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BRIEF COMMUNICATION

Perinatal Exposure to Cannabinoids Alters Neurochemical Development in Rat Brain

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WALTERS, D. E. AND L. A. CARR. *Perinatal exposure to cannabinoids alters neurochemical development in rat brain.* PHARMACOL BIOCHEM BEHAV 29(1) 213–216, 1988.—Adult female rats received daily oral doses of Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-THC and cannabidiol (CBD) throughout gestation and lactation. The offspring were sacrificed at various ages and tissue samples of cerebral cortex and striatum were assayed for α_1-adrenergic and D_2-dopaminergic receptors, respectively. In addition, tyrosine hydroxylase activity was determined in the striatum. The K_d for ligand binding to α_1 receptors in the cerebral cortex was significantly increased in 10-day-old offspring exposed to CBD. Significant increases in the B_{max} of these receptors occurred at 20 days of age following perinatal exposure to Δ^9-THC or Δ^8-THC. Exposure to CBD increased the K_d of D_2 receptors in the striatum of 10 and 20-day-old offspring compared to control. There were no significant treatment effects on the B_{max} of D_2 receptors in the striatum at any age. Tyrosine hydroxylase activity was significantly decreased only at 60 days of age in offspring exposed to Δ^8-THC or CBD. These results differ from those previously reported with a crude marihuana extract, suggesting that changes in the development of brain catecholamine mechanisms resulting from perinatal exposure to marihuana extracts may be due to an additional constituent of the extract, interactions between specific cannabinoids or other unknown factors.

Δ^9-Tetrahydrocannabinol Δ^8-Tetrahydrocannabinol Cannabidiol Tyrosine hydroxylase
Dopamine receptors Adrenergic receptors Striatum Cerebral cortex Perinatal exposure

CANNABINOIDS can be transferred from adult females to the central nervous system of developing offspring via placental transport and the maternal milk supply [16]. Perinatal exposure to marihuana or specific cannabinoids has been found to alter behavioral development and learning ability [9,10]. A recent study [19] has shown that chronic perinatal exposure to a crude marihuana extract can also alter the development of brain catecholamine mechanisms. In this study, tyrosine hydroxylase activity and the maximum binding capacity (B_{max}) of D_2-dopamine receptors were significantly decreased at certain ages in the striatum of rat offspring exposed to a crude marihuana extract throughout gestation and lactation. Administration of cannabinol or cannabidiol (CBD) to female mice on day 18 of pregnancy [5] or within 12 hours of parturition [6] decreased catecholamine levels in the brain of adult offspring. A decrease in steady-state levels of brain catecholamines which correlated with behavioral changes was reported in adult rats following acute administration of Δ^9-THC [2,3]. These results suggest that alterations in the development of brain catecholamine mechanisms may be responsible for the behavioral changes associated with perinatal exposure to Δ^9-THC.

The purpose of this study was to determine whether changes in the development of brain catecholamine mechanisms associated with perinatal exposure to a cannabis extract could be attributed to specific cannabinoids found in marihuana.

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 per cage, in a temperature-controlled room (25°C±2°C) with lights on from 0500 to 1900 hr. Food and water were supplied ad lib.

Drugs

The following cannabinoids were obtained from the National Institute on Drug Abuse: Δ^9-THC (98.8%), Δ^8-THC in absolute ethanol (10.5 mg/ml) and CBD. All cannabinoids were stored in the dark at −20°C.

Procedure

The treatment protocol and experimental design were

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TABLE 1

EFFECTS OF CANNABINOIDS ON BINDING OF ^{3}H-SPIPERONE TO STRIATAL D_2-DOPAMINE RECEPTORS

	Age (Days)			
	10	20	40	60
B_{max} (fmol/mg tissue)				
Control	6.3 ± 0.1 (4)*	12.7 ± 2.3 (6)	19.0 ± 2.9 (6)	18.1 ± 2.5 (4)
Delta-9-THC	6.7 ± 1.5 (6)	17.2 ± 2.3 (5)	23.5 ± 0.7 (5)	12.3 ± 2.3 (4)†
Delta-8-THC	5.4 ± 0.5 (6)	14.5 ± 1.2 (4)	19.3 ± 1.0 (6)	17.4 ± 1.4 (6)
CBD	4.6 ± 0.3 (4)	16.7 ± 0.6 (4)	17.4 ± 1.9 (4)	13.4 ± 3.2 (4)
K_d (nM)				
Control	0.08 ± 0.02 (4)	0.16 ± 0.04 (4)	0.10 ± 0.03 (6)	0.10 ± 0.03 (4)
Delta-9-THC	0.06 ± 0.02 (4)	0.12 ± 0.04 (5)	0.09 ± 0.01 (6)	0.10 ± 0.02 (6)
Delta-8-THC	0.05 ± 0.01 (6)	0.18 ± 0.05 (6)	0.07 ± 0.01 (6)	0.17 ± 0.05 (4)
CBD	0.46 ± 0.01 (4)‡	0.39 ± 0.08 (4)‡	0.14 ± 0.01 (4)	0.07 ± 0.02 (4)

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

similar to that previously reported [19]. Four different groups of female rats received daily oral administration of Δ^9-THC (10 mg/kg), Δ^8-THC (1 mg/kg), CBD (10 mg/kg) or sesame oil vehicle. Each solution was administered in a volume of 2.5 ml/kg. Treatment began two weeks prior to mating. Pregnant females were individually housed and treatment was continued throughout gestation and lactation. On day 20 postpartum, the offspring were weaned and on day 35, littermates were segregated by sex. At 10, 20, 40 or 60 days of age, the offspring were sacrificed, the brains removed and tissue samples of cerebral cortex and striatum from either sex were dissected for subsequent assay of α_1-adrenergic and D_2-dopaminergic receptors, respectively. In addition, the striatum was assayed for tyrosine hydroxylase activity.

Chemical Assays

Tyrosine hydroxylase activity, ^{3}H-prazosin binding to α_1-adrenergic receptors and ^{3}H-spiperone binding to D_2-dopamine receptors were determined as previously described [19]. The ^{3}H-spiperone binding assay was modified by the addition of 100 nM ketanserin to correct for binding of the radioligand to striatal serotonin receptors [12].

Statistical Analysis

All data were subjected to hierarchical multivariate analysis of variance to partition treatment and litter effects [17]. Significance implies $p<0.05$.

RESULTS

Effects of Cannabinoids on Pregnancy and Offspring

None of the cannabinoids had an effect on maternal weight gain during pregnancy. Furthermore, there were no significant differences in the average litter size, the average number of live or stillborn pups per litter or birth weight as a result of exposure to any of the cannabinoids.

Effects of Cannabinoids on Development of Catecholamine Receptors

The B_{max} for D_2 receptors increased with age between 10 and 40 days postpartum in the striatum of control and treated offspring, at which time peak levels occurred (Table 1). The binding capacity at 60 days of age was consistently less than 40 day values for every group. However, only those offspring exposed to Δ^9-THC showed a significant decrease in B_{max} between these two ages. Exposure to the cannabinoids had no effect on B_{max} in the striatum at any age when compared to controls. The apparent affinity of the ligand for the receptor (K_d) in the striatum of control offspring and offspring exposed to Δ^9-THC or Δ^8-THC showed no consistent age-related changes nor were there any significant effects due to treatment with these two cannabinoids. Exposure to CBD, however, caused a significant increase in K_d at 10 and 20 days of age compared to controls.

There was also an age-related increase in B_{max} for α_1-adrenergic receptors in the cerebral cortex of control animals (Table 2). The greatest increase in binding capacity occurred between 10 and 20 days of age. Exposure to each of the cannabinoids appeared to enhance the increase in binding between these two ages. Both Δ^8 and Δ^9-THC caused a significantly greater B_{max} at 20 days of age compared to controls. The K_d for prazosin binding in the cerebral cortex tended to decrease with age in each of the treatment groups, at least up to 40 days. Exposure to CBD increased the K_d at every age, although the difference was significant only at 10 days.

Effects of Cannabinoids on Development of Tyrosine Hydroxylase Activity in Striatum

An age-related increase in striatal tyrosine hydroxylase activity was observed during the first 40 days postpartum in all treatment groups, although the pattern was more variable in animals exposed to Δ^9-THC (Fig. 1). Compared with day 40 levels, most of the groups showed a decrease in activity at 60 days. The only significant effect caused by cannabinoids was a decrease in enzyme activity at 60 days of age in animals exposed to Δ^8-THC or CBD.

DISCUSSION

It was previously reported that chronic perinatal exposure to a crude marihuana extract (CME) decreased B_{max} for

TABLE 2

EFFECTS OF CANNABINOIDS ON BINDING OF ^{3}H-PRAZOSIN TO α_1-ADRENERGIC RECEPTORS IN CEREBRAL CORTEX

	Age (Days)			
	10	20	40	60
B_{max} (fmol/mg tissue)				
Control	4.5 ± 0.3 (4)*	7.1 ± 0.8 (4)	8.2 ± 1.0 (5)	9.4 ± 0.6 (4)
Delta-9-THC	3.7 ± 0.9 (6)	13.9 ± 1.7 (5)†	6.3 ± 1.2 (6)	9.1 ± 0.6 (4)
Delta-8-THC	1.8 ± 0.2 (6)	9.5 ± 0.4 (6)†	8.5 ± 0.6 (6)	9.4 ± 0.6 (6)
CBD	2.6 ± 0.1 (4)	8.2 ± 0.9 (4)	7.6 ± 0.4 (4)	10.6 ± 1.5 (4)
K_d (nM)				
Control	0.38 ± 0.08 (4)	0.34 ± 0.03 (4)	0.10 ± 0.01 (5)	0.18 ± 0.01 (4)
Delta-9-THC	0.27 ± 0.08 (4)	0.39 ± 0.09 (5)	0.24 ± 0.09 (6)	0.13 ± 0.03 (4)
Delta-8-THC	0.28 ± 0.01 (5)	0.30 ± 0.06 (6)	0.14 ± 0.02 (5)	0.31 ± 0.08 (5)
CBD	0.87 ± 0.03 (4)†	0.48 ± 0.03 (4)	0.15 ± 0.01 (3)	0.25 ± 0.08 (4)

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

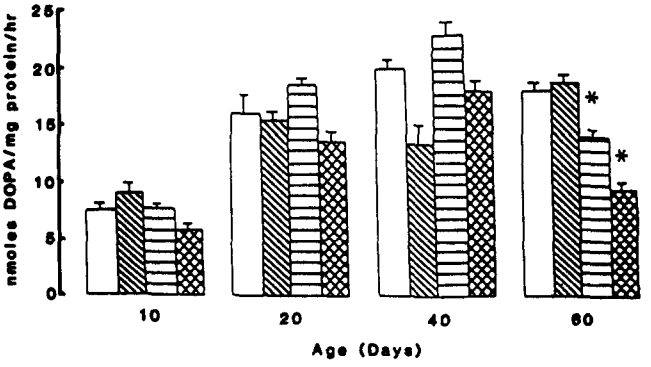

FIG. 1. Tyrosine hydroxylase activity in the striatum of offspring exposed to vehicle (open bars), Δ^9-THC (diagonally striped bars), Δ^8-THC (horizontally striped bars) or CBD (cross hatched bars) during gestation and lactation. Each bar represents the mean value from 6–12 tissue samples. *Significantly different from control ($p<0.05$).

D_2 dopamine receptors in the striatum of 10- and 20-day-old offspring [19]. The present results would seem to suggest that these effects were due to a constituent of CME other than Δ^9-THC, Δ^8-THC or CBD since administration of these compounds throughout gestation and lactation had no effect on B_{max} at any age. However, another possible explanation for the contrasting results of these studies was a difference in doses of the cannabinoids administered in the present study compared to the concentrations contained in the CME (e.g., 10 mg/kg Δ^9-THC alone vs. 20 mg/kg administered as CME). A reduction in the doses of individual compounds was required in the present study since equal doses of Δ^9-THC were more toxic when given singly than when administered in CME (unpublished results). Another possibility is that the cannabinoids may have interacted when administered together in CME so that they produced a greater effect than when administered individually. Evidence for such interactions has been reported previously. For example, CBD was found to potentiate the hypothermic and analgesic effects [8,14] and the anticonvulsant activity [18] of Δ^9-THC. However, this explanation must be tempered by the fact that CBD also inhibits several actions of Δ^9-THC [7]. Finally, it is possible that the inclusion of ketanserin in the present study could account for the lack of cannabinoid effects on the B_{max} of D_2 receptors. Since 5-HT_2 receptors were not excluded from ^{3}H-spiperone binding in the earlier study, some of the actions of CME may have involved striatal serotonergic receptors.

The decrease in binding affinity for ^{3}H-spiperone at 10 and 20 days of age during exposure to CBD was probably due to a direct effect on the receptors since it has been shown that CBD increases the K_d for spiperone binding in mouse striata *in vitro* without altering the B_{max} [4]. As was observed with CME [19], no alterations in D_2 receptor binding occurred after drug exposure was terminated.

Exposure to CBD also increased the K_d for ^{3}H-prazosin binding to α_1-adrenergic receptors in the cerebral cortex of 10-day-old offspring, suggesting that the effects of this cannabinoid on catecholamine receptors may be due to a nonspecific membrane effect. However, CBD does not increase membrane fluidity and it has contrasting effects on different catecholamine receptor types [15], which would argue against the production of membrane disturbances.

Although a decrease in tyrosine hydroxylase activity in the striatum of rats exposed to Δ^8-THC and CBD was observed at 60 days of age, it is unlikely that this was due to direct drug effects since the pharmacokinetics of these compounds follows the same pattern as Δ^9-THC [1], which rapidly disappears from the brain in a matter of hours [11]. These results confirm the previous report with CME [19] which showed that an alteration of tyrosine hydroxylase activity may occur several days or weeks following drug exposure. The present results also suggest that the decrease in brain dopamine levels reported in adult rats following perinatal exposure to CBD [5,6] may have been caused by a decrease in synthesis. This possibility is further supported by the observation that Δ^9-THC had no effect on either dopamine levels [5,6] or tyrosine hydroxylase activity following perinatal treatment. As was the case with D_2 receptor binding, the lack of an effect of any of the cannabinoids on tyrosine hydroxylase activity at earlier ages, in contrast to the effects seen with CME, may have been due to drug interactions or exposure to different doses.

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