# EFFECT OF LUTEINIZING HORMONE ON 3',5'-CYCLIC AMP AND TESTOSTERONE PRODUCTION IN ISOLATED INTERSTITIAL TISSUE OF RAT TESTIS

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Received 29 March 1973

## 1. Introduction

It has been shown that human chorionic gonadotrophin (HCG) and luteinizing hormone (LH) will stimulate steroidogenesis in testes *in vivo* [1]. The intracellular mediator of this trophic hormone action is thought to be 3',5'-cyclic AMP (cAMP) because *in vitro* experiments have shown that i) HCG or LH stimulates cAMP and testosterone production in testes [2–7], ii) the increase in cAMP production precedes the increase in testosterone production [3] and iii) dibutyryl-cAMP stimulates testicular testosterone synthesis *in vitro* [2, 3, 5] and *in vivo* [1]. However, because of the different cell types present in testes only tentative conclusions can be drawn. It is possible, for example, that cAMP production is stimulated in cell types that are not involved in steroidogenesis.

*In vitro* studies with separated testis tissues have shown that LH specifically stimulates cAMP production in the interstitial tissue [7] and that this tissue is the main site of testosterone biosynthesis [8]. It was therefore decided to investigate the effect of LH on the relationship between cAMP and testosterone synthesis in isolated interstitial tissue *in vitro*.

The results obtained are in accordance with cAMP being an intracellular mediator of LH action. Both cAMP and testosterone production in interstitial tissue were stimulated by LH and the increase in cAMP preceded the increase in testosterone production. The addition of glucose was found to increase the production of testosterone in LH-stimulated interstitial tissue. The magnitude of the observed increased testosterone production in interstitial tissue was, however, lower than might be expected from the relatively higher number of Leydig cells in this tissue compared with the total testis.

## 2. Materials and methods

Ovine LH (NIH-LH-S18, 1 unit/mg) was a gift from the Endocrinology Study Section, National Institute of Health, Bethesda, Maryland. Testis tissue was obtained from 10–13 weeks old rats (Wistar strain). Some rats were used 11–15 days after hypophysectomy, starting on the day after hypophysectomy these rats received daily subcutaneous injections of 10 $\mu$g LH. The isolation of the tissues, incubation conditions and the extraction procedure were as published previously [3, 7], except that in all experiments 50 $\mu$g $\gamma$ globulin but no theophylline and in some experiments 0.2% glucose were present in the incubation medium. The following amounts of tissue (expressed as weight of protein per volume incubation medium) were used: unteased testis 70 mg/2 ml, teased testis 5–10 mg/0.5 ml and interstitial tissue 0.3–1.0 mg/0.5 ml. cAMP was isolated as described previously [3] and assayed by saturation analysis [9]. Testosterone was measured by radioimmunoassay essentially as described by Furuyama et al. [10], except that the tissue extracts were not chromatographed. Samples were incubated with antiserum at 4° for 16 hr and separation of free and bound testosterone was achieved with dextran coated charcoal (0.5 ml containing 250 mg charcoal

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and 25 mg dextran T250 per 100 ml borate buffer).

Evaluation of the procedure for testosterone estimation showed that the coefficient of variation of the within assay precision was approx. 13% for samples containing between 0.3 and 50 ng (n=114). The coefficient of variation of the between assay precision for mean values of duplicate determinations was approx. 14% for samples containing between 1 and 30 ng (n=32). The specificity and accuracy of the method under the experimental conditions used was evaluated by comparing the results of estimations by radioimmunoassay and gas-liquid chromatography [11]. The correlation coefficients between estimations by radioimmunoassay and gas-liquid chromatography of total testis tissue extracts (n=54) and interstitial tissue extracts (n=12) were 0.95 and 0.94, respectively.

## 3. Results and discussion

The time course relationship for cAMP and testosterone production during incubations of interstitial tissue in the presence of 200 ng LH/ml is given in fig. 1. The first detectable increase in cAMP levels was 5 to 10 min after the addition of LH while stimulation of testosterone production was not noticeable until 30 to 60 min. These results are similar to observations with total testis tissue [3]. It is striking, however, that cAMP in interstitial tissue continuously increased during 4 hr incubation whereas in total testis tissue a decrease was found already after 30 min incubation. The difference may be explained if cAMP is released in the intact gland from the interstitial cells and metabolized elsewhere in the testis e.g. in the seminiferous tubules. During incubation of whole testis *in vitro*, release of cAMP into the incubation medium has been shown by Dufau et al. [5] and phosphodiesterase activity has been detected in seminiferous tubules [12].

The dose–response relationship between LH and testosterone has been investigated with interstitial tissue and was compared with results from incubations with teased and unteased testes (fig. 2). With interstitial tissue from some rats a stimulation of testosterone production was found with 0.002 μg LH/ml but stimulation was consistently obtained only with 0.02 μg LH/ml. The amount of testosterone formed, varied from one rat to another with higher doses of LH (0.2–2.0 μg/ml) especially in the teased testis tissue and interstitial tissue. In this series of experiments the testosterone production in stimulated interstitial tissue and in total testis tissue in the absence of glucose was between 4 and 12 ng testosterone/mg protein/4 hr. Glucose was added to the incubation medium when investigating the time course relationship for cAMP and testosterone production and it was found that the amount of testosterone produced in LH-stimulated interstitial tissue was much higher (72.8 ± 20.8; mean value ± S.D., n=6) than the production by tissues in the absence of glucose. The amount of testosterone produced in total testis tissue in the

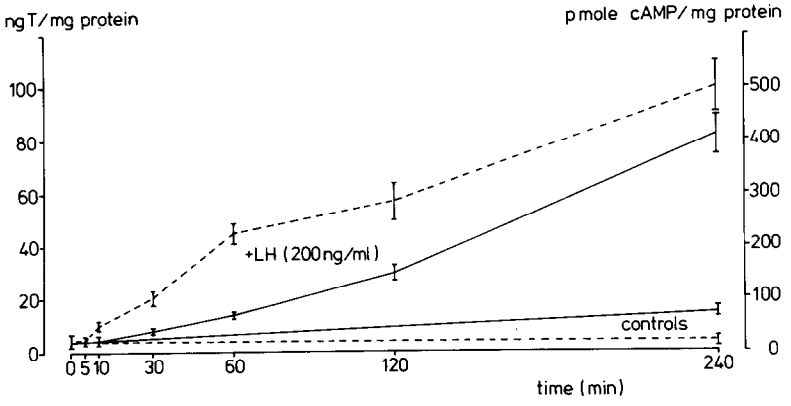


Fig. 1. Time course relationship for cAMP (– – – –) and testosterone (——) production in interstitial tissue in the presence of 200 ng LH/ml. The values presented are means ± S.E.M. from 3 different duplicate incubations with tissue from 3 different rats. Tissues were incubated in the presence of 0.2% glucose.

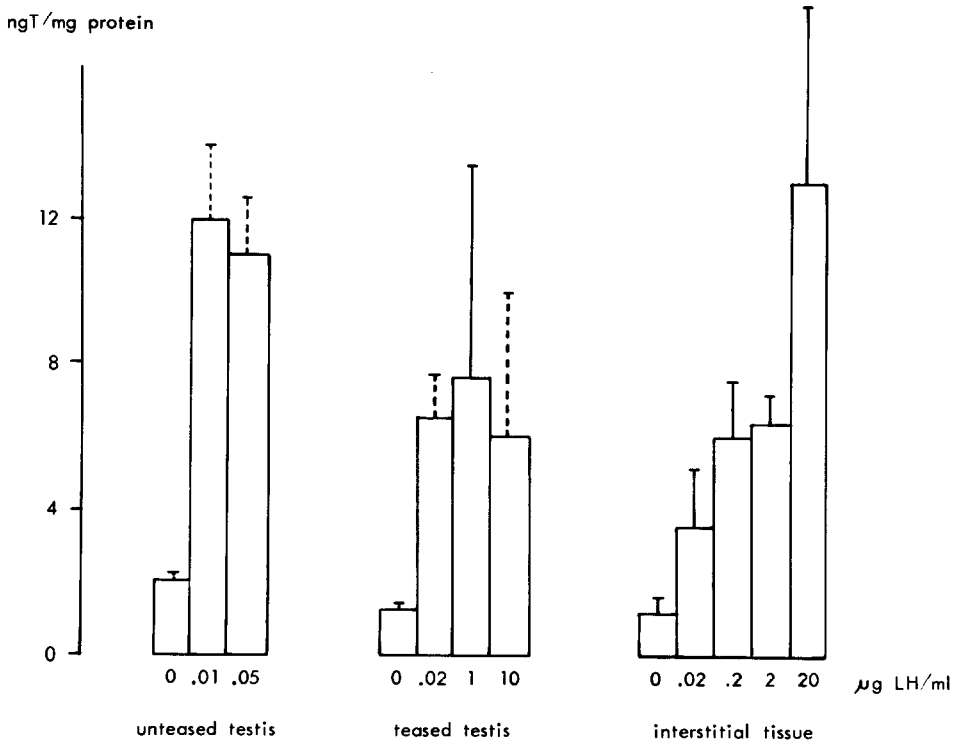


Fig. 2. Effect of various amounts of LH *in vitro* on testosterone production by unteased testis, teased testis and interstitial tissue. Tissues were incubated for 240 min at 32°. Zero time values were subtracted. Mean values ± S.E.M. (——) for n=3 to 6 or mean value and the range (– – – –) for n=2 to 3 are indicated, n is the number of observations with tissues from different rats. Tissues were incubated without glucose added to the incubation medium.

Table 1
Effect of glucose on production of testosterone during incubations of interstitial tissue *in vitro*.

| Rat | Testosterone production (ng/mg protein/4 hr) | |
|---|---|---|
| | Without glucose | With glucose (0.2%) |
| 1 | 5.3 | 20.0 |
| 2 | 5.0 | 36.1 |
| 3 | 2.7 | 12.7 |

Interstitial tissue was obtained from 3 normal rats (1, 2 and 3). Zero time values (2.7–3.6 ng T/mg protein) have been subtracted. Each value is a mean from duplicate incubations. Incubations were carried out over a period of 4 hr in the presence of 200 ng LH/ml.

presence of LH was also increased when glucose was added (26.6 ± 5.6, n=3). It may be concluded, therefore, that the testicular preparations used in the two series of experiments produced different amounts of steroids, presumably because of the addition of glucose. The effect of glucose on steroid production was therefore investigated within one experiment (table 1). It was confirmed that a higher testosterone production is obtained in the presence of glucose thus clearly indicating the necessity of this compound in addition to LH for a high steroid production. This is somewhat surprising because Gomes [14] has reported that glucose had no effect on oxygen uptake by isolated interstitial tissue and he therefore concluded that glucose was not utilized by this tissue. From these observations it may be concluded therefore that oxygen uptake does not correlate with the effect of glucose on steroid production in interstitial tissue.

Table 2
Production of cAMP and testosterone during incubations of interstitial tissue *in vitro*.

| Incubation time (min) | LH concentration (ng/ml) | Testosterone (ng/mg protein) | | | cAMP (pmole/mg protein) | | |
|---|---|---|---|---|---|---|---|
| | | 4 | 5 | 6 | 4 | 5 | 6 |
| 0 | 0 | 0.3 | 0.5 | 1.8 | 4.0 | 4.0 | 7.0 |
| 120 | 0 | 0.8 | 1.8 | 3.0 | 5.2 | 3.9 | 14 |
| 120 | 20 | 1.3 | 3.6 | 5.5 | 4.8 | 5.0 | 17 |
| 120 | 200 | 3.2 | 3.5 | 17 | 22 | 21 | 158 |

Interstitial tissue was obtained from hypophysectomized rats which were injected daily for 11–15 days with 10 μg LH. Each value is a mean from duplicate incubations with tissue from rat 4, 5 or 6 carried out in the presence of 0.2% glucose.

Approx. 17% of the total amount of protein in the testis is present in the interstitial tissue [13], therefore the isolated interstitial tissue should theoretically produce approx. 6 times more testosterone per mg protein when compared to the total testis, stimulated with the same amount of LH. The absence of a proportionally higher production by isolated interstitial tissue (fig. 2) may reflect a decreased steroid production in this isolated tissue. This low steroid production may be explained by destruction of the tissue during dissection. However, this is not reflected in the relatively high cAMP production in isolated interstitial tissue compared with total testis tissue [7]. Another explanation may be a lack of essential factors from the tubules which might be required for optimal steroid production.

When cAMP and testosterone production in interstitial tissue from hypophysectomized rats were studied (table 2), it was found that with 20 ng LH/ml only testosterone production was stimulated, whereas with 200 ng LH/ml both cAMP and testosterone production were stimulated. Other studies with theophylline added to the incubation medium to inhibit metabolism of the cAMP, have shown that 100 ng LH/ml was required to detect a change in cAMP production in isolated interstitial tissue from normal rats (reference 7 and unpublished observations). It has also been reported [15] that trophic stimulation of adrenal cortex may result in an increased corticosteroid production without effects on the cAMP production. The absence of an effect on cAMP production when steroid production is stimulated may reflect a non-obligatory role of cAMP in the control of steroidogenesis. It is possible, however, that with the analytical techniques used, small differences in cAMP levels which could have stimulated steroid production remain undetectable.

In conclusion, the results of the present study clearly demonstrate that the testosterone production by isolated interstitial tissue *in vitro* can be stimulated by LH. This is in contrast to the results of Dufau et al. [2] who reported that testosterone production of an interstitial cell fraction could not be stimulated *in vitro* and they therefore suggested that the intact testis was required for the synthesis of testosterone. Although our results do not support the latter suggestion, the reason for low production of testosterone in interstitial tissue remains to be elucidated.

**Acknowledgement**

The authors wish to thank the NIH Endocrinology Study Section for gifts of the LH.

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