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FSH STIMULATION OF CYTOSOLIC PROTEIN SYNTHESIS IN CULTURED PIG SERTOLI CELLS

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Summary—The effects of FSH on the cytosolic protein synthesis by a primary culture of immature porcine Sertoli cells were studied. Cells were cultured in a chemically-defined medium for 3 days and, on day 3, they were incubated for 40 h in another medium containing labelled amino-acids either in the presence or absence of 50 ng/ml porcine FSH (pFSH). In control cells, about 107 spots (pI in the range of 5 to 8) were identified by a two-dimensional polyacrylamide gel electrophoresis of the radiolabelled cytosolic proteins. pFSH treatment produced a marked increase of seven proteins whose molecular weights and isoelectric points are respectively comprised between 25 to 58 K and 5.5 to 5.8. In addition, pFSH treatment induced a slight but constant increase of two other proteins (mol. wt: 24 and 12 K and isoelectric point: 5.3).

INTRODUCTION

Follitropin (FSH) is the main peptide hormone regulating Sertoli cell function [1]. Its effects are mediated *via* cAMP [1] and it also produces an increase in RNA and intracellular protein synthesis [2], and the secretion of proteins [3]. In order to understand the mechanism by which FSH acts on Sertoli cells, we looked for intracellular protein markers induced by this hormone. FSH treatment was performed in primary culture of 20-day old 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 [4]. In this paper we report the effects of FSH on the synthesis of the cytosolic proteins whose pI is in the range of 5–8.

EXPERIMENTAL

Materials

Porcine follicle stimulating hormone (NIH-FSH-P2) was a gift from NIADDK, National Pituitary Agency. [^{35}S]Methionine (sp. act. 1400 to 1500 Ci/mmol) was purchased from Amersham, England. [^{3}H]Leucine (sp. act. 100 Ci/mmol) was purchased from Saclay, France. Collagenase-dispase was obtained from Boehringer, Mannheim. Dulbecco 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, desoxyribonuclease type I were supplied by Sigma Chemicals. Earle's-salt medium without L-methionine and L-glutamine was obtained from Gibco Europe.

The molecular weight protein standards were: transferrin and actin from Sigma Chemicals, chymotrypsin and cytochrome *c* from Boehringer kit S.A., and phosphorylase B and carbonic anhydrase from Bio-Rad.

Methods: Sertoli cell culture

Testes of 20-day old pigs were decapsulated, minced and treated in 500 ml of a 1:1 mixture of Ham's F12 medium and Dulbecco modified Eagle's medium (Ham F12/DME) containing Collagenase-dispase (400 mg/l) and soybean trypsin inhibitor (20 mg/l), at 37°C as described by Mather *et al.*[5]. The digested tissue was filtered through Nytex (mesh ≃ 400) and diluted with 2 vol of 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 and then incubated in trypsin–EDTA buffer containing 0.02% desoxyribonuclease 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 a 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's F12/DME.

Fifteen million Sertoli cells were cultured in Falcon flasks (75 cm^2) at 35°C in a 5% CO_2 atmosphere in the Ham F12/DME medium containing sodium bicarbonate 1.2 mg/l, Hepes 10 mM, gentamycine 20 μg/l, fetal calf serum 0.1%, insulin 5 μg/ml, transferrin (5 μg/ml), vitamin E (10 μg/ml), mycostatin (40 U/ml) and penicillin–streptomycin (100 U/ml). The medium was changed every day.

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FSH treatment

On day 3 (cells are prepared on day 0), cells were incubated with [^{35}S]methionine (25 μCi/ml) and [^{3}H]leucine (25 μCi/ml) for 40 h in Earle's salts medium without L-methionine and L-glutamine, supplemented as above, 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–HCl buffer (pH 7.4) containing methionine 5 mM, leucine 5 mM. 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–Elvehjem Teflon glass homogenizer [AA] (50 strokes at maximum speed). Cytosol fractions were prepared as detailed elsewhere [6].

Electrophoresis

Two-dimensional gel electrophoresis of the cytosolic proteins was performed. In the first dimension, proteins were separated by isoelectric-focusing within the pH range of 5–8 according to the method of O'Farrell[7]. About 10 μg of proteins containing about 300,000 cpm of [^{35}S] and 100,000 cpm of [^{3}H] were applied to these gels in a total volume of 50 μl.

In the second dimension, proteins were separated according to their molecular weights in slab gels at pH = 7.2 using acrylamide at a concentration of 20% in system J3561 as described by Neville and Glossman[8] in the presence of 0.1% SDS in the upper reservoir buffer. The slab gels were treated by sodium salicylate, dried and exposed to Kodak films (X-Omat AR-5). For the comparison of cytosolic preparations from control and FSH treated cells, the same amount of radioactivity was loaded on the gels.

RESULTS AND DISCUSSION

After 4 days of culture, cells had the aspect shown in Fig. 1. No differences in the cell shape were observed between control (Fig. 1a) and pFSH-treated cells (Fig. 1b). It has been reported, however, that FSH at high concentration (0.3 μg/ml) was able to change the shape of cultured pig Sertoli cells after 6 days of culture [9]. This effect was attributed to a modification of the cytoskeleton. The absence of pFSH-induced cell shape changes in our culture conditions may be due either to a shorter period of pFSH treatment, or to the lower pFSH concentration used, or to both.

The two-dimensional polyacrylamide gel electrophoresis allowed the detection of 107 cytosolic proteins under control conditions (Figs 2a and 2c). Porcine FSH treatment did not induce the appearance of any new spots, but, the hormone produced a marked increase of seven proteins (proteins 33, 64, 65, 73, 77, 79, 80). In addition, FSH also stimulated the synthesis of two other proteins but to a lesser extent (proteins 96 and 102). The molecular weights and the isoelectric points of these proteins are given in Table 1. It must be pointed out that the effects reported in this study were consistently found in three different experiments while the changes in the other proteins were not reproducible.

It is a well known fact that FSH is able to stimulate both RNA and protein synthesis of rat Sertoli cells [2] and also the secretion of specific proteins [11]. Most of the studies dealing with FSH-stimulation of proteins have been conducted by the analysis of the medium of the cells. Moreover, most of such studies have been conducted in the rat model [3, 12] whereby it was reported that the hormone stimulated the synthesis of two intracellular polypeptides, SCc_1 and SCc_2. These two polypeptides have identical molecular weights, 25 K_d, but differ in their pI (5.8 and 5.9 respectively). However, their exact intracellular localizations have not been studied [12]. As far as the pig is concerned, we recently showed that FSH stimulated acidic nuclear proteins in Sertoli cells [13]. Moreover, in this study, we now show that FSH also has a stimulatory effect on the cytosolic proteins.

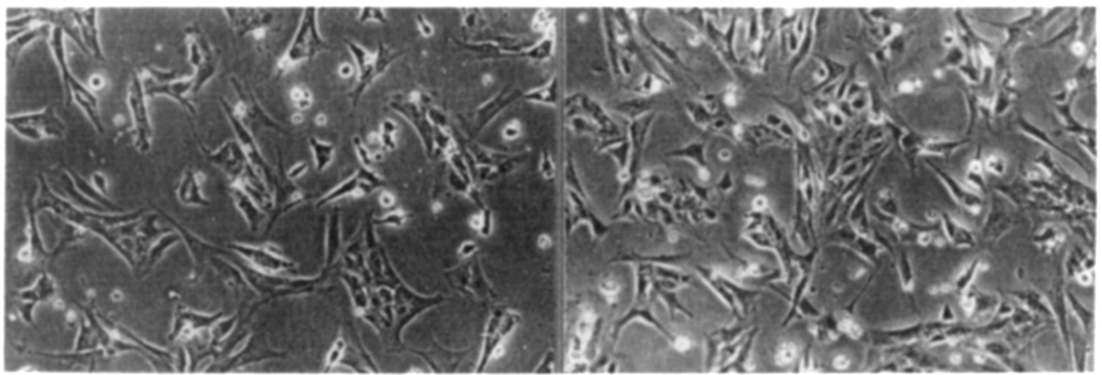

Fig. 1. Morphological appearance of pig Sertoli cells grown in the absence (a) or presence of pFSH (b). Photomicrographs were taken under phase-contrast microscopy (×200) after 4 days in culture.

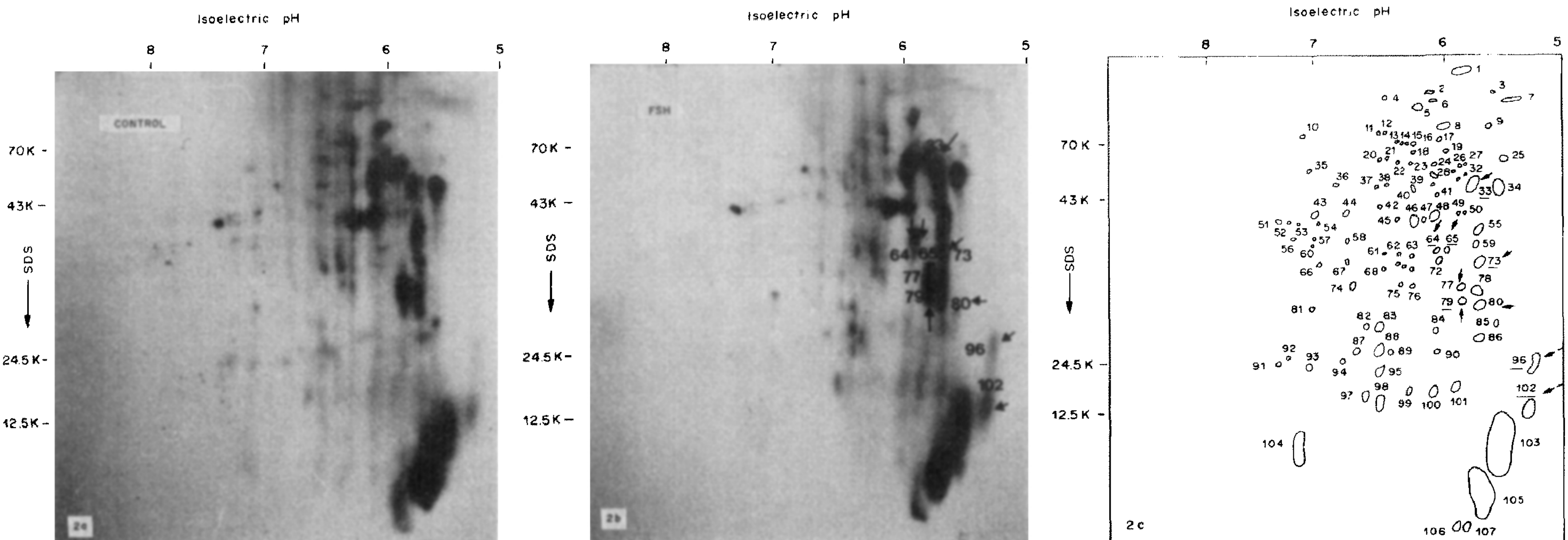

Fig. 2. Effect of FSH on Sertoli cytosolic protein synthesis after 40 h of FSH treatment. 2a: Control conditions. 2b: FSH treatment. 2c: Diagrammatic representation of 2a. The concentration of acrylamide is 20%. The molecular weight standards are: Transferrin (70 K), actin (43 K), chymotrypsin (24.5 K) and cytochrome *c* (12.5 K). These standards are ^{14}C radiolabeled according to the method of Dottavio-Martin [10]. The arrows → show proteins which are markedly increased by FSH treatment. The arrows ---→ show proteins which are only slightly increased by FSH treatment.

Table 1. Molecular weights and isoelectric points of the pFSH-stimulated cytosolic proteins

Proteins	33	64	65	73	77	79	80	96	102
pI	5.8	6.1	5.9	5.5	5.8	5.8	5.5	5.3	5.3
Mol. wt (K_d)	57.5	37.5	37.5	35	28	26	25	24	12

The molecular weights were determined by comparison with the standards: transferrin (70 K_d), actin (43 K_d), chymotrypsin (24.5 K_d) and cytochrome *c* (12.5 K_d). In addition to these, carbonic anhydrase (29 K_d) and phosphorylase B (92.5 K_d) were also used in order to determine the exact molecular weights of proteins 73, 77, 79 and 80. A standard curve was plotted and the molecular weights determined. Estimates of isoelectric point (pI) were determined by comparison to the pH gradient in a blank isoelectrofocusing gel following electrophoresis.

According to their pI and molecular weights, pig Sertoli cell cytosolic proteins 77 and 79 seem to be analogues of the two rat Sertoli cell intracellular polypeptides, SCc_1 and SCc_2. The identity and the role of these pFSH-stimulated cytosolic proteins are still unknown, but they might be useful markers of FSH action in Sertoli cells.

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