Effect of neonatal testosterone and oestradiol treatment on the development of the hypothalamo-hypophyseal axis in the female rat

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Summary. The hypothalamic LH-RH content and the concentrations of pituitary and plasma LH were measured at various ages in female rats treated daily with 10 µg testosterone propionate or 10 µg oestradiol-17β from birth to Day 15. Persistent vaginal oestrus was induced in all the treated rats. Both hormones significantly reduced the hypothalamic LH-RH content and pituitary and plasma LH concentrations. Hypothalamic LH-RH increased after cessation of treatment but pituitary LH did not return to normal levels. Plasma LH levels were significantly lower than those in control rats. It is concluded that testosterone propionate and oestradiol-17β (1) have a direct negative feed-back influence on the hypothalamus in the neonatal female rat; (2) alter the normal pattern of plasma and pituitary LH in developing female rats; (3) prevent the cyclic secretion of plasma LH after maturity; and (4) probably cause a chronic impairment in the release of LH-RH.

Introduction

An extensive literature indicates that the differentiation of the hypothalamus is determined by androgens during the neonatal period (Barraclough, 1968; Gorski, 1971). The presence of endogenous or exogenous androgens leads to the development of a masculine pattern of gonadotrophin secretion. Administration of aromatizable androgens or oestrogens to neonatal female rats alters the sexual differentiation of the neuroendocrine mechanisms and results in the development of an 'anovulatory, persistent oestrus syndrome' (Harris, 1964; Barraclough, 1966a, b; Flerko, 1968; Gorski, 1971). The failure of non-aromatizable androgens to influence cyclic activity when administered to neonatal female rats (Luttage & Whaler, 1970; Brown-Grant, Munck, Naftolin & Sherwood, 1971) and the ability of antioestrogens to antagonize the action of testosterone (Doughty & McDonald, 1974) support the concept that testosterone may exert its influence on the differentiation of the hypothalamus by its conversion to oestrogen.

Segal & Johnson (1959) reported that androgenized females had more pituitary LH than normal female rats, but Gorski & Barraclough (1962) refuted these findings and showed that androgenized female rats have pituitary LH levels as low as those in normal oestrous females. However, Dupon & Schwartz (1971) reported that postpubertal androgenized females had more pituitary LH than normal females, the values being slightly less than in normal male rats. Barraclough (1968) and Barraclough & Haller (1970) found higher levels of plasma LH in androgenized females compared to those in normal female rats, while Brown-Grant *et al.* (1971) could not demonstrate elevated serum LH levels in androgen-treated females.

Data relating to pituitary and plasma gonadotrophin levels in persistent-oestrous rats are therefore controversial. The purpose of the present study was to investigate the possible mechanisms involved in the alteration of sexual differentiation; the effects of neonatal treatment of female rats with testosterone and oestradiol on the levels of hypothalamic LH-RH and pituitary and plasma LH were studied.

Materials and Methods

Rats of the Holtzman strain were maintained in temperature- ($26 \pm 1^\circ\text{C}$) and light- (14 h light/24 h) controlled rooms, were fed a standard pellet diet (Hindustan Lever) and had free access to water. Rats of known ages were killed between 10.00 and 11.00 h by decapitation and blood was collected in heparinized tubes, centrifuged and the plasma stored at -20°C until analysed. Each female was given a daily s.c. injection of 10 μg testosterone propionate or 10 μg oestradiol-17 β in 0.05 ml olive oil from the day of birth (Day 0) until Day 15 of age. Control animals were not given any treatment. The animals were weaned on Day 21, and examined daily for vaginal opening after which vaginal smears were taken daily. Rats which showed an oestrous smear for 10 days were considered to be in constant oestrus. Experimental and untreated control rats were killed on Days 5, 7, 10, 14, 17, 22, 25, 30, 37, 45, 52 and 60. For prepubertal rats (<30 days of age) each plasma pool was derived from 8 or 9 animals; plasma was collected from 3 or 4 of the older animals and pooled. Each observation was based on the results obtained with 3 or 4 pools.

Hypothalami were dissected and homogenized in ice-cold 0.1 N-HCl, and centrifuged at 800 g for 30 min at 4°C . The supernatant was used for the measurement of LH-releasing activity as described by Crichton, Schneider & McCann (1970) with some modifications.

Anterior pituitaries were incubated in Medium 199 (Burroughs Wellcome, London) containing synthetic LH-RH (NIH, Bethesda, U.S.A.) or hypothalamic extract for 6 h at 37°C in a metabolic shaker, preceded by preincubation of pituitaries for 30 min without standard or samples. LH released into the medium was determined by radioimmunoassay. A standard curve for synthetic LH-RH was constructed on the basis of the amount of LH released/mg pituitary.

Concentrations of LH were measured by a double-antibody radioimmunoassay procedure similar to that described by Niswender, Midgley, Monroe & Reichert (1968) using the kit supplied by the Rat Pituitary Distribution Program, NIAMDD, Bethesda, U.S.A., and LH levels are expressed in terms of ng equivalents of the LH-RP1 preparation. Plasma samples were analysed at three dose levels using 20, 100 and 200 μl aliquots. Pituitary and medium samples were diluted 1:20 with phosphate-buffered saline, pH 7.2. The inter- and intra-assay coefficients of variation were 12 and 7% respectively.

The significance of differences between means was determined by Student's paired *t* test: a level of 5% was taken to be significant.

Results

Vaginal changes

Oestradiol treatment hastened the day of vaginal opening: 70% of the rats had a perforate vagina by Day 20 and 98% by Day 30. In the control and testosterone-treated rats vaginal opening was observed by Day 37. All the treated rats exhibited persistent vaginal oestrus by 40–50 days of age.

Testosterone treatment

Hypothalamic LH-RH. A marked reduction in the content of hypothalamic LH-RH was observed during the period of treatment, Days 1–15 (Text-fig. 1a). Immediately after the withdrawal of testosterone, i.e. after Day 14, there was a significant increase in hypothalamic LH-RH content. Levels were high until Day 25 and were thereafter low, except on Day 45.

Pituitary LH. The concentration of pituitary LH in the testosterone-treated rats was significantly lower than that of control female rats throughout the period studied (Text-fig. 1b). After withdrawal of testosterone, the concentration of pituitary LH increased significantly after Day 22. Thereafter, the levels fluctuated in a manner similar to that of the hypothalamic LH-RH levels.

Plasma LH. Testosterone treatment markedly reduced the concentration of plasma LH in neonatal female rats compared with those of normal females (Text-fig. 1c). The LH levels remained almost static after Day 37.

Text-fig. 1. Changes in (a) the hypothalamic LH-RH content and in the concentrations of (b) pituitary LH and (c) plasma LH in rats from 5 to 60 days of age when untreated (●), treated with testosterone propionate (×, 10 µg/day daily from Days 1–15) or treated with oestradiol-17β (○, 10 µg/day daily from Days 1–15).

Oestradiol treatment

Hypothalamic LH-RH. Administration of oestradiol significantly reduced the content of hypothalamic LH-RH on Days 5 and 7 but not on Day 10 (Text-fig. 1a). There was a significant increase on Day 30 and levels thereafter remained significantly higher than those of the control rats.

Pituitary LH. After Day 10 the concentrations of pituitary LH were significantly lower in all groups of oestradiol-treated rats than in the control animals (Text-fig. 1b). After Day 30 LH levels rose and subsequently remained significantly higher than in younger rats.

Plasma LH. After an initial fall, plasma LH levels remained fairly constant, albeit significantly lower in all groups than those of control rats (Text-fig. 1c).

Discussion

In the present study relatively high doses of testosterone propionate (10 µg) and oestradiol-17β (10 µg) were used over 15 days. This was necessary because Sheridan, Zarrow & Denenberg (1973) had observed that when 1–3 µg testosterone propionate were administered to neonatal rats for 10 days 85–90% of the females failed to exhibit constant oestrus by 50 days of age.

Our results substantiate and provide direct evidence for the concept that the hypothalamo-hypophysial axis of neonatal rats is sensitive to feed-back effects of gonadal steroids. Administration of testosterone propionate or oestradiol-17β to neonatal female rats significantly decreased the content of hypothalamic LH-RH, indicating that both hormones have a direct negative feed-back effect on the hypothalamus. The inhibition of FSH-RH by short-term administration of sex steroids in the adult rat (Mittler & Meites, 1966; Martini, Fraschini & Motta, 1968a, b) supports our finding.

The increased content of hypothalamic LH-RH, compared with control levels, after the withdrawal of testosterone or oestradiol treatment while the levels of pituitary and plasma LH fail to reach normal levels indicates that these steroids cause a chronic impairment of the release of LH-RH. It is apparent that synthesis of LH-RH is not permanently altered because the hypothalamic LH-RH content does rise above the control levels. However, the possibility of an LH-RH accumulation because of its impaired release cannot be ruled out.

The present results show that neonatal testosterone or oestradiol treatment significantly decreases the concentration of LH in the pituitary of rats at all the ages studied. Matsuyama, Weisz & Lloyd (1966) and Barraclough & Haller (1970) also reported markedly reduced pituitary LH concentrations in persistent-oestrous rats. The characteristic increased pituitary LH concentrations observed on Days 7 and 17 in normal prepubertal rats are not seen in oestradiol-treated rats. The LH increase on Day 17 in the testosterone-treated rats may be an immediate effect of testosterone withdrawal rather than a characteristic peak. Failure of pituitary LH to reach control levels after the withdrawal of testosterone or oestradiol treatment indicates that neonatal exposure of female rats to these steroids permanently alters the mechanisms controlling the synthesis of LH in the pituitary.

Treatment of neonatal female rats with testosterone or oestradiol therefore permanently alters the mechanism responsible for release of LH-RH, prevents the normal synthesis and storage of LH in the pituitary, and the cyclic release of LH into the plasma.

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