Androgen Effect on Gonadotropin Secretion by Organ-Cultured Anterior Pituitary[1] (36628)

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It is well established in rats that testicular hormones act through feedback mechanisms to inhibit the secretion of gonadotropins by the anterior pituitary. Since castration results in rapid and dramatic increases in plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations (1–3) and because treatment with testosterone prevents or reverses some castration responses (1, 3, 4), it is generally concluded that testosterone is an important component of this gonadotropin-inhibiting activity of the testis.

On the other hand, Bogdanove (4) found that whereas castration of male rats resulted in increased pituitary FSH stores, androgen treatment caused further increases in pituitary FSH content. Kingsley and Bogdanove (personal communication) observed that minute unilateral implants of testosterone propionate into the pituitary glands of castrated male rats consistently increased the ratio of FSH content to LH content on that side as compared to the contralateral side. Also, the data of Johnson and Naqvi (5, 6) imply a stimulatory effect of androgen upon FSH release in prepubertal female rats.

In the present study, a positive effect of androgen on rat pituitary FSH secretion was observed under conditions chosen to minimize possible participation of other organs.

Materials and Methods. Organ cultures of rat anterior pituitaries were performed for 5 days as described previously (7). Male Sprague-Dawley rats (Camm Research Institute) weighing 150–200 g were used as tissue donors. Each anterior pituitary was removed and cut into 4 to 6 explants. The fragments were mounted in petri dishes at the interface between a 95% O_2–5% CO_2 atmosphere and 5 ml of medium consisting of 10% newborn calf serum and 90% medium 199 (Microbiological Associates, Bethesda, MD). Penicillin (25 units/ml), streptomycin (25 μg/ml), and dibutyryl cyclic AMP (4×10^{-5} *M*) were added also. A temperature of 36° was maintained.

Opposite sides from the same pituitaries provided matched control and experimental tissue preparations. In each experiment, fragments from 3 pituitaries were distributed to 2 dishes. Experimental sides were exposed to testosterone (Sigma Chemical Co., 30 ng/ml) or to dihydrotestosterone (Sigma, 30 ng/ml). One microliter of absolute ethanol per milliliter of medium was used as vehicle for addition of steroids and was always added to controls. Viability was confirmed by histological examination of glands from additional rats maintained separately but treated similarly in each experiment. Experiments designed to test stability of FSH and LH revealed no evidence of inactivation with prolonged incubation.

Medium was replaced daily and the previous day's medium was stored frozen until radioimmunoassayed for FSH and LH using the materials provided by the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Hormone Distribution Program. FSH assays (8) were done with NIAMD anti-rat FSH serum-2 and are expressed in terms of micrograms of NIAMD rat FSH RP-1. LH assays (9) utilized NIAMD anti-rat LH serum-2 and are expressed as micrograms of NIAMD rat LH RP-1. Samples from each experiment were assayed in duplicate in the optimal portion of the standard

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TABLE I. FSH and LH Release (μg/Pituitary) During Final 3 Days of 5 Day Period *in Vitro*.

Treatment	No. of expts.	FSH in medium	LH in medium	FSH:LH ratio
Control	6	14.7 ± 1.4[a]	14.5 ± 2.0	1.10 ± 0.12
Testosterone		23.9 ± 2.5[b]	12.4 ± 1.5	2.16 ± 0.41[c]
Control	3	12.9 ± 0.8	14.8 ± 2.0	0.90 ± 0.07
Dihydrotestosterone		17.9 ± 2.2	11.3 ± 0.02	1.52 ± 0.18[c]

[a] Mean ± standard error.
[b] $p < .01$.
[c] $p < .05$.

curve. Results are expressed as reference standard hormone per two pituitary halves or as ratio of FSH to LH (μg) with calculations of mean and standard error of the mean based on one value for each experiment. Probability was calculated using the *t* test for paired observations.

Results. In six separate experiments with testosterone, combined for analysis, control tissues released 53.7 ± 3.6 μg (mean ± standard error) of FSH and 95.0 ± 6.2 μg of LH/pituitary during 5 days. Experimental tissues released 67.0 ± 9.7 and 83.7 ± 6.1 μg, respectively. Although these differences are not statistically significant, testosterone treatment produced a consistent rise in FSH:LH ratios from a control mean of 0.568 ± 0.033 to a treated level of 0.786 ± 0.059 ($p < .01$). At the end of culture, the mean control pituitary FSH content was 189 ± 12 μg; the mean LH content was 132 ± 11 μg. Experimental tissues contained 227 ± 24 μg of FSH and 137 ± 11 μg of LH/pituitary (not significantly different from control).

During the final 3 days of the 5 day period, testosterone was associated with an increase in release of FSH from 14.7 to 23.9 μg ($p < .01$) and in medium FSH:LH ratio from 1.10 to 2.16 ($p < .05$) as shown in Table I. In three experiments, dihydrotestosterone exposure also significantly elevated the FSH:LH ratio ($p < 0.5$). LH release was not significantly influenced by androgen.

Discussion. The observed increase in radioimmunoassayable FSH release from tissues during the last 3 days of a 5 day period of exposure to testosterone, and in FSH:LH ratio, are consistent with the work of Kingsley and Bogdanove (personal communication) who observed that local implantation of testosterone propionate into the pituitaries of castrated rats resulted in increased FSH:LH ratio locally in the tissue. Dihydrotestosterone also significantly elevated the FSH:LH ratio; therefore the possibility remains that these testosterone effects followed conversion to dihydrotestosterone. Differential effects of androgen upon gonadotropin secretion were reported also by Gay and Bogdanove (10) and by Swerdloff *et al.* (11) who found that high doses of testosterone propionate which suppressed LH in castrated animals failed to suppress FSH to levels found in intact animals.

Without question, these observed effects were by direct action of androgen upon the pituitary, in contrast with the generally accepted concept of "negative feedback" by way of the hypothalamus (12). The possible physiological significance of these positive effects of androgen remains to be determined.

Summary. Effects of testosterone (30 ng/ml) on gonadotropin release were studied using isolated male rat pituitaries maintained for 5 days. Testosterone exposure significantly elevated the FSH:LH ratio in medium as measured by radioimmunoassay. During the last 3 days of culture, FSH release was significantly greater in the presence of testosterone.

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