Effect of estrogens on LH- and FSH-levels in prepuberal male and female androgenized rats[1]

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Summary. This paper has demonstrated that neonatal androgenization increases in prepuberal male and female rats the FSH-levels without changes in LH, indicating that the sexual differences in the control of FSH is a relevant process that takes place during the differentiation of the hypothalamus.

It is currently assumed that testosterone, which is secreted by the testes around the time of birth, affects certain hypothalamic areas, so that the cyclic hypothalamic control of gonadotrophin secretion typical of the female is suppressed and a masculine or 'tonic' development ensues[3]. This tonic control characteristically shows higher levels of FSH[4]. Moreover, neonatal administration of androgens produced in the male the development of an hypertonic hypothalamus with higher levels of FSH than in normal males[5]. In a previous paper[6] it has been demonstrated that the administration of estrogens to prepubertal female rats, 31 days old, produced an increase in the ovarian weight in the normals and a decrease in the neonatal androgenized rats; and we proposed that androgenization affects the development of the hypothalamic mechanisms implicated in the positive feed-back effect of estrogens on gonatrodophin secretion. Since such positive action of estrogens appears to be a very important mechanism in the female, implicated in the ovulatory process, it seems to be of interest to compare the effect of estrogens on LH- and FSH-secretion, separately, in prepuberal androgenized female with normal male and female rats. These studies were also extended to androgenized male rats, in which alterations in the hypothalamic control of gonadotrophin secretion were also described[8].

Material and methods. Immature male and female rats of the strain of the Institute of Physiology, Buenos Aires Medical School, maintained in temperature and light (12 h light/day) controlled quarters with free access to Purina Laboratory chow and tap water were used. Androgenization was accomplished by injecting the pups with 1 mg of testosterone propionate dissolved in 0.1 ml of sesame oil on the 2 nd postpartum day. Controls were injected with the vehicle. Normal and androgenized rats were injected s.c. at 29 days old either with 10 µg of estradiol benzoate dissolved in 0.1 ml peanut oil, or with the vehicle (control groups). Animals were sacrificed by decapitation 24 h after the injection, and blood was taken from the trunk. Blood samples were allowed to clot at 4 °C, centrifuged and serum separated and kept frozen until assayed. LH- and FSH-concentration in serum was assayed at 2 dose levels, using a radioimmunoassay kits supplied by the NIAMD-Rat Pituitary Program. Pure rat LH and FSH were iodinated using the cloramine T-method[6]. Concentrations of gonadotrophins were expressed in ng/ml on the basis of the standard supplied (RP-1).

The significance of the difference between the groups was calculated by variance and by the Tukey's method[8].

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Table 1. Effect of estrogens on LH- and FSH-secretion in normal and androgenized prepuberal female rats

Groups	LH (ng/ml)	FSH (ng/ml)
A. Control	33.1 ± 5.2* (9)	161.7 ± 14.4 (9)
B. Control + estrogens	51.7 ± 6.3 (9)	228.2 ± 18.3 (9)
C. Androgenized	36.1 ± 5.3 (10)	260.8 ± 30.3 (10)
D. Androgenized and estrogens	13.6 ± 2.0 (10)	145.3 ± 22.2 (10)
Ratio	$p < 0.01$	$p < 0.01$
$p < 0.01$ between	A vs B A vs D B vs C B vs D C vs D	A vs B A vs C B vs D C vs D

*Mean ± SE. Number of animals are in parenthesis.

Table 2. Effect of estrogens on LH- and FSH-secretion in normal and androgenized prepuberal male rats

Groups	LH (ng/ml)	FSH (ng/ml)
A. Control	30.8 ± 2.1 (10)	408.2 ± 43.3 (12)
B. Control and estrogens	15.5 ± 3.1 (10)	174.3 ± 26.7 (12)
C. Androgenized	33.8 ± 6.4 (11)	538.0 ± 30.2 (11)
D. Androgenized and estrogens	14.3 ± 3.1 (11)	59.3 ± 6.0 (11)
Ratio	$p < 0.01$	$p < 0.01$
$p < 0.01$ between	A vs B A vs D B vs C C vs D	A vs B A vs C A vs D B vs C B vs D C vs D

*Mean ± SE. Number of animals are in parenthesis.

Results. As can be seen in the tables 1 and 2, neonatal androgenization increases the FSH-levels without altering LH-concentrations in prepuberal male and female rats of 30 days of age. Table 1 shows that, while normal female rats treated with estrogens, showed 24 h later higher levels of LH and FSH than the controls, the administration of estrogens induced in the androgenized group a significant decrease in the levels of both gonadotrophins. On the other hand, the administration of estrogens to prepuberal male rats, 30 days old (table 2), produced a reduction in the LH- and FSH-levels in normal and androgenized rats, but the inhibition in the FSH-levels observed in the androgenized group, was significantly greater than in the controls (89% vs 57% respectively). Consequently, FSH-concentrations were lower in the androgenized male rats treated with estrogens than in the controls with a similar treatment.

Discussion. The results here reported showed that neonatal androgenization increases the FSH-levels in prepuberal male and female rats. According to the current concepts on the sexual differentiation[3], it could be postulated that the increase in the FSH-levels observed in the androgenized female rats is connected with the development of a tonic or male control of gonadotrophin secretion, since the prepuberal stage that characteristically shows higher levels of FSH than the female type of control[4]. On the other hand, the higher levels of FSH observed in the androgenized male rats as compared with the normals, gives an additional support to the hypothesis that the presence of androgens in the first days of life is responsible for the higher levels of FSH that characterize the male control of gonadotrophin secretion, and also shows that an additional dose of testosterone during the first days of life is able to develop in the prepuberal male a type of pituitary control with higher levels of FSH than those observed when physiological levels of androgens are present. While the administration of estradiol to prepuberal female 30-day-old rats induced an increase in the LH and FSH, there was a significant reduction of both gonadotrophins in androgenized female rats as well as in normal and androgenized male rats. These results appear to indicate that neonatal androgenization in the female rats, besides increasing FSH-levels, altered the normal mechanisms implicated in the development of the positive feed-back effect of estrogens on gonadotrophins secretion. Since estrogens produced a similar reduction in FSH- and LH-levels in normal male and androgenized female, it could be considered that the positive feed-back effect of estrogens is also a sexually differentiated function and its development is impaired by the presence of testosterone in the first days of life.

The androgenization of male rats increased the FSH-sensibility to the negative feed-back effect of estrogens. This fact could be additional evidence that the mechanism of control of FSH is a relevant process that takes place during the sexual differentiation of hypothalamus, influenced by the presence of sexual hormones in the first days of life.

Radioprotective effects of cyproterone acetate

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Summary. The pretreatment of irradiated mice by cyproterone acetate had a better radioprotective effect in comparison with orchidectomy, although the weight of the thymus was lower in the cyproterone acetate group. The radioprotective mechanisms involved in both cases were discussed.

The reduced immunocompetence accompanying the thymolytic effect of androgens is expected to enhance the sensitivity of mammals to ionizing radiation[2]. Nevertheless, the reports on the radiosensitizing effects of androgens (and on the radioprotection provided by estrogens) are rather controversial[3]. This may be due to the role of the time-interval between irradiation and the intervention in the hormonal status – during this period, the regeneration of the thymus and the immunocompetence may be restored. Male mice were therefore pretreated by the antiandrogen cyproterone acetate. After a subsequent irradiation, the mortality was compared with that of irradiated mice having no pretreatment. The expected radioprotective effect of cyproterone acetate was obtained, but the administration of this drug to irradiated mice was accompanied at the age of 14 weeks by a decrease in weight of the thymus, not observed in irradiated mice pretreated by orchidectomy. The mechanism of radioprotection induced by cyproterone acetate therefore remains to be explained.

Materials and methods. 5-week-old male mice (Velaz breeding) were kept in groups of 10–20 animals in plastic boxes. The mice were daily exposed to light for 12 h, the room temperature was maintained at 24 ± 2 °C. In group 1 castration was performed in ether narcosis at the age of 5 weeks. After the ligation of the ductus deferens, the testes and epididymis were removed and the wound was powdered with neomycin and bacitracin (Framykoin, Spofa). In the control group (2), a sham operation (opening of the scrotum) was executed. In group 3 cyproterone acetate (6-chloro-17-hydroxy-1,2-methylene-pregna-4,6-dien-3,20-dione acetate, Androcur Schering AG, Berlin) was added to the food at the age of 7 weeks in a daily dose of 1 mg/100 g b.wt. The drug was administered for 14 days, the amount of food consumed by 1 animal was in general 3 g a day. At the age of 10 weeks (5 weeks after castration, 3 weeks after the beginning of cyproterone acetate administration and 1 week after stopping this medication), all 3 groups were irradiated by ^{60}Co. A 4th group with no other intervention was also irradiated at the age of 10 weeks. The exposure was 700 rad with a

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