GONADOTROPHIN-INDUCED REDUCTION IN THE STEROIDOGENIC RESPONSIVENESS OF THE IMMATURE RAT TESTIS

R.M. Sharpe
MRC Unit of Reproductive Biology,
2 Forrest Road, Edinburgh EH1 2QW

Received May 4,1977

SUMMARY: Injection of immature male rats with 10 IU human chorionic gonadotrophin resulted in a decrease in the steroidogenic capacity of the testis in vitro; this effect was apparent at 20 and 40 hours, but not at 65 hours after injection, and was not attributable to the loss of gonadotrophin receptors that occurs. This reduction in steroid output was strictly local as intra-testicular injection of gonadotrophin affected only the treated testis. It is concluded that the rat testis is capable of actively regulating its responsiveness to repeated hormonal stimulation.

The ability of various hormones to regulate the availability of their specific receptors in the target tissue now seems well established (1). In the rat testis, receptors for LH/hCG[1] show a dramatic reduction in numbers after exposure to hCG in vivo; this has been shown for both the adult (2) and the immature rat (3), and in the case of the latter this effect is dependent on protein synthesis (4). Occupancy of less then 1% of rat testis LH/hCG receptors will elicit maximal testosterone secretion, the so called phenomenon of 'spare receptors' (5,6). A loss of receptors following exposure to hCG might therefore be expected to decrease the sensitivity of the testis to further hormonal stimulation, whilst retaining the capacity for maximum steroid output (1). The present paper shows that the steroidogenic capacity of the testis is in fact reduced following exposure to hCG, suggesting that the testis can regulate not only its sensitivity but also its responsiveness to repeated hormonal stimulation.

MATERIALS AND METHODS

PVG male rats aged 21 days 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 20, 40 or 65 hours after injection. Their testes were removed and

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

ISSN 0006-291X

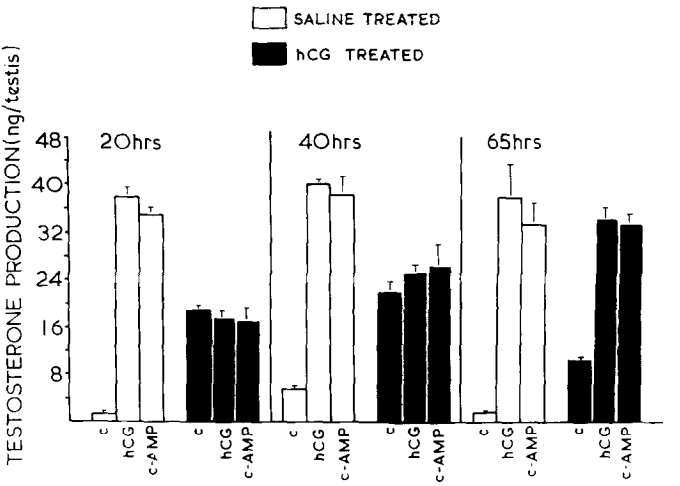

Fig. 1. Testosterone production in vitro by testes from saline- and hCG-treated rats at various times after injection. Each column represents the mean (+ s.d.) of values obtained from 3 or 4 testes. Additions made to the incubation medium are indicated at the base of each column; c = baseline (control) testosterone production.

decapsulated in Krebs-Ringer bicarbonate solution containing 1 mg/ml glucose (KRBG). Individual testes were then placed into 7 ml polystyrene tubes containing 0.25 ml KRBG, and one of the following three additions made in 0.05 ml 0.01 M phosphate buffered saline (pH 7.5) containing 1% bovine serum albumin, fraction V:- (1) hCG[1], to give a final concentration of 16.6 ng/ml, (ii) dibutryl c-AMP[2] (Sigma) to give a final concentration of 10 mM, or (iii) the buffer only. The amounts of hCG and c-AMP added were calculated as being ten times the concentration required to stimulate maximal testosterone secretion by adult rat testes in vitro (5). Testes were incubated for 4 hours at 37°C in a shaking water bath and the medium then aspirated and centrifuged at 1500 g for 5 min; 0.1 ml of the supernatant was then removed and diluted for measurement of testosterone directly by radioimmunoassay (7, 8).

In other experiments, rats were killed at 20 hours after subcutaneous injection of varying doses of hCG or after intra-testicular injection (4) of the right testis with 1 IU hCG and subjected to the procedures described above.

RESULTS

In the absence of in vitro stimulation (baseline output), testes from saline-treated (control) rats produced 1-6 ng testosterone/testis, while the

[1] hCG CR119 (11,600 IU/mg)
[2] N^6, $O^{2'}$-dibutryl adenosine 3′ : 5′-cyclic monophosphate

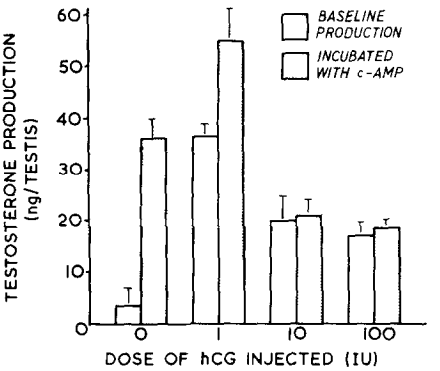

Fig. 2. Relationship between dose of injected hCG and in vitro testicular testosterone production at 20 h after treatment. Other details are as given for Fig. 1.

addition of either hCG or c-AMP stimulated output to 33-40 ng/testis (Fig. 1). The baseline output of testosterone by testes from hCG-treated rats was always significantly higher ($P<0.001$) than by testes from saline-treated controls, although much higher levels were obtained with testes from rats killed at 20 and 40 hours after injection of hCG (19-22 ng/testis) than at 65 hours (10 ng/testis; Fig. 1). Incubation of testes from hCG-treated rats with either hCG or c-AMP failed to increase testosterone output significantly above baseline at 20 hours and 40 hours (Fig. 1) so that the maximum steroidogenic response obtained was considerably lower ($P<.0.001$) than in controls; however, at 65 hours after injection both hCG and c-AMP provoked a similar steroid output to that found with control rat testes (Fig. 1).

Testosterone production by testes from rats injected with 10 or 100 IU hCG was similar at 20 hours after injection, and the response to c-AMP stimulation was significantly reduced ($P<0.01$) compared to control rat testes (Fig. 2) as in the first experiment (Fig. 1). In contrast, testes from rats injected with only 1 IU hCG showed a baseline production of testosterone which was equal to the maximum (stimulated) output by saline-treated controls, and the addition of c-AMP increased secretion significantly ($P<0.01$) above that seen in the controls (Fig. 2).

Intra-testicular injection of 1 IU hCG resulted in a significant decrease ($P<0.001$) in testosterone production by the injected testis when compared to

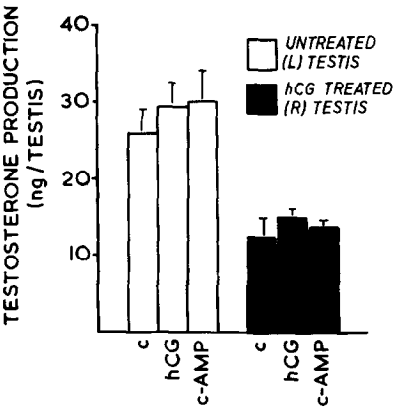

Fig. 3. Effect of intra-testicular injection of hCG on testosterone production in vitro by the treated and untreated testis at 20 h after injection. Other details are as given for Fig. 1.

the contralateral, untreated testis (Fig. 3); addition of hCG or c-AMP did not increase steroid output above baseline in either testis.

DISCUSSION

These results show that the ability of the testis to secrete testosterone in vitro is reduced after injection of 10 IU hCG; this effect was most marked at 20 hours after treatment, but was still evident at 40 hours. At these times, testes from hCG-treated rats showed maximum steroid output in both the presence and absence of in vitro stimulation, but the level of testosterone achieved was considerably lower than by control rat testes. This effect can not be explained by the loss of LH/hCG receptors that occurs after hCG treatment (3, 4) as c-AMP and hCG were equally ineffective at provoking steroid output at 20 and 40 hours after injection.

A reduction in testosterone secretion by the testis following hCG treatment might be due simply to depletion of an enzyme or steroid precursor. This seems unlikely, as the present data show that maximum steroid output can be maintained, perhaps even enhanced, following injection of 1 IU hCG while testicular cholesterol levels are similar (55-57 μg/testis) at 20 hours after injection of saline or 10 IU hCG (unpublished data).

Intra-testicular injection of hCG caused a reduction in the steroido-

genic capacity of the treated but not the untreated testis; this localisation of the effect is also true for the effect of hCG on testis LH/hCG receptors (unpublished data). However, in other ways these two parameters appear to respond differently to hCG treatment. Firstly, the steroidogenic responsiveness of the testis has completely recovered by 65 hours after injection whereas the restoration of normal receptor numbers takes considerably longer (2, 3) and secondly, subcutaneous injection of 1 IU hCG did not reduce the steroidogenic capacity of the testis but does reduce receptor availability (3).

It appears, therefore, that exposure to hCG may affect both the sensitivity and the responsiveness of the immature rat testis. Sensitivity is, at least in theory, likely to be reduced as a result of receptor loss (1) while responsiveness is reflected by a reduction in the steroidogenic capacity of the testis. Presumably, both these changes are initiated by the occupancy of receptors but other than this, little is known. However, it is well established for the adult rat that with increasing receptor occupancy there is a progressive rise in c-AMP production, despite the fact that above 1% occupancy there is no further increase in testosterone production (5, 6). Perhaps this 'excess' of c-AMP, by activation of protein kinase (9, 10), results in the synthesis of proteins which mediate the observed changes in membrane receptors (4) and steroidogenesis. It may be significant that stimulation of Leydig cells with LH has been shown to result in the synthesis of a specific protein which is apparently not directly involved in steroidogenesis (11).

ACKNOWLEDGEMENTS: I am grateful to Dr. A. S. McNeilly for helpful discussions and advice and to the NIAMDD, U.S.A. for hCG CR119.

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