990). The present studies extend to the endocrine system the consideration of HU-210 as the most potent synthetic cannabinoid available.

Plasma prolactin levels were affected in a biphasic fashion (Fig. 1), the lower dose of HU-210 (4 μg/kg) increasing and the higher ones (20 and 100 μg/kg) decreasing, the levels of this hormone. This particular biphasic profile also appeared in the time course experiments (Fig. 2), in which the short increase in plasma prolactin levels observed 30 min after acute administration of HU-210 was followed by a profound and long-lasting decrease in plasma prolactin. This biphasic profile had been observed 20 min after acute i.p. administration of Δ^9-tetrahydrocannabinol (Fernández-Ruiz et al., 1997), after intracerebroventricular infusion of Δ^9-tetrahydrocannabinol (Rettori et al., 1988) and after i.v. injection of Δ^9-tetrahydrocannabinol to female rats with rostral deafferentation of the medial basal hypothalamus (Tyrey, 1986). Interestingly, the inhibitory effects of HU-210 (20 μg/kg) on plasma prolactin levels were seen to be associated with a decrease in the medium-basal hypothalamic contents of dopamine, the proposed prolactin-inhibiting factor (Ben-Jonathan, 1985) and with an increase in the DOPAC/dopamine ratio, an index of dopaminergic activity (Table 1). These findings suggests that HU-210 administration increases dopamine release to the portal veins, resulting in tonic inhibition of prolactin release from the pituitary. The stimulatory effect observed after acute administration of the low doses of HU-210 (4 μg/kg) was not found to affect dopaminergic activity in the medium basal hypothalamus (data not shown). These results support the proposed hypothesis of a different origin for the stimulatory and the inhibitory actions of Δ^9-tetrahydrocannabinol on plasma prolactin levels (Tyrey, 1986; Fernández-Ruiz et al., 1997). HU-210 also suppressed the plasma prolactin surge that occurs in female rats during the afternoon of the proestrus phase (Fig. 3). This effect had been observed after acute administration of Δ^9-tetrahydrocannabinol (Ayalon et al., 1977; Murphy et al., 1990a) and was also associated with a decrease in hypothalamic contents of dopamine (Table 1). The fact that neither dopaminergic activity nor plasma prolactin levels were altered after the administration of HU-210 during the morning of the proestrus phase (11.00 a.m., Table 1) has also been described previously for Δ^9-tetrahydrocannabinol (Bonnin et al., 1993) and could be related to the influence of the sex-steroids on hypothalamic cannabinoid CB_1 receptors (Rodríguez de Fonseca et al., 1994a). Finally, plasma prolactin levels were altered during early postnatal development after acute HU-210 challenge only on postnatal day 40 (Fig. 4), which is in agreement with the ontogenic profile of hypothalamic dopaminergic neurons (Rodríguez de Fonseca et al., 1991a). The picture for plasma corticosterone was similar. Interestingly, the behavioral response to HU-210 appeared before the endocrine effects observed during postnatal development (Fig. 4), suggesting a differential ontogenic profile in motor behavior-related areas, such as the neostriatum, with respect to the hypothalamic nuclei involved in the regulation of the pituitary hormones. The different timing of the behavioral and endocrine responses could be related to the brain area-dependent ontogenic pattern of brain cannabinoid CB_1 receptors (Rodríguez de Fonseca et al., 1993)

Plasma growth hormone secretion was dose-dependently inhibited by HU-210 (Fig. 1), an effect also described for Δ^9-tetrahydrocannabinol (Rettori et al., 1988) and one that has been proposed to occur through the cannabinoid-induced portal release of somatostatin (Rettori et al., 1988). Plasma gonadotropins were also dose-dependently inhibited after acute HU-210 (Fig. 1) an effect also observed in female rats (Fig. 2), in which acute HU-210 administration blocked the afternoon surges of luteinizing hormone and follicle-stimulating hormone. In a previous study using HU-210 (Rodríguez de Fonseca et al., 1995) or Δ^9-tetrahydrocannabinol (Fernández-Ruiz et al., 1992) we found this effect to be associated to a decrease in hypothalamic contents of noradrenaline, a neurotransmitter controlling the pulsatility of gonadotropin-releasing hormone neurons (Steger et al., 1983). In the present study the results showed the same tendency (Table 1) although they did not reach statistical significance.

The administration of HU-210 resulted in marked activation of the pituitary–adrenal axis; this was dose-dependent (Fig. 1), rapid (Fig. 2) and was also observed in both females (Fig. 3) and developing rats (Fig. 4). Plasma adrenocorticotropic hormone levels were found to be elevated after both the 4 and 20 μg/kg doses of HU-210, but not after 100 μg/kg. However plasma corticosterone levels were dose dependently increased by HU-210 (Fig. 1). The apparent lack of effect of the higher dose of HU-210 on plasma adrenocorticotropic hormone might therefore reflect the onset of rapid negative feedback mechanisms as a result of the high levels of corticosterone. It has been proposed that the effects of cannabimimetics on pituitary–adrenal responses are centrally mediated (Kubena et al., 1971; Rodríguez de Fonseca et al., 1991b, 1996, 1997), probably through an increased release of corticotropin-releasing factor to the portal veins, as described for both Δ^9-tetrahydrocannabinol and anandamide (Weidenfeld et al., 1993) and also through the alteration of hypothalamic dopaminergic neurons (Kitchen et al., 1988; Rodríguez de Fonseca et al., 1995). The fact that acute HU-210 exposure activates not only extrapyramidal nuclei (Glass and Dragunov, 1995) but also stress-responsive nuclei in the brain (Rodríguez de Fonseca et al., 1997), together with the induction of anxiety-like responses in the rat (Rodríguez de Fonseca et al., 1996), which has also been reported after acute Δ^9-tetrahydrocannabinol exposure (Navarro et al., 1993), suggests that extrahypothalamic inputs from stress-responsive limbic nuclei might contribute to the pituitary–adrenal activation observed after acute exposure to this cannabinoid.

In conclusion, the results of the present study showed that the synthetic cannabinoid, HU-210, induces profound alterations in the endocrine system that can be compared to

those described for other cannabinoids. This suggests that HU-210 could be a useful tool for studying the role of cannabinoid receptors in neuroendocrine regulation.

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References

Ayalon, D., Nir, I., Cordova, T., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., Lindner, H.R., 1977. Acute effect of Δ^1-tetrahydrocannabinol on the hypothalamo–pituitary–ovarian axis in the rat. Neuroendocrinology 23, 31–42.

Ben-Jonathan, N., 1985. Dopamine: A prolactin inhibiting hormone. Endocr. Rev. 6, 564–589.

Bonnin, A., Fernández-Ruiz, J.J., Martín, M., Rodríguez de Fonseca, F.A., de Miguel, R., Ramos, J.A., 1992. Estrogenic modulation of Δ^9-tetrahydrocannabinol effects on the nigrostriatal dopaminergic activity in the female rat brain. Mol. Cell. Neurosci. 3, 325–325.

Bonnin, A., Ramos, J.A., Rodríguez de Fonseca, F.A., Cebeira, M., Fernández-Ruiz, J.J., 1993. Acute effects of Δ^9-tetrahydrocannabinol on tuberoinfundibular dopamine activity, anterior pituitary sensitivity to dopamine and prolactin release very as a function of the estrous cycle. Neuroendocrinology 58, 280–286.

Devane, W.A., Dysarz III, F.A., Johnson, M.R., Melvin, L.S., Howlett, A.C., 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34, 605–613.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, R.G., Griffin, G., Gibson, D., Mandelbaum, L.A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Dewey, W.L., 1986. Cannabinoid pharmacology. Pharmacol. Rev. 38, 151–178.

Eldridge, J.C., Landfield, P.W., 1990. Cannabinoid interactions with glucocorticoid receptors in rat hippocampus. Brain Res. 534, 135–141.

Esquifino, A.I., Moreno, M.L., Agrasal, C., Villanúa, M.A., 1995. Effects of cyclosporine on ovarian function in sham-operated and pituitary-grafted young female rats. Proc. Soc. Exp. Biol. Med. 208, 397–403.

Fernández-Ruiz, J.J., Navarro, M., Hernández, M.L., Vaticón, D., Ramos, J.A., 1992. Neuroendocrine effects of an acute dose of Δ^9-tetrahydrocannabinol: Changes in hypothalamic biogenic amines. Neuroendocrinol. Lett. 14, 349–355.

Fernández-Ruiz, J., Muñoz, R.M., Romero, J., Villanúa, M.A., Makriyannis, A., Ramos, J.A., 1997. Time-course of the effects of different cannabimimetics on prolactin and gonadotropin secretion: Evidence about the presence of CB_1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. Biochem. Pharmacol. 53, 1919–1927.

Gamallo, A., Villanúa, M.A., Trancho, G., Fraile, A., 1986. Stress adaptation and adrenal activity in isolated and crowded rats. Physiol. Behav. 36, 217–221.

Glass, M., Dragunov, M., 1995. Induction of the Korx 24 transcription factor in striosomes by a cannabinoid agonist. Neuroreport 6, 241–244.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA 87, 1932–1936.

Howlett, A.C., Champion, T.M., Wilken, G.H., Mechoulam, R., 1990. Stereochemical effects of 11-OH-Δ^{-8}-tetrahydrocannabinol-dimethylheptyl to inhibit adenylate cyclase and bind to the cannabinoid receptor. Neuropharmacology 29, 161–165.

Howlett, A.C., Champion-Dorow, T.M., McMahon, I.L., Westlake, T.M., 1991. The cannabinoid receptor: Biochemical and cellular properties in neuroblastoma cells. Pharmacol. Biochem. Behav. 40, 565–569.

Hughes, C.L., Everett, J.W., Tyrey, L., 1981. Delta-9-tetrahydrocannabinol suppression of prolactin secretion in the rat: Lack of direct pituitary effect. Endocrinology 109, 876–880.

Kitchen, I., Kelly, M., Turner, M., 1988. Dopamine modulation of corticosterone secretion in neonatal and adult rats. J. Pharm. Pharmacol. 40, 580–581.

Kramer, J., Ben-David, M., 1978. Prolactin suppression by (−)-delta-9-tetrahydrocannabinol (Δ^9-THC): Involvement of serotoninergic and dopaminergic pathways. Endocrinology 103, 452–457.

Kubena, R.K., Perhack, J.C., Barry, H., 1971. Corticosterone elevation mediated centrally by Δ^1-tetrahydrocannabinol in rats. Eur. J. Pharmacol. 14, 89–92.

Little, P.J., Compton, D.R., Mechoulam, R., Martin, B.R., 1989. Stereochemical effects of 11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl on mice and dogs. Pharmacol. Biochem. Behav. 32, 661–666.

Mailleux, P., Vanderhaeghen, J.J., 1992. Distribution of neuronal cannabinoid receptor in the adult rat brain: A comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience 48, 655–668.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Murphy, L.L., Steger, R.W., Bartke, A., 1990a. Psychoactive and non psychoactive cannabinoids and their effects on reproductive neuroendocrine parameters. In: Watson, R.R. (Ed.), Biochemistry and Physiology of Substance Abuse, vol. 2. CRC Press, Boca Raton, FL, pp. 73–93.

Murphy, L.L., Steger, R.W., Smith, M.S., Bartke, A., 1990b. Effects of Δ^9-tetrahydro-cannabinol, cannabidiol and cannabinol, alone and in combinations, on luteinizing hormone and prolactin release and on hypothalamic neurotransmitters in the male rat. Neuroendocrinology 52, 316–321.

Murphy, L.L., Rodríguez de Fonseca, F.A., Steger, R.W., 1991. Δ^9-Tetrahydrocannabinol antagonism of the anterior pituitary response to estradiol in immature female rats. Steroids 56, 97–102.

Navarro, M., Fernández-Ruiz, J.J., de Miguel, R., Hernández, M.L., Cebeira, M., Ramos, J.A., 1993. An acute dose of Δ^9-tetrahydrocannabinol affects behavioral and neurochemical indices of mesolimbic dopaminergic activity. Behav. Brain. Res. 5, 37–46.

Ovadia, H., Wohlman, A., Mechoulam, R., Weidenfeld, J., 1995. Characterization of the synthetic cannabinoid HU-210 in the rat. Relation to the adrenergic system and endogenous pyrogens. Neuropharmacology 34, 175–180.

Rettori, V., Wenger, T., Snyder, G., Dalterio, S., McCann, S.M., 1988. Hypothalamic action of delta-9-tetrahydrocannabinol to inhibit the release of prolactin and growth hormone in the rat. Neuroendocrinology 47, 498–503.

Rodríguez de Fonseca, F., Cebeira, M., Fernández-Ruiz, J.J., Navarro, M., Ramos, J.A., 1991a. Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons. Neuroscience 43, 713–723.

Rodríguez de Fonseca, F.A., Fernández-Ruiz, J.J., Eldridge, J.C., Steger, R.W., Bartke, A., Murphy, L.L., 1991b. Effects of the exposure to delta-9-tetrahydrocannabinol on the adrenal medullary function: Evi-

dence of an acute effect and development of tolerance in chronic treatments. Pharmacol. Biochem. Behav. 40, 593–598.

Rodríguez de Fonseca, F., Ramos, J.A., Bonnin, A., Fernández-Ruiz, J.J., 1993. Presence of cannabinoid binding sites in the brain from early postnatal ages. Neuroreport 4, 135–138.

Rodríguez de Fonseca, F.A., Cebeira, M., Ramos, J.A., Martín, M., Fernández-Ruiz, J.J., 1994a. Cannabinoid receptors in rat brain areas: Sexual differences, fluctuations during estrous cycle and changes after gonadectomy and sex steroid replacement. Life Sciences 54, 159–170.

Rodríguez de Fonseca, F., Martín-Calderón, J.L., Mechoulam, R., Navarro, M., 1994b. Repeated stimulation of D-1 dopamine receptors enhances (−)-11-hydroxy-Δ^8-tetrahydro-cannabinol-dimethylheptyl-induced catalepsy in male rats. Neuroreport 5, 761–765.

Rodríguez de Fonseca, F., Villanúa, M.A., Muñoz, R.M., San-Martin-Clarke, O., Navarro, M., 1995. Differential effects of chronic treatment with either dopamine D1 or D2 receptor agonists on the acute neuroendocrine actions of the highly potent synthetic cannabinoid HU-210 in male rats. Neuroendocrinology 61, 714–721.

Rodríguez de Fonseca, F.A., Rubio, P., Menzaghi, F., Merlo-Pich, E., Rivier, J., Koob, G.F., Navarro, M., 1996. Corticotropin-releasing factor (CRF) antagonist [D-Phe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist (−)-11-hydroxy-Δ9-tetrahydrocannabinol-dimethylheptyl on defensive-withdrawal behavior in rats. J. Pharmacol. Exp. Ther. 276, 56–64.

Rodríguez de Fonseca, F., Carrera, M.R.A., Navarro, M., Koob, G.F., Weiss, F., 1997. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 276, 2050–2054.

Romero, J., García, L., Ramos, J., Fernández-Ruiz, J.J., 1994. The putative cannabinoid receptor ligand, anandamide, stimulates hypothalamic tyrosine hydroxylase activity and inhibits prolactin release. Neuroendocrinol. Lett. 16, 159–164.

Steger, R.W., De Paolo, L.V., Asch, R.H., Silverman, A.Y., 1983. Interactions of delta-9-tetrahydrocannabinol with hypothalamic neurotransmitters controlling luteinizing hormone and prolactin release. Neuroendocrinology 37, 361–370.

Tyrey, L., 1986. Reversal of the delta-9-tetrahydrocannabinol inhibitory effect on prolactin secretion by rostral deafferentation of the medial basal hypothalamus. Neuroendocrinology 44, 204–210.

Thomas, B.F., Wei, X., Martin, B.R., 1992. Characterization and autoradiographic localization of the cannabinoid binding site in rat brain using [3H]-11-OH-Δ^8-tetrahydrocannabinol-DMH. J. Pharmacol. Exp. Ther. 263, 1383–1390.

Venance, L., Piomelli, D., Glowinski, J., Giaume, C., 1995. Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. Nature 376, 590–594.

Weidenfeld, J., Feldman, S., Mechoulam, R., 1993. Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo–pituitary–adrenal axis in the rat. Neuroendocrinology 59, 110–112.