LH INDUCTION OF A SPECIFIC PROTEIN (LH-IP) IN RAT TESTIS LEYDIG CELLS

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1. Introduction

The available evidence suggests that LH stimulation of testosterone production in rat testis Leydig cells involves protein(s) with a short half life. This evidence is based on the effects of inhibitors of protein and RNA synthesis on LH stimulated testosterone production [1–3], particularly the rapid effect of cycloheximide, which causes a decrease in testosterone synthesis following first order kinetics with a half life of 13 min [4]. Recent work in our laboratory has shown that two proteins which are synthesized in rat testis Leydig cells and which can be detected using polyacrylamide gel electrophoresis, may be important in the regulation of testosterone production by LH; one of these proteins has a short half life (about 11 min) and is present in the particulate fraction of the Leydig cell, but is not under the influence of LH; the other protein (referred to as LH-IP, LH-induced protein) can be detected 2 h after the addition of LH to Leydig cells and has a half life longer than 30 min [5]. We now wish to report that the second protein (LH-IP) can be induced by LH or dibutyryl-cAMP but not by testosterone or follicle stimulating hormone (FSH). Dose response studies have also shown that the induction of LH-IP and the stimulation of testosterone production require approximately the same concentrations of LH. Incubation of the Leydig cells with actinomycin D prevented the induction of LH-IP by LH.

2. Materials and methods

Ovine FSH (NIH-FSH-S9) and ovine LH (NIH-LH-S18) were gifts from the Endocrinology Study Section, National Institute of Health, Bethesda, Maryland, USA. [^{35}S]Methionine (280 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, England. Elipten-phosphate (an inhibitor of cholesterol side-chain cleavage) was a gift from CIBA, Basel, Switzerland.

Leydig cell suspensions from rat testis were prepared and purified by centrifugation through Ficoll and Dextran solutions as described before [6]. Leydig cells were incubated in Krebs-Ringer solution pH 7.4 containing 0.2% glucose, 0.1% bovine serum albumin fraction V and amino acid mixture lacking in methionine under an atmosphere of 95% O_2/5% CO_2 with LH as indicated in the text and then proteins were labelled by addition of [^{35}S]methionine for 30 minutes.

In order to control whether the cells were stimulated by LH, Leydig cells were incubated in parallel experiments with or without added LH (100 ng/ml) for 2 h. After this incubation period testosterone was extracted and determined as described before [7]. Testosterone production (mean ± S.E.M.) in the absence of added LH was 4.0 ± 0.4 ng/10^6 nucleated cells (n = 15), in the presence of LH 115.8 ± 14.4 ng/10^6 nucleated cells (n = 15) and in the presence of LH and elipten-phosphate 3.3 ± 1.5 ng/10^6 nucleated cells (n = 3).

After incubation of the cells, 10 vol. cold (0°C) Krebs-Ringer buffer without bovine serum albumin was added and the cells were sedimented by centrifugation for 10 min at 100 × g at 4°C. The supernatant was discarded and the sedimented cells were resuspended in a glycine–sodium dodecyl sulphate buffer (0.1 M glycine, 0.1 M NaCl, 0.01 M EDTA, 0.1% sodium dodecyl sulphate and 0.01 M β-mercapto-

ethanol) pH 8.5. The suspension was heated at 100°C for 10–15 min in glass tubes and after cooling, acetone (4 vol.) was added. The water–acetone mixture was stored overnight at –20°C. The precipitated proteins were sedimented by centrifugation for 10 min at 1500 × *g* at 4°C and dissolved in Tris–glycerol buffer (0.05 M Tris, 10% glycerol, 1% sodium dodecyl sulphate and 1% β-mercaptoethanol) pH 6.8 and heated at 100°C for 2 min. Electrophoresis was carried out in 10% and 15% discontinuous SDS polyacrylamide slab-gels according to Laemli [8]. Before drying the gels, they were impregnated with 2,5-diphenyl oxazole [9]. The gels were then exposed to Kodak X-ray film RP 14 for 1–2 weeks.

3. Results

3.1. *Influence of incubation time*

Two hours after addition of LH to Leydig cells increased incorporation of [^{35}S]methionine could be observed in a protein band with a mol. wt of approximately 21 000 (LH-IP) (fig.1). Only in one out of 6 experiments with intact and hypophysectomized animals was this LH-increased incorporation detectable one hour after addition of LH to the cells. About four hours after addition of LH the labelling of LH-IP seemed to be at a maximal level.

3.2. *Effect of different doses of LH*

When Leydig cells were incubated with 1 ng LH/ml for 3 h the incorporation of [^{35}S]methionine in LH-IP was only slightly increased compared with the control. With 10 ng LH/ml a clear increase in ^{35}S-incorporation in LH-IP was evident while maximal labelling was obtained with 100–1000 ng LH/ml (fig.2).

3.3. *Effect of testosterone and FSH*

To investigate whether the increase in LH-IP labelling after addition of LH was due to the higher level of testosterone in LH-stimulated Leydig cells, testosterone (100 ng/10^6 nucleated cells) was added to Leydig cells and incubated for 3 h. No change in ^{35}S-incorporation of LH-IP as compared with control cells was observed (fig.3). In other experiments

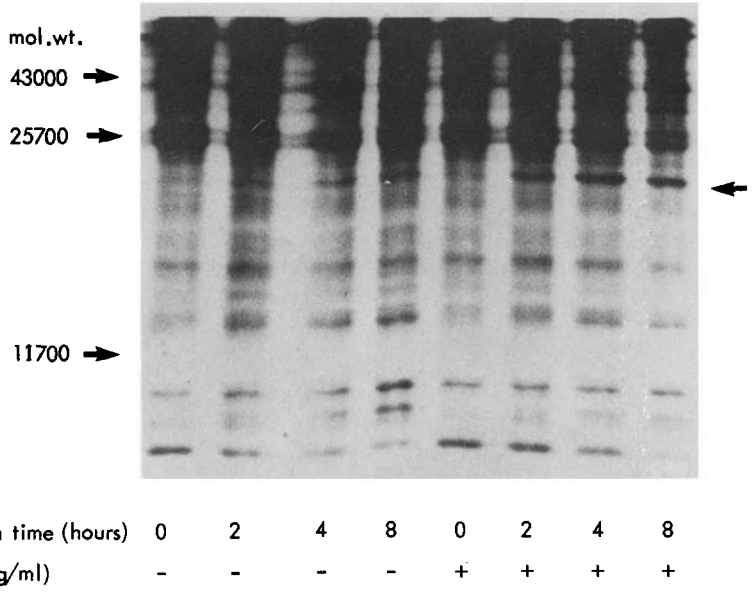

Fig.1. Time course of LH induction of LH-IP. Leydig cells were incubated with or without added LH (100 ng/ml) for 0, 2, 4, and 8 h followed by incubation with [^{35}S]methionine for 30 min. Proteins were separated by electrophoresis on 10–15% discontinuous SDS–polyacrylamide gel. The following mol. wt markers were used: ovalbumin, chymotrypsinogen and cytochrome *c*. In this figure only the 15% part of the gel is shown.

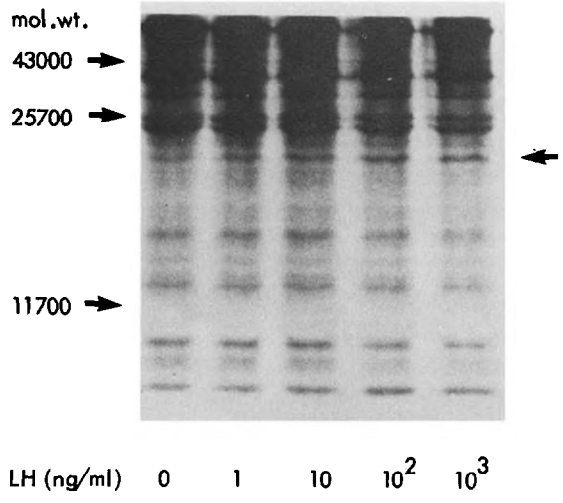

Fig.2. LH dose response relationship of LH-IP synthesis. Leydig cells were incubated with different doses of LH for 3 h followed by incubation with [^{35}S]methionine for 30 min.

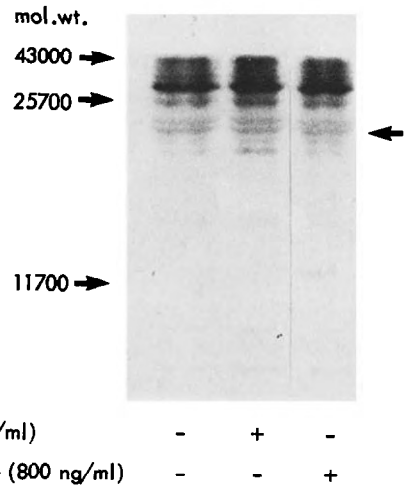

Fig.3. Effect of testosterone on LH-IP synthesis. Leydig cells were incubated without or with LH (100 ng/ml) or with testosterone (± 100 ng/10^6 nucleated cells) for 3 h followed by incubation with [^{35}S]methionine for 30 min.

elipten-phosphate (300 μg/ml) was added to LH-stimulated Leydig cells to prevent the synthesis of testosterone [10]. However, under these conditions the LH-stimulated labelling of LH-IP was not inhibited. Addition of FSH (100 ng/ml) to the Leydig cells instead of LH did not stimulated the labelling of LH-IP.

3.4. *Effect of dibutyryl-cAMP*

Addition of 0.1 mM dibutyryl-cAMP instead of LH

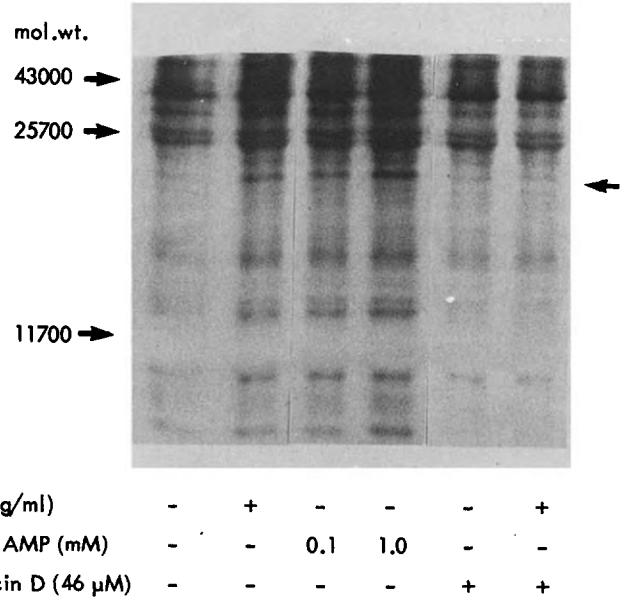

Fig.4. Effect of dibutyryl-cAMP or actinomycin D on synthesis of LH-IP. Leydig cells were incubated with or without LH alone or in combination with actinomycin D or with dibutyryl-cAMP for 3 h followed by incubation with [^{35}S]methionine for 30 min.

to Leydig cells gave a submaximal stimulation of testosterone production while addition of 1 mM dibutyryl-cAMP gave about the same stimulation of testosterone production as 100 ng LH/ml. As can be seen in fig.4 both concentrations of dibutyryl-cAMP stimulated the ^{35}S-incorporation in LH-IP.

3.5. *Effect of actinomycin D*

Addition of actinomycin D (46 μM) to Leydig cells inhibits 89–93% of RNA synthesis [2]. Addition of this amount of actinomycin D to LH-stimulated Leydig cells prevented the LH-stimulated labelling of LH-IP (fig.4).

4. Discussion

From the present results it may be concluded that an increased incorporation of [^{35}S]methionine in a protein with a mol. wt of 21 000 (LH-IP) can be detected 2 h after addition of LH to Leydig cells. This increase of ^{35}S-incorporation most probably reflects protein synthesis and was not due to the effects of increased synthesis of testosterone or contaminating amounts of FSH in the LH preparation. Dibutyryl-cAMP also increases the incorporation of the methionine into LH-IP, therefore it is probable that the effect of LH on the synthesis of LH-IP is mediated by increased cAMP production. Actinomycin D was found to inhibit the LH-stimulated synthesis of LH-IP, which suggests that this stimulation of protein synthesis is probably mediated by increased synthesis of new mRNA. In another study [5] it has been shown that LH-IP is located specifically in the Leydig cells and not in other cell types of the rat testis and that its half life is longer than 30 min.

These results raise the question about the possible role of LH-IP in the Leydig cell. Certain aspects would support a role of this protein in the LH-stimulation of testosterone production in Leydig cells, namely:

1. The close correlation between the LH dose response relationship of LH-IP synthesis and testosterone production; the lowest dose of LH required for stimulation of the synthesis of this protein as well as for stimulation of the testosterone production was about 1 ng/ml and maximum response was obtained with about 100 ng/ml LH [6].
2. RNA synthesis is apparently required for LH stimulation of testosterone production up to 150 min after addition of LH to Leydig cells [3].

However 2 points may be raised against an obligatory role of LH-IP in testosterone production:

1. The long half life (more than 30 min) of LH-IP, which is not in accordance with the rapid effect of cycloheximide [4].
2. LH-IP could be detected only 2 h after addition of LH Leydig cells, which is much later than the first stimulation of testosterone production, which can already be detected within 5–30 min [11] after addition of LH.

Therefore further work will be necessary to investigate the possible role of LH-IP in the effect of LH on testosterone production in rat testis Leydig cells.

Acknowledgements

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