Effects of FSH on acidic nuclear protein synthesis in cultured pig Sertoli cells

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Received 14 January 1984

We studied the effect of FHS on nuclear protein synthesis by a primary culture of immature porcine Sertoli cells. The cells were cultured in a chemically defined medium and treated with FSH (50 ng/ml) for various periods (4, 8, 25 and 64 h). About 65 spots ($pH_i < 8$) were identified by two-dimensional polyacrylamide gel electrophoresis of the radiolabelled nuclear proteins. After 25 h of FHS treatment, we observed an increase of 5 proteins, a slight decrease of two and a disappearance of one. For the short periods of FSH treatment (4 and 8 h), no effect of FHS on nuclear protein synthesis was observed. After 64 h of FSH treatment, the synthesis of all nuclear proteins appears to be decreased.

Sertoli cell *FSH* *Nuclear protein* *Primary culture*

1. INTRODUCTION

Follitropin (FSH) is the main peptide hormone regulating Sertoli cell function [1]. Some of the effects of the hormone are expressed by an increase of RNA, intracellular protein synthesis [2] and secretion of proteins [3]. However, the effect of the FSH on the synthesis of nuclear proteins is unknown. Since non-histone proteins seem to be implicated in gene expression and the control of transcription [4,5] and since many polypeptide hormones have been shown to affect nuclear proteins (TSH, hCG, insulin) [6–9], we studied the effects of FSH on the synthesis of nuclear proteins whose pH_i values are lower than 8. FSH treatment was performed in primary culture of pig Sertoli cells maintained in a chemically defined medium, since it has been previously shown that the pig model is probably closer to man than the rat in many aspects [10].

2. MATERIALS AND METHODS

2.1. *Materials*

Porcine follicle stimulating hormone (NIH-FSH-P2) was obtained from NIAMDD, National Pituitary Agency. [^{35}S]Methionine (spec. act. 1400–1500 Ci/mmol) was purchased from Amersham, England. [^{3}H]Leucine (spec. act. 100 Ci/mmol) was purchased from Saclay, France. Collagenase was obtained from Boehringer, Mannheim. Dulbecco's modified Eagle's medium and Ham's F12 medium in powdered form and trypsin–EDTA were obtained from Grand Island Biologicals Co. Soybean trypsin inhibitor, vitamin E, transferrin, insulin, Hepes, deoxyribonuclease type I were supplied by Sigma. Earle's-salt medium without L-methionine and L-glutamine was obtained from Gibco Europe.

2.2. *Methods*

2.2.1. Sertoli cell culture

Porcine testes were decapsulated, minced and treated by collagenase at 37°C as in [11]. The digested tissue was filtered through Nytex (mesh ≃400) and diluted with 2 vols of a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (Ham F12/DME). The digested tissue was left at room temperature for 20 min. The sedimented portion, which contained mainly tubules, was washed twice with Ham F12/DME

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00145793/84/$3 00

and then incubated in trypsin–EDTA buffer containing 0.02% deoxyribonuclease at 37°C for 20 min. The tubule fragments were then teased apart using a pipet tip.

The dispersed cells were filtered through Nytex (mesh ≃160): only Sertoli cells and small clumps were filtered. Following centrifugation (800 × *g* for 5 min), cells were resuspended in Ham F12/DME containing trypsin inhibitor 0.1% and counted. The cells were subsequently washed and resuspended in Ham F12/DME.

Sertoli cells (15×10^6) were cultured in Falcon flasks (75 cm^2) at 37°C in a 5% CO_2 atmosphere in Ham F12/DME medium containing 1.2 mg/l sodium bicarbonate, 10 mM Hepes, 20 μg/l gentamycin, 0.1% fetal calf serum, 5 μg/ml insulin, 100 U/ml streptomycin. The medium was changed every day.

2.2.2. FHS treatment

On day 3 (cells were prepared on day 0) some dishes were treated with pFSH (50 ng/ml). Different periods of FSH treatment were used (see figures): 2, 6, 9 and 52 h. Thereafter, the cells were incubated with [^{35}S]methionine (25 μCi/ml) and [^{3}H]leucine (25 μCi/ml) for different periods (see figures) in Earle's salts medium without L-methionine and L-glutamine supplemented, as above, and in the presence or absence of pFSH (50 ng/ml).

At the end of the incubation period, the incorporation of [^{35}S]methionine and [^{3}H]leucine was stopped at 4°C with several washes with Tris buffer (pH 7.4) containing 5 mM methionine, 5 mM leucine. Cells were scraped using a disposable cell scraper and resuspended in saline solution (9‰ NaCl) at 4°C. They were then homogenized at 4°C in a Potter Elvejehm Teflon glass homogenizer (AA) (50 strokes at maximum speed), and centrifuged at 800 × *g* for 15 min. The pellet obtained was referred to as 'nuclear preparation' as in [9].

2.2.3. Electrophoresis

Two-dimensional gel electrophoresis of the nuclear proteins was performed. In the first dimension, a basic system described in [12] with migration toward the anode, permitted the analysis of proteins whose pH_i values were lower than 8. Ten μg of proteins containing about 300000 cpm of ^{35}S and 100000 cpm of ^{3}H were applied to these gels in a total volume of 60 μl.

In the second dimension, proteins were separated according to their molecular masses in slab gels at pH 7.2 using acrylamide at a concentration of 11% in system J 3561 as in [13] in the presence of 0.1% SDS in the upper reservoir buf-

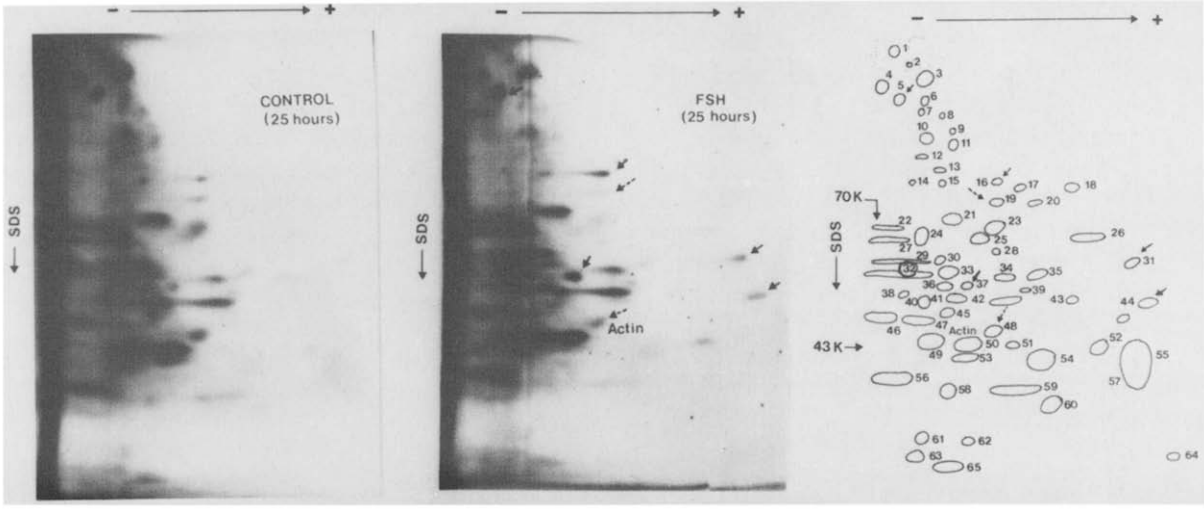


Fig.1. Effect of FSH on Sertoli nuclear protein synthesis after 25 h of FSH treatment (9 h of FSH treatment alone and then, 16 h FSH treatment in the presence of labelled amino acids). (a) Control conditions, (b) FSH treatment, (c) diagrammatic representation of a. The arrows ⟶ show proteins which are increased by FSH treatment. The arrows – – → show proteins which are decreased by FSH treatment. 32, protein 32 which disappeared. K, kDa.

fer. The slab gels were treated by sodium salicylate, dried and exposed to Kodak films (X-Omat AR-5). For the comparison of nuclear preparations from control and FSH-treated cells, the same amount of protein as determined by the method in [14] was applied to each electrophoresis. Similar results were obtained if the same amount of radioactivity was loaded on the gels.

3. RESULTS

Stimulation of nuclear protein synthesis by FSH was measured after 25 h of treatment: fig.1 shows the pattern of nuclear proteins under control conditions (a) (this pattern is different from that of cytosolic proteins and microsomal proteins: not shown), and after FSH stimulation (b). Sixty-five spots were identified: see fig.1c which represents the gel pattern. Three different significant effects of FSH on nuclear protein synthesis were consistently observed in 3 different experiments:

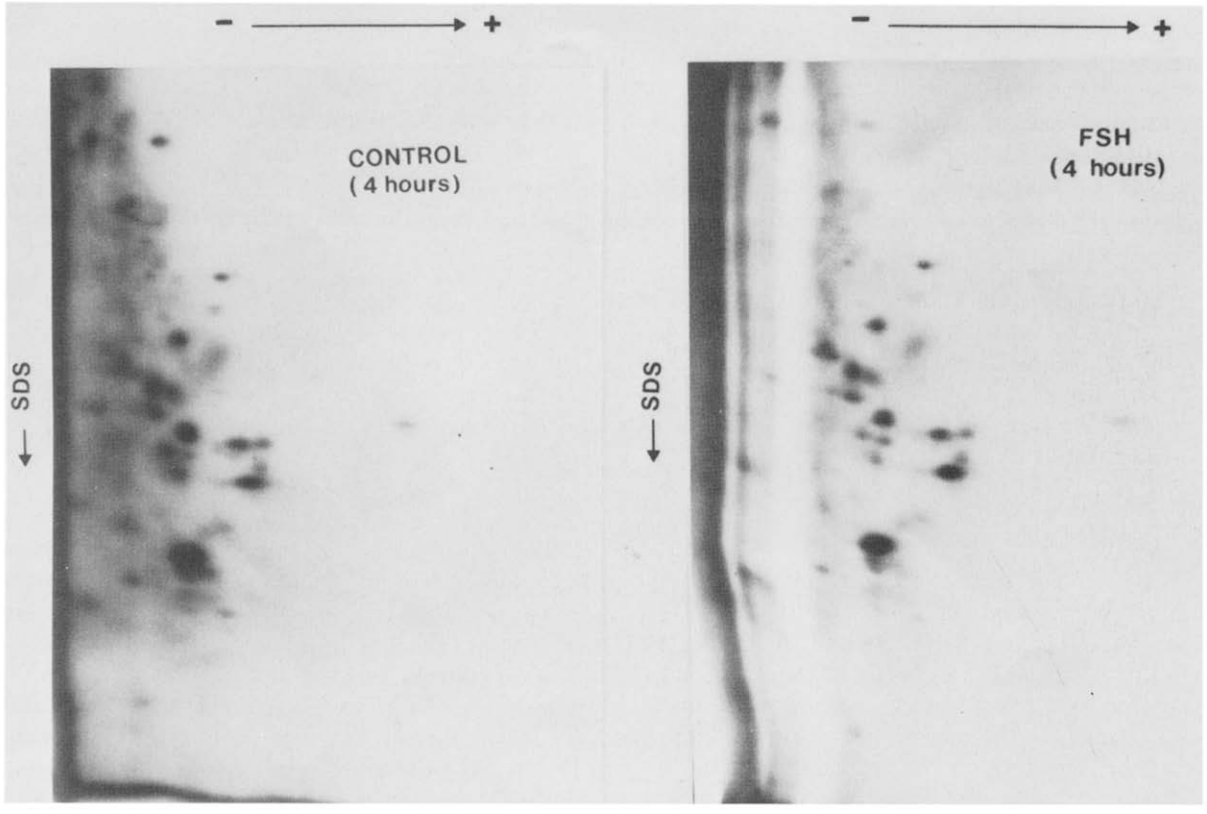
(i) Five proteins were stimulated: proteins 5, 16, 31, 37, 44 (molecular mass in the range of 220 kDa (protein 5), 93 kDa (protein 16), 58 kDa (protein 31), 53 kDa (protein 37), 50 kDa (protein 44));

(ii) While one protein consistently disappeared: protein 32 (molecular mass in the range of 58 kDa);

(iii) And two proteins were always slightly but significantly diminished: proteins 19 and 48 (molecular mass in the range of 82 and 45 kDa, respectively).

No changes in the other proteins were observed.

Two experiments showed that, after 4 h of FSH treatment, there was no effect of the hormone on nuclear proteins (see fig.2). Whether under control or FSH conditions, the same pattern as that in



a b

Fig.2. Effect of FSH on Sertoli nuclear protein synthesis after 4 h of FSH treatment (2 h of FSH treatment alone and then, 2 h FSH treatment in the presence of labelled amino acids). (a) Control conditions, (b) FSH treatment.

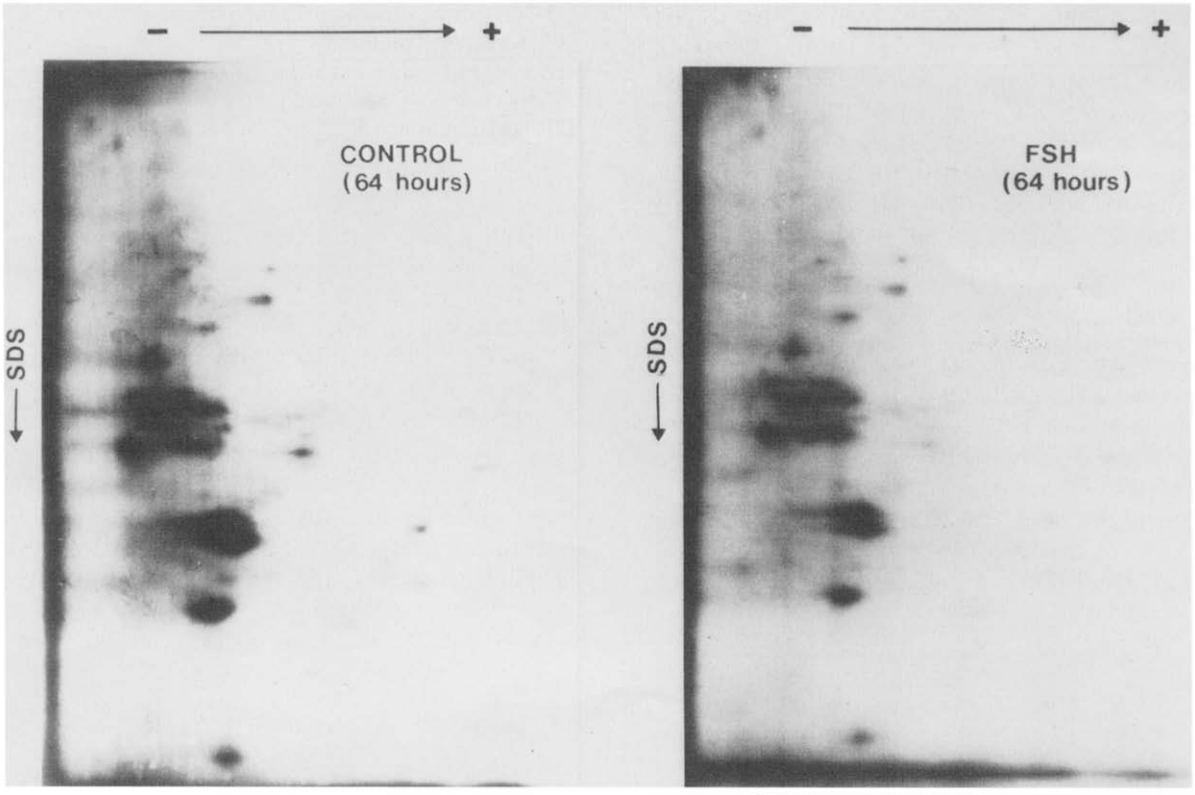


Fig.3. Effect of FSH on Sertoli nuclear protein synthesis after 64 h of FSH treatment (52 h of FSH treatment alone and then, 12 h FSH treatment in the presence of labelled amino acids). (a) Control conditions, (b) FSH treatment.

fig.1a was observed. Similar results were obtained after 8 h of FSH treatment (not shown).

Fig.3 shows the pattern obtained after 64 h of FSH treatment. The synthesis of nuclear proteins appears to be decreased. This pattern was also consistently found in 3 such experiments.

4. DISCUSSION

It is well known that FSH is able to stimulate rapid RNA and protein syntheses of cultured rat Sertoli cells [2] and their secretion of specific proteins [3]. The hormonal control of these effects has been reported [15]. To our knowledge, there are no data concerning the stimulation of nuclear protein synthesis after FSH or other hormonal tretament. However, it has been shown that other polypeptide hormones [6,7] affect the phosphorylation or acetylation of non-histone proteins.

Our data showed that 25 h of FSH treatment induced differential effects on synthesis of nuclear proteins whose pH_i values are lower than 8: 4 groups of proteins could be distinguished: 5 proteins were increased, 2 proteins were decreased, one disappeared while the others were not affected. These effects were not observed either after short- or long-term treatments. In the case of short-term FSH treatment, there was no effect of the hormone which is not surprising since we are dealing with the synthesis of nuclear proteins. After 64 h of FSH treatment, all proteins were decreased. Two hypotheses are therefore possible: firstly, during the 52 h of FSH treatment (without labelled amino acids), a great amount of non-labelled proteins have been synthesized. Therefore, after the subsequent addition of labelled amino acids there may be an isotopic dilution of the newly synthesized proteins. Secondly, this lack of stimulation of nuclear synthesis may be due to a desensitization phenomenon [16,17].

In conclusion, FSH affects the synthesis of pig Sertoli cell nuclear proteins. We are probably dealing with acidic proteins since we have only looked at the proteins whose pH_i values are lower than 8. Since non-histone proteins are involved in gene expression and in the control of transcription, our data suggest that FSH could act at the gene expression level via a mechanism whereby nuclear acidic proteins are modified.

ACKNOWLEDGEMENTS

I wish to thank Joelle Bois for her secretarial work and Dominique Langlois-Gallet for her technical help. This work was supported by MIR grant 82 L 0853.

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