Pharmacology Biochemistry & Behavior, Vol. 14, pp. 911–914, 1981. Printed in the U.S.A.

Modifications of Brain Steroidogenesis and Plasma Steroids After p-Chlorophenylalanine-methyl-ester Treatment in Male Rats and Rabbits

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Received 10 November 1980

FARABOLLINI, F., C. LUPO DI PRISCO AND F. DESSÌ-FULGHERI. *Modifications of brain steroidogenesis and plasma steroids after p-chlorophenylalanine-methyl-ester treatment in male rats and rabbits.* PHARMAC. BIOCHEM. BEHAV. **14**(6)911–914, 1981.—The effect of p-chlorophenylalanine (p-CPA) on testosterone (T) hypothalamic metabolism and on plasma levels of T, estradiol (E_2) and corticosterone (B) was studied in male rats and rabbits: both were sacrificed 48 hours after the last injection. In both rats and rabbits a significant increase in T→E_2 transformation (aromatization) was evident after p-CPA treatment. This increase could be responsible for the stimulatory effect on sexual behavior which has been described in literature following p-CPA administration. Moreover p-CPA caused a decrease of circulating T and E_2 in both species.

p-CPA Testosterone aromatization Brain steroidogenesis Serotonin depletion Plasma steroid levels

IT has been reported that p-chlorophenylalanine (p-CPA), a drug that blocks the synthesis of catecholamines (CA) and 5-hydroxytryptamine (5-HT) [10], stimulates male sexual behavior in several species [1, 8, 15]. This stimulatory effect is seen in intact as well as in castrated testosterone (T)-treated animals [14,18]. Most remarkable, p-CPA stimulates sexual behavior in castrated rats even without concomitant treatment with T [5,18].

The main hormone stimulating the expression of masculine sexual behavior is T, which is thought to exert its behavioral effect in several species after its transformation into aromatized metabolites in the brain [11]. On this basis it could be speculated that p-CPA is active in modifying T metabolism in the brain, providing in such a way a neuroendocrine explanation of behavioral effects of p-CPA.

Starting from this assumption in the present experiment we studied in two different species the effect of p-CPA administration on T hypothalamic metabolism. We also considered T, estradiol (E_2) and corticosterone (B) plasma levels.

METHOD

Animals

Twenty-two male Sprague Dawley rats (mean body weight 250 g) were divided into two groups. The animals in the first group (N=11) were injected IP with p-CPA methyl-ester dissolved in water in a single dose (400 mg/kg body weight). The animals of the second group (N=11) were injected with saline. Both groups were sacrificed by decapitation 48 hours after the injection between 6.00 and 7.00 p.m. Heparinized blood and brain were taken.

Seventeen male New Zealand rabbits (mean body weight 2650 g) were divided into two groups. The animals in the first group (N=9) were injected with p-CPA methyl-ester dissolved in water (600 mg/kg body weight) for three days (300,150,150 mg) and they were sacrificed 48 hours after the last injection between 8.30 and 9.30 a.m. The animals in the second group (N=8) were used as controls. Blood samples were taken from the ear before and after treatment, and brain was taken after sacrifice.

Plasma

Heparinized blood was centrifuged and plasma was used for the determination of T, E_2 and B. Testosterone was determined by radioimmunoassay without preliminary chromatography according to the method of Collins *et al.* [3]. Estradiol was determined by radioimmunoassay without preliminary purification. The antiserum used was prepared in rabbit against estradiol conjugated in position 6 with human tireoglobulin. This antiserum showed a 3% cross-reaction with estrone and 0.4% with estriol. The incubation and the separation between free and conjugated fraction were performed according to the method of Emment *et al.* [6]. Corticosterone was determined by competitive protein binding using 1,2,6,7-^{3}H- B (specific activity 81 Ci/mmole, Amersham) and pure lyophilized transcortin, following a modification of the method described for cortisol [16], which we have previously reported [13].

Brain

Three regions were dissected from the rabbit brain: midbrain, septal region and hypothalamus. The three areas were obtained with the following procedure: the hypothalamic sample was first dissected from the ventral surface of the brain between the opticum tractum and mammillary bodies, for a deepness of 2.5–3 mm. The cerebral hemispheres were then pulled away from the midline by a sagittal section of the corpus callosum. Septum sample was obtained by bilateral sagittal sections through the lateral ventriculi, and coronal sections at the level of the rostral boundary of the anterior commissura and at the level of the tractus opticus. Mesencephalon was a cuneiform sample delimited dorsally by superior and inferior colliculi and ventrally by mammillary bodies and pons. In the case of the rat only the hypothalamus was utilized. In the midbrain and septal regions the activity of tryptophan hydroxylase was assayed in the particulate fraction (17000×g pellet) according to the method of Knapp and Mandel [9]. Hypothalamus was incubated for 1 hour in a Dubnoff metabolic shaker at 37°C in air with 0.25 μCi of 1α,2α-^{3}H-T (for the rat) and with 1 μCi of 1α,2α-^{3}H-T (for the rabbit). The incubation medium consisted in 5 ml of Krebs-Ringer phosphate 0.1 M pH=7.4 with 1 mg of NAD and 1 mg of NADP as cofactors. The metabolites were extracted three times with methylene chloride (10 volumes each time) and then once with 10 volumes of ethyl acetate. Extracts were pooled and evaporated, and the residue was dissolved in 40 ml of benzene and the phenolic fraction was separated from the neutral one with NaOH N (40,30,30 ml) and, after neutralization, it was extracted with diethyl ether. Neutral steroids were chromatographed on thin layer in the system chloroform: acetone 90/10, in which T has an R_f=0.38, dihydrotestosterone (DHT) 0.55, androstenedione 0.70 and androstenediol 0.20. Phenolic steroids were separated by thin layer chromatography in a system of benzene; ethanol 90:10, in which E_2 has an R_f=0.30. The zones corresponding to standard steroids were eluted and the eluates were crystallized to constant specific activity after addition of 4 mg of carrier.

RESULTS

Figure 1 shows the activity of tryptophan hydroxylase in the rabbit brain after treatment with p-CPA. The reduction was to 25% of control values in midbrain and to 35% of control values in septal region. Table 1 shows plasma steroid levels in the rat. A significant decrease after p-CPA treatment was observed for B ($p<0.01$), T ($p<0.01$) and E_2 ($p<0.02$). Table 2 shows the effect in the rabbit. Testosterone ($p<0.05$) and E_2 ($p<0.02$) significantly decreased while B did not change. The specific activities of T metabolites in rat hypothalamus are reported in Table 3: only T→E_2 transformation was enhanced by p-CPA treatment ($p<0.01$), while other metabolites did not change. Table 4 shows specific activities of T metabolites in rabbit hypothalamus: aromatization increased after treatment ($p<0.05$), while the conversion T→androstenedione decreased ($p<0.01$).

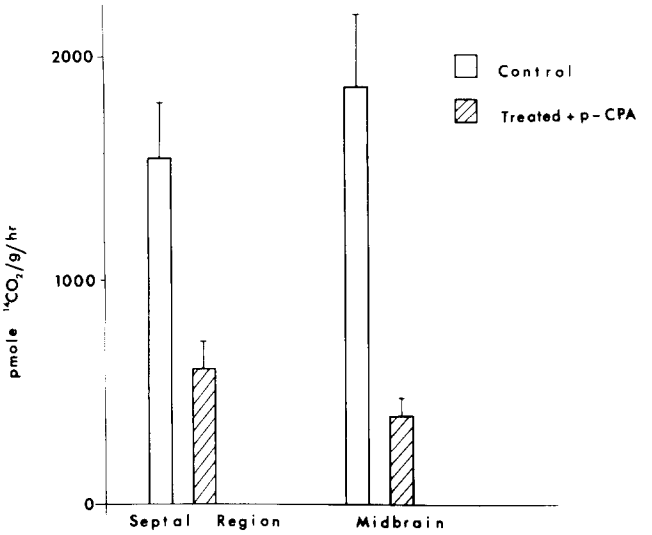

FIG. 1. Effect of p-CPA treatment on tryptophan-hydroxylase activity assayed in particulate fraction (17000×g) of the brain of the male rabbit (Means and SD). Controls: N=8. Treated: N=9.

TABLE 1

EFFECT OF p-CPA TREATMENT ON PLASMA STEROID LEVELS IN MALE RATS (MEANS AND SD)

	Controls (N=11)	Treated (N=11)
Corticosterone (μg/100 ml)	25.03 ± 11.24	13.93 ± 10.58†
Testosterone (ng/ml)	1.45 ± 0.79	0.39 ± 0.52†
Estradiol (ng/ml)	0.49 ± 0.26	0.16 ± 0.11*

Student t test: *$p<0.02$; †$p<0.01$.

DISCUSSION

The finding that p-CPA enhances hypothalamic T aromatization in rats and rabbits is of interest since in both species the conversion of T to estrogen appears to be important for the expression of male sexual behavior [2,11]. This increase in aromatase activity provides an explanation for

TABLE 2

EFFECT OF p-CPA TREATMENT ON PLASMA STEROID LEVELS IN MALE RABBITS (MEAN DIFFERENCES (N=10))

	Before treatment	After treatment	Differences	t*	$p<$
Corticosterone (μg/100 ml)	10.00	7.88	2.21 ± 1.57	1.40	n.s.
Testosterone (ng/ml)	1.56	0.82	0.74 ± 0.31	2.35	0.05
Estradiol (ng/ml)	0.43	0.25	0.18 ± 0.05	3.23	0.02

*t Test for paired samples [17].

TABLE 3

IN VITRO TESTOSTERONE METABOLISM IN THE HYPOTHALAMUS OF p-CPA TREATED MALE RATS (MEANS ± SE)

	dpm/mg/100 mg of Tissue	
	Controls (9)	Treated (11)
Estradiol	48 ± 10	208 ± 46*
Dihydrotestosterone	1,043 ± 306	1,544 ± 347
Androstenedione	21,651 ± 3,752	15,708 ± 1,771
Androstenediol	1,225 ± 374	1,949 ± 499
Testosterone	63,137 ± 7,909	71,875 ± 7,703
Total recovered radioactivity	87,113 ± 8,654	92,197 ± 7,499

*Student t test, $p<0.01$.

TABLE 4

IN VITRO TESTOSTERONE METABOLISM IN THE HYPOTHALAMUS OF p-CPA TREATED MALE RABBITS (MEANS ± SE)

	dpm/mg/100 mg of Tissue	
	Controls (8)	Treated (9)
Estradiol	8,651 ± 1,997	21,264 ± 3,555*
Dihydrotestosterone	43,105 ± 12,581	36,687 ± 6,735
Androstenedione	65,436 ± 12,635	20,813 ± 3,310†
Androstenediol	88,515 ± 26,754	65,973 ± 12,532
Testosterone	1,369,482 ± 277,179	1,281,497 ± 177,386
Total recovered radioactivity	1,901,934 ± 634,686	1,430,944 ± 195,821

Student t test, *$p<0.05$; †$p<0.01$.

the stimulatory effect of p-CPA on sexual behavior. Although p-CPA produced a reduction of plasma T level, it is not clear whether the enhancement of aromatization is related to the reduction of T secretion. No necessary relationship exists, however, between T plasma level and brain T aromatization, as evidenced by our observation that castration does not influence hypothalamic aromatase activity (Dessì-Fulgheri *et al.*, unpublished results). The observation that a drug stimulating sexual behavior reduces T secretion may be surprising, but there is no direct correlation between sexual capacity and plasma T level in the rat [4].

The reduction of plasma B observed in p-CPA treated rats may reflect an influence of this drug on the pituitary-adrenal function, as suggested by Van Delft *et al.* [19], who found that a change in the circadian rhythm of B induced by p-CPA resulted in a decrease of the peak value at 8 p.m.

In regard to the mechanism mediating the p-CPA effects observed in our experiments, it is important to indicate that under the present conditions of dose and drug administration the brain CA levels are restored within 48 hours after p-CPA administration, while the 5-HT level remains lowered for several weeks [10]. Thus, 5-HT depletion can account for the effect seen in the present experiment. In fact, depletion of brain 5-HT, either produced by treatment with p-CPA [8] or by intracerebral injection of the neurotoxin 5,7-dihydroxytryptamine [12], has been found to facilitate the expression of male rat sexual behavior. Moreover, it has been recently reported that castration enhances the synthesis of monoamines in the brain, and that this effect can be counteracted by treatment with T [7].

It must be acknowledged, however, that our results do not rule out the possibility that the observed effects of p-CPA may be due to a mode of action not directly related to 5-HT depletion, such as a direct action of p-CPA or its metabolites on steroidogenesis enzymes.

ACKNOWLEDGEMENT

We especially wish to thank Knut Larsson for many valuable discussions and helpful criticism.

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