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Changes in Brain Catecholamine Mechanisms Following Perinatal Exposure to Marihuana

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WALTERS, D. E. AND L. A. CARR. *Changes in brain catecholamine mechanisms following perinatal exposure to marihuana.* PHARMACOL BIOCHEM BEHAV 25(4) 763–768, 1986.—Adult female rats received daily oral doses of a crude marihuana extract (CME; equivalent to 20 mg/kg Δ^9-THC) throughout gestation and lactation. The offspring were sacrificed at 10, 20, 40 or 60 days postpartum and tissue samples of cerebral cortex and striatum were dissected and assayed for α_1-adrenergic and D_2-dopaminergic receptors, respectively, and tyrosine hydroxylase activity. The body weight at birth and 10 days of age was reduced as was brain weight at 10 and 60 days of age in offspring exposed to CME. Perinatal exposure to CME reduced the binding capacity (B_{max}) of D_2 receptors in the striatum of 10 and 20-day-old offspring. The B_{max} for α_1 receptors in the cerebral cortex was not altered at any age. Tyrosine hydroxylase activity was significantly decreased in the striatum of 20 and 40-day-old offspring exposed to CME. The results indicate that chronic perinatal exposure to CME can selectively alter the development of specific catecholamine mechanisms in rat brain.

Marihuana Tyrosine hydroxylase Dopamine receptors Adrenergic receptors Striatum Cerebral cortex Perinatal exposure

SEVERAL studies have indicated that Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of marihuana, can be transferred from adult females to the central nervous system of developing animals via placental transport and the maternal milk supply. For example, administration of ^{3}H-Δ^9-THC to pregnant dogs just prior to parturition led to the distribution of labeled compounds to fetal brain tissues [29] and injection of ^{14}C-Δ^9-THC to lactating rats was followed by the appearance of labeled compounds in the brain of suckling neonates [20]. Prenatal exposure to marihuana or its constituents has also been shown to alter behavioral development and learning ability [9, 15, 16, 39].

Acute administration of Δ^9-THC to adult animals caused a decrease in steady-state levels of brain norepinephrine and dopamine which correlated with behavioral changes [5,6]. Pretreatment of adult mice with Δ^9-THC led to increased uptake of norepinephrine and dopamine into brain synaptosomes [17]. Synthesis and turnover of brain norepinephrine and dopamine were also increased following acute treatment with Δ^9-THC [7,28]. Chronic administration of Δ^9-THC to adult mice has also been shown to alter the binding characteristics of β-adrenergic receptors in the cerebral cortex [18]. Prenatal administration of other psychoactive drugs such as haloperidol [35] and ethanol [27] has been shown to affect ^{3}H-spiperone binding in the striatum of rat offspring. These results suggest that the behavioral effects associated with perinatal exposure to Δ^9-THC may be due to alterations in the binding characteristics of brain catecholamine receptors or to changes in catecholamine synthesis or turnover.

The purpose of this study was to determine if the development of pre- and postsynaptic noradrenergic and dopaminergic neuronal mechanisms in the cerebral cortex and striatum, respectively, of neonatal rats is altered following chronic perinatal exposure to a crude marihuana extract (CME).

METHOD

Animals

Mature male and female Sprague-Dawley rats weighing 180–200 g (Laboratory Supply Co., Indianapolis, IN) were segregated by sex and housed, 3–5 to a cage, in a temperature-controlled room (25±2°C) with lights on from 0500 hr to 1900 hr. They were supplied with food and water ad lib.

Drugs

CME containing 21.31% Δ^9-THC, 0.38% Δ^8-THC and 1.90% cannabinol was obtained from the National Institute

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on Drug Abuse and stored in the dark at −20°C. A stock solution of CME (37.6 mg/ml) was prepared in sesame oil vehicle for administration.

Procedure

The female rats received daily oral administration of CME (equivalent to 20 mg/kg Δ^9-THC daily) or sesame oil. This dose is an extrapolation from current estimates of moderate exposure to Δ^9-THC in humans, correcting for differences in route of administration and body surface area [33]. After two weeks of treatment, a mature male rat was placed in each cage of females for mating. Once pregnancy had been confirmed, each female was housed individually. Treatment was continued daily throughout gestation and lactation. Of the 15 pregnant rats treated with CME, 10 had litters consisting of at least 8 live pups. Nine of these litters were culled to eight pups for subsequent study. Twelve of the 13 pregnant control rats had at least 8 live pups. Eleven of these litters were culled to 8 pups. On Day 20 postpartum, the offspring were weaned from their mothers and on Day 35, male and female littermates were segregated. At 10, 20, 40 or 60 days of age, the offspring were sacrificed by decapitation. Tissue samples of cerebral cortex and striatum from either sex were dissected from the brains and assayed for α_1-adrenergic and D_2-dopaminergic receptors, respectively. Tissue homogenates were prepared as described below and the assays performed on the day of sacrifice. In addition, samples of cerebral cortex and striatum were sonicated in 100 or 500 μl, respectively, of Tris-Triton buffer and stored at −20°C until assayed for tyrosine hydroxylase activity.

Chemical Assays

Tyrosine hydroxylase activity was determined in 50 μl of supernatant fluid following centrifugation of the tissue homogenate at 10,000 × g for 10 minutes by a method previously described [38] with the following modifications. The samples were incubated under saturating conditions with 0.2 M tyrosine and 1 mM DL-6-methyl 5, 6, 7, 8 tetrahydropterine (Sigma Chemical Co.) for 45 minutes. Protein content of the tissue sonicate was determined in a 10 μl aliquot [26].

Binding characteristics of α_1-adrenergic receptors in the cerebral cortex were determined as previously described [30] with the following modifications. Tissue samples from two animals were pooled (300 mg) and homogenized in 20 volumes (w/v) of cold homogenization buffer (0.25 M sucrose, 5 mM Tris HCl, 1 mM $MgCl_2$, 0.05% ascorbic acid, pH 7.4) and centrifuged at 2,000 × g for 2 min at 4°C. The supernatants were centrifuged at 49,000 × g, washed in fresh buffer and recentrifuged. The final pellets were resuspended in 20 volumes of cold incubation buffer (50 mM Tris HCl, 10 mM $MgCl_2$, 0.05% ascorbic acid, pH 7.4). Membrane suspensions were incubated in triplicate at 25°C for 60 min with varying concentrations of ^{3}H-prazosin (0.5–10 nM, New England Nuclear, 17.4 Ci/mmole) in the absence and presence of 10 μM phentolamine to determine total and nonspecific binding, respectively. The total volume of the incubation mixture was 450 μl. Incubations were terminated by addition of 2 ml cold incubation buffer and immediate filtration through Whatman GF/C glass fiber filters. The filters were subsequently washed with three 5 ml aliquots of cold incubation buffer. The radioactivity retained on the filters was determined by liquid scintillation spectrometry.

The binding characteristics of D_2-dopaminergic receptors in striatal tissue were determined by a modification of a method previously described [12]. Tissue samples from two animals were pooled (100 mg) and homogenized in 100 volumes (w/v) of cold 50 mM Tris buffer, pH 7.7 at 25°C. Following a preliminary centrifugation at 3,000 × g for 2 min at 4°C, the supernatants were centrifuged at 49,000 × g for 10 min, washed in fresh buffer and recentrifuged. The final pellets were resuspended in 100 volumes of cold buffer containing 50 mM Tris, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% ascorbic acid, pH 7.1 at 37°C. Membrane suspensions were incubated at 37°C for 10 min with varying concentrations of ^{3}H-spiperone (0.1–1.6 nM, New England Nuclear, 21.0 Ci/mmol). Nonspecific binding was determined in the presence of 0.5 μM (+)-butaclamol HCl (Research Biochemicals Inc.). The total volume of the incubation mixture was 500 μl. Incubations were terminated, filtered, washed and radioactivity counted as described above.

Specific binding was defined as the difference between total and nonspecific binding. The data were analyzed by the method of Scatchard [36] to determine the maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_d) of each ligand for its receptor.

Statistical Analysis

The data pertaining to maternal weight, litter size and pup weight and mortality at birth were analyzed by Student's t-test. All other data were subjected to hierarchial multivariate analysis of variance to partition treatment and litter effects [32]. Significance implies $p<0.05$.

RESULTS

Effects of CME on Pregnancy and Offspring

Mother rats treated with CME showed a smaller weight gain compared to controls during the first and third weeks of pregnancy (Table 1). There was no significant difference in the average litter size or the average number of stillborn pups between control groups and those exposed to CME. However, there were significantly fewer live pups per litter at birth in groups exposed to CME. The average birth weight and body weight at 10 days of age was also significantly reduced in offspring exposed to CME. There were no significant differences in body weight at subsequent ages for either sex. The brain weight of pups of either sex exposed to CME was significantly decreased at 10 and 60 days of age (Table 2).

Effects of CME on Development of Catecholamine Receptors

There was an age-related increase in B_{max} for D_2 receptors in the striatum of control offspring between 10 and 40 days of age, at which time peak levels occurred (Table 3). Subsequently, there was a slight, but significant, decrease in binding capacity between 40 and 60 days of age. A similar age-related pattern of development in B_{max} was observed in offspring exposed to CME. However, the decrease between 40 and 60 days of age was not significant. Exposure to CME throughout gestation and lactation significantly decreased the B_{max} in the striatum of 10 and 20-day-old offspring compared to controls. There were no consistent age-related changes in K_d in the striatum of either control offspring or

TABLE 1

EFFECTS OF CME ON PREGNANT RATS AND THEIR OFFSPRING*

	Control	CME
Cumulative maternal weight gain during pregnancy (g)		
Week 1	24.7 ± 2.1†	12.9 ± 1.7‡
Week 2	59.2 ± 3.0	50.1 ± 3.7
Week 3	133.6 ± 4.7	99.5 ± 6.2‡
Litter size (live+dead)	11.7 ± 0.9	9.5 ± 0.8
Live pups/litter at birth	11.5 ± 0.8	8.3 ± 1.0‡
Stillborn pups/litter	0.2 ± 0.2	1.3 ± 0.6
Birth weight of culled pups (g)	7.49 ± 0.09 (64)	6.62 ± 0.07‡ (56)
Body weight at 10 days of age (g)	22.4 ± 0.3 (24)	18.9 ± 0.3‡ (13)

*All data pertaining to litters include animals from both sexes; numbers in parentheses represent number of animals.
†All values expressed as mean ± 1 S.E.M.
‡Significantly different from control ($p<0.05$).

TABLE 2

EFFECTS OF CME ON BRAIN WEIGHT OF OFFSPRING

Age (days)		Control	CME
10	(both sexes)	0.763 ± 0.008 (24)*	0.672 ± 0.008† (8)
20	M	1.121 ± 0.013 (13)	1.146 ± 0.009 (3)
	F	1.069 ± 0.013 (11)	1.107 ± 0.013 (4)
40	M	1.272 ± 0.022 (10)	1.221 ± 0.009 (9)
	F	1.239 ± 0.024 (6)	1.175 ± 0.011 (7)
60	M	1.390 ± 0.010 (11)	1.319 ± 0.014† (11)
	F	1.331 ± 0.006 (12)	1.222 ± 0.036† (4)

*Mean weight (g) ± S.E.M., excluding cerebellum and brainstem; numbers in parentheses represent number of animals.
†Significantly different from control ($p<0.05$).

TABLE 3

EFFECTS OF CME ON BINDING CHARACTERISTICS OF STRIATAL D_2-DOPAMINE RECEPTORS

		Age (days)			
		10	20	40	60
B_{max} (fmol/mg tissue)	Control	6.3 ± 0.4 (6)*	19.9 ± 1.1 (6)	27.9 ± 1.2 (4)	21.8 ± 2.5† (4)
	CME	5.0 ± 0.2‡ (4)	14.4 ± 1.1‡ (6)	28.2 ± 0.4 (4)	26.0 ± 1.2 (4)
K_d (nM)	Control	0.05 ± 0.01 (4)	0.03 ± 0.01 (6)	0.06 ± 0.01 (4)	0.05 ± 0.01 (4)
	CME	0.04 ± 0.01 (4)	0.03 ± 0.01 (4)	0.06 ± 0.01 (4)	0.08 ± 0.02 (4)

*Mean value ± S.E.M.; numbers in parentheses represent number of pooled tissue samples.
†Significantly different from 40 day control ($p<0.05$).
‡Significantly different from control ($p<0.05$).

TABLE 4

EFFECTS OF CME ON BINDING CHARACTERISTICS OF α_1-ADRENERGIC RECEPTORS IN CEREBRAL CORTEX

		Age (days)			
		10	20	40	60
B_{max} (fmol/mg tissue)	Control	2.6 ± 0.4 (6)*	10.2 ± 0.4 (4)	11.3 ± 0.4 (4)	13.3 ± 1.1 (5)
	CME	2.6 ± 0.3 (3)	8.7 ± 0.5 (6)	10.5 ± 0.8 (4)	10.4 ± 1.1 (4)
K_d (nM)	Control	0.55 ± 0.07 (6)	0.23 ± 0.05 (5)	0.27 ± 0.03 (4)	0.17 ± 0.03 (5)
	CME	0.55 ± 0.19 (3)	0.12 ± 0.01† (6)	0.33 ± 0.05 (4)	0.22 ± 0.08 (4)

*Mean value ± S.E.M.; numbers in parentheses represent number of pooled tissue samples.
†Significantly different from control ($p<0.05$).

offspring exposed to CME nor were there any significant effects related to treatment with CME.

In the cerebral cortex there was a marked increase in B_{max} for α_1-adrenergic receptors between 10 and 20 days of age in both control offspring and offspring exposed to CME (Table 4). Binding capacity tended to level off and showed only a gradual increase between 20 and 60 days of age. In contrast to the striatum, there were no significant treatment effects on B_{max} in the cerebral cortex at any age. The apparent K_d tended to decrease with age in both control offspring and offspring exposed to CME. The K_d at 10 days of age was consistently greater than the K_d at later ages in both groups. The only significant effect of CME on K_d was a decrease observed at 20 days.

Effects of CME on Tyrosine Hydroxylase Activity in Developing Rats

There was a steady age-related increase in tyrosine hydroxylase activity in the striatum of both control offspring and offspring exposed to CME (Fig. 1a). However, enzyme activity was significantly lower in 20 and 40-day-old animals exposed to CME compared with controls at these ages. Tyrosine hydroxylase activity in the cerebral cortex of both control and treated offspring also showed an age-related increase between 20 and 60 days of age (Fig. 1b). Although activity was lower at every age in animals exposed to CME, the effect was significant only at 40 days of age.

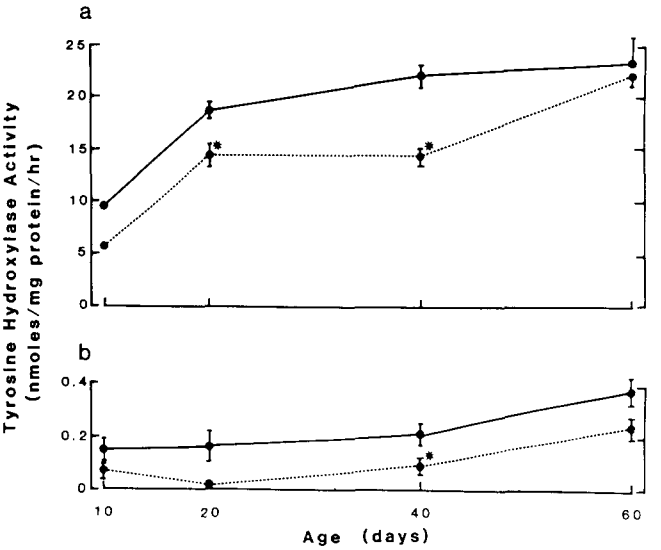

FIG. 1. Tyrosine hydroxylase activity in the striatum (a) and cerebral cortex (b) of offspring exposed to CME during gestation and lactation. Each control group (solid line) and groups exposed to CME (dotted line) consisted of 4–9 pooled tissue samples. *Significantly different from control ($p<0.05$).

DISCUSSION

The decrease in maternal weight gain and pup birth weight observed in this study following chronic treatment with CME is in agreement with others [2,3]. A decrease in food consumption and/or total litter weight, although not determined in this study, may have been responsible for the effect on maternal weight [3]. It seems unlikely, however, that the effect on birth weight was due to maternal malnutrition since food restriction *per se* has been reported to have no effect on the birth weight of the offspring compared to ad lib controls [2,3]. The effects on maternal and pup weights may be related directly to the Δ^9-THC content of the CME since administration of Δ^9-THC to rats throughout pregnancy also reduced maternal weight gain and pup weights [1,10]. The lack of effect of CME exposure on litter size or number of stillborn pups suggests that the dose of CME used in this study does not cause significant fetal toxicity. However, the reduced brain weight at certain ages in both sexes of offspring exposed to CME indicates some postnatal organ toxicity during and following exposure.

The pattern of development of D_2 receptors in the striatum of control offspring was similar to that in a previous report [34]. Interestingly, the K_d for ^{3}H-spiperone binding remained relatively constant during the 60 day postnatal period. Similar results have been reported for whole brain [22]. Thus, maximal receptor affinity for the ligand, in contrast to the maximal number of binding sites, is attained prior to 10 days of age.

This is the first known study in which an alteration of dopamine receptor binding was found as a result of chronic perinatal exposure to marihuana. It is unlikely that the decrease in B_{max} was a compensatory response to an increase in dopamine synthesis and release since both the receptor concentration and tyrosine hydroxylase activity were decreased at 20 days of age. It is possible that the decrease in

receptor binding could have been caused indirectly by perinatal undernutrition since body weight and brain weight were decreased postnatally up to 10 days of age. Others have shown that severe pre- and postnatal diet restriction can decrease brain catecholamine receptors [23]. However, the fact that the decrease in B_{max} of striatal D_2 receptors persisted after return of brain and body weight to normal levels suggests that this effect resulted from a direct action of CME on receptors. This is further supported by recent *in vitro* [8] and chronic *in vivo* [4] studies which found evidence for direct effects of cannabinoids and cannabis extracts on striatal D_2 receptors in adult rats. Although these reports indicated an alteration in K_d rather than B_{max}, this may have been due to marked differences in design of the studies. No significant changes in D_2 receptor binding were apparent at 40 or 60 days of age, 20 and 40 days, respectively, after weaning. This suggests that the effects on D_2 receptors may depend on presence of the drug. Previous studies have indicated that Δ^9-THC and its metabolites disappear from the whole rat with a half-life of approximately 17 hours [24]. It is unlikely, therefore, that significant amounts of cannabinoids would have been present in the brain of offspring at 40 or 60 days of age.

The greatest increase in cortical α_1-adrenergic receptors in control offspring occurred between 10 and 20 days of age. Subsequently, the B_{max} leveled off to adult values. This developmental pattern is generally consistent with a previous report [31], with the exception that the number of receptors in the present study did not decrease after 20 days of age. The inverse relationship between K_d and increasing age agrees with an earlier report [14] and indicates a possible difference in the rate of maturation or development of these receptors compared with striatal D_2 receptors. Exposure to CME had virtually no effect on cortical α_1-receptors, suggesting that its effects in the striatum were somewhat selective.

Previous reports involving the effects of marihuana and cannabinoids on brain catecholamine synthesis or turnover have dealt only with changes in adult animals. Acute *in vivo* [13] and *in vitro* [7] studies indicated that dopamine turnover and synthesis, respectively, were increased by cannabinoids. Chronic treatment with Δ^9-THC increased brain tyrosine hydroxylase activity [19]. The apparent discrepancy between these results and the present study could be explained on the basis that acute alterations in transmitter synthesis and turnover generally involve changes in the affinity of tyrosine hydroxylase for substrate, cofactors or end-product [11], whereas the present study was concerned only with the total amount of enzyme present during postnatal development. Thus, it would appear that marihuana or its constituents impair development of dopamine nerve terminals and/or tyrosine hydroxylase but in the adult animal the major effect may be an alteration of kinetic properties of the enzyme, leading to enhanced catalytic activity. It is unlikely that the presynaptic effects of CME in this study were due indirectly to undernutrition of the offspring since perinatal diet restriction results in an increase in brain tyrosine hydroxylase activity [21,37]. It is also doubtful that the decrease in B_{max} of the D_2 receptors was a contributing factor to the decrease in striatal tyrosine hydroxylase activity since chronic receptor blockade with haloperidol causes a kinetic activation of the enzyme [25].

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