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# Extrahypothalamic Serotonergic Modification After Masculinization Induced by Neonatal Gonadal Hormones

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GONZÁLEZ, M. I. AND M. L. LERET. *Extrahypothalamic serotonergic modification after masculinization induced by neonatal gonadal hormones.* PHARMACOL BIOCHEM BEHAV **41**(2) 329-332, 1992.—Exposure to gonadal steroids during the critical period exerts an organizational effect on the CNS. This hormonal effect could be mediated, at least in part, by neurotransmitters. Traditionally, the main place involved in the aminergic sexual differentiation has been the hypothalamus. The aim of this work was to examine the possible long-term effect of cerebral administration of testosterone or estradiol on sexual behavior and hypothalamic/extrahypothalamic monoaminergic systems in the adult rat. For this purpose, female Wistar rats were intraventricularly injected during the first 24 h of life with testosterone (T) or estradiol benzoate (EB) (200 μg/kg) (male and female control groups were vehicle treated) and sexual behavior and monoaminergic mediobasal hypothalamic, striatal, and limbic metabolism in adult rats were studied. Receptive behavior was not affected, whereas a masculinizing effect (% mounts) was observed in the animals treated with both gonadal hormones. Only testosterone-treated females showed a male-like serotonergic ratio in corpus striatum and limbic system. A possible extrahypothalamic serotonergic role could be suggested in the mechanisms of sexual differentiation.

Sexual behavior Catecholamines Serotonin Neonatal Sexual hormones Extrahypothalamic Intraventricular Masculinization

IT is well known that exposure to gonadal steroids during the critical period exerts an organizational influence upon the developing CNS (11,25).

Estrogens (mainly converted from androgens within the brain) are believed to be involved in the differentiation of the "sex centers" (6). Estrogens and androgens are known to exert an organizational influence on the "mating centers" (21), and "gender role centers" seem to be organized only by androgens (19).

It is thought likely that steroids influence neuronal organization since there are steroid-dependent sexually dimorphic neuronal structures in specific hypothalamic areas (1,12), but extrahypothalamic areas have also been implicated in sexual differentiation (17). There is physiological, biochemical, pharmacobehavioral, and clinical evidence suggesting that the corpus striatum is a site of neuroendocrine interface (23). The limbic system (especially the septum and the hippocampus) is also involved in sexual behavior and hypothalamic and pituitary secretion (27).

The biochemical mechanisms by which steroids exert their effects on the CNS are still obscure. The biogenic amines noradrenaline (NA), dopamine (DA), and serotonin (5-HT) are involved in normal neuroendocrine function and a direct effect of androgens on the development of one or more of these neurotransmitter systems could be expected to have profound and permanent consequences. Sex differences have been found in neurotransmitter content (26) and monoaminergic receptors (9,22). It follows, then, that neurotransmitters may be involved in sexual differentiation. This hypothesis is supported by the fact that administration of pharmacological agents known to alter neurotransmitter activity can affect sexually differentiated behavior in adulthood (8,16).

The present study was designed to examine the possible influence of central neonatal gonadal steroid administration on sexual behavior and both hypothalamic or extrahypothalamic monoaminergic differentiation patterns.

## METHODS

Female Wistar rats were injected with estradiol benzoate (EB) 200 μg/kg ($n$ = 19) or testosterone (T) 200 μg/kg ($n$ = 10) during the first 24 h of postnatal life. Male ($n$ = 17) and

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female ($n$ = 15) groups were injected with an equivalent volume of vehicle alone (controls). All injections were given via 1 $\mu$l corn oil vehicle administered intraventricularly. Injections were carried out under cold anesthesia, which was induced by placing the 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.

All animals were kept under controlled conditions of 12L: 12D (lights on at 0800) and temperature (21 ± 1°C). They were given free access to food and water.

Rats were given behavioral tests at 80–85 days of age. Feminine sexual activity was tested 8 h into the dark period under red illumination by placing animals with vasectomized vigorous males in the heterotypical behavior test and noting their lordotic responses to 10 mounts. The results were expressed as a lordosis quotient [LQ = (no. of lordoses/no. of mounts) × 100]. Only receptive females whose natural estrous had been verified by vaginal smears were used. All animals were cycling regularly. We avoided any manipulation (ovariectomy, hormonal-induced estrous) that might have disguised the potential physiological or hormonal modifications induced by the treatments. Masculine behavior was observed by placing each animal in an arena and 5 min later introducing a sexually receptive female. The percentage of females showing mounting behavior was noted. The test was terminated after 10 min.

All animals were decapitated at 90–95 days of age, at the same time of the day at which behavioral testing had been performed. The mediobasal hypothalami, corpus striata, and limbic structures (hippocampus, septum, olfactory cortex) were dissected (3), weighed, and stored at −40°C until assayed for biogenic amines (max. storage time 3 weeks). Tissue weights varied between 2–20%. Samples were homogenized in 1 ml cold 0.2 N perchloric acid containing 0.4 mM odium bisulphite and 0.4 mM EDTA. 3,4-Dihydroxybenzylamine was also added to each sample as an internal standard to control for procedural losses. The homogenates were centrifuged (15000 rpm, 5 min at 3–5°C) and supernatants frozen pending amine determination using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Limbic structures were assayed together. Separation of amines was effected with an RP-18 column (22 cm, 4.6 mm, 5 $\mu$m 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 [DA, NA, 5-HT], 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenilacetic acid (DOPAC), homovanillic acid (HVA), and 4-hydroxy-3-metoxyphenylglycol (MHPG)] were measured at a potential of 0.80 V relative to a 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*

Monoaminergic contents were tested for significant differences by Scheffé's test after one-way analysis of variance (ANOVA) using metabolic index (metabolite concentration/amine concentration). Behavioral results were compared by means of Mann–Whitney test after Kruskall–Wallis one-way ANOVA.

TABLE 1

SEXUAL BEHAVIOR IN FEMALES: FEMININE AND MASCULINE BEHAVIOR

| Neonatal Treatment | $n$ | LQ | % Mount | Mounts |
|---|---|---|---|---|
| Control females | 15 | 100 ± 0 | 0% | 0 ± 0 |
| T Females | 10 | 100 ± 0 | 6.0%* | 5.3 ± 1.3* |
| EB Females | 19 | 100 ± 0 | 4.2%* | 4.6 ± 2.5* |

LQ in heterotypical behavior test; percentage of females showing mounting behavior in homotypical behavior test, as well as number of mounts (mean ± SEM). *$p < 0.05$. $n$, number of animals.

## RESULTS

### *Behavioral Tests*

Receptivity (LQ) was not affected by the hormonal treatments (Table 1). Typical male behavior (mounts) was observed either in EB-treated females or in T-treated animals.

### *Monoaminergic Metabolism*

Neither EB nor T neonatal treatments affected noradrenergic metabolism in the areas studied (Fig. 1). No significant increments appear in the noradrenergic ratio in limbic system, and a very low ratio is shown in female controls in hypothalamus (Fig. 1).

Serotonergic ratio (Fig. 2) was significantly higher in males than females, both in the corpus striatum and the limbic system. In these two extrahypothalamic areas, but not in the medial basal hypothalamus, T-treated females showed levels similar to males, while EB-treated females remained at control female levels.

Dopaminergic metabolism (Fig. 3) was not modified by the treatments.

## DISCUSSION

A sexual dimorphism has been described in hypothalamic noradrenergic systems, but some authors report higher levels in males (4), while others found higher levels in females (15).

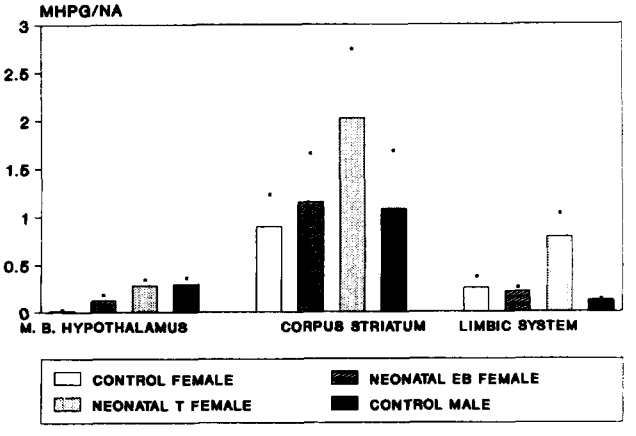


FIG. 1. Noradrenergic metabolism (NA/MHPG level) in the mediobasal hypothalamus, corpus striatum, and limbic structures of female adult rats neonatally intraventricularly injected with oil, estradiol benzoate, or testosterone, and control males. Values are mean ± SEM. No differences were shown.

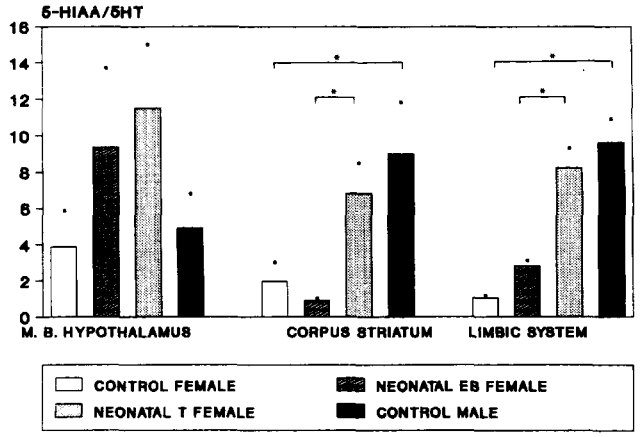


FIG. 2. Serotoninergic metabolism (5-HIAA/5-HT level) in the mediobasal hypothalamus, corpus striatum, or limbic structures of female adult rats neonatally intraventricularly injected with oil, estradiol benzoate, or testosterone, and control males. Values are mean ± SEM. *$p < 0.05$.

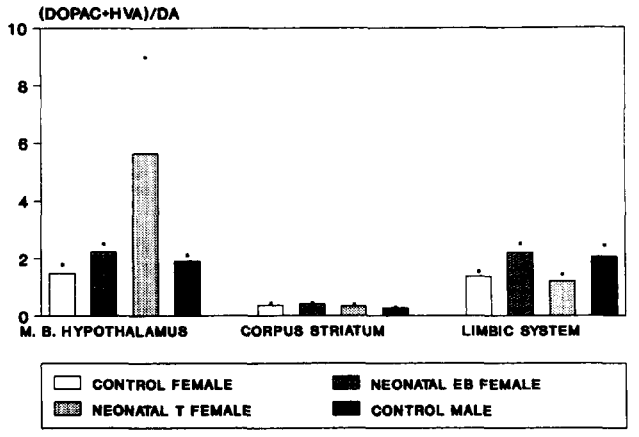


FIG. 3. Dopaminergic metabolism (DOPAC + HVA/DA level) in the mediobasal hypothalamus, corpus striatum, or limbic structures of female adult rats neonatally intraventricularly injected with oil, estradiol benzoate, or testosterone, and control males. Values are mean ± SEM. No differences were shown.

We did not find a sexual noradrenergic dimorphism between control male and control female groups in terms of activity index, and neither the EB nor the T neonatal treatment showed any effect in the adult noradrenergic metabolism.

Hypothalamic dopamine has been reported to be differently affected by neonatal treatment, depending on the region studied. The tuberoinfundibular dopaminergic system is permanently affected by neonatal androgen (5), but a different sexual differentiation exists between the tuberoinfundibular and the incertohypothalamic dopaminergic systems. In the mediobasal hypothalamic dopaminergic system studied in this work, no sexual differences were found. These results are supported by in vitro diencephalic studies that did not reveal any sex steroid influence (24), suggesting that the possible sexual dimorphism described by other authors in the diencephalic dopaminergic system could be mediated in a mechanism independent of gonadal hormones.

Extrahypothalamic areas have been previously reported to be involved in catecholaminergic sexual differentiation after neonatal castration (17), but there is no evidence about sexual differences of DA or NA in these areas. In our study, no significant differences were found between sexes or after the steroid administration neither in the corpus striatum nor in the limbic system in the DA or NA ratios.

Hypothalamic serotonergic metabolism was not affected by neonatal steroid treatment. A sexual dimorphism has been described in this system during the second week of life (10), this difference disappearing at adulthood. In the corpus striatum and limbic system, a sexual difference developed, the males showing a higher serotonergic ratio than the females. Neonatally androgenized females showed levels similar to males, but, interestingly, EB-treated females remained at control female levels. There is a reported relationship between testosterone and serotonin in terms of differentiation of sexual behavior (28), and our data verified this finding, but only in extrahypothalamic areas.

There have been different critical periods proposed for higher mammals in which testosterone or estrogen each play a distinct role (2). This reported specificity of hormonal influence agrees with our results. A specific cerebral localization required for sexual differentiation of the brain was also suggested in the present investigation.

Concerning sexual behavior, a number of authors have reported a stimulatory effect on receptivity of either EB or T (13,18). In our results, neonatal IV administration of both sex steroids resulted in total receptivity, but as control females were already totally receptive we cannot attribute a stimulatory effect to sex hormone administration. Neonatal androgenization produced an elevated incidence of male-like behavior in females, a result consistent with those reported by other authors (14,20). EB treatment resulted in similar behavioral changes, suggesting the importance of aromatization in this process.

Both hormonal treatments showed a masculinizing effect in terms of mounting behavior, but only neonatally administered T seemed to masculinize the serotonergic ratio. Consequently, there is not a clear relationship between behavioral and neurochemical data. Various factors seem to influence sexual differentiation of the several facets involved in reproductive function (7). Our study suggests the existence of different mechanisms modulating behavioral or serotonin masculinization. A clear masculinization of sexual behavior appeared both after intraventricular estrogen or androgen neonatal administration. Only T-treated females showed a male-like serotonergic ratio in the corpus striatum and limbic system; a possible extrahypothalamic serotonergic role could be suggested in the sexual differentiation mechanisms.

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