RELATIONSHIP BETWEEN TESTOSTERONE, FLUID CONTENT AND LUTEINIZING HORMONE RECEPTORS IN THE RAT TESTIS

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SUMMARY: Injection of immature male rats with human chorionic gonadotrophin resulted in a decreased ability of the testis to bind /^{125}I/-labelled human chorionic gonadotrophin in vitro, and a marked, but transient increase in testis weight; the latter was apparently due to the accumulation of fluid containing high levels of testosterone. Intra-testicular injection of cycloheximide significantly inhibited all these changes, thus demonstrating their dependence on protein synthesis. It is concluded from this and other data that either testosterone itself or a steroidogenic protein intermediary may be responsible for the gonadotrophin-induced reduction in availability of gonadotrophin receptors.

It is becoming increasingly clear that protein hormones may be involved in the regulation of their own receptors in the target tissue; this phenomenon, termed receptor autoregulation, has been demonstrated in tissue cultures for growth hormone (1) and insulin (2). Receptors for LH/hCG[1] in the immature rat testis can be modulated in a similar way (3), as injection of hCG results in decreased binding of /^{125}I/hCG to testis homogenates for up to 90 hours after injection, an effect which cannot be explained by receptor-occupancy. The mechanism whereby such changes in the availability of receptors is induced is at present unknown. The present paper describes changes in fluid content and testosterone levels in the testes of hCG-treated rats and relates these to the

[1] LH = luteinizing hormone; hCG = human chorionic gonadotrophin

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concomitant reduction in availability of LH/hCG receptors, and shows how all these changes are dependent on protein synthesis.

MATERIALS & METHODS

Treatments & preparation of samples: 21-day old Liverpool hooded rats were injected subcutaneously with either 10 IU hCG (Chorulon) in 0.5 ml 0.9% saline, or with the vehicle, and killed with ether at intervals after injection. Animals were weighed and the testes individually removed and cleaned of extraneous material. Each testis was then placed in a tared tube, the capsule slit open and peeled off, and the testis weighed (W1). After addition of 0.2 ml 0.9% saline to each tube, samples were centrifuged at 1500g for 5 mins, the supernatant aspirated and kept, and the testis reweighed (W2). The difference between first and second testis weights (W1-W2) was taken as a measure of the fluid content of the testis. The addition of 0.2 ml saline prior to centrifugation facilitated easier removal of the very small quantities of fluid normally present in the testis.

Binding of ^{125}I-hCG: Testes were stored at -20°C until all samples had been collected; after thawing, 0.25 ml Krebs-Ringer bicarbonate solution (KRB) was added to one testis from each animal, followed by 30,000 cpm ^{125}I-hCG in 0.05 ml KRB containing 1% bovine serum albumin, fraction V. The ^{125}I-hCG[1] was prepared as described previously (3) and had a specific activity of 22 μCI/μg. Tubes were then incubated for 3 hours at 37°C. Non-specific or non-displaceable binding of ^{125}I-hCG was determined by similar incubation of the remaining testis from each rat together with 200 IU hCG. Incubation was terminated by addition of 6.0 ml cold 0.9% saline, the tubes centrifuged at 1500g for 20 mins, the supernatant aspirated, and radioactivity in the pellet measured in a gamma spectrometer. Specific binding of ^{125}I-hCG to testes from saline-treated control rats ranged from 20-29% of the total counts added.

Testis testosterone levels: The saline-diluted fluid aspirated from each testis was extracted, and assayed for testosterone by radioimmunoassay (4,5); as extracted and unextracted samples gave similar results, in experiments with cycloheximide (see below) samples were assayed directly. Testosterone levels were corrected for the volume of fluid recovered (including the added saline) and the total testosterone obtained from each testis calculated.

Effect of cycloheximide: 28-day old male rats were lightly anaesthetised with ether and injected subcutaneously with saline or 10 IU hCG as described. Equal numbers of control and hCG-treated rats were then injected intra-testicularly through the scrotal wall with either 30 μg cycloheximide (Sigma) in 25 μl 0.9% saline or with the vehicle; the right testis was injected, the left remaining untreated. Animals were killed with ether at 16 hours after injection and subjected to the procedures described above.

[1] HCG CR119 (11,600 IU/mg)

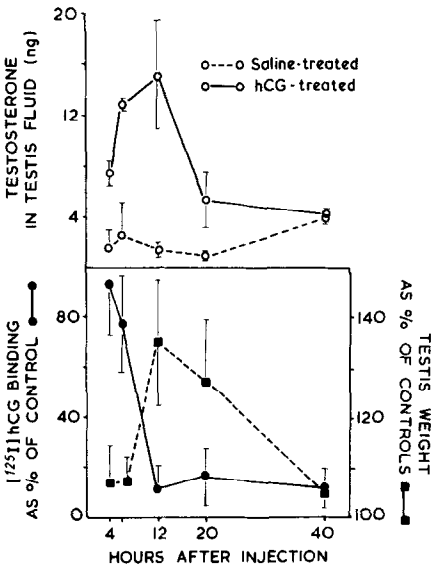

Fig. 1. Specific binding of ^{125}I-hCG to testes from hCG-treated rats, in relation to testis weight and testosterone levels in testis fluid. There were 4 to 5 saline-treated or hCG-treated rats killed at each of the times indicated. Specific binding of ^{125}I-hCG was calculated as cpm/mg testis, using the previously recorded weight (W2) and after subtraction of the non-specific binding. Results are plotted as ^{125}I-hCG binding in hCG-treated rats as a % of the mean binding by testes from the respective controls. Testis weight (W1) in hCG-treated rats was expressed relative to body weight (mg/g) and is plotted as a % of the mean value for the respective controls. Vertical bars represent ± s.d.

RESULTS AND DISCUSSION

The specific binding of ^{125}I-hCG to whole testes from hCG-treated rats showed a similar pattern to that found previously with testis homogenates (3); at 4 and 6 hours after hCG-injection, binding of ^{125}I-hCG, although reduced, was not significantly different from controls, whereas at 12, 20 and 40 hours binding was significantly reduced ($P<0.001$) to less than 20% of control levels (Fig. 1). This effect cannot be explained by saturation of receptors with injected hCG as, *in vivo* receptor occupancy falls by 90% or more between 8 and 40 hours after injection of ^{125}I-hCG alone or together with 10 IU hCG (3). Initial testis

weight (W1) in hCG-treated rats increased dramatically between 6 and 12 hours after injection from 108% ($P>0.05$) to 135% ($P<0.01$) of control values (Fig. 1), was still elevated at 20 hours ($P<0.01$) but had returned to near control levels at 40 hours. This transient increase in testis weight appeared to be largely due to the accumulation of fluid as removal of this by centrifugation halved the difference in testis weight between control and hCG-treated rats at 12 and 20 hours, resulting in the removal of 56 ± 14 (mean ± S.D.) and 41 ± 19 mg fluid respectively; the same procedure had only minor effects (1-5 mg) on control testes. The testosterone content of the fluid aspirated from the testis was higher ($P<0.01$) in hCG-treated than in saline-treated rats at 4-20 hours, with peak values at 6-12 hours (Fig. 1), paralleling the changes seen in serum testosterone levels in hCG-treated rats (3).

In a preliminary experiment, cycloheximide (30 μg) injected into the right testis significantly inhibited ($P<0.01$) the hCG-induced reduction in ^{125}I-hCG binding to both testes at 16 hours after injection, but was without significant effect in control rats. In a more detailed study (Table 1), cycloheximide significantly inhibited fluid accumulation ($P=0.01$) and testis weight increase ($P<0.001$) in hCG-treated rats and partially prevented the hCG-induced elevation of testosterone levels in testis fluid ($P<0.001$) and the concomitant reduction in ^{125}I-hCG binding ($P<0.01$). In control rats, cycloheximide non-significantly ($P>0.05$) reduced fluid testosterone levels and increased ^{125}I-hCG binding (Table 1).

These effects of cycloheximide were not due to the physical disturbance caused by intratesticular injection as this procedure was without significant effect ($P>0.05$), other than to reduce

Table 1 The effect of cycloheximide on hCG-induced changes in the immature rat testis

Treatment Peripheral/ testicular	Testis weight(W1) (mg/g BW)	Testis fluid (mg/testis)	^{125}I-hCG bound (cpm/mg)	Testosterone in testis fluid (ng)
NS/-	3.5 ± 0.8	1.0 ± 1.0	26.6±3.8	2.6 ± 1.5
NS/NS	3.1 ± 0.6	1.7 ± 1.5	28.9±6.3	2.8 ± 1.9
NS/-	3.5 ± 0.5	1.7 ± 2.1	31.0±5.8	1.2 ± 1.2
NS/CHX	3.4 ± 0.8	2.3 ± 1.5	32.9±6.9	1.0 ± 0.8
hCG/-	5.0 ± 0.2	61.3±11.4	12.5±4.4	17.3± 2.3
hCG/NS	4.9 ± 0.3	35.3± 7.4	11.1±1.4	17.3± 1.5
hCG/-	4.1 ± 0.8	20.7± 5.1	18.3±4.6	9.6± 0.6
hCG/CHX	3.7 ± 0.7	16.3± 2.1	19.9±3.5	10.2± 1.4

Results are shown as the mean ± s.d. of values for the untreated testis and treated testis (NS = saline; CHX = cycloheximide) for 3 animals in each of the 4 treatment groups. Details are as given in the text except that testes were used fresh for uptake studies and non-specific binding was estimated by incubation of comparable testes from untreated rats in the presence of 200 IU hCG. Results were analysed by two-factor analysis of variance, in such a way that local (i.e. between testes within animals) and systemic (i.e. between animals in different treatment groups) effects of treatments were distinguished; probability values are given in the text.

($P<0.01$) the accumulation of fluid in the testes of hCG-treated rats (Table 1). Interestingly, this reduction was not accompanied by a fall in the testosterone content, suggesting that levels of the latter are unrelated to fluid accumulation. Therefore the levels of testosterone found in this fluid are probably best considered as an indication of the testis content of testosterone.

Again, as cycloheximide had similar effects on both injected and uninjected testes (Table 1), its action would appear to be systemic rather than local.

The finding that cycloheximide partially prevented the ability of hCG to induce changes in ^{125}I-hCG binding, fluid accumulation and fluid testosterone levels shows that all these changes are dependent on protein synthesis. However, as cycloheximide will presumably reduce all protein synthesis within the testis, it is uncertain in what way these three parameters are related to one another. Although there was a distinct negative correlation between ^{125}I-hCG binding and fluid testosterone levels ($r = -0.89$, $P<0.001$) using the data for the four treatment groups shown in Table 1, there was no consistent correlation between these two variables within the treatment groups; the correlation between these two factors is therefore more likely due to their mutual correlation with some other factor(s).

In vitro, cycloheximide prevents testosterone synthesis by rat interstitial Leydig cells in response to hCG, but does not prevent c-AMP[1] formation (6-8). Although there is still some question as to the involvement of c-AMP in steroidogenesis (9,10), there seems little doubt that activation of protein kinase and consequent synthesis of protein(s) are intermediary steps between receptor-binding and testosterone secretion, and it is presumably at this stage that cycloheximide interferes (8). Therefore, it seems reasonable to assume that either this intermediary protein(s) or some factor dependentent on it, perhaps testosterone, is the agent via which hCG induces a reduction in rat testis LH/hCG receptors. The associated increase in fluid

[1] adenosine 3':5'-cyclic monophosphate

content of the testis following hCG stimulation is at present inexplicable, but may be related to increased testicular blood flow (3,11).

The significance of receptor autoregulation is uncertain; as a means of preventing over-stimulation of a target organ its function is self-evident, but its involvement in normal physiological processes is less obvious. Identification of the factors responsible for the hCG-induced changes in LH/hCG receptors in the rat testis may help to answer this question.

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