Pharmacology Biochemistry and Behavior, Vol. 61, No. 3, pp. 229–238, 1998
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0091-3057/98 $19.00 + .00

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PII S0091-3057(98)00099-9

Maternal Exposure to Low Doses of Δ^9-Tetrahydrocannabinol Facilitates Morphine-Induced Place Conditioning in Adult Male Offspring

PILAR RUBIO,* FERNANDO RODRÍGUEZ DE FONSECA,* JOSÉ LUIS MARTÍN-CALDERÓN,* IGNACIO DEL ARCO,*† SANDRA BARTOLOMÉ,* MARÍA ANGELES VILLANÚA† AND MIGUEL NAVARRO*

*Instituto Universitario de Drogodependencias (Departamento de Psicobiología, Facultad de Psicología), and †Departamento de Fisiología, Facultad de Medicina, Universidad Complutense, 28223-Madrid, Spain

Received 21 September 1997; Revised 24 February 1998; Accepted 4 March 1998

RUBIO, P., F. RODRÍGUEZ DE FONSECA, J. L. MARTÍN-CALDERÓN, I. DEL ARCO, S. BARTOLOMÉ, M. A. VILLANÚA AND M. NAVARRO. *Maternal exposure to low doses of Δ^9-tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring.* PHARMACOL BIOCHEM BEHAV **61**(3) 229–238, 1998.—The possible existence of an increased susceptibility to the reinforcing properties of morphine was analyzed in male and female rats born from mothers exposed to Δ^9-tetrahydrocannabinol (THC, 1, 5, or 20 mg/kg) during gestation and lactation. Maternal exposure to low doses of THC (1 and 5 mg/kg), relevant for human consumption, resulted in an increased response to the reinforcing effects of a moderate dose of morphine (350 μg/kg), as measured in the place-preference conditioning paradigm (CPP) in the adult male offspring. These animals also displayed an enhanced exploratory behavior in the defensive withdrawal test. However, only females born from mothers exposed to THC 1 mg/kg exhibited a small increment in the place conditioning induced by morphine. The possible implication of the hypothalamo–pituitary–adrenal axis (HPA) was analyzed by monitoring plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone in basal and moderate-stress conditions (after the end of the CPP test). Female offspring perinatally exposed to THC (1 or 5 mg/kg) displayed high basal levels of corticosterone and a blunted adrenal response to the HPA-activating effects of the CPP test. However, male offspring born from mothers exposed to THC (1 or 5 mg/kg) displayed the opposite pattern: normal to low basal levels of corticosterone, and a sharp adrenal response to the CPP challenge. The present study reveals that maternal exposure to low doses of THC results in an increased sensitivity to the reinforcing effects of morphine in the adult male offspring, and in sexually dimorphic behavioral and endocrine alterations in the adaptative responses to stressors such as novelty or place-preference testing. These results support the growing evidence of the importance of monitoring the long-term consequences of maternal consumption of cannabis derivatives. © 1998 Elsevier Science Inc.

Rat Cannabinoids Perinatal exposure Morphine Reinforcement Conditioning ACTH Corticosterone

ANIMAL models have revealed the existence of long-term behavioral consequences of maternal exposure to drugs of abuse during gestation and lactation (30–32,34,50). However, understanding the real impact of maternal exposure to drugs of abuse on the development and adult expression of cognitive and behavioral functions in humans is far from being achieved. In this regard we are just beginning to understand the nature of the long-term effects of maternal exposure to cannabis sativa preparations (Hashish, Marijuana), which remain the most widely used illicit drugs during pregnancy in western countries (6,11,35). Cannabinoids can be transferred from the mother to the offspring through placental blood during gestation (16) and through maternal milk during lactation (19). The presence of psychoactive cannabinoids in the devel-

Requests for reprints should be addressed to Miguel Navarro, Departamento de Psicobiología, Facultad de Psicología, Universidad Complutense, 28223-Madrid, Spain.

oping brain might interfere as epigenetic factors with the rigidly ordered temporal sequences of events that occur during the ontogeny of the central nervous system, leading to the onset of neurodevelopmental alterations (27).

An important question on the behavioral teratology of drugs of abuse is the possible role as a vulnerability factor for drug-seeking behavior in the adult. Several studies have shown that maternal exposure to either morphine (12) or psychostimulant (20) resulted in a sensitization to the reinforcing effects of these compounds in the adult offspring. Because most drugs of abuse are potent activators of the hypothalamo–pituitary–adrenal axis (HPA), it has been proposed that maternal stress induced by these drugs might underlie the behavioral sensitization observed (26,28,43). As suggested recently (7,20), both prenatal stress and prenatal drug exposure seem to play an important role in the individual predisposition to psychostimulant self-administration in rodents, through the induction of long-term changes in the activity of mesocorticolimbic- projecting dopamine neurons, and HPA activity in the adult offspring (3). In this regard, there is little information on the possible long-term vulnerability-inducing effects of maternal exposure to marijuana or its psychoactive compounds. Natural cannabinoids, such as Δ^9-tetrahydrocannabinol, are potent activators of the HPA axis through their interaction with hypothalamic brain cannabinoid receptors (35,37,40). Perinatal exposure to cannabinoids might then result in increased maternal circulating levels of corticosterone and could interfere with the development of the HPA axis in the fetuses. The fact that brain cannabinoid receptors are present early in the development (39), adds another important biological substrate for the actions of maternally delivered cannabinoids on brain development.

The present study was designed to analyze the role of maternal exposure to THC as a vulnerability factor to opiate reinforcement in rats. We have selected morphine as the drug to be tested because endogenous opioid systems (peptides and their receptors) have also been found to be altered after maternal exposure to either stress (18,21) or perinatal cannabinoid treatments (22). Moreover, it has been recently proposed that the functional status of the HPA axis might be relevant for opiate-seeking behavior in the rat (45). Additionally, recent findings have revealed the existence of convergent mechanisms for opiates and cannabinoids in brain areas relevant to reward (35,45), and have linked cannabinoid receptor gene expression to IV heroin abuse (4). Experiments were conducted to assess the sensitivity to the reinforcing properties of a moderate dose of morphine (350 μg/kg, which induces place conditioning only in 50% of the control animals), in adult offspring born from mothers exposed during gestation and lactation to several doses of THC closely related to human consumption. The functional status of the HPA axis was monitored by measuring plasma levels of ACTH and corticosterone in basal and moderate stressing conditions (after the adaptative challenge of the place preference testing). Because it has been described that the increased sensitivity to the reinforcing effects of abused drugs is associated to changes in the pattern of locomotor and exploratory activity (33), we have also evaluated the behavioral response to novelty in the defensive withdrawal test.

METHOD

Animals

Morphine dose response. Fifty male Wistar rats (Panlab, Barcelona, Spain), weighing 300–400 g, were housed in groups of four per cage, in a room with controlled photoperiod (0800–2000 lights on), and temperature (23 ± 1°C). They had free access to standard food and water.

Perinatal studies. Female virgin rats of the Wistar strain (>8 weeks old; 200–250 g) were housed in a room with controlled photoperiod (lights on: 0800–2000) and temperature (23 ± 1°C). They had free access to standard food (Panlab, Barcelona) and water. Daily vaginal smears were taken between 1000–1200 h, and only those animals exhibiting three or more consistent 4-day estrous cycles were used in this study. Females in the proestrus phase were allowed to stay with a male for mating, and a new vaginal smear was taken on the next day. Those animals showing the presence of sperm cells were accepted as probably pregnant and used for Δ^9-tetrahydrocannabinol exposure studies. The day on which sperm plugs were found was designated the first day of gestation. After weaning, the animals were separated and housed four to five animals of the same sex and treatment per cage. One hundred seventy-two animals were used for the behavioral studies, distributed as follows: vehicle (50 animals), THC 1 mg/kg (46 animals), THC 5 mg/kg (40 animals), and THC 20 mg/kg (36 animals). For both place-preference and defensive-withdrawal studies, two to three male offspring and two to three female offspring were chosen randomly per litter at adult age (>70 days). Female rats were studied in the estrous phase of the cycle. All the procedures were carried out according to the European Communities Council directive of 24 November 1986 (86/609/EEC) regulating animal research.

Experimental Designs

In the first experiment, a full dose–response study was performed for evaluating the place conditioning properties of morphine, under the 3-day schedule of conditioning. Six doses of morphine were selected (0.062, 0.125, 0.25, 0.5, 1, and 2 mg/kg). In the second series of experiments, adult animals of both sexes (>70 days), born from mothers exposed to the different experimental treatments, were studied in the defensive withdrawal test under novelty conditions (41). One week after the testing procedure they were divided into two groups. Rats of the first group were left undisturbed for 2 additional weeks. They were killed after habituating the animals to the handling procedure, and plasma samples were obtained (basal group). The second group was used for studying the effects of perinatal exposure to THC on the reinforcing properties of a moderate dose of morphine (350 μg/kg), which was selected based on the results obtained in the dose–response experiment. These animals were killed at the end of the 45-min CPP test, and plasma samples were collected (place preference group).

Drugs and Treatments

Morphine treatment. Morphine hydrochloride was used for the place preference studies. It was supplied by Centro Nacional de Estupefacientes y Psicótropos, prepared daily using isotonic saline as vehicle, and injected IP at the doses described in the experimental designs section, in a volume of 0.3 ml. None of the morphine doses tested induced physical dependence, as evaluated using naloxone (1 mg/kg) after the 3-day conditioning sessions (39).

Perinatal THC exposure. Δ^9-Tetrahydrocannabinol of greater than 95% purity was provided in an ethanol solution by the National Institute on Drug Abuse (Project 4886-OB). Immediately before use, the ethanol was evaporated and the residue was emulsified in sesame oil as vehicle. Pregnant females received a daily single oral dose of THC (1, 5, or 20 mg/kg

b.wt., given between 1000 and 1200 h) or vehicle in a volume of 0.1 ml. The treatment started in the fifth day of gestation and was maintained until the day 24 after birth, the day on which pups were weaned. The doses of THC chosen were an extrapolation from current estimates of moderate to heavy exposure to this compound in humans, and were corrected considering the differences in route of administration and body surface area (42). We have previously observed that this dosage resulted in plasma THC levels within the range of those reported to cause behavioral and physiological effects in animal models (29,30,37). To assess the possible toxic effects of the treatment (2,17) several gestational and lactational parameters were controlled (31).

Behavioral Testing

Conditioning. Morphine-induced place preference (CPP) studies were performed as previously described (39) in a three-arm apparatus similar to that described by Hand et al. (14). The apparatus consisted of three interconnected rectangular boxes of 40 × 35 × 35 cm situated at 1200 angles from each other. In the middle there was a triangular area with a smooth glass floor, from which any of the three compartments were accessible. Each compartment was equipped with a set of different sensory stimuli: compartment A was equipped with a sand floor, plain walls, and a small container with a drop of 10% acetic acid. Compartment B contained a removable soft plastic floor, walls painted with white dot circles (7.5 cm), and a small container with a drop of anise extract. Finally, compartment C had a cork floor, alternating white strips (5 cm wide) painted on the walls, and no odor (a container with distilled water). The apparatus was placed in an isolated room dimly illuminated (110 lx). Each compartment was equipped with eight photocells that allowed us to monitor the position of the animal, and to automatically register the time spent in each compartment. After testing each animal, the floors were changed and washed to avoid odor cues.

Each CPP experiment consisted of a 5-day schedule, with three phases: preconditioning, conditioning, and testing. Animals exhibiting strong unconditioned aversions (<10% of the session) or preferences (>60% of the session) for any compartment during the 45-min preconditioning session were discarded for the conditioning procedures. Those two compartments to which the animals exhibited the most similar time of preference were randomly assigned for the conditioning procedure. This consisted of a 3-day schedule of double conditioning sessions. The first day involved a morning session (0900–1300 h) in which animals received a single dose of morphine (a full dose response (Experiment 1) or 350 μg/kg, in THC-exposed animals, IP) and were immediately placed in one of the compartments. During this 30-min conditioning sessions, the animals were not allowed to explore the other compartments of the apparatus. In the evening session (1600–1900) the animals received a single IP injection of saline, and were placed for 30 min. in the other compartment chosen for conditioning. On the second day of conditioning the rats received the saline injections in the morning session and the drug administration in the evening session. The third day of conditioning had the same schedule as the first one. We have chosen this schedule to avoid circadian variability (morning/evening), based on preliminary studies in our laboratory. After three days of conditioning, the animals were allowed again to freely explore the three compartments, exactly as in the preconditioning phase (testing session). The absolute time spent in each compartment was automatically registered and used for the evaluation of the CPP.

Defensive withdrawal. The defensive withdrawal test was conducted as previously described (41). The apparatus consisted of an opaque open field (100 × 100 × 40 cm), the floor of which was marked with 20 × 20 cm squares. The field contained a cylindrical polyethylene chamber measuring 17 cm deep and 10 cm in diameter. The chamber was opened at one end and situated alongside the wall running lengthside and 20 cm away from a corner of the open field. The open field was illuminated using a 500-W ceiling halogen light that was regulated to yield 350 lx at the center of the open field. Testing was conducted only under novelty conditions, without a previous habituation to the open field (41). The rats were placed inside the chamber, in the open field, and the following behaviors were scored by trained observers, who were blind to experimental conditions: the latency to leave the chamber (emergence latency), defined as placement of all four paws in the open field; the total time spent in the chamber; the mean time spent in the chamber (i.e., the total time spent in the chamber divided by the total number of entries); motor activity, defined as the total number of lines on the floor of the open field crossed outside the chamber (crossings); and the number of rears performed outside the chamber. The test length was 15 min. After testing each animal, the apparatus was cleaned with a weak acid solution (1% acetic acid), to prevent olfactory cues from affecting the behavior of subsequently tested rats.

Hormonal Determinations

Rats were killed by rapid decapitation using a guillotine. 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. Plasma corticosterone levels were measured by a radioimmunoassay system (RIA), using a specific rabbit policlonal antibody commercially available from Bio Clin (Cardiff, UK). 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 (41). The intraassay variability of the method was 15.3%, and the detection limit was 62 pg/ml. Plasma ACTH levels were measured using a commercial kit (CIS-Biointernational, Gif-Sur-Yvette, France), as previously described (40). The intraassay coefficient of variation was 5%, and the sensitivity was 10 pg/ml. All samples were measured in the same assay to avoid interassay variations.

Statistics

Two levels of analysis were performed in the present study. Data from individual animals were assessed by multifactorial analysis of variance, as required. Following a significant *F*-value, post hoc analysis (Newman–Keuls) were performed for assessing specific group comparisons. Litter analysis was performed following Holson and Pearce's (15) suggestions to asses the presence of litter effects. To this end, ANOVA analysis were performed using the mean litter value observed as the unit for analysis. All calculations were performed using the statistical package BMDP. Data were considered statistically significant if $p < 0.05$.

RESULTS

Morphine Dose–Response Analysis

Figure 1 displays the changes in preference to the morphine compartment as a function of conditioning dose. Mor-

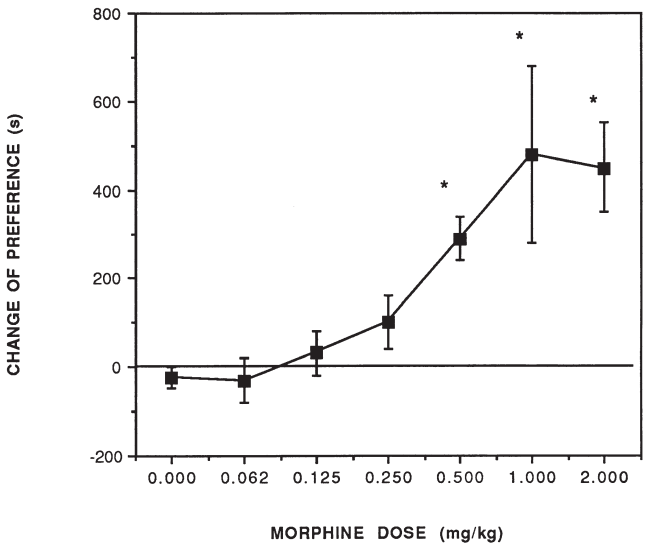

FIG. 1. Place conditioning produced by several doses of morphine given IP to male rats in a 3-day schedule of conditioning. Ordinates are means ± SEM of the difference between the time spent the day of testing and the time spent on preconditioning session. *$p < 0.05$ vs. vehicle-treated animals.

phine produced a dose-dependent marked place preference for stimuli associated with its administration, $F(6, 48) = 8.89$, $p < 0.05$. Post hoc analysis revealed that the effect was present starting at the 500 μg/kg dose ($p < 0.05$, Newman–Keuls). Based on this data, a morphine dose of 350 μg/kg was selected for the conditioning studies after perinatal THC. This dose induced conditioning in 50% of the control animals, as revealed in a preliminary experiment, previously published (43).

Effects of Perinatal Exposure to THC on Several Gestational and Lactational Parameters

To assess the possible toxic and nutritional effects of THC administration (2,17), we recorded several parameters throughout the gestation and lactation [Table 1; (32)]. Our results showed that treatment with 20 mg/kg THC reduced maternal food intake during the first (simple effect of dose, $F(3, 12 = 7.4$, $p < 0.05$, and second, $F(3, 12) = 8.7$, $p < 0.05$, days of treatment, disappearing thereafter. Moreover, overall ANOVA analysis did not result in differences in maternal food intake throughout the entire gestation, $F(3, 12) = 1.2$, $p = 0.35$, NS). Mothers exposed to 1 mg/kg THC drank more water during gestation, $F(3, 12) = 4.6$, $p < 0.05$, although this effect disappeared during the lactational period. Perinatal THC exposure did not result in differences either in maternal weight gain, or in the size and weight of the litters. Weight gain of the offspring, measured at postnatal days 10, 15, 20, 30, and 40 was equal in all the experimental groups (data not shown).

Effects of Maternal Exposure to THC on Morphine Place Preference in Adult Offspring

Maternal exposure to 1 and 5 mg/kg THC resulted in an enhanced sensitivity to the reinforcing properties of morphine 350 μg/kg displayed by the adult offspring, as measured in the place-preference paradigm, $F(1, 62) = 12.72$, $p < 0.05$, Fig. 2A and B). This effect appeared as a clear interaction between the absolute time spent in the morphine compartment and the maternal treatment group, the results of which male, $F(3, 62) = 3.07$, $p < 0.05$, and female offspring, $F(3, 33) = 3.21$, $p < 0.05$, spent more time in morphine-paired compartment than in a saline-paired one. This effect was significant in male offspring from the 1 mg/kg and 5 mg/kg THC groups and in female offspring from mothers exposed to 1 mg/kg THC. Neither control offspring nor those born from mothers exposed to 20 mg/kg

TABLE 1

PARAMETERS MEASURED DURING PERINATAL EXPOSURE TO Δ^9-THC. VALUES ARE MEANS ± SEM OF SELECTED GESTATIONAL AND LACTATIONAL VARIABLES

Parameters	Mother Treatment			
	Vehicle	TCH 1 (mg/kg)	THC 5 (mg/Kg)	THC 20 (mg/kg)
Mother food intake (g)†				
Gestation	24.1 ± 0.5	25.5 ± 0.6	23.9 ± 0.8	22.9 ± 1.4
Lactation	38.9 ± 4.7	38.5 ± 3.1	37.6 ± 3.9	39.6 ± 5.8
Mother water intake (ml)†				
Gestation	34.9 ± 1.5	42.4 ± 1.3*	40.2 ± 1.4	37.3 ± 1.5
Lactation	57.5 ± 6.3	58.6 ± 5.4	56.7 ± 5.5	57.3 ± 7.8
Mother weight gain (g)‡	107.7 ± 4.4	107.2 ± 11.9	92.7 ± 14.2	98.6 ± 6.9
Gestational length (days)	22.7 ± 0.2	23.2 ± 0.2	23.3 ± 0.3	23.3 ± 0.3
Litter size	12.5 ± 1.0	11.5 ± 1.8	10.0 ± 2.0	12.3 ± 0.8
Litter weight	97.0 ± 3.0	89.3 ± 12.3	89.3 ± 12.3	88.3 ± 5.0
Number of males	6.5 ± 0.6	7.8 ± 1.2	4.3 ± 2.3	6.7 ± 2.0
Number of females	5.8 ± 1.0	3.8 ± 0.4	5.7 ± 1.4	5.7 ± 1.2
Sex ratio (males/females)	1.3 ± 0.3	2.2 ± 0.3	1.1 ± 0.6	1.4 ± 0.6
Postnatal mortality	0.2 ± 0.2	0.5 ± 0.5	0 ± 0	0 ± 0

*$p < 0.06$, Student–Newman–Keul's
†Average value per day.
‡Difference between the weight in the day before delivery and the weight before mating.

THC exhibited a clear preference for the morphine-paired compartment when compared to the saline-paired one. The same effects appeared when the analysis was done using the change of preference (Fig. 4A and B), a more sensitive measure of place preference, as suggested by Hand et al. (14). Male offspring exposed to 1 and 5 mg/kg THC displayed an increased preference for morphine-paired compartment [interaction treatment × compartment, $F(3, 124) = 5.6, p < 0.05$, with a clear sexually dimorphic distribution of the effects on both saline and morphine compartments, $F(1, 124) = 10.2, p < 0.05$]. Post hoc analysis (Newman–Keuls) revealed that male offspring exposed perinatally to 1 and 5 mg/kg THC displayed a higher change of preference to the morphine-paired compartment when compared to female offsprings of the same treatment groups. To discard underlying alterations in spontaneous locomotor activity, an additional experiment was performed to evaluate locomotor activity in animals habituated to the CPP-maze compartments, but which were not previously conditioned with morphine or saline injections. Data reflected that under these conditions, spontaneous horizontal locomotor activity was similar in all the different groups. Data expressed as photocell beam breaks were: 1—(Oil): males 179 ± 7.7, females 164 ± 26.4; 2—(1 mg/kg THC): males 162 ± 18.4, females 194.5 ± 18.4; 3—(5 mg/kg THC): males 148 ± 14.6, females 216.3 ± 16.4; 4—(20 mg/kg THC): males 169 ± 14.3, females 200.1 ± 17.7.

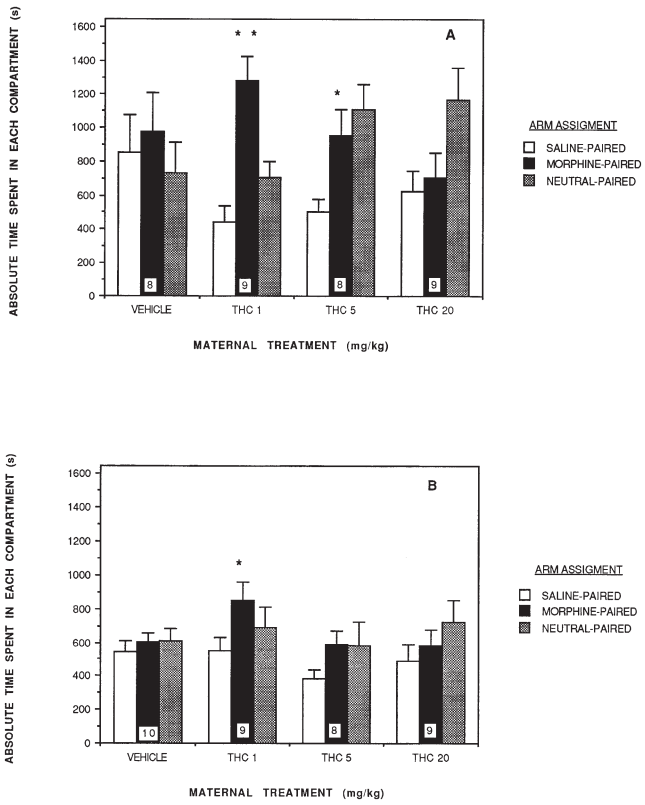

FIG. 2. Absolute time spent in the different compartments during the testing session of the morphine-induced place preference study by males (A) and females (B) animals born from mothers exposed to different doses of THC. Numbers in columns indicate the animals tested in each experimental group. $^{**}p < 0.005$, $^{*}p < 0.05$ morphine vs. the saline compartment.

Effects of Exposure to THC on the Performance of Adult Offspring in the Defensive Withdrawal Test Under Novelty Conditions (Fig. 3A and B)

The pattern of behavior displayed in the defensive-withdrawal test was clearly sexually dimorphic, as reflected in the several parameters scored: female animals emerge before males, $F(1, 81) = 23.9, p < 0.05$, they remained in the tube for less time, $F(1, 81) = 29.7, p < 0.05$, and they exhibited higher motor activity scores than males for crossings, $F(1, 81) = 69.2$, $p < 0.05$, and for rearings, $F(1, 81) = 39.7, p < 0.05$, data not shown. Maternal exposure to 1 or 5 mg/kg THC, but not 20 mg/kg THC, resulted in a decreased emergence latency in male offspring [sex × treatment interaction, $F(3, 81) = 3.01$, $p < 0.05$]. Male offspring of the 1 mg/kg THC group remained inside the tube for a less time (425 ± 76.9 s vs. 660.5 ± 67.4 s) and exhibited a lower mean time spent in the small chamber

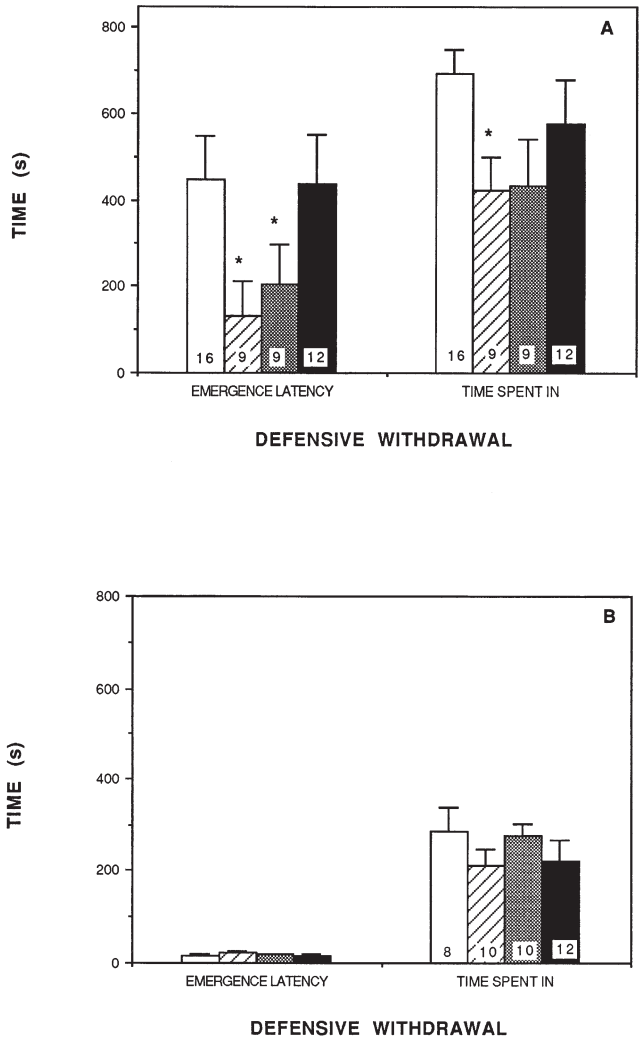

FIG. 3. Effects of maternal exposure to THC on the exploratory behavior displayed in the defensive withdrawal test under novelty conditions by males (A) and females (B) animals born from mothers exposed to different doses of THC. Numbers in columns indicate the animals tested in each experimental group. $^{*}p < 0.05$ vs. the control group.

when compared to control animals (412.7 ± 102.7 s vs. 162.5 ± 81.6 s). A significant sex × treatment interaction was observed also in both, the number of crossings, $F(3, 81) = 4.72$, $p < 0.05$, and rearings, $F(3, 81) = 4.2$, $p < 0.05$, scored during the test.

Effects of Maternal Exposure to THC on Plasma ACTH and Corticosterone After Morphine Place Preference

Maternal exposure to THC resulted in alterations in the pattern of ACTH secretion in both basal conditions and after the end of the CPP test (Fig. 5A and B). Plasma ACTH were sexually dimorphic, $F(1, 149) = 3.85$, $p < 0.05$, and rose clearly after the CPP test, $F(1, 149) = 52.05$, $p < 0.05$. There was a clear interaction between maternal treatment and condition [basal or after CPP test, $F(1, 149) = 8.9$, $p < 0.05$], in which the group exposed to 1 mg/kg THC did not exhibit the increase in ACTH as a result of the exposure to the CPP test. There was no interaction between the sex and treatment factors, $F(1, 149) = 0.06$, $p = 0.80$, NS). Basal corticosterone levels (Fig. 6A and B) were also sexually dimorphic, $F(1, 83) = 12.03$, $p < 0.05$, and they were clearly affected by maternal exposure to THC [sex × treatment interaction, $F(1, 83) = 7.66$, $p < 0.05$]. Maternal exposure to THC resulted in high basal corticosterone levels in female offspring, and reduced basal levels of this steroid in male offspring of the 1 mg/kg THC group. The exposure to the place-preference test increased the levels of this adrenal steroid [test effect, $F(1, 141) = 10.2$, $p < 0.05$]. This response was also sexually dimorphic, $F(1, 141) = 15.03$, $p < 0.05$, and exhibited a sex × treatment interaction, $F(1, 141) = 4.95$, $p < 0.05$. Thus, males exhibited a marked corticosterone response following the conditioning test, which was not observed in the female offspring exposed to 1 mg/kg THC. Regression analysis revealed that change of preference correlates positively with plasma corticosterone levels in males of the 1 mg/kg THC groups, $F(1, 8) = 6.75$, $p < 0.04$, $r = 0.49$. This positive correlation was not observed in the remaining experimental groups. Interestingly, the ratio [corticosterone levels (CPP groups)]/[Average basal corticosterone levels], displayed a marked sexual dimorphism, $F(1, 62) = 16.7$, $p < 0.05$, showing a clear effect of maternal treatment, $F(3, 62) = 6.8$, $p < 0.05$, and a marked sex × treatment interaction, $F(3, 62) = 12.85$, $p < 0.05$, which revealed again that male offspring from mothers exposed to 1 mg/kg THC displayed a higher increase in corticosterone levels as result of the exposure to the CPP test. (Average corticosterone change in % over basal levels was: 1—(Oil): males 151%, females 155%; 2—(1 mg/kg THC): males 405%, females 88%; 3—(5 mg/kg THC): males 140%, females 73%; 4—(20 mg/kg THC): males 140%, females 156%).

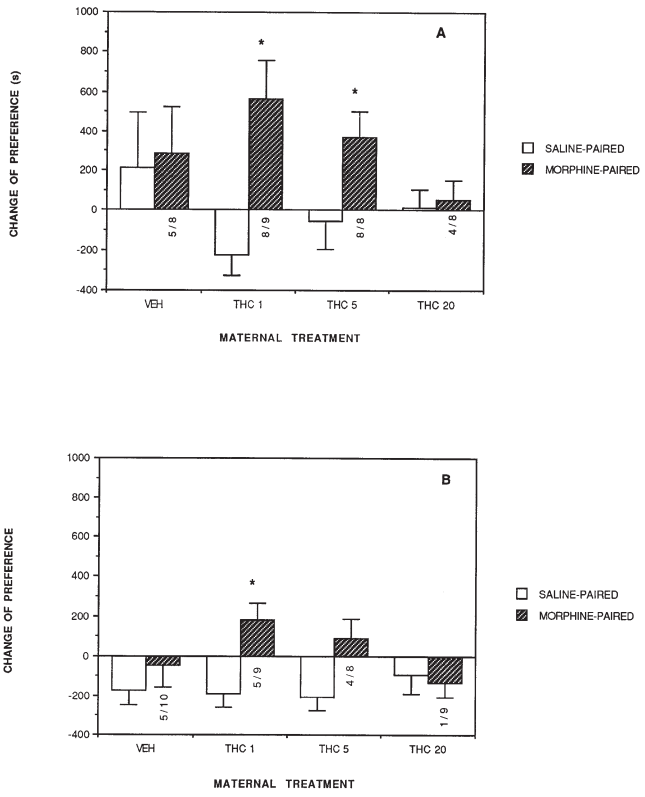

FIG. 4. Effects of maternal exposure to THC on the change of preference to either the saline-paired or the morphine-paired compartment after conditioning with morphine by males (A) and females (B) animals born from mothers exposed to different doses of THC. Numbers in columns indicate the animals tested in each experimental group. $^{**}p < 0.005$, $^{*}p < 0.05$ vs. the control group.

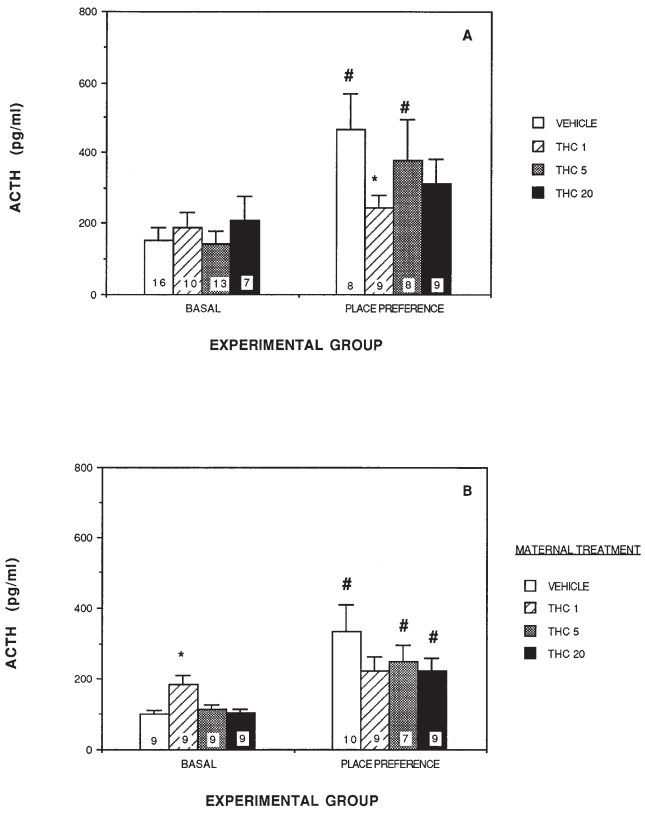

FIG. 5. Effects of maternal exposure to THC on plasma ACTH levels in males (A) and females (B) animals born from mothers exposed to different doses of THC. Two different groups were analyzed: undisturbed adult offspring (basal) and animals exposed to the place conditioning (place preference). Numbers in columns indicate the animals tested in each experimental group. $^{*}p < 0.05$ vs. the control group; $\#p < 0.05$, place preference vs. basal treatment.

Litter Analysis of the Effects of Perinatal Exposure to THC

Litter analysis showed that in male rats, perinatal exposure to 1 or 5 mg/kg THC resulted in a significant decrease in the

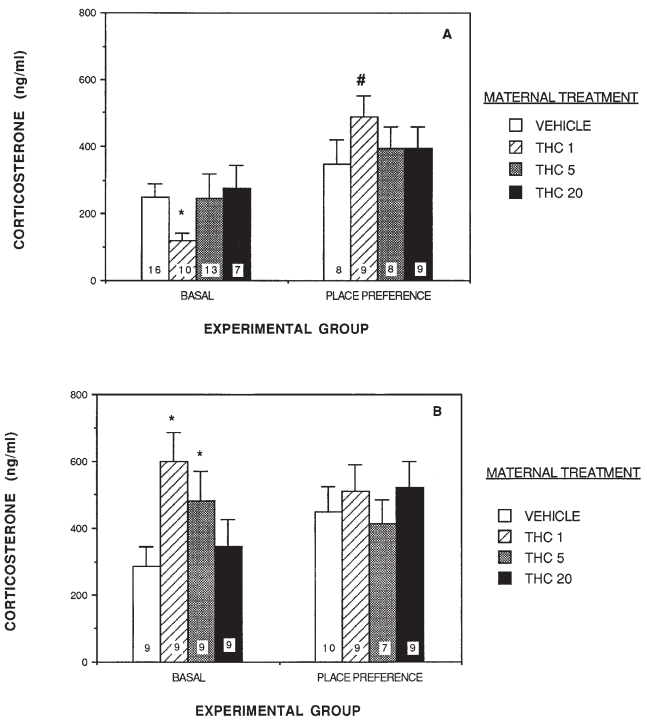

FIG. 6. Effects of maternal exposure to THC on plasma corticosterone levels in males (A) and females (B) animals born from mothers exposed to different doses of THC. Two different groups were analyzed: undisturbed adult offspring (basal) and animals exposed to the place conditioning (place preference). Numbers in columns indicate the animals tested in each experimental group. *$p < 0.05$ vs. the control group; #$p < 0.05$, place preference vs. the basal treatment.

emergence latency and in a tendency to exhibit a decrement in the total time spent in the compartment of the defensive withdrawal test, $F(3, 22) = 3.91$, $p < 0.05$, and $F(3, 22) = 2.52$, $p < 0.08$, respectively. There was a marked sexual dimorphism in the performance of this test, as described above, and perinatal THC exposure failed to induce alterations in female offspring. The effects of perinatal treatment with 1 mg/kg THC on morphine-induced change of place preference were present when the mean litter value was considered as the unit for analysis, $F(3, 19) = 3.8$, $p < 0.05$. These effects were again sexually dimorphic, $F(1, 9) = 8.7$, $p < 0.05$, and were significant in males exposed to 1 mg/kg THC. ACTH analysis revealed again the presence of increased plasma ACTH levels as result of the place-preference testing, $F(1, 44) = 35.7$, $p < 0.05$. There was a significant test × treatment interaction, $F(3, 44) = 5.4$, $p < 0.05$, revealing a disrupting effect of maternal exposure to 1 mg/kg THC in the pattern of ACTH levels displayed by both the basal and place-preference group. Litter analysis confirmed the sexually dimorphic effects of maternal THC exposure on basal corticosterone levels (sex × treatment interaction, $F(3, 22) = 4.3$, $p < 0.05$, and the different response to the CPP test, $F(3, 44) = 3.8$, $p < 0.05$, in males and females as result of the exposure to THC.

DISCUSSION

The present study reveals that maternal exposure to the natural cannabinoid THC results in an altered sensitivity to the reinforcing properties of a moderate dose of morphine in the adult offspring, as measured in the conditioned place-preference paradigm. This sensitivity was greater in animals exposed to low doses of this cannabinoid (1 and 5 mg/kg) and exhibited a sexual dimorphism. A similar finding has been recently reported using morphine self-administration (24). The place conditioning induced by morphine 350 —g/kg in male offspring perinatally exposed to 1 mg/kg THC was similar to that obtained in control animals using doses three to six times greater (see Figs. 1 and 4A). These effects were associated with sexually dimorphic changes in the pattern of exploratory behaviors under novelty conditions, as evaluated in the defensive withdrawal test. Because the spontaneous horizontal locomotor activity displayed by habituated animals was similar in all groups, we can exclude a motor impairment as an underlying factor for the differences found in the conditioning properties of morphine. Additionally, clear sexually dimorphic alterations of the activity of the hypothalamo–pituitary–adrenal axis were observed: female offspring perinatally exposed to THC (1 or 5 mg/kg) displayed high basal levels of corticosterone and a blunted adrenal response to the HPA-activating effects of the place-preference test. However, male offspring born from mothers exposed to THC (1 or 5 mg/kg) displayed the opposite pattern: normal to low basal levels of corticosterone, and an enhanced adrenal response to the CPP challenge. The results observed in males are consistent with previous findings, which reflect that early life experiences, like prenatal stress or perinatal exposure to morphine or psychostimulant, might be a vulnerability factor for drug abuse (7,12,20,23).

The pattern of behavioral and endocrine responses found in male offspring of mothers exposed to 1 mg/kg THC partially resembles that described in adult animals predisposed to develop psychostimulant self-administration: increased behavioral responses to novelty, and a sharp adrenal response to adaptative challenges (33). Male animals born from mothers exposed to 1 or 5 mg/kg THC exhibited an increase in both exploratory and locomotor activities in response to the exposure to novelty (defensive-withdrawal test). However, adult male animals exposed to low doses of THC displayed an opposite pattern, and increased their rate of exploration, indicating a lower anxiety state. In these animals, the response to the CPP test was shifted to exhibit both an increased sensitivity to the reinforcing properties of a ED_{50} dose of morphine (resulting in close to a 100% positive change of preference in perinatal-exposed animals), and a clear rise in plasma corticosterone levels, which were positively correlated. Although the ACTH levels are reduced in these animals, it is possible that this finding may reflect an alteration of hypothalamic mechanisms involved in corticosterone-mediated feedback regulation of ACTH secretion, as previously suggested (23). This differential regulation of the activity of the HPA axis has also been found to be a relevant factor in the vulnerability for developing amphetamine self-administration (23). At the present moment we do not know the time course of the HPA response in the animals perinatally exposed to cannabinoids. However, the enhanced behavioral response to novelty, the higher reactivity of the HPA axis, and the increased sensitivity to morphine are consistent with this previously proposed model of vulnerability to drugs of abuse in male rats (33).

Several mechanisms have been proposed in the elicitation of the behavioral effects of perinatal cannabinoid exposure [for review, see (9)]. They include changes in opioid peptides and their receptors (22), prenatal stress-like effects (43), direct effects on developing monoaminergic systems (1,32,36,48), or the activation of brain cannabinoid receptors that are present at birth (38). Besides the previously described actions of can-

nabinoids on the developmental profile of dopaminergic cells, a possible cannabinoid-induced developmental alteration in opioid peptides and their receptors must be considered as a factor underlying the increased sensitivity to morphine-induced place preference. It has been previously described that perinatal cannabinoid exposure is able 1) to alter the developmental expression of opioid peptides in the rat brain (22), and 2) to induce changes in opioid-related behaviors (47). For example, naloxone (5 mg/kg)-induced opioid-like abstinence syndrome in weanling males exposed to the cannabinoid, and developmental alterations in both pain sensitivity and the analgetic properties of morphine. The sexually dimorphic nature of the behavioral and endocrine alterations associated to early cannabinoid exposure is a common finding in perinatal cannabinoid studies (5,9,31,36), and in the present study might be reflecting the sex-dependent developmental profile of both, opioid receptors (13), and cannabinoid receptors (38) in rat brain.

An additional explanation for the effects described after perinatal THC might be a possible cannabinoid-induced stress-like effects, which might contribute to both the disruption of the behavioral responses to novelty and to a possible sensitization to the rewarding properties of morphine, as previously described for psychostimulant (7). This prenatal stress-like effect might occur through a THC-induced activation of the maternal HPA axis, resulting in a rise of plasma corticosterone levels in the fetuses, which are dependent on maternal levels (49). However, this possibility remains to be conclusively determined. A recent study (25) has described that maternal-restraint stress resulted in a permanent increase in basal corticosterone levels in the adult female offspring, which is exactly the same finding that we have observed after perinatal exposure to THC, and which has been found also after perinatal exposure to alcohol in rats (46). In any case, the effects of perinatal THC on morphine sensitivity and HPA activity might be independent under most circumstances except for male animals exposed to the lower dose of THC. This independent effect is particularly relevant if we consider the lack of correlation between a morphine-induced change of preference and HPA responses in female rats of the 1 mg/kg THC group. It remains to be determined whether the association between morphine reinforcement and HPA activity is sexually dimorphic in naive animals, particularly because the studies that have set a role for glucocorticoid on opiate reinforcement (44) have been performed only in males. Other gender-related findings arose from the present study: male rats born from mothers exposed to THC (1 or 5 mg/kg) displayed a greater exploratory behavior in the defensive-withdrawal paradigm, which might be considered as resembling a female pattern of behavior. However, they displayed a greater sensitivity to the reinforcing properties of morphine than did females from the same experimental groups. In addition, although females showed also an altered response to morphine, when compared to controls, they did not display an enhanced adrenal response to the CPP testing, but exhibited permanently elevated basal levels of corticosterone. These data suggest that perinatal THC resulted neither in a feminization nor in a masculinization of the behavioral and endocrine parameters studied. We might conclude from these findings that the proposed role of the HPA in the vulnerability to opiate reinforcement is sexually dimorphic, and that differential mechanisms might be underlying this response in both sexes.

The surprisingly efficacy of the low doses of THC for eliciting long-term behavioral alterations might be related to the well-documented biphasic actions of THC (8), which commonly induces opposing actions at low doses (0.2–2.0 mg/kg) when compared to higher ones (5–50 mg/kg). This has also been observed using anandamide, the proposed endogenous ligand for the brain cannabinoid receptor (10). In any case, we observed that offspring of female rats exposed to 20 mg/kg THC, a dose 10–20-fold times higher than that estimated for human consumption of two to three marijuana cigarettes per day (42), did not exhibit either the increased sensitivity to the reinforcing properties of morphine or the altered response to novelty. However, they did have alterations in locomotor activity (data not shown). Moreover, we have previously described that perinatal exposure to a hashish crude extract (at a dose containing 20 mg/kg THC) induced motor disturbances and altered the behavioral pattern in both the social interaction test and the sociosexual approach test (32). Regardless of the lack of effects observed in the CPP paradigm, it is important to remark that other mechanisms, such as alterations in the ontogeny of cannabinoid receptors, nutritional deficits, or other adaptative processes, might preclude the appearance of the effects observed with the lower doses.

In summary, perinatal exposure to THC doses related to human consumption produced a clear shift in the sensitivity to the reinforcing properties of morphine in male offspring, indicating that maternal exposure to cannabis derivatives might be considered as a possible vulnerability factor in drug abuse. These results support the growing evidence of the importance of monitoring the long-term behavioral consequences of maternal consumption of marijuana.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the expert assistance of Raquel Gómez, Elena Hernández, Yolanda Martín, and Cristina Meseguer, and they thank José Flórez de Uría for his technical support. They thank Frederique Menzaghi, Lisa H. Gold, and Charles Heyser for helpful comments on the manuscript, and Raúl M. Muñoz for his generous support in hormonal determinations. The authors are indebted to the National Institute on Drug Abuse for providing Δ^9-THC. This work has been supported by Comisión Interministerial de Ciencia y Tecnología (CICYT, Grant PM-96/0047), Universidad Complutense de Madrid (Proyecto Multidisciplinar PR218/94-5670), Comunidad de Madrid (proyecto CAM-AE00340/95), Fondo de Investigaciones Sanitarias de la Seguridad Social, (FISSS, Grant 94/0299) and Plan Nacional Sobre Drogas.

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