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# Role of Monoamines in the Male Differentiation of the Brain Induced by Androgen Aromatization

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GONZÁLEZ, M. I. AND M. L. LERET. *Role of monoamines in the male differentiation of the brain induced by androgen aromatization.* PHARMACOL BIOCHEM BEHAV **41**(4) 733–737 1992.—Cerebral androgen aromatization has been described as a mechanism responsible for masculinization of the brain, and monoamines seem to be involved in sexual differentiation of the brain. The aim of this study was to investigate the possible implication of monoamines in the masculinization of the brain induced by cerebral androgen aromatization not only in the classic hypothalamic areas but also in some extrahypothalamic ones. For this purpose, 1-day-old male Wistar rats were injected intraventricularly with 5 mg/kg of a suspension of an aromatase inhibitor, LY43578. Saline was administered to male and female control groups. At adulthood, open-field, heterotypical, and homotypical sexual behavior tests were performed and cerebral amines were determined by HPLC-ED. Behavioral tests revealed feminine-like exploratory activity and defecation rate in the treated group, as well as an 89% lordotic response and decreased number of mounts plus intromissions. Testosterone levels were not affected by the treatment. Striatal and limbic serotonergic metabolism showed a sexual dimorphism, higher in males than females, that disappeared in the treated group. From these results, we suggest a possible role of extrahypothalamic serotonin in the mediation of the estrogen-induced mechanisms of behavioral sexual differentiation.

Catecholamines Serotonin Aromatization Sexual differentiation Sexual behavior Open field Intraventricular Extrahypothalamic

EXPOSURE to sex hormones during the critical period of sexual differentiation exerts an organizational influence upon the developing CNS (5,17). Estrogens, mainly aromatized from androgens within the brain (11), are believed to be involved in the differentiation of the "sex centers" (3).

The biochemical mechanisms by which steroids exert their effect on the central nervous system are still obscure. Serotonin (5-HT) and catecholamines [dopamine (DA) and noradrenaline (NA)] seem to be involved in sexual differentiation of the brain (7,8), and a direct effect of either androgens or estrogens or both on the development of these neurotransmitter systems could be expected to have profound and permanent consequences.

The present study was designed to examine the possible implication of monoamines in the masculinization of the brain induced by cerebral androgen aromatization. Cerebral areas not directly involved in the control of sexual behavior, like corpus striatum and limbic structures, have also been studied.

## METHOD

Wistar rats were used in the present work. Litters of five mothers were randomized and groups were injected intraventricularly during the first 24 h after birth with either 1 μl saline or 5 mg/kg LY43578 (an inhibitor of aromatase, Eli Lilly and Co., Indianapolis, IN) administered as a suspension in 1 μl saline.

Injections were carried out under cold anesthesia that was induced by placing pups at −20°C for 10–15 min. A microsyringe was introduced to the lateral ventricle 1.5 mm lateral from bregma (seen through the skin) and 2 mm ventral from the surface of the skin. These coordinates were determined by dye injections. There were 3 treatment groups: 12 males receiving LY43578 and 2 control groups receiving saline, one consisting of 10 males and the other of 10 females.

After injection, animals were kept under controlled conditions of light/dark cycles (12 : 12, lights on at 0800 h) and temperature (21 ± 1°C). They were fed ad lib.

At the age of 80–85 days, rats were submitted to behavioral testing. Open-field testing was performed during a 3-min test. Exploratory activity (ambulations as number of line crossings), rearing, and defecation rate were noted. Sexual behavior was tested 8 h into the dark period under red illumination. Masculine behavior was observed by placing each animal in an arena and 5 min later introducing a sexually receptive fe-

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male in natural estrous state determined by vaginal smear. The number of mounts, intromissions, and ejaculation latency were noted in a test period of 10 min. Feminine sexual activity was tested by placing animals with vigorous males and noting their lordotic responses to 10 mounts. The results were expressed as the lordotic percentage [(lordosis:mounts) × 100]. All animals were sexually naive prior to the test.

All animals were decapitated at 90–95 days old at the same hour at which behavioral testing had been performed. Female rats were sacrificed in diestrous as determined by vaginal smear.

Blood samples were taken and testosterone contents determined using a direct radioimmunoassay kit with coated tubes (Sorin, Biomedica). The radioactivity of the tubes was measured in a Gamma Packard Multiprias 1 counter (United Technologies, Packard).

The encephalons were extracted and the mediobasal hypothalamus, corpus striatum, and limbic structures (hippocampus, septum, olfactory cortex) were dissected (2), weighed, and stored at −40°C until assayed for amines (maximum time 3 wk).

Samples were homogenized in 1 ml cold 0,2-*N*-perchloric acid containing 0.4 mM sodium bisulfite and 0.4 mM EDTA. 3,4-Dihydroxybenzylamine was also added to each sample as an internal standard to control for procedural losses. Homogenates were centrifuged (15,000 rpm, 5 min at 3–5°C) and supernatants frozen until amine determination using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Separation of amines [DA, NA, 5-HT, 5-hydroxyindolacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 4-hydroxy-3-metoxyphenylglycol (MHPG)] was performed by a RP-18 column (22 cm, 4.6 mm, 5 μm particle size, Brownlee Labs) after precolumn, then eluted with a mobile phase consisting of 0.05 M monopotassium phosphate, 0.1 mM EDTA, 1 mM heptane sulphonate, and 8–10% methanol (pH adjusted to 3.8 with acetic acid). Amines were measured at a potential of 0.80 V relative to an Ag/AgCl reference electrode. Standards were run concurrently and concentrations of unknowns were determined by comparison to peak areas of standards after correction for recovery of the internal standard.

### *Statistics*

Behavioral results were compared by means of Mann-Whitney test after Kruskal-Wallis one-way analysis of variance (ANOVA). Aminergic ratios (5-HIAA/5-HT; DOPAC/DA; MHPG/NA) were used as a turnover measure to give a functional approach of the amines' activity. Aminergic ratios, as well as testosterone levels, were tested for significant differences by Scheffe's test after one-way ANOVA.

## RESULTS

### *Open Field*

Significant differences were found in exploratory activity (ambulations) and defecation rate between control male and control female groups (Table 1). The aromatase inhibitor treated group showed female-like exploratory activity and defecation rate, and were significantly different from control males. No differences were found in rearing.

### *Sexual Behavior*

Neonatal aromatase inhibitor administered in brain significantly decreased masculine behavior (mounts plus intromissions) and resulted in feminine sexual behavior (lordotic quotient) (Table 1). No ejaculations were shown during the test time.

### *Aminergic Contents*

Sexual differences were found in the ratio of 5-HIAA/5-HT (Fig. 1) in the corpus striatum and the limbic system, with a greater value in males compared to females. Males treated with the aromatase inhibitor possessed a ratio of 5-HIAA/5-HT significantly lower than control males and similar to that observed in females.

DOPAC/DA ratio (Fig. 2) was similar among the three groups in the studied areas.

Noradrenergic index (MHPG/NA, Fig. 3) showed an increment in the treated group in the three studied regions compared to that of the male and female controls, which showed no significant differences.

## DISCUSSION

The results clearly showed a female-like behavior in the aromatase-inhibitor-treated-males. As observed both from the open-field test and from the sexual activity test, a feminized, as well as demasculinized behavior appeared in adult male rats when they were neonatally administered with a product that prevents aromatization of androgens in the brain. This would suggest an important role for aromatization in the normal development of the male brain. Both testosterone and estradiol seem to prevent neonatally castrated male brain from feminiza-

TABLE 1

EFFECT OF NEONATAL ICV ADMINISTRATION OF THE AROMATASE INHIBITOR LY43578 OR SALINE ON PLASMA TESTOSTERONE, OPEN FIELD, AND SEXUAL BEHAVIOR

| | Open Field (3 min) | | | Sexual Behavior | | Testosterone |
|---|---|---|---|---|---|---|
| | Ambulations | Boluses | Rearing | Mounts & Intromissions | Lordotic Quotient | (pg/ml) |
| Control males | 55.3 ± 3.1 * | 4 ± 0.6 * | 8.1 ± 1.3 | 5.7 ± 1.7 * | 1 ± 0.8 * | 1229 ± 168 |
| Aromatase-inhibited males | 67.5 ± 2.6 * | 1.9 ± 0.5 * | 6.9 ± 0.7 | 1.2 ± 0.8 * | 89 ± 8.5 * | 2379 ± 541 |
| Control females | 67.4 ± 2.8 * | 1.3 ± 0.5 * | 7.6 ± 0.7 | – | – | – |

Number of ambulations and boluses noted in the open-field test; number of mounts + intromissions and lordotic quotient shown in the sexual behavior tests; plasmatic concentration (pg/ml) of testosterone. Groups were adult males receiving neonatally an intraventricular injection of LY43578 and control groups of males and females receiving saline in the same conditions. Values are means ± SEM. *$p < 0.05$.

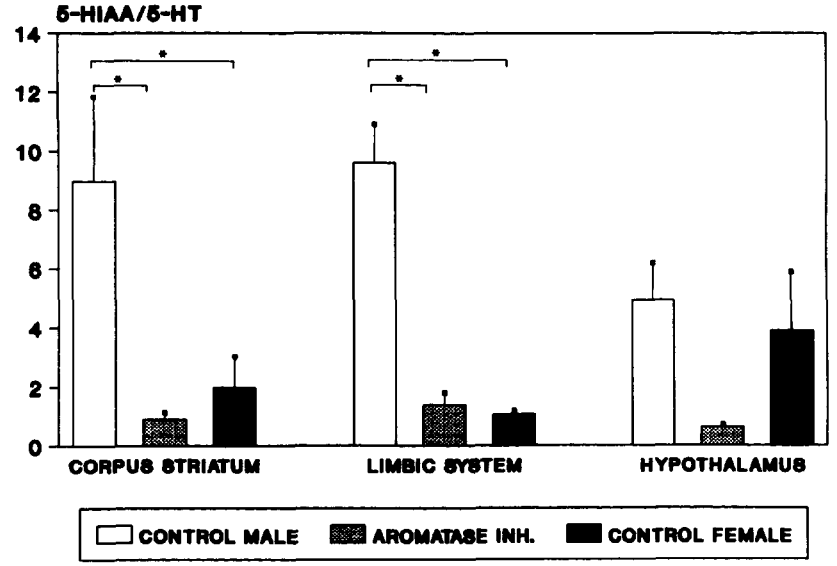


FIG. 1. Serotonergic metabolism (5-HIAA/5-HT ratio) in the corpus striatum, limbic structures, and mediobasal hypothalamus of adult male rats neonatally intraventricularly injected with LY43578 (5 mg/kg) or saline and control females injected with saline in the same conditions. Values are means ± SEM. *$p < 0.05$.

tion (6,10,16), while nonaromatizable androgens, such as 5-α-dihydrotestosterone (5-α-DHT), fail to prevent feminization or induce masculine behavior (1). However, other authors have found a masculinizing effect of nonaromatizable androgens such as 5-α-DHT (12,15).

From our data, it appears that intraneurally aromatized estrogen is an essential step in the development of the masculine organization of the brain and prevention of feminization. But, at the same time, only a partial inhibition of masculine behavior was observed, but was not as dramatic as the total inhibition described for gonadectomized males. This lack of complete masculinization also suggests a possible role for androgens themselves in brain masculinization, perhaps acting together with estrogens in the normal organization of male sexual behavior. At the same time, we cannot forget the possible effect of prenatal aromatization (9), which was not modified in this study. A former determination of the different critical periods within the critical period could be a next step in this investigation.

The changes in sexual behavior do not seem to be related to the hormonal levels, which were not affected by the treatment.

A connection might exist between the behavioral changes

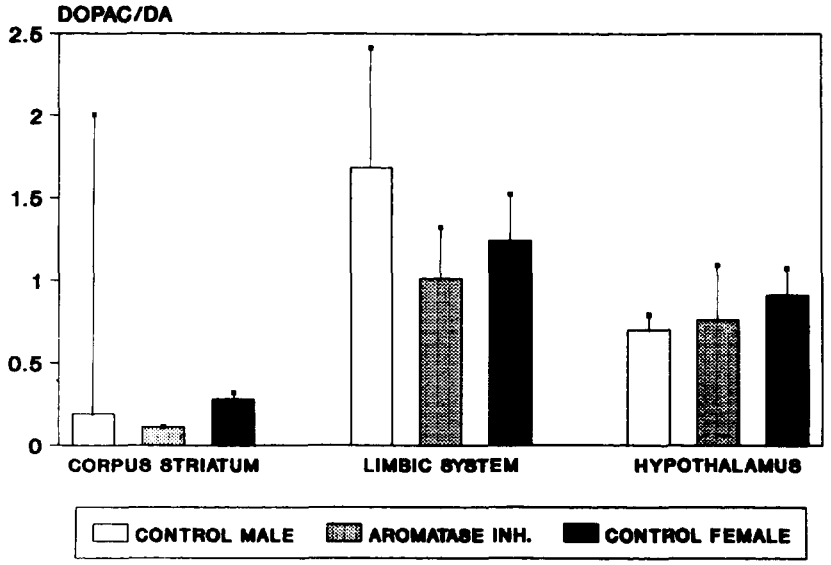


FIG. 2. Dopaminergic metabolism (DOPAC/DA ratio) in the corpus striatum, limbic structures, and mediobasal hypothalamus of adult male rats neonatally intraventricularly injected with LY43578 (5 mg/kg) or saline and control females injected with saline in the same conditions. Values are means ± SEM.

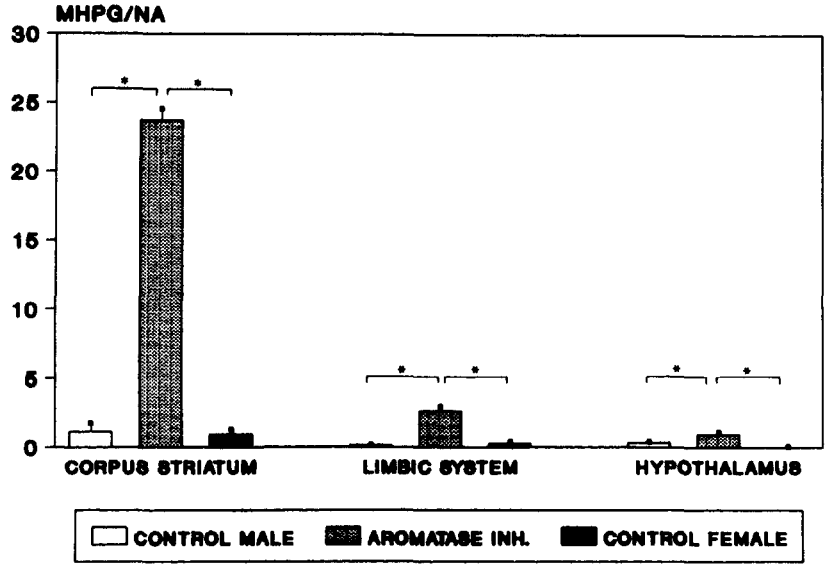


FIG. 3. Noradrenergic metabolism (MHPG/NA ratio) in the corpus striatum, limbic structures, and mediobasal hypothalamus of adult male rats neonatally intraventricularly injected with LY43578 (5 mg/kg) or saline and control females injected with saline in the same conditions. Values are means ± SEM. *$p < 0.05$.

and the indolaminergic alterations produced by this neonatal treatment. Serotonin metabolism showed a sexual dimorphism between control males and control females in terms of 5-HIAA/5-HT ratio, and treatment with the aromatase inhibitor decreased this serotonergic index to female-like levels not in the hypothalamus but in the two extrahypothalamic studied areas.

Serotonin has been proposed as a neurotransmitter involved in sexual differentiation mechanisms, mainly in the hypothalamus (4). Our results confirm that a certain concentration of cerebral estrogen is required for normal serotonergic development, which may be related to masculinization of the brain. The cathecholaminergic contribution to this mechanisms is less clear from our results, and serotonin appears to be the main neurotransmitter involved in sexual masculinization induced by aromatization.

Extrahypothalamic areas such as the corpus striatum and the limbic system have previously been reported to be involved in sexual differentiation (8). Striatal contribution to sexual differentiation can be supported by data reported by other authors (13) that provide evidence that the striatum is a site for neuroendocrine interactions. The limbic system (especially the septum and hippocampus) is also involved in sexual behavior and hypothalamic and pituitary secretion (18), providing a possible implication of this area in sexual differentiation of the brain. Interestingly, hypothalamic tissue did not show a sexual difference or a significant effect of the drug. Traditionally, this has been the main area associated with sexual behavior, and high aromatase activity has been detected in hypothalamic areas (14). From this study, extrahypothalamic areas seem to be more involved in monoaminergic sexual differentiation, which suggests a wide field to study many cerebral areas not directly associated with sexual behavior.

An increase in the noradrenergic ratio (MHPG/NA) occurred in the aromatase-inhibited males, compared both to control males and females, in the three studied areas. This activatory effect could depend on a direct effect of the LY43578, but does not seem to be sexually dimorphic. A comprobation of the effect of this compound on females would be necessary.

From this study, we conclude that aromatization of intraneural androgen is of importance in normal masculinization of the brain, and we suggest a possible involvement in this process of serotonergic extrahypothalamic cerebral areas.

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