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Early Changes in the Development of Dopaminergic Neurotransmission After Maternal Exposure to Cannabinoids

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RODRÍGUEZ DE FONSECA, F., M. L. HERNÁNDEZ, R. DE MIGUEL, J. J. FERNÁNDEZ-RUIZ AND J. A. RAMOS. *Early changes in the development of dopaminergic neurotransmission after maternal exposure to cannabinoids.* PHARMACOL BIOCHEM BEHAV **41**(3) 469–474, 1992.—Perinatal exposure to cannabinoid derivatives has been shown to affect brain development. In this work, we studied the changes induced by maternal exposure to cannabinoids during gestation and lactation on the dopaminergic activity in the prosencephalic area of offspring of several days of development. This brain area contains an increasing population of dopaminergic terminals from the different dopaminergic pathways that become differentiated in the adult rat. We measured the endogenous content of dopamine and its intraneuronal metabolite, L-3,4-dihydroxyphenylacetic acid, and the activity of tyrosine hydroxylase as indices of dopaminergic activity. Results showed that perinatal exposure to cannabinoids caused several changes in the evolution of the dopaminergic indices studied. These changes were mainly observed in males. The only alteration in females occurred on the tenth day of development: An increase in dopamine content was observed with no changes in either the content of L-3,4-dihydroxyphenylacetic acid or tyrosine hydroxylase activity. In males, the content of both dopamine and L-3,4-dihydroxyphenylacetic acid were decreased on the day previous to birth in the animals exposed to cannabinoids. Although the reduction in its metabolite disappeared on the fifth day, the decrease in dopamine was maintained and it was correlated with a decrease in tyrosine hydroxylase activity. However, this decrease in the activity of tyrosine hydroxylase was followed by an increase on the tenth day. These results allow us to conclude that perinatal exposure to cannabinoids produces changes in the normal development of several indices of the activity of dopaminergic neurons in the brain area containing the most important population of dopaminergic endings. These changes were mainly observed in males. They could be responsible for a long-term alteration in the neurological processes in which these neurons are involved in the adult.

Dopamine DOPAC Tyrosine hydroxylase Cannabinoids Development

CANNABIS sativa preparations (hashish, marijuana) are among the most widely used psychoactive drugs in the western world (27). Their abuse produces multiple effects on a variety of physiological processes controlled by the brain, such as the neuroendocrine control of pituitary function and extrapyramidal motor behavior. Cannabinoid consumption has been reported to produce decreases in anterior pituitary hormone release (21,39), antidystonic effects (7), and enhancement of psychotic disorders (2). Many experimental works have related these neurological and psychiatric effects of cannabinoids to alterations in the activity of some neurotransmitter systems in the brain areas involved in the control of these processes (3). These modifications were reflected by changes in some biochemical indices of neurotransmitter activity [for review, see (12)]. The administration of marijuana or delta-9-tetrahydrocannabinol (THC), its main psychoactive constituent, to experimental animals was followed by: a) changes in the hypothalamic content of biogenic amines (43,44); b) stimulation of nigrostriatal dopaminergic neurotransmission (36,42); and c) increase in the content of homovanillic acid in the prefrontal cortex and the olfactory tubercle (5).

Cannabinoids can be transferred from the mother to the offspring through the placental blood during gestation (25) and through the maternal milk during lactation (23). This suggests the possibility of alterations in the developing pup caused by maternal consumption of cannabinoid derivatives during perinatal age. Both clinical and experimental studies have shown that perinatal exposure to marijuana or its purified constituents produced a variety of long-term abnormalities in brain, endocrine, immune, and hepatic functions [for

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review, see (9,32)]. Thus, prenatal exposure affected mainly the embryonic and fetal organogenesis, whereas postnatal exposure resulted in damage to the growing offspring (22,32). Many of these perinatal effects were produced on the brain. It has been proposed that one of the mechanisms of the neuroteratological disturbance induced by cannabinoid exposure could be the neurotransmitter disturbance (28) since neurotransmitters may have important "trophic" and "plastic" influences on the development of the brain (24). Thus, perinatal exposure to cannabinoids resulted in alterations in the normal development of brain neurotransmission (10,40,41,46). Although it is difficult to determine whether any of these changes are specifically related to long-term effects on behavioral parameters, some studies have shown alterations in adult male copulatory behavior (9), in learning ability (1,45) and in open-field activity (4) following perinatal exposure to cannabinoids.

In recent papers, we reported the existence of alterations in the activity of striatal and limbic dopaminergic neurons in late postnatal stages after perinatal exposure to hashish crude extract (HCE) (41), as well as the effects of withdrawal caused by weaning (40). The aim of the present study is to investigate the effects of maternal cannabis exposure on the development and maturation of brain dopaminergic neurotransmission on the days around the birth. To this end, pregnant rats were daily fed HCE from fifth day of gestation up to and including the lactation period. This period of treatment was chosen based on a previous work of Mirmiran and Swaab (28) showing that the last week of prenatal and the first 3 weeks of postnatal life in rats are the periods of most vulnerability of the neurotransmitters to the drug action, with the following results: 1) development of brain neurotransmitter target areas is affected and 2) activity of the neurotransmitter system itself and its receptor sensitivity become permanently hampered, leading to behavioral abnormalities. Studies were carried out in late prenatal (first day before birth: -1) and early postnatal days (fifth and tenth days after birth). These days were chosen on the basis of previous studies performed by other authors (17) concerning the development of dopaminergic neurotransmission. We measured dopamine (DA) and L-3,4-dihydroxyphenylacetic acid (DOPAC) contents and the ratio between both (DOPAC/DA) as an index of development of dopaminergic activity. Although it is not specific for dopaminergic neurons, the activity of tyrosine hydroxylase (TH) was also measured and partially used as an additional index of the development of this neurotransmission. Measurements were carried out in the prosencephalic area of offspring of both sexes. This brain area contains an increasing population of dopaminergic terminals of the different dopaminergic pathways that are differentiated in the adult rat (35).

METHODS

Animals

Female virgin rats of the Wistar strain were housed from birth in a room with controlled photoperiod (0800–2000 h light) and temperature (23 ± 1°C). They had free access to standard food (Panlab, Barcelona, Spain) and water. At adult age (>8 weeks of life; 150–200 g), daily vaginal smears were taken between 1000–1200 h, and only those animals exhibiting three or more consistent 4-day cycles were used in this study. Females in the proestrous 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 the cannabinoid exposure studies. The day on which sperm plugs were found was designated the first day of gestation.

Cannabinoid Treatment

Hashish was obtained from the Spanish Administration (Servicio de Restricción de Estupefacientes y Psicótropos, Dirección General de Farmacia y Productos Sanitarios, Ministerio de Sanidad y Consumo, Madrid, Spain). A crude extract was obtained by maceration with methanol and subsequently dried under a nitrogen flow. The extract contained 11.8% THC, 5.7% cannabinol, and 9.7% cannabidiol, measured by gass chromatography-mass spectrometry (19). This was prepared in a sesame oil solution for administration. Pregnant females received a daily dose of HCE (equivalent to 20 mg/kg THC daily) from the fifth day of gestation. This dose is an extrapolation from current estimates of moderate exposure to this compound in humans, correcting for differences in route of administration and body surface area (31). HCE was given orally with the help of a cannula. Control rats were fed vehicle alone. This treatment was maintained until the last day studied. During the whole period, we recorded the weight of the mothers and the food intake in both groups.

Sampling

Studies were performed at three different ages of development. In a first group, mothers treated or nontreated with HCE were decapitated during the morning of the twenty-first day of gestation (the day before birth). Fetuses were quickly removed and sacrificed and brains were obtained and immediately frozen at -70°C until assay. They correspond to day -1 of development taking the day of birth as zero. In other groups, offspring of 5 and 10 days of life, born from mothers treated with HCE or controls, were decapitated and similarly processed as described above. In all development days, rats of both sexes were obtained and analyzed separately. On the day of analysis, brains were thawed and the prosencephalic area dissected (18) and used for dopaminergic measurements. We also checked several parameters such as the duration of the pregnancy, the number of pups per litter, the ratio between males and females, and the postnatal mortality.

Dopamine and DOPAC Determinations

DA and DOPAC contents were analyzed using HPLC with electrochemical detection. Tissues were homogenized in 10–20 vol ice-cold 0.2 N perchloric acid with 0.5 mM sodium bisulfite and 0.45 mM EDTA. Dihydroxybenzylamine was added as an internal standard. The homogenates were then centrifuged and the supernatants injected into the HPLC system. Details of this system have been previously published (13). Values are expressed as pg/mg of tissue weight.

Tyrosine Hydroxylase Determination

Tissues were weighed and homogenized in 5 vol 0.25 M sucrose and processed according to the method described by Nagatsu et al. (30). The amounts of L-dopa formed were evaluated by HPLC according to our previously reported method (14). Values are expressed as ng/mg of tissue weight/h of incubation.

Statistics

Data were assessed by Student's *t*-test or analysis of variance (ANOVA) as required.

RESULTS

Cannabinoid Effects on Several Gestational Parameters

As can be seen in Table 1, maternal exposure to HCE increased the average duration of pregnancy. Mothers treated with HCE showed also a decrease in total weight gain compared to vehicle-fed animals. This decrease was not caused by a reduction in the food intake, but can be related to the decrease in the number of pups per litter observed in mothers treated with HCE. The females/males ratio was not affected by the maternal treatment with HCE, although there was a marked increase in the postnatal mortality in this group.

Cannabinoid Effects on Developing Dopaminergic Neurotransmission

Results showed that perinatal exposure to HCE caused several changes in the normal evolution of dopaminergic indices throughout the developmental period studied. These changes were mainly observed in males. However, the only alteration observed in females was on the tenth day of development and consists in an increase in the DA content (Fig. 1), without changes in the DOPAC/DA ratio (Table 2) and in TH activity (Fig. 2). In the case of males, the contents of both DA and DOPAC decreased on the day previous to birth in animals exposed to HCE (Figs. 1 and 3). The reduction in DOPAC content disappeared on the fifth day (Fig. 3), although the decrease in DA (Fig. 1) was maintained and was correlative to a decrease in TH activity on this day (Fig. 2). However, the decreased TH activity on this day was followed by an increase on the tenth day (Fig. 2).

DISCUSSION

The effects of HCE exposure on gestational parameters studied agree with previous reports (22,32,46). The length of gestation was slightly higher in mothers fed with HCE, correlative to a decrease in litter size and an increase in postnatal mortality in accordance with Walters and Carr (46). Moreover, the maternal weight gain was decreased, although this effect was not related to a decrease in the food intake, which was not significantly altered, but to a reduction in the litter size. Some discrepancies between these observations and previous reports could be attributed to the different cannabinoid preparation used in the treatment.

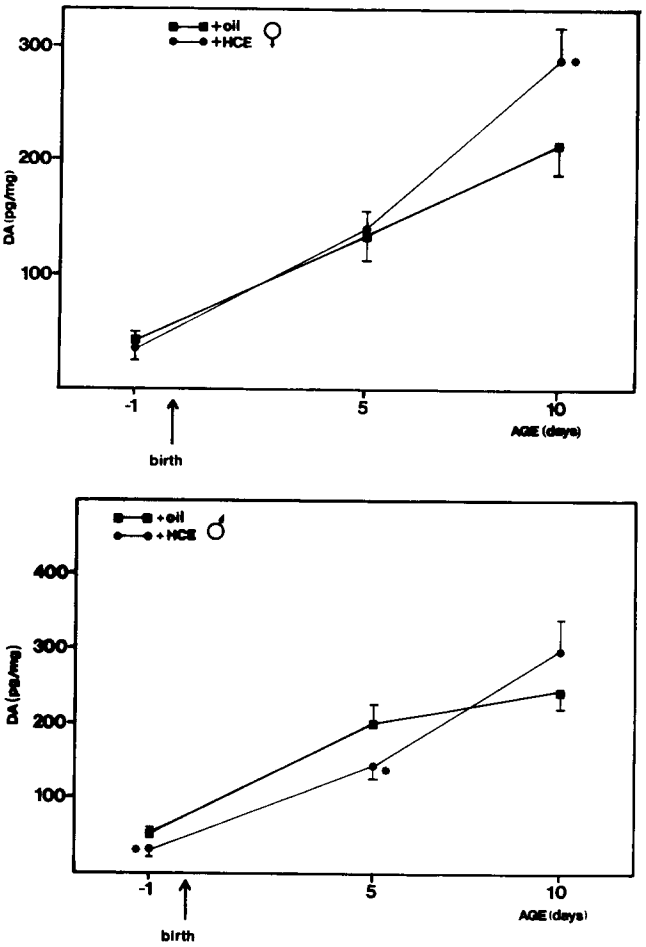

FIG. 1. DA content in the prosencephalic area of females (upper) and males (lower) of different days of development born from mothers perinatally exposed to HCE. Values are means ± SEM of six to eight determinations per group. Statistical differences were obtained by ANOVA (*$p < 0.05$ vs. the corresponding oil-treated group).

TABLE 1

GENERAL DATA ABOUT THE EFFECTS OF PERINATAL CONSUMPTION OF HCE ON GESTATIONAL PERIOD AND BIRTH

Parameters	+ Vehicle	+ HCE
Gestational length (days)	22 ± 0	22.6 ± 0.2*
Mother weight gain (g)†	117.5 ± 7.1	87.4 ± 5.6*
Mother weight gain/ litter size (g)	8.2 ± 0.4	9.4 ± 1.1
Mother food intake (g)	20.4 ± 0.6	20.5 ± 0.7
Litter size	14.4 ± 0.6	10.8 ± 1.3*
Females/males ratio	1.2 ± 0.3	1.0 ± 0.2
Postnatal morality (%)	10.9 ± 5.6	27.4 ± 7.3*

Values are means ± SEM of 6–10 determination per group. Statistical differences were obtained by Student's *t*-test.

*$p < 0.05$.

†This parameter was obtained as the difference between the weight in the day before delivery and the weight in the day of mating.

TABLE 2

DOPAC/DA RATIO IN THE PROSENCEPHALIC AREA OF FEMALES AND MALES OF DIFFERENT DAYS OF DEVELOPMENT BORN FROM MOTHERS PERINATALLY EXPOSED TO HCE

Days of Development		+ Oil	+ HCE
−1	Females	1.32 ± 0.23	1.40 ± 0.38
	Males	1.17 ± 0.28	1.10 ± 0.13
5	Females	0.60 ± 0.05	0.52 ± 0.07
	Males	0.46 ± 0.05	0.60 ± 0.08
10	Females	0.16 ± 0.03	0.14 ± 0.02
	Males	0.19 ± 0.03	0.14 ± 0.03

Values are means ± SEM of six to eight determinations per group. Statistical differences were obtained by ANOVA.

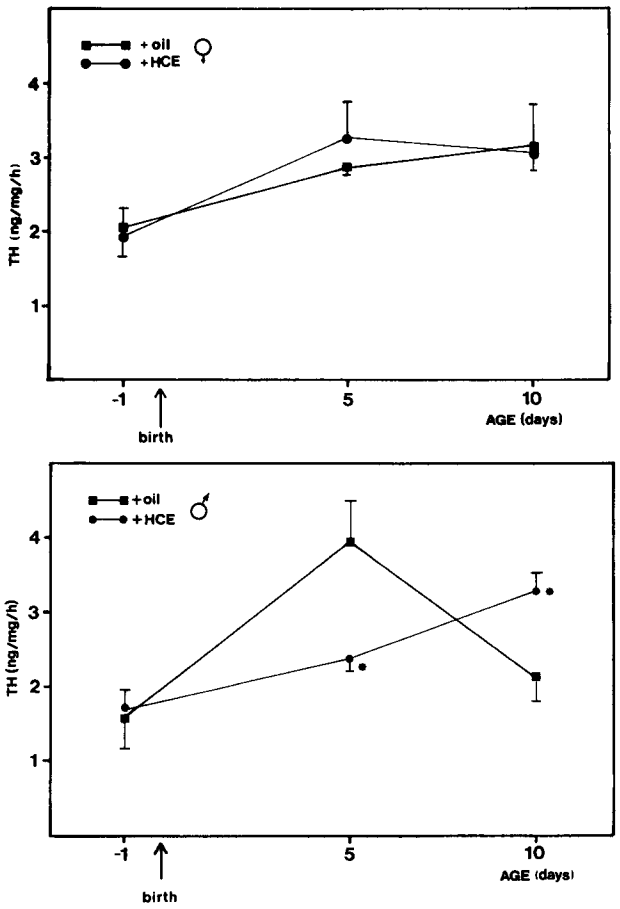

FIG. 2. TH activity in the prosencephalic area of females (upper) and males (lower) of different days of development born from mothers perinatally exposed to HCE. Values are means ± SEM of six to eight determinations per group. Statistical differences were obtained by ANOVA (*$p < 0.05$ vs. the corresponding oil-treated group).

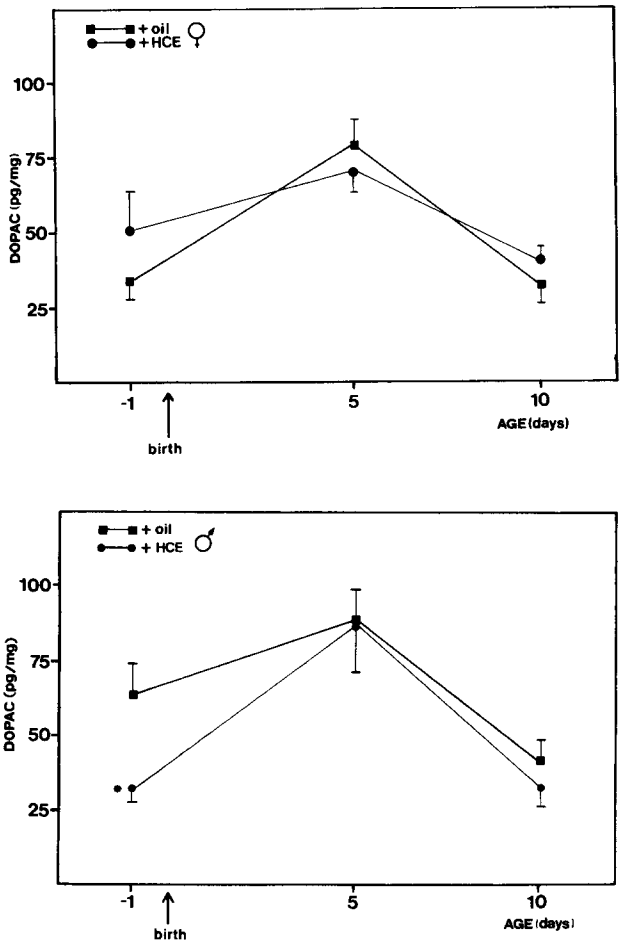

FIG. 3. DOPAC content in the prosencephalic area of females (upper) and males (lower) of different days of development born from mothers perinatally exposed to HCE. Values are means ± SEM of six to eight determinations per group. Statistical differences were obtained by ANOVA (*$p < 0.05$ vs. the corresponding oil-treated group).

Regarding the development of dopaminergic neurotransmission, it is important to note that the evolution of the dopaminergic indices studied in control animals agrees with previous reports on the development of dopaminergic neurotransmission (8,33). It has been shown that the normal evolution is not linear because there are phases of maximum growth (33). This phenomenon occurred in the case of the evolution of TH activity in males, which reached a maximum on the fifth day after birth. Similarly, DOPAC contents were high on the fifth day but decreased on the tenth day, whereas DA contents were continuously increasing throughout the period studied. Therefore, a constant reduction was originated in the DOPAC/DA ratio indicating a dopaminergic terminal maturation. In this respect, dopaminergic neurons differentiate on the fourteenth embryonic day and their projections reach the maturation in the first month after birth, with differences between areas (33). Since phenotypic expression of a neurotransmitter seems to be dependent on the presence of several factors in the microenvironment of the neurons (16), the presence of cannabinoids may lead to alterations in the development of dopaminergic indices during both prenatal and postnatal periods. Thus, exposure to cannabinoids affected the pattern of evolution of TH activity in males. It produced a progressive increase in this enzyme, which abolished the drop in its activity observed in controls. This caused maternal treatment with HCE to produce an initial transitory decrease in the TH activity in the prosencephalic area, corresponding to a low DA content on the fifth day of development, but followed by a rapid catch-up with an increase on the tenth day, accompanied by a recovery to normal contents in DA.

In a previous report, we found that perinatal exposure to cannabinoids alters several dopaminergic indices at late postnatal stages in a sex-dependent manner (40,41). Our present data also show a sex-dependent alteration of this neurotransmitter even before complete differentiation and maturation of dopaminergic projections into their target areas. The effects observed in males were more marked, whereas only slight changes were observed in females, which exhibited only an increase in the DA content on the tenth day after birth. Hence, the hormonal milieu seems to play an important role in the cannabinoid effects on the perinatal development of dopaminergic neurons, even more if one considers that the period of cannabinoid treatment included the critical developmental periods for sexual differentiation of the brain—late prenatal and

early postnatal days—and that THC and/or other marijuana ingredients could have estrogenic properties. Various observations can be argued at this respect.

First, several studies have shown the existence of sex differences in brain development following perinatal exposure to various psychoactive drugs, such as alcohol, nicotine, and cocaine [for review, see (15)]. The perinatal exposure to these drugs mainly affected the development of male offspring, abolishing several sexually dimorphic behaviors. These effects have been related to a drug-induced deficit in the fetal production of testosterone that leads to an incomplete masculinization or, even, originates a demasculinizing effect (15). Although a similar cannabinoid-induced decrease in fetal testosterone production has been shown after perinatal THC administration (9), the possibility that this decrease was the responsible of our observed sex differences in the perinatal dopaminergic development is unknown at the present.

Second, cannabinoids have been reported to have estrogenic (38), antiestrogenic (6), and antiandrogenic effects (37). Studies in progress also suggest the possibility of an interaction between estrogens and THC at the pituitary (29) and brain levels (Fernández-Ruiz et al., unpublished observations), similar to that observed at gonadal level (38).

Third, a possible explanation for the differences observed in the present study between males and females is that the neurological effects of cannabinoid derivatives were produced using a physiological mechanism that could have a heterogeneous distribution in brain and may be related to the gonadal status. In support of this possibility is the recent description of cannabinoid binding sites in the brain with different properties (11,34) whose structure and genomic expression have just been characterized (26).

On the other hand, although it has not been demonstrated whether these receptors are present or not in dopaminergic neurons, the recent observation of their high presence in the nuclei of the basal ganglia (20) suggests that they could be probably related to the dopaminergic nigrostriatal activity. However, the possibility that cannabinoid effects were mediated through modifications in other neurotransmitters cannot be discarded (9,46).

In summary, these results allow us to conclude that perinatal exposure to cannabinoids produces changes in the normal evolution of several parameters, indicating the activity of dopaminergic neurons in the brain area containing the most important population of dopaminergic endings. These effects involve changes on the DA synthesis and are mainly observed in males, which suggests that the hormonal milieu plays an important role in the cannabinoid effects. They could affect the neurological processes in which these neurons are involved in the adult. Further experimental studies of the molecular mechanisms involved in the effects of perinatal exposure to cannabinoids are presently underway.

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