Effects of Progesterone or Cyproterone Acetate on Androgen Uptake in the Brain, Pituitary and Peripheral Tissues[1] (37519)

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The uptake and distribution of radioactivity in specific regions of the brain as well as in the peripheral target tissues after the injection of labeled testosterone have been studied using both biochemical and histological techniques (1–7). The extensive research of Neumann and colleagues (8–9) has shown that the synthetic steroid cyproterone acetate (2-methylene pregna-4,6-dione-3,20-dione acetate) is a potent anti-androgen. This compound when implanted into the hypothalamus, significantly enhanced gonadotropin secretion (10), but no effects were observed on male sexual behavior (11). Progesterone has also been shown to antagonize a number of the actions of androgen on both central and peripheral tissues involved in reproduction (12). Since the effect of the anti-androgen cyproterone upon the uptake and retention of testosterone in the central nervous tissue is controversial (13), we studied the distribution or retention of radioactivity in the brain, the anterior pituitary, and in peripheral target tissues after the injection of 1,2-^{3}H-testosterone into orchiectomized male rats pretreated with cyproterone acetate or progesterone.

Materials and Methods. Twenty-five 35-day-old male Sprague–Dawley rats weighing approximately 160 g were orchiectomized and injected subcutaneously daily for 5 days with the following substances dissolved in 0.2 ml sesame oil: Group I ($n = 9$) was injected with sesame oil only, Group II ($n = 8$) with 4 mg of progesterone and Group III ($n = 8$) with 10 mg of cyproterone acetate. On Day 6, the rats were injected intravenously with 0.5 μg/100 g body weight, of 1,2-^{3}H-testosterone, specific activity 45 Ci/m*M* (New England Nuclear), and decapitated after 1 hr. The animals were orchiectomized in order to prevent the competition of endogenous androgens with the exogenous radioactive androgen or its metabolites. Chronic treatments were employed to study the effects of progesterone or cyproterone acetate upon the radioactivity uptake, since anti-androgenic effects of these steroids have been shown after prolonged treatment (8, 9).

The brain was removed and placed on a Petri dish cooled with ice. The hypothalamus was dissected. The sample was defined anteriorly by the frontal border of the optic chiasm, laterally by the hypothalamic fissure and posteriorly by the posterior margin of the mamillary body. The hypothalamic sample was then divided into 3 parts, the "preoptic region," the "central hypothalamus," and the "posterior hypothalamus." Samples of the midbrain, amygdala, hippocampus, septum, cerebellum, neocortex, ventral prostate, seminal vesicles, and adrenals were also dissected. The pituitary was removed from the sella turcica and the anterior pituitary was separated from the posterior pituitary. The tissue samples were freeze-dried and oxidized individually in a Packard Tissue Oxidizer, Model 305. After the end of the oxidation of a tissue sample, tritiated H_2O was collected in a scintillation vial containing 15 ml of Permafluor ^{Tm}II (Packard Liquid Scintillator). The scintillation vial was immediately capped to prevent the evaporation of

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TABLE I. Effects of Progesterone or Cyproterone Acetate Treatment on the Radioactivity Uptake in Brain Tissue, Anterior Pituitary and Peripheral Tissues 1 hr After Injection of 1,2-^{3}H-Testosterone into Castrated Male Rats.

Tissue	Oil control dpm/mg dried tissue[a]	Progesterone dpm/mg dried tissue[a]	Cyproterone acetate dpm/mg dried tissue[a]	df^F	p, based on analysis of variance F-table
Preoptic region	540.9 ± 51.7 (9)	434.17 ± 23.1* (8)	362.54 ± 39.8*** (8)	4.872 22,2	0.017
Central hypothalamus	564.6 ± 56.3 (8)	427.4 ± 25.1** (8)	359.6 ± 45.6*** (8)	5.569 21,2	0.011
Post hypothalamus	446.7 ± 38.3 (8)	418.5 ± 22.6 (8)	338.6 ± 36.2 (8)	2.868 21,2	0.079
Neocortex	445.9 ± 45.5 (9)	392.6 ± 25.9 (8)	379.1 ± 38.3 (8)	2.371 22,2	0.116
Hippocampus	474.6 ± 47.8 (8)	458.1 ± 32.3 (8)	315.8 ± 37.5** (8)	4.830 21,2	0.018
Amygdala	463.6 ± 45.1 (9)	428.7 ± 35.9 (8)	333.9 ± 35.7 (8)	2.846 22,2	0.079
Septum	459.8 ± 41.9 (9)	409.0 ± 26.4 (8)	320.7 ± 35.6** (8)	3.870 22,2	0.036
Cerebellum	456.3 ± 53.9 (9)	428.9 ± 26.1 (8)	398.7 ± 59.5 (8)	0.347 22,2	0.710
Midbrain	460.1 ± 98.3 (9)	450.8 ± 22.3 (7)	432.6 ± 73.5 (8)	0.829 21,2	0.450
Anterior pituitary	1924.7 ± 170.1 (9)	1125.3 ± 116.6† (8)	697.7 ± 62.3†† (8)	24.077 22,2	0.001
Ventral prostate	3295.9 ± 599.8 (8)	2424.1 ± 422.5 (8)	1184.3 ± 128.2† (8)	6.745 21,2	0.005
Seminal vesicles	4054.4 ± 353.3 (8)	3491.5 ± 334.3 (8)	1284.6 ± 228.6†† (8)	22.270 21,2	0.001
Adrenals	1701.8 ± 241.4 (8)	1436.3 ± 72.3 (8)	1127.3 ± 115.5 (8)	1.376 21,2	0.253

[a] Mean ± SE. Number in parentheses indicates the number of animals in each group. Significance level between control vs progesterone or cyproterone acetate treated group based on t test. * = < 0.05; ** = < 0.025; *** = < 0.01; † = < 0.005; †† = < 0.001.

tritiated H_2O. The radioactivity in each vial was counted in a scintillation counter (Nuclear Chicago, Mark II) using external standard channel-ratio techniques. The counting efficiency for tritium, as determined by external standard channel-ratio techniques, ranged from 45 to 50%. The radioactivity was expressed as "dpm."

Statistical analysis. The data were analyzed in an IBM 1130 Computer by analysis of variance, and the significance level between the control and progesterone or cyproterone acetate treated group was determined by the t test.

Results. Table I shows the radioactivity concentration (uptake) in different brain regions, anterior pituitary and peripheral tissues of 35-day-old male orchiectomized rats without and with pretreatment of progesterone or cyproterone acetate for 5 days, one hour after the injection of 1,2-^{3}H-testosterone.

Cyproterone acetate produced a significant reduction in the accumulation of radioactivity in the "preoptic region," "central hypothalamus," "septum," and "hippocampus," a slight reduction in the "amygdala" and "posterior hypothalamus" ($p < 0.10$), but no

change in the "neocortex," "cerebellum," and "midbrain." On the other hand, progesterone significantly reduced the accumulation of radioactivity in the "central hypothalamus" and "preoptic area," but no significant effect was observed on the other brain regions. Cyproterone acetate significantly reduced the accumulation of radioactivity in the anterior pituitary, ventral prostate, and seminal vesicles, but not in the adrenals. Pretreatment with progesterone also significantly reduced the accumulation of radioactivity in the anterior pituitary, but in the ventral prostate and seminal vesicles only a slight inhibition of radioactivity uptake was observed.

Discussion. The present results indicate that treatment with the anti-androgen cyproterone acetate (CA) for 5 days reduces the retention of radioactivity after ^{3}H-testosterone injection in hypothalamic and extrahypothalamic sites of the brain, similar to peripheral target tissues such as the ventral prostate and seminal vesicles. This is in agreement with the findings of other investigators (2–5). In contrast, however, Whalen *et al.* (13) did not find any effect of CA upon the uptake and retention of radioactivity in hypothalamic, preoptic-diagonal band, or cortical brain structures, although these authors noticed a significant reduction of radioactivity in the seminal vesicles and pituitary. They argue "that CA did not pass the blood–brain barrier" in adult male rats. Our results do not support such a conclusion. The differences between our results and those of Whalen *et al.* (13) may be attributable to differences in treatment schedule, dissection technique, number of animals used, as well as the amount of ^{3}H-testosterone injection.

Among these factors, differences in the dissection technique may be most important, since the areas of neurons which concentrate steroids are restricted and small. These areas have been defined by our autoradiographic studies (14, 15). Using this topographical information as a guide for tissue dissection for obtaining tissue blocks with a maximal number of hormone concentrating neurons and a minimal amount of nontarget tissue, the background noise is reduced and the probability of detecting specificity of radioactivity uptake and competition is enhanced. Deficiencies in dissection techniques may account for the low radioactivity counts and the failure to demonstrate specificity in the studies of Whalen *et al.* (13). Similarly, Whalen and Maurer (16) failed to show specific uptake of estrogen in the hypothalamus, although published radioassay studies and our autoradiographic experiments had established the existence of specific estrogen binding sites in this area.

Our observation that progesterone reduces the uptake of radioactivity in the hypothalamus after the administration of ^{3}H-testosterone is supported by a report in the literature according to which progesterone pretreatment prevents the testosterone induced "masculinizing effect" in neonatal animals on gonadotropin secretion and behavior (12). It is not surprising that progesterone has "anti-androgenic" properties since the anti-androgen CA is a progesterone derivative.

Summary. Effects of progesterone or cyproterone acetate on the accumulation of radioactivity in the brain, anterior pituitary and peripheral tissues after the injection of 1,2-^{3}H-testosterone were studied in castrated male rats.

Cyproterone acetate significantly reduced the accumulation of radioactivity in the central hypothalamus, preoptic region, hippocampus, septum, pituitary, ventral prostate, and seminal vesicles, but not in other brain tissues or the adrenals. Progesterone reduced the accumulation of radioactivity significantly in the anterior pituitary and to a lesser extent in the central hypothalamus and preoptic region. The results suggest that cyproterone acetate has anti-androgenic effects on the central nervous system and that progesterone has less anti-androgenic effects when compared to cyproterone acetate.

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