Short-term effect of cyproterone acetate on testicular FSH binding in immature rats

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Summary. Male rats, aged 19 days, were injected with 1 mg cyproterone acetate, an antiandrogen, and killed 24 h later. In 9 out of 10 experiments this increased the apparent FSH-binding capacity by testicular tissue *in vitro*. In 4 out of 5 similar experiments, injection of 500 µg testosterone propionate caused a significant reduction in FSH binding. Observed changes were small but this does not preclude the possibility that androgens contribute to the physiological control of FSH receptor numbers.

Introduction

Both FSH and androgens apparently influence the production of androgen-binding protein by the Sertoli cells (Elkington, Sanborn & Steinberger, 1975; Louis & Fritz, 1979) and their inter-relationship is of interest. We have therefore injected rats with the anti-androgen, cyproterone acetate, and studied the subsequent short-term binding of FSH to the testicular receptors *in vitro*. Longer term effects have been reported by Neumann, Diallo, Hasan, Schenck & Traore (1975).

Materials and Methods

The animals and general procedures for studying the binding of iodinated rat FSH were as described previously (O'Shaughnessy & Brown, 1978) except that homogenates were of total testis tissue. Male Porton rats, aged 19 days, were weaned and injected subcutaneously with 1 mg cyproterone acetate (Schering) in 0.1 ml arachis oil containing benzyl benzoate or with the vehicle alone. In subsidiary experiments, rats of this age were injected with 500 µg testosterone propionate in oil containing ethyl oleate or with the vehicle. Rats were killed 24 h later and blood was pooled within treatment groups for radioimmunoassay of serum FSH by using the NIAMDD kits and recommended method as previously described (Sharpe & Shahmanesh, 1974): the method fails to detect FSH in the serum of hypophysectomized rats.

In one series of experiments, homogenates were made from the pooled testes of all animals in the treatment group, either at a fixed weight of tissue/ml or at a fixed volume/testis. Specific binding of ¹²⁵I-labelled rat FSH was measured during 1.5 h at 37°C in a Tris buffer, pH 7.5, at two or more concentrations of homogenate. Measurements were at least in triplicate and each incubation tube was paired with another to measure non-specific binding (O'Shaughnessy & Brown, 1978). In the other series of experiments, a separate homogenate was made for each rat at a fixed volume/testis: these homogenates were tested in triplicate at one concentration of homogenate to give a single value for specific binding for each animal.

Results

Binding to homogenates of pooled tissue from rats treated with cyproterone acetate

Table 1 shows the results of Exps 1–4. The percentage of added ^{125}I -labelled rat FSH bound by the lowest concentration of homogenate varied between experiments. Analysis of variance showed that in Exps 1 and 3 the specific binding of FSH was significantly ($P < 0.01$) greater per unit weight of tissue after cyproterone acetate treatment. This suggested an increase in total testicular binding capacity as the weight of tissue recovered from each testis was not apparently altered by cyproterone acetate treatment. For Exps 1–7, 59.3 ± 5.2 mg testicular tissue was recovered from controls and 58.4 ± 3.4 mg from those treated with cyproterone acetate. Similarly, there was no significant change in serum FSH levels: in Exps 2–4, 6 and 7 values in rats treated with 1 mg cyproterone acetate 24 h previously, expressed as a percentage of that in controls (with 95% fiducial limits), were 108 (97–121), 84 (67–105), 98 (78–122), 95 (75–121) and 92 (72–116), respectively.

In Exps 5–7 (Table 1) maximum binding capacity per testis was increased by treatment with cyproterone acetate and there was no apparent change in the slope of the lines to suggest any change in affinity, K_d ranging from 7.9 to 9.8×10^{-11} M.

Table 1. Binding of FSH by homogenates prepared from pooled testes of immature rats (at least 3/group) treated with 1 mg cyproterone acetate (CPA) or with vehicle alone (controls)

Specific binding of ^{125}I -labelled rat FSH to fixed weight of homogenate						
Exp.	Combined data using all concentrations of homogenate		At lowest conc. of homogenate: % of added FSH bound (mean $\pm$ s.e.m.)		Maximum binding capacity from Scatchard plots (pg ^{125}I -labelled rat FSH/testis)	
	After treatment as % control	Significance of difference from control	Control	CPA-treated	Control	CPA-treated
1	126	$P < 0.01$	5.1 ± 0.1	6.1 ± 0.2	—	—
2	113	$P > 0.05$	2.4 ± 0.2	2.7 ± 0.1	—	—
3	131	$P < 0.01$	10.3 ± 0.4	13.0 ± 0.4	—	—
4	95	$P > 0.05$	11.7 ± 0.02	11.9 ± 1.5	—	—
5	—	—	—	—	756	1000
6	—	—	—	—	2221	2578
7	—	—	—	—	1912	3550

Differences in control binding capacity in Exps 5–7 are due mainly to the use of a different batch of iodinated FSH for Exp. 5.

Binding to homogenates of testes of individual animals treated with cyproterone acetate

In Exps 8–10 (Table 2) homogenates were prepared from the testes of individual treated or control animals, and in all 3 experiments, testes from the treated rats bound more FSH. This effect was statistically significant in Exps 8 and 9 but not in Exp. 10 in which one of the control rats showed uncharacteristically high binding.

Effects of testosterone

In Exps 11–13, injection of 500 μg testosterone propionate reduced specific binding of FSH by homogenates of pooled testicular tissue 24 h later. Reductions were small, binding being 91, 87 and 96% of binding shown by control homogenates, but analysis of variance showed that the

Table 2. Binding of FSH by fixed aliquots of testicular tissue from individual rats treated with cyproterone acetate (CPA) or vehicle alone (controls)

Control animals			CPA-treated animals			Significance of differences from control group
Exp.	No. of rats	Specific binding as % of added FSH (mean $\pm$ s.e.m.)	No. of rats	Specific binding as % of added FSH (mean $\pm$ s.e.m.)	As % of binding in control group	
8	3	22.1 $\pm$ 0.3	3	24.8 $\pm$ 0.7	112	$P < 0.05$
9	4	25.6 $\pm$ 0.5	5	28.2 $\pm$ 0.4	110	$P < 0.01$
10	5	23.9 $\pm$ 6.2	4	25.9 $\pm$ 0.8	108	$P > 0.2$

Binding was measured in each animal using 1/20th of the total testicular tissue. The s.e.m. values represent variation among animals.

reduction summed over all concentrations of homogenate was significant in Exp. 11 ($P < 0.05$) and Exp. 12 ($P < 0.01$). In Exp. 13 the overall effect was not significant, but the reduction of binding at the lowest concentration of homogenate was ($P < 0.05$). This reduction in binding/unit weight of tissue was not due to any change in the weight of testicular tissue recovered as this was almost identical in control and treated animals. In Exps 14 and 15 homogenates were prepared from individual treated and control rats and FSH binding was measured using a fixed aliquot of total testicular tissue. In Exp. 14 testosterone propionate caused a significant ($P < 0.05$) reduction in binding to 93% of the control value but there was no significant effect in Exp. 15 (101%).

Discussion

In 9 out of 10 experiments, treatment with 1 mg cyproterone acetate caused an increase in FSH binding 24 h later. The increase was small but statistically significant in 4 out of the 7 experiments in which significance could be assessed. The remaining 3 experiments (Nos. 5–7) showed increases in apparent receptor numbers ranging from 16 to 86%. The effect was therefore usually small and variable, but demonstrable on all but one occasion. Increases in FSH binding were also observed with more prolonged administration of cyproterone acetate in immature rats but are not reported here as the accompanying changes in testicular weight and probably morphology made interpretation uncertain. Neumann *et al.* (1975) showed an opposite effect of cyproterone acetate but this was produced by the treatment of adult rats over several weeks.

The short-term effect of cyproterone acetate was presumed to be due to its anti-androgenic effect, although circulating androgen would be expected to be low at this age (Ketelslegers, Hetzel, Sherins & Catt, 1978). It was therefore anticipated that treatment with androgen would reduce FSH binding, and injection of testosterone propionate was shown to cause a small but statistically significant reduction in 4 out of 5 experiments.

These findings suggest that androgens may tend, directly or indirectly, to reduce the number of FSH receptors in the testes of maturing rats. The effects demonstrated were small under the conditions of these experiments but such an effect might make some contribution to the physiological control of testicular function.

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