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Short communication

Effect of maternal Δ^9-tetrahydrocannabinol on developing serotonergic system

Francisco Molina-Holgado [1], Alberto Amaro, M. Isabel González [2], Francisco J. Alvarez, Maria L. Leret *

Departamento Biologia Animal II, Facultad CC. Biologicas, Universidad Complutense, 28040 Madrid, Spain

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Abstract

In this study we investigated the effects of maternal Δ^9-tetrahydrocannabinol on the developing serotonergic system. A daily dose of Δ^9-tetrahydrocannabinol (5 mg/kg body weight) was administered p.o. to pregnant rats from gestational day 5 to postnatal day 1. Levels of indolamines were measured in four brain areas of the offspring on the day before or after birth. Levels of indolamines depended on the cerebral area, sex and pre- or postnatal age. Maternal exposure to Δ^9-tetrahydrocannabinol decreased diencephalic levels of 5-hydroxytryptamine (5-HT), males being more susceptible than females. These perinatal changes could be responsible for the long-term neurophysiological alterations produced by cannabinoids.

Keywords: Δ^9-Tetrahydrocannabinol; 5-HT (5-hydroxytryptamine, serotonin); 5-HTP (5-hydroxy-L-tryptophan); 5-HIAA (5-hydroxyindole-3-acetic acid); High-performance liquid chromatography with electrochemical detection (HPLC-ED); Perinatal

1. Introduction

Δ^9-Tetrahydrocannabinol, the main psychoactive component of marihuana and hashish, has well known effects on different neurochemical systems, affecting several neurophysiological processes (Dewey, 1986; Hollister, 1986; Kumar et al., 1990). Some of the dysfunctions produced by Δ^9-tetrahydrocannabinol have been connected to alterations in the serotonergic system (Bideaut-Russell et al., 1990), since administration of Δ^9-tetrahydrocannabinol has been reported to modify the brain levels of 5-hydroxytryptamine (5-HT) (Sofia et al., 1971; Dewey, 1986; Ouellet et al., 1973). It is well known that cannabinoids are capable of passing across the placenta (Vardaris et al., 1976), and they are also present in maternal milk (Jakubovic et al., 1973), and therefore, maternal administration of Δ^9-tetrahydrocannabinol might affect the differentiation of the brain during early stages of development. The neonatal changes could determine certain effects in adulthood that, in turn, could explain the long-term effects of cannabinoids (Dalterio, 1986).

In this study we aimed to investigate the effects of Δ^9-tetrahydrocannabinol administered throughout gestation on the development of the serotonergic system of the male and female offspring, as assessed in four immature cerebral areas, before and after birth.

2. Materials and methods

Eight pregnant female Wistar rats (250–300 g; Ifa-Credo S.L., Madrid, Spain) were individually housed and maintained on a dark/light cycle (lights off at 20:00 h) with food and water ad libitum. In the experimental group, each dam ($n = 4$) received a daily dose of Δ^9-tetrahydrocannabinol (5 mg/kg body weight) administered orally through a buccopharyngeal cannula, from gestational day 5 to the day before or after birth (days -1 and $+1$). The dose administered is known to be non-teratogenic and is equivalent to the doses used by humans (Rosenkrantz and Braude, 1976). Control pregnant rats ($n = 4$) received the same volume of vehicle. The Δ^9-tetrahydrocannabinol (Sigma,

* Corresponding author. Tel.: (34-1) 394-4990; Fax: (34-1) 394-4935; e-mail: lverdu@eucmax.sim.ucm.es

[1] Present address: Cajal Institute, CSIC, Avenida Dr. Arce 37, 28002 Madrid, Spain.

[2] Present address: Department of Obstetrics and Gynaecology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK.

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London, UK) was kept and prepared as described before (Molina-Holgado et al., 1993). The pups were killed by decapitation one day before or one day after birth, forming groups −1 and +1, respectively. The whole brains were quickly (< 45 s) removed and frozen at −80°C. The metencephalon, mesencephalon, diencephalon and telencephalon were dissected (Glowinski and Iversen, 1966). The samples were prepared as described before (Molina-Holgado et al., 1993) and supernatants were used for the determination of endogenous levels of 5-HT, of its precursor 5-hydroxy-L-tryptophan (5-HTP), of the metabolite 5-hydroxyindole-3-acetic acid (5-HIAA), and of the turnover ratio (5-HIAA : 5-HT), using high-performance liquid chromatography with electrochemical detection (HPLC-ED) (Molina-Holgado et al., 1993).

2.1. Statistical analysis

Data were analysed with three-way analysis of variance (ANOVA), factor 1 being Age (2 levels: day −1, day +1), factor 2 being Treatment (2 levels: control, Δ^9-tetrahydrocannabinol) and factor 3 being sex (2 levels: male, female). Whenever appropriate, specific cells in the design were compared by Planned Comparisons. The analysis was carried out separately for each area of the brain.

3. Results

3.1. Telencephalon

Three-way ANOVA showed a significant effect for the factor sex on the levels of 5-HTP ($F = 12.437$, $P = 0.0009$) and 5-HT ($F = 16.498$, $P = 0.0001$) (Table 1). Independent of treatment and age, Planned Comparisons demonstrated that females had higher levels of 5-HTP and 5-HT than males.

3.2. Mesencephalon

ANOVA resulted in a significant effect for the factor sex on the concentration of 5-HTP ($F = 7.775$, $P = 0.008$) and 5-HIAA ($F = 4.419$, $P = 0.040$), and in a significant effect for the main factor Age on the levels of 5-HIAA ($F = 8.81$, $P = 0.0045$) and on the turnover ratio ($F = 4.277$, $P = 0.0445$) (Table 1). Independent of treatment and age, females had higher levels of 5-HTP and 5-HIAA than males. With respect to the factor Age, 5-HIAA was greater ($P < 0.05$) on day +1 than on day −1, and this difference was significantly reflected in the turnover ratio.

3.3. Metencephalon

ANOVA revealed a significant effect for the factor Age on the levels of 5-HTP ($F = 5.596$, $P = 0.0221$), 5-HT ($F = 25.643$, $P = 0.00001$) and 5-HIAA ($F = 6.795$, $P = 0.0112$) (Table 2). The three indolamines were lower on day +1 than on day −1, independent of treatment and sex.

3.4. Diencephalon

Age had a significant effect on the levels of 5-HIAA ($F = 13.04$, $P = 0.0006$), which were higher on day +1 than on day −1 (Table 2). The factor sex had a significant effect on the content of 5-HTP ($F = 9.612$, $P = 0.0036$), of 5-HT ($F = 11.291$, $P = 0.0013$) and on the turnover ratio ($F = 4.845$, $P = 0.0328$). The results of Planned Comparisons revealed higher levels of 5-HTP and a higher turnover ratio in males than in females, while females

Table 1
Levels of indolamines in neonatal male and female telencephalon and mesencephalon, following gestational treatment with Δ^9-tetrahydrocannabinol (TCH) or vehicle and measured on the day before (−1) or after birth (+1)

	Day −1				Day +1			
	Control		TCH		Control		THC	
	Males	Females	Males	Females	Males	Females	Males	Females
Telencephalon								
5-HTP	2.08 ± 0.55 [c]	2.67 ± 0.45	2.05 ± 0.63 [c]	2.95 ± 0.62	2.02 ± 0.58	2.29 ± 0.66	2.15 ± 0.66	2.38 ± 0.40
5-HT	2.63 ± 0.47	3.28 ± 0.97	2.58 ± 0.53	3.07 ± 0.88	2.57 ± 0.62 [c]	3.31 ± 0.65	2.64 ± 0.54 [c]	3.58 ± 0.27
5-HIAA	6.16 ± 0.89	6.18 ± 0.86	5.70 ± 0.96	6.02 ± 0.89	5.62 ± 1.21	5.94 ± 0.87	5.77 ± 1.04	5.61 ± 0.81
5-HIAA : 5-HT	2.13 ± 0.29	1.67 ± 0.30	1.82 ± 0.27	1.81 ± 0.35	1.86 ± 0.63	1.76 ± 0.17	2.12 ± 0.38	1.60 ± 0.21
Mesencephalon								
5-HTP	2.12 ± 0.63	2.75 ± 0.50	1.99 ± 0.47	2.68 ± 0.85	2.01 ± 0.32 [c]	2.61 ± 0.30	2.26 ± 0.84	2.28 ± 0.63
5-HT	10.7 ± 1.30	11.2 ± 1.59	14.0 ± 1.91	13.2 ± 1.65	13.2 ± 2.08	15.2 ± 2.26	11.2 ± 2.09	12.9 ± 1.38
5-HIAA	11.6 ± 1.10	12.5 ± 2.25 [a]	11.7 ± 1.10	11.8 ± 2.35	14.4 ± 2.78	16.3 ± 2.46	13.3 ± 1.93	13.9 ± 1.54
5-HIAA : 5-HT	1.65 ± 0.19	1.11 ± 0.25	0.92 ± 0.06	0.89 ± 0.28	1.10 ± 0.15	1.18 ± 0.23	1.28 ± 0.45	1.07 ± 0.08

The values are the means ± S.D. ($n = 8$) in ng/mg protein.
Planned Comparisons ($P < 0.05$): [a] vs. same sex, same treatment, day +1; [b] vs. same age, same sex, Δ^9-tetrahydrocannabinol; [c] vs. same age, same treatment, females.

Table 2
Levels of indolamines in neonatal male and female metencephalon and diencephalon, following gestational treatment with Δ^9-tetrahydrocannabinol (TCH) or vehicle and measured on the day before (−1) or after birth (+1)

	Day −1				Day +1			
	Control		TCH		Control		THC	
	Males	Females	Males	Females	Males	Females	Males	Females
Metencephalon								
5-HTP	1.85 ± 0.70	1.86 ± 0.50	2.12 ± 0.28 [a]	2.17 ± 0.89	1.63 ± 0.22	1.58 ± 0.43	1.64 ± 0.39	1.96 ± 0.47
5-HT	4.03 ± 0.92	4.38 ± 0.41 [a]	4.75 ± 0.51 [a]	4.43 ± 0.73	3.17 ± 0.66	3.73 ± 0.77	3.37 ± 0.93	3.69 ± 0.48
5-HIAA	6.12 ± 0.68	5.75 ± 1.21	6.34 ± 1.29	5.03 ± 0.82	5.08 ± 1.92	5.03 ± 0.84	5.13 ± 1.10	5.27 ± 0.57
5-HIAA : 5-HT	1.39 ± 0.28	1.29 ± 0.35	1.38 ± 0.25	1.10 ± 0.14	1.53 ± 0.34	1.32 ± 0.23	1.52 ± 0.46	1.40 ± 0.36
Diencephalon								
5-HTP	3.55 ± 0.33	2.97 ± 0.22	3.47 ± 0.24	2.70 ± 0.26	3.20 ± 0.25	2.88 ± 0.31	3.01 ± 0.63	2.13 ± 0.38
5-HT	5.87 ± 0.3 [b]	6.25 ± 0.35	4.7 ± 0.42 [c]	6.07 ± 0.34	6.24 ± 0.35 [b]	6.96 ± 0.24	5.2 ± 0.19 [c]	6.37 ± 0.46
5-HIAA	5.87 ± 0.30	6.16 ± 0.19 [a]	6.62 ± 0.23	6.31 ± 0.18	6.76 ± 0.35	7.38 ± 0.48	7.31 ± 0.40	7.20 ± 0.44
5-HIAA : 5-HT	1.12 ± 0.07	1.07 ± 0.12	1.24 ± 0.10	1.05 ± 0.06	1.16 ± 0.13	1.03 ± 0.08	1.41 ± 0.08	1.17 ± 0.09

The values are the means ± S.D. ($n = 8$) in ng/mg protein.
Planned Comparisons ($P < 0.05$): [a] vs. same sex, same treatment, day +1; [b] vs. same age, same sex, Δ^9-tetrahydrocannabinol; [c] vs. same age, same treatment, females.

showed higher levels of 5-HT. ANOVA showed a significant effect for the factor Treatment on the content of 5-HT ($F = 9.959$, $P = 0.0024$). Independent of age or sex, Δ^9-tetrahydrocannabinol decreased diencephalic levels of 5-HT, compared to the control groups. However, a closer inspection of the data indicated that females contributed very little to this effect, which was mainly due to males. In the same manner, the significant effect of sex was mainly due to the Δ^9-tetrahydrocannabinol-treated groups, the control groups contributing very little to this effect.

4. Discussion

The results obtained in the present investigation show differences in the levels of indolamines in the rat brain, depending on age (pre- or postnatal), sex, and cerebral region studied. Administration of Δ^9-tetrahydrocannabinol during gestation altered diencephalic 5-HT levels, there being an interesting relationship between the effects of Δ^9-tetrahydrocannabinol and the sex of the pup.

It is possible to detect monoaminergic neurons from the 13th day of gestational life (Parés-Herbuté et al., 1989), and from early stages they may exert a trophic action and are capable of modulating ontogenic processes (Lauder, 1983). From gestational day 16 some pathways are completely developed, but from gestational day 19 to 21 postnatal day the whole organization of the terminal fields in this system is finished (Lidov and Moliver, 1981). In this investigation we found an interesting effect of age on the serotonergic system. There were differences in the levels of indolamines depending on the pre- or postnatal state of the brain. These results point out the importance of the day of birth in the organization of the serotonergic system. Critical neuroendocrine changes take place on the day of birth, including a surge in testosterone, and these factors could contribute to the differences observed between days −1 and +1. At the same time, there is a caudal to rostral maturational pattern documented in other ontogenic studies in postnatal rat brain (Hedner and Lundberg, 1980), making it possible that different cerebral structures are in different states of maturation, thus making the effects of the treatment potentially different.

One of the most evident effects shown in this study was the effect of sex, females showing higher levels of indolamines than males. The role of serotonin in the sexual differentiation of the brain is a well accepted fact (Wilson et al., 1992) and it is known that there is a transient reduction in hypothalamic levels of serotonin in females during the second week of life (Ladosky and Gaziri, 1972).

Perinatal exposure to cannabinoids alters the normal development of serotonergic neurons and these changes may be sex-related. The last week of prenatal life and the first three weeks of postnatal life in rat are the period of greatest vulnerability of neurotransmitters to drug action.

The possible involvement of sex steroids in the mediation of the developmental effects of Δ^9-tetrahydrocannabinol was also considered in this study. The fact that the effects observed in males were more marked that those in females could indicate that hormonal levels play an important role in the cannabinoid's effects. Cannabinoids might be able to alter brain development, probably by mimicking or modifying the action of endogenous steroid hormones, which seem to play a very important role in neuronal fate during early stages of brain development (McEwen, 1987). It has been demonstrated that drugs of abuse can modify the distribution of steroids during brain ontogeny (Dalterio, 1986). Therefore it is possible that the effects of perinatal exposure to Δ^9-tetrahydrocannabinol on the development of serotonergic neurons might be mediated by alterations in steroid hormones. The possible contribution of sex steroids to the neurodevelopmental alterations induced by

perinatal and adult Δ^9-tetrahydrocannabinol exposure has been previously addressed (Murphy et al., 1991; Wenger et al., 1992). Moreover, Δ^9-tetrahydrocannabinol has been reported to have both estrogenic and antiestrogenic effects and also antiandrogenic effects (Wenger et al., 1992). A possible explanation for the differences between males and females could be that the neurological effects of cannabinoids are mediated by physiological mechanisms.

It is known that the central 5-HT system has a higher potential in female than in male rats, and that this sex difference is not restricted to a specific region but seems to exist throughout in the brain (Carlsson and Carlsson, 1988).

In summary, the present results show that the developing serotonergic system in the rat brain is affected by the sex and the pre- or postnatal state of the animal, with effects depending on the cerebral area investigated. Maternal exposure to Δ^9-tetrahydrocannabinol affects the organization of the diencephalic serotonergic system, and this effect seems to be sexually dimorphic, males being more susceptible than females. These early changes could lead to permanent neurophysiological and behavioral changes in which sexual dimorphism could be present.

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