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In Vitro Regulation of Pig Sertoli Cell Growth and Function: Effects of Fibroblast Growth Factor and Somatomedin-C[1]

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ABSTRACT

The effects of insulin, somatomedin-C (Sm-C), epidermal growth factor (EGF), fibroblast growth factor (FGF), vitamin E, and retinoic acid on growth and function of immature cultured pig Sertoli cells were investigated. All these factors, except vitamin E, stimulated Sertoli cell DNA synthesis and proliferation. The mitogenic effects of insulin observed only at micromolar concentrations were similar to those induced by nanomolar concentrations of Sm-C or EGF, but significantly less than those induced by FGF. The effects of EGF and Sm-C were almost additive, whereas those of Sm-C and FGF were synergistic. After a 6-day treatment, FGF and retinoic acid induced a significant increase in the number of follicle-stimulating hormone (FSH) receptors per cell, and in FSH-induced cyclic adenosine 3',5'-monophosphate (cAMP) production. Sm-C, which alone had no effect on these two parameters, potentiated FGF action. Basal plasminogen activator activity was enhanced after the 6-day treatment with EGF plus insulin and, particularly, with FGF plus insulin. Similarly, the response of the latter group to FSH was significantly higher than in any other group of cells. FGF was also able to stimulate cell multiplication and enhanced the FSH receptor number of Sertoli cells isolated from 15- and 26-day-old rats. Thus, FGF is the most potent known mitogenic factor for cultured Sertoli cells, and it stimulates the phenotypic expression of these cells.

INTRODUCTION

The development of chemically defined media has made it possible not only to culture established cell lines under more reproducible conditions, but also to establish new cell lines and to investigate primary cultures of several cell types (Mather and Sato, 1979; Barnes and Sato, 1980). These serum-free media have allowed the culture of both isolated Leydig and Sertoli cells for several days (for review, Mather and Phillips, 1984) and have contributed markedly to a better understanding of the biochemical properties of these cells. Concerning Sertoli cell primary cultures, most studies have been orientated to investigate the effects of hormone-supplemented serum-free media on Sertoli cell functions (Tung et al., 1975, 1980; Verhoeven, 1980; Skinner and Griswold, 1982; Conti et al., 1983; Rich et al., 1983; Wright et al., 1983; Perrard et al., 1985; Saez and Jaillard, 1986), but the mitogenic effect of such media has been investigated in only a few cases (Griswold et al., 1976; Tung et al., 1980; Rich et al., 1983; Borland et al., 1984). Moreover, all of the above studies were limited to Sertoli cells from rats.

In the last few years, pig as well as rat Sertoli cells have been extensively used to investigate their secretory products and their role in the regulation of the function of other testicular cells (review in Parvinen, 1982; Saez et al., 1985). It therefore seemed of interest to determine the optimal culture conditions for these porcine cells. In the present work, we have investigated the effect of several factors on pig Sertoli cell growth and function and show that fibroblast growth factor (FGF) is the most active of the factors studied. In addition, we have shown that somatomedin-C (Sm-C) at nanomolar concentrations or insulin at micromolar concentrations have a synergistic effect with FGF.

MATERIALS AND METHODS

Materials

Transferrin, bovine insulin, soybean trypsin inhibitor, vitamin E, retinoic acid, fibrinogen,

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plasminogen, and 4-(2-hydroxyethyl)-l-piperazinethanesulfonate (Hepes) were purchased from Sigma Chemical Co. (St. Louis, MO), and collagenase was purchased from Boehringer (Mannheim, Germany). Ham's F-12 medium and Dulbecco's modified Eagle's medium in powder form, and trypsin-ethylenediaminetetraacetic acid (EDTA) were obtained from Grand Island Biological Co. (Grand Island, NY). Porcine follicle-stimulating hormone (NIH-FSH-P_2) and human follicle-stimulating hormone (hFSH-2) were provided by NIADDK, National Pituitary Agency; ^{125}I-Na (carrier-free) was obtained from Amersham. Epidermal growth factor (EGF) from mouse submaxillar gland was prepared as described by Savage and Cohen (1972). An aliquot of the purified material was iodinated with ^{125}I-Na as described by Bernier et al. (1986a) and analyzed under reduction conditions in 0.1% sodium dodecyl sulfate (SDS)/15% polyacrylamide gel. Autoradiography of the gel revealed a single band of molecular weight 6000. Bovine brain basic FGF was purified as described by Gospodarowicz et al. (1984), using heparin-Sepharose affinity chromatography. An aliquot (1 μg of protein) of this purified material was solubilized in 0.1 ml 0.1 tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7, containing 2% SDS and 100 mM dithiothreitol, and applied to a 0.1% SDS/15% polyacrylamide gel. After staining with silver nitrate, a single band of molecular weight 18,000 was observed. Sm-C was purified from acromegalic plasma by a modification of the method described by Chernausek et al. (1985). The purified material migrates as a single narrow band on polyacrylamide gel in the same position as pure recombinant DNA Sm-C (Am-Gen Biologicals, Thousand Oaks, CA) and has the same potency as pure plasma Sm-C in the specific Sm-C radioimmunoassay.

Preparation and Culture of Pig Sertoli Cells

Sertoli cells were prepared with a slight modification of the methods described previously (Perrard et al., 1985; Saez and Jaillard, 1986). In brief, testes from 3- to 4-wk-old piglets were decapsulated, minced, and incubated in Ham's F-12 medium and Dulbecco's modified Eagle medium (1:1) containing 1.2 g/l sodium bicarbonate, 15 mM Hepes, 20 μg/ml gentamycin (DME/Ham's F-12) and 0.4 mg/ml collagenase at 32°C for 90–120 min. At the end of incubation, tubules were allowed to sediment. After several washings, the tubule fraction was treated with 1 M glycine buffer (pH 7.2) for 10 min to remove peritubular cells, then diluted 10-fold with the above medium without collagenase and sedimented again. After washing, the tubular fraction was submitted to a final collagenase treatment (30 min). Dispersed cells were filtered successively through 160-mesh Nitex. The same methods were used to prepare Sertoli cells from 15- or 25-day-old rats.

Sertoli cells were cultured at 33°C in a humidified atmosphere in DME/Ham's F-12 supplemented with penicillin (20 U/ml), streptomycin (50 μg/ml), transferrin (5 μg/ml), insulin (5 μg/ml), and vitamin E (5 μg/ml). The medium was changed after 24 h and every 48 h thereafter. After 3 to 5 days, the cells were subcultured by incubation with 0.5% trypsin and 1 mM EDTA in phosphate-buffered saline (Ca^{2+}, MG^{2+}-free) for 3–4 min at 33°C. The detached cells were diluted with Ham's F-12:DME medium containing either 5% fetal calf serum (FCS) or 1 mg/ml of soybean trypsin inhibitor. After 5 min, the cells were centrifuged, washed twice with Ham's F-12/DME, and plated with the culture medium described above. The next day, the medium was removed and replaced by fresh DME/Ham's F-12 medium supplemented only with antibodies. After 36 to 48 h, fresh medium with the appropriate growth factors was added.

We chose to work with secondary cultures because in primary cultures considerable variations may be seen in the measurement of biochemical parameters made on different preparations of cells, whereas on secondary cultures, the variability between different cell preparations is far less pronounced. The reason for such differences is not clear. It must be pointed out, however, that in preliminary experiments using primary cultures, and within the same preparation, the pattern of response to several growth factors was similar to that observed with secondary cultures (see Results).

[^{3}H]Thymidine Incorporation into DNA

About 8 h after addition of growth factors, [^{3}H]thymidine (1 μCi/ml) was added and the incubation continued for 18–20 h. At the end of this period, the medium was removed. The cells were washed three times with 5% trichloroacetic acid and solubilized in 0.5 N NaOH, 0.4% deoxycholate. Counting was carried out for 10 min in a liquid scintillation spectrometer with a yield of 45%.

Cell Growth Measurements

After 36 to 48 hours in DME/Ham's F-12 medium and before the addition of the growth factors, some dishes were trypsinized and cells counted to determine the initial number of cells. The growth factor was added every other day without change of the media, and the incubation continued for 6–7 days. At the end of the experimental period, the cells were washed three times with ice-cold saline. In addition, when cells were pretreated with FSH, the cells were also washed with low pH glycine buffer to remove the membrane-bound hormone (Saez and Jaillard, 1986). Then the dishes were divided into three groups: one group was trypsinized and the cell number was determined with a coulter counter, the second was used for [^{125}I]-hFSH binding measurement, and the third was used for cyclic adenosine 3',5'-monophosphate (cAMP) and plasminogen activator activity measurement.

Binding of [^{125}I]-Labeled hFSH

hFSH was iodinated with ^{125}I by the lactoperoxidase method (Ketelslegers and Catt, 1974). The specific activity varied from 60 to 80 μCi/μg. For each batch, the maximum specific bindable [^{125}I]-labeled hFSH in the presence of an excess of receptor was determined. This varied from 13 to 22%. All the binding assays were performed in sextuplicate, three containing only [^{125}I]-labeled hFSH ($\simeq 5 \times 10^{-10}$ M), the other three a 500-fold excess of hFSH to correct for the nonspecific binding that accounted for 5–15% of the total binding, depending on the experimental conditions. The incubation was performed at 33°C for 4 h. Then the medium was removed, and the monolayers were quickly washed twice with ice-cold 150 mM sodium chloride containing 1% bovine serum albumin (NaCl-BSA) and once with ice-cold 150 mM saline. The cells were then dissolved in 0.5 M NaOH 0.4% deoxycholate, and the radioactivity was measured in a gamma counter.

cAMP and Plasminogen Activator Activity Productions

After the washing procedure at the end of the experimental periods, the cells were incubated in the same medium used during the pretreatment (except when the cells were pretreated with FSH, in which case the hormone was deleted) without or with maximal stimulating concentration of porcine FSH (pFSH; 1 μg/ml). After 30 min, one aliquot was taken for cAMP determination, and a second was taken 24 h later for plasminogen activator activity. cAMP was measured by specific radioimmunoassay as described elsewhere (Steiner et al., 1972). Plasminogen activator activity was measured by determining plasminogen-dependent degradation of [^{125}I]-fibrin (Lacroix et al., 1977). Several dilutions of the medium were used (1:1 to 1:80), and each dilution was assayed in triplicate. Aliquots were taken at different periods of time and the solubilized [^{125}I]-fibrin degradation was expressed as percentage of the fibrinolysis produced by a 0.4% trypsin solution.

Statistics

Statistical analyses were performed with Student's *t*-test for comparison of two groups or by analysis of variance with Dunnett's test for comparison of data from multiple groups. Differences were considered significant when $p<0.05$.

RESULTS

Effects of Several Growth Factors on Pig Sertoli Cell DNA Synthesis and Cell Multiplication

When Sertoli cells are cultured at high density ($\simeq 2 \times 10^5$ cells/cm^2) in a chemically defined medium containing transferrin (5 μg/ml) and insulin (5 μg/ml), both DNA synthesis and cell multiplication are very low (data not shown). At low density, however, insulin, but not transferrin, significantly stimulates both DNA synthesis and cell multiplication (see below).

To investigate whether the mitogenic effects of insulin were mediated by its own receptors or through Sm-C/insulin-like growth factor (IGF) type I receptors, the effects of both peptides on DNA synthesis were investigated (Fig. 1). Sm-C stimulated Sertoli cell DNA synthesis at low concentrations ($ED_{50} \simeq 7.5 \pm 1.2$ ng/ml, n = 3), whereas the stimulatory effect of insulin was seen only at very high concentrations ($ED_{50} \simeq 1$ μg). Moreover, at maximal concentrations, the effects of Sm-C and insulin were not additive (Fig. 2). At maximal concentrations, the stimulatory effects of EGF alone were similar to those of insulin or Sm-C, but less than those induced by FGF (Fig. 2). When EGF was associated with insulin or Sm-C, the stimulation was significantly higher than that observed with each factor alone. Again, the effects of FGF and

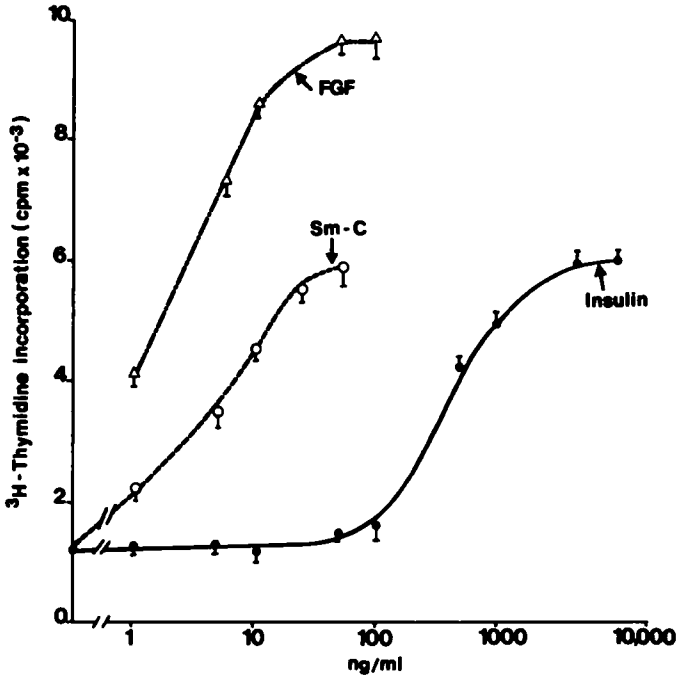

FIG. 1. Effects of somatomedin-C (Sm-C), insulin, or fibroblast growth factor (FGF) on pig Sertoli cell DNA synthesis. Sertoli cells were cultured for three days in complete medium (transferrin [TR] + insulin [Ins.] + vitamin E [Vit E]), divided and cultured in the same medium for 24 h. The medium was removed and replaced by fresh medium containing only TR. After two days, the medium was replaced by fresh medium containing TR and the indicated concentrations of Sm-C (○), Ins. (●), or FGF (△). [^{3}H]Thymidine (≃ 1 μCi/ml) was added 6 h later, and the incubation continued for 18 additional h. The incorporation of [^{3}H]thymidine into DNA was measured as indicated in *Materials and Methods*. The results are the mean ± SD of quadruplicate determinations. Similar results were obtained in two other experiments.

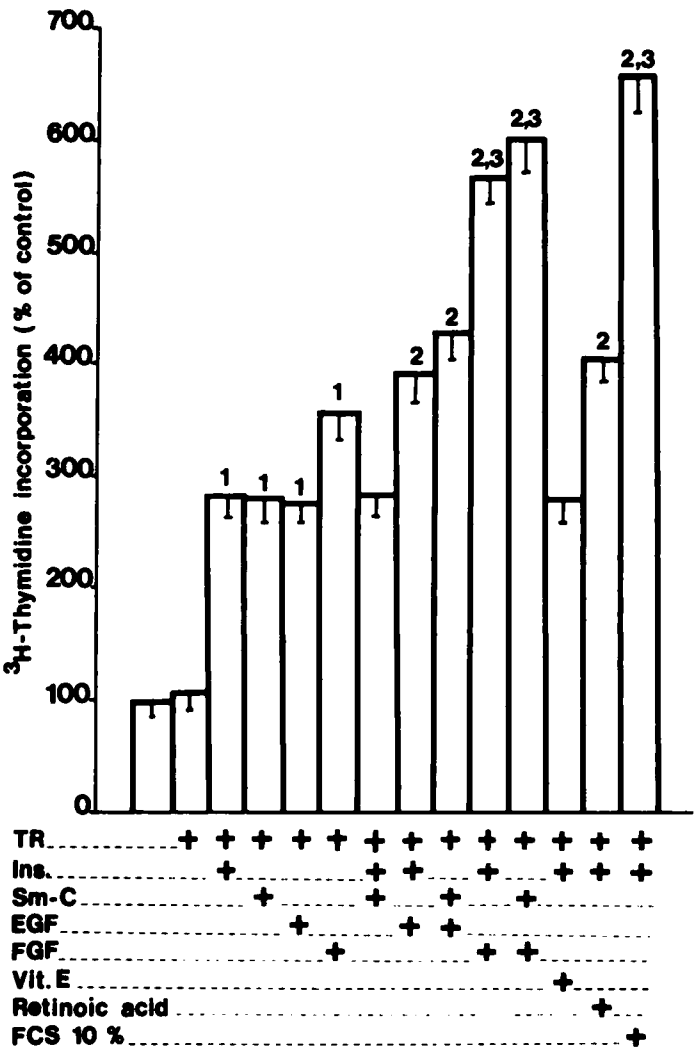

FIG. 2. Effects of several factors on pig Sertoli cell DNA synthesis. The experimental protocol was similar to that described in the *legend of Figure 1*. The concentrations of the factors were: 5 μg/ml (transferrin, *TR*), 5 μg/ml (insulin, *Ins.*), 50 ng/ml (somatomedin-C, *Sm-C*; epidermal growth factor, *EGF*; fibroblast growth factor, *FGF*), 200 ng/ml (vitamin E, *Vit E*), and 300 ng/ml (*retinoic acid*). The results are expressed as percentage of medium alone and represent the mean ± SEM of quadruplicate determinations of 2 to 8 different experiments. 1:$p<0.05$ compared to control; 2:$p<0.05$ compared to the values observed with each factor alone; 3:$p<0.05$ compared to EGF associated with Ins. or Sm-C.

insulin or Sm-Sm-C were significantly higher than those of EGF. Neither vitamin E nor retinoic acid alone had any effects. However, in the presence of insulin, retinoic acid, but not vitamin E, had a significant stimulatory effect. It is interesting to note that the stimulatory effect of FGF plus insulin or Sm-C was similar to that induced by 10% FCS.

When the mitogenic activity was evaluated by the number of cells after a 6-day treatment, the same pattern of response was obtained (Fig. 3) except that the mitogenic activity of 10% FCS was more potent than that of the other factors, whether alone or combined. This discrepancy might be related either to the fact that the factors were added only every other day and that Sertoli cells were able to degrade some of these factors very rapidly (EGF [Bernier et al., 1986a] and Sm-C [unpublished results]) or that some of the cells detached from the plastic in the presence of the growth factors, but not in the presence of FCS.

In addition, it is possible that 10% FCS, but not FGF, stimulated the growth of the small percentage of contaminant cells. As described before (Perrard et al., 1985), our Sertoli cell preparations contain about 6–8% myoid cells, and the growth of these cells is stimulated by FCS (Tung et al., 1984). Indeed, at the end of the 6-day treatment, the percentage of myoid cells, as determined by the Feulgen staining procedure, was 3–6% (n = 3) after FGF or FGF plus insulin

treatment and 19 and 22% (n = 2) in dishes cultured with 10% FCS.

The effects of pFSH on Sertoli cell multiplication were investigated in two independent experiments. The results (Table 1) clearly indicate that the hormone enhanced the stimulatory effect of insulin and FGF on DNA synthesis, but apparently blocked the effects of both factors on cell multiplication (Table 2).

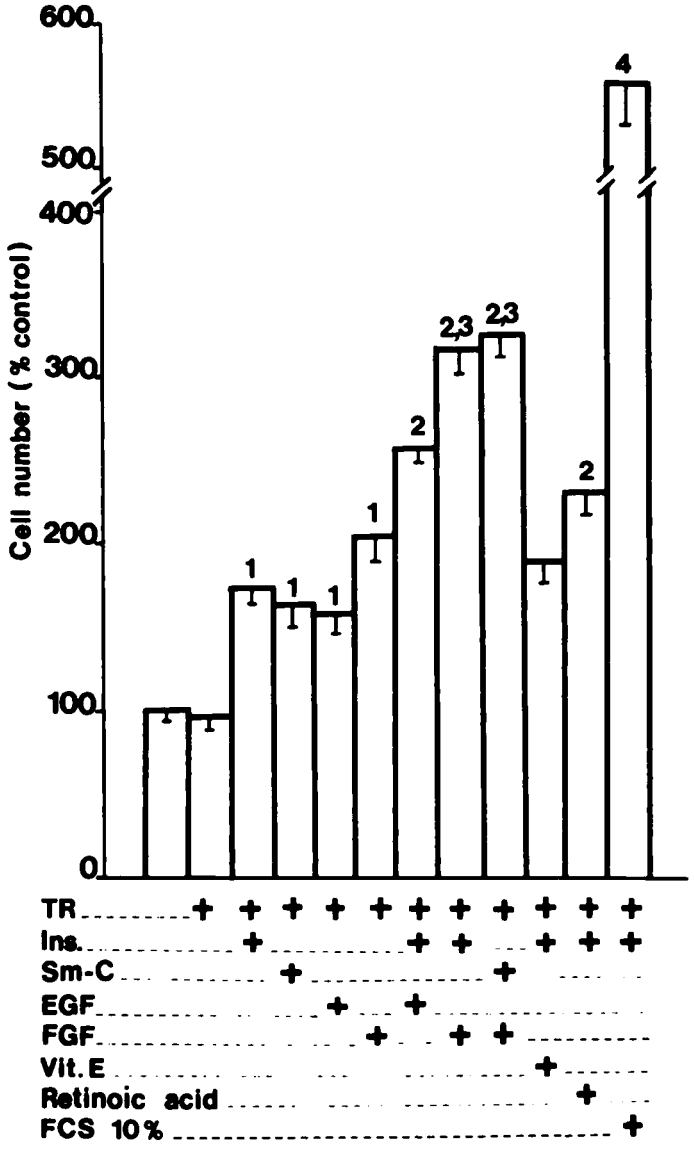

FIG. 3. Effects of several factors on Sertoli cell multiplication. The cells were prepared as indicated in the *legend* to *Figure 1* and the concentration of factors was identical to that indicated in *Figure 2*. The medium and the factors were renewed every other day and the cells counted after six days of treatment. The results are the mean ± SEM of quadruplicate determinations of 3 to 9 different experiments. 1: $p<0.05$ compared to media alone; 2: $p<0.05$ compared to the values observed with each factor alone; 3: $p<0.05$ compared to EGF plus Ins. or Sm-C; 4: $p<0.05$ compared to all other values.

TABLE 1. Effects of follicle-stimulating hormone (FSH) and/or fibroblast growth factor (FGF) on pig Sertoli cell DNA synthesis and multiplication.*

Treatment	[^{3}H]Thymidine incorporation	Cell number
	% of control	
Control	100	100
Insulin	255 ± 10	160 ± 12
Insulin + pFSH	300 ± 20	70 ± 4
Insulin + FGF	450 ± 45	260 ± 20
Insulin + FGF + pFSH	520 ± 40	65 ± 10

*Subcultured Sertoli cells were incubated two days in medium containing only transferrin (control). The medium was replaced by fresh medium containing insulin (5 μg/ml), pFSH (500 ng/ml), FGF (50 ng/ml) alone or together. The incorporation of [^{3}H]thymidine into DNA was measured after 24 h, and the cell number after six days. In this case, the factors were added every other day. The results are the mean ± SEM of quadruplicate determinations of two experiments.

Indeed, the cell number in dishes treated with pFSH was even lower than in those treated only with transferrin. This apparent discrepancy in the effect of FSH on DNA synthesis and cell number was probably due to the fact that after FSH treatment, Sertoli cells retracted and "rounded-up," and these morphological modifications were followed by detachment of some of the cells during the 6-day treatment.

Effects of Growth Factors on Pig Sertoli Cell FSH Receptors and Responsiveness

When Sertoli cells are cultured in a chemically defined medium containing transferrin and insulin, they maintain both FSH binding sites and the cAMP and plasminogen responses to FSH at a fairly constant level for at least two weeks (Saez and Jaillard, 1986). Figure 4 shows that the only factors able to increase the binding of ^{125}I-FSH were FGF alone or in combination with insulin or Sm-C, and retinoic acid with insulin. On the other hand, vitamin E and FCS—at the concentrations used—significantly decreased the apparent number of FSH binding sites. However, since 10% FCS stimulated myoid cell multiplication (see above), the diminution of FSH binding sites might be related to a dilution of Sertoli cells by myoid cells rather than to a decrease of receptor number per cell.

The effect of growth factors on Sertoli cell responsiveness to FSH was investigated by measuring the cAMP and plasminogen activator activity responses to this hormone after a 6-day treatment. The basal cAMP production was always very low, less than 1

TABLE 2. Effects of long-term treatment with follicle-stimulating hormone (FSH) and/or fibroblast growth factor (FGF) on pig Sertoli cell responsiveness.*

Pretreatment	[^{125}I]-FSH binding	cAMP production % of control	Plasminogen activator
Control	100	100	100
Insulin	92 ± 10	98 ± 6	162 ± 12
Insulin + pFSH	27 ± 6	11 ± 2	27 ± 6
Insulin + FGF	193 ± 12	209 ± 16	522 ± 32
Insulin + pFSH + FGF	58 ± 8	33 ± 4	205 ± 18

*Subcultured Sertoli cells were incubated with the indicated factor for six days (insulin 5 μg/ml; pFSH 500 ng/ml, and FGF 50 ng/ml). The factors were added every other day. At the end of incubation, the binding of [^{125}I]-FSH and the cyclic adenosine 3',5'-monophosphate (cAMP) and plasminogen responses to FSH were measured as indicated under Materials and Methods. The values are the mean ± SD of two different experiments.

pmole/10^6 cells/30 min, and no significant difference was observed in cells treated with FGF, either alone or with insulin or Sm-C, and also was seen in cells treated with retinoic acid with insulin (Fig. 4). This increase was parallel to the effect of these factors on FSH binding. A similar parallelism was observed in the inhibitory effects of vitamin E on FSH binding and FSH-induced cAMP response. Since the values for both FSH binding and cAMP production were corrected by the total number of cells present after each treatment, the modification described above probably expresses specific changes in the activity of Sertoli cells.

The effects of several factors on plasminogen activator activity are shown in Figure 5. Under basal conditions, the activity was very low, except in cells treated with FGF alone or with insulin and with EGF and insulin. The response to FSH was enhanced by previous treatment with insulin or EGF, but again FGF was several times more potent than the other two factors. Insulin potentiated the effect of EGF on FSH responsiveness, but this potentiation of insulin was more pronounced in the case of FGF. The response to FSH of cells treated with insulin and retinoic acid or FCS was higher than that of cells treated only with insulin. FSH pretreatment of Sertoli cells induced receptor loss and desensitization (Table 2). However, simultaneous treatment with FGF partially prevented these two phenomena.

Effects of FGF on Rat Sertoli Cells

Since most studies concerning Sertoli cell growth and function have been performed with rat Sertoli cells, we wondered whether FGF would have in these cells similar stimulatory effects as in pig Sertoli cells. The results in Table 3 show that FGF alone stimulated rat Sertoli cell multiplication and enhanced the FSH receptor number, and that insulin at high concentrations potentiated the effect of FGF.

TABLE 3. Effects of fibroblast growth factor (FGF) on rat Sertoli cell growth and functions.*

Treatment	15 days old		29 days old	
	Cell number (X 10^5)	[^{125}I]-FSH binding (cpm X $10^3/10^6$ cells)	Cell number (X 10^5)	[^{125}I]-FSH binding (cpm X $10^3/10^6$ cells)
Control	2.1 ± 0.1	25 ± 3	1.9 ± 0.08	22 ± 2
T + Ins	2.5 ± 0.2	23 ± 2	2.4 ± 0.1	21 ± 2
T + FGF	3.5 ± 0.2	42 ± 4	3.2 ± 0.2	36 ± 3
T + Ins + FGF	5.8 ± 0.3	41 ± 3	5.4 ± 0.3	38 ± 4

*Sertoli cells from 15- or 29-day-old rats were cultured for five days with DME/Ham's F-12 (see Materials and Methods for composition) medium containing insulin (Ins; 5 μg/ml) and transferrin (T; 10 μg/ml). Then the cells were divided and seeded (≃ 2 X 10^5 cells/well) and cultured in DME/Ham's F-12 supplemented with T and 0.5% fetal calf serum (FCS). The next day, the medium was removed and replaced by the same medium without FCS without or with 5 μg/ml Ins, 50 ng/ml FGF, or both. The culture was continued for seven days without changing the medium, but the factors were added every other day. At the end of the culture, the cell number and specific binding of [^{125}I]-hFSH were measured. The results are the mean ± SD of quadruplicate determinations.

DISCUSSION

The present results demonstrate that several factors including insulin, Sm-C, EGF, FGF, and retinoic acid are able to stimulate both DNA synthesis and cell multiplication of cultured pig Sertoli cells. Insulin was only active at micromolar concentrations, whereas maximal effects were observed with nanomolar concentrations of Sm-C. These results suggest that the mitogenic action of insulin in pig Sertoli cells, as well as in rat Sertoli cells (Borland et al., 1984) are mediated through Sm-C/IGF type I receptors. This conclusion is in agreement with the fact that both rat (Borland et al., 1984) and pig (Perrard-Sapori et al., 1987a) contain IGF type I, but not insulin receptors.

Previous work has shown that EGF is mitogenic for both mouse (Mather and Sato, 1979) and rat (Rich et al., 1983) Sertoli cells. Pig Sertoli cells contain specific EGF binding sites (Bernier et al., 1986a) and

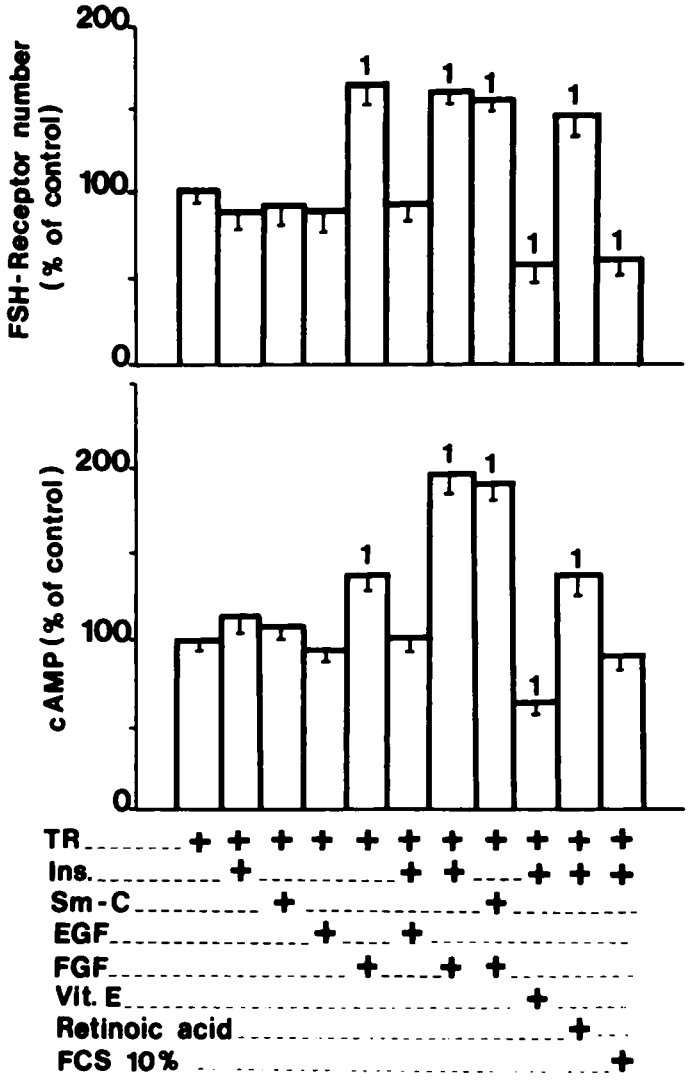

FIG. 4. Effects of several factors on Sertoli cell follicle-stimulating hormone *(FSH)* binding sites and FSH-induced cyclic adenosine 3',5'-monophosphate *(cAMP)* production. Cell preparation and culture conditions were identical to those described in *legends* to *Figures 2* and *3*. After they were washed, some dishes were used to measure the binding of [^{125}I]-hFSH whereas others were treated with 1 μg/ml pFSH to measure the cAMP production. The results are expressed as percentage of cells cultured with transferrin *(TR)* and represent mean ± SEM of quadruplicate determinations of 3 to 8 different experiments. The number of cells at the end of the cultures has been taken into account for calculations. 1:$p<0.05$ compared to cells pretreated with TR.

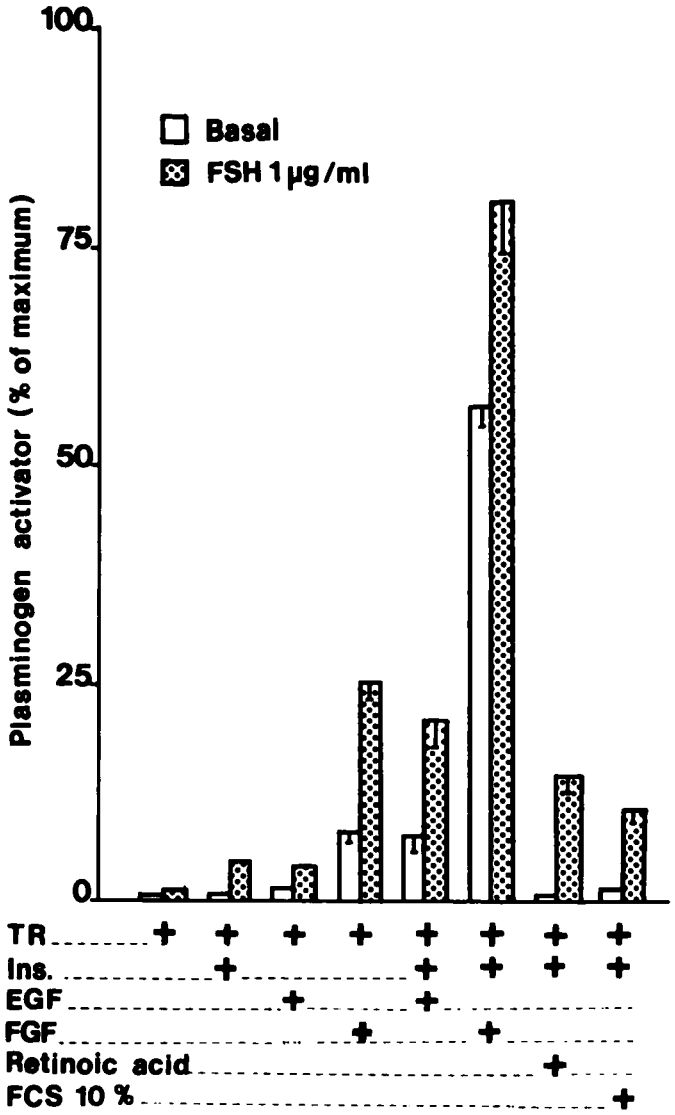

FIG. 5. Effects of several factors on plasminogen activator activity secretion by Sertoli cells. Cells were cultured in conditions identical to those described in *Figure 3*. At the end of the 6-day pretreatment, cells were washed and incubated in the same medium used during the pretreatment without (*open bars*) or with (*stippled bars*) 1 μg/ml pFSH. After 24 h, the medium was removed and saved for plasminogen activator activity measurements. The values are the mean ± SD of triplicates. Similar results were obtained in three other experiments.

this factor is mitogenic for these cells. Moreover, the mitogenic effect of EGF is almost additive with that induced by micromolar concentrations of insulin or nanomolar concentrations of Sm-C. However, 6 days of treatment with insulin, Sm-C, or EGF alone had no significant effect on the number of FSH binding sites or on the cAMP response to this hormone. Only when insulin and EGF were associated was the FSH-induced plasminogen activator activity significantly increased.

The most interesting finding from the present study was the effect of basic FGF. This factor has been shown to be a potent mitogen for mesoderm-derived cells (Gospodarowicz et al., 1986). In some cases, FGF inhibits cell differentiation (Spizz et al., 1986), but in most cases FGF induces cell differentiation (Broad and Ham, 1983; Togari et al., 1985; Walicke et al., 1986) and stabilizes the phenotypic expression of differentiated cells (Gospodarowicz et al., 1986). FGF is the most potent of the mitogenic factors studied for pig Sertoli cells. In addition, FGF enhances their responsiveness to FSH. Of particular interest is the effect of FGF on plasminogen activator activity. It has been reported that in cultured rat Sertoli cells, this protease is a specific marker of FSH action and that this effect is mediated by cAMP (Lacroix et al., 1977; Lacroix and Fritz, 1982). However, FGF—without affecting cAMP production—was able, after 6 days treatment, to enhance both basal and FSH-stimulated plasminogen activator activity secretion. Moreover, insulin at high concentrations, which alone had small effects on this parameter, potentiates the effect of FGF. A stimulatory effect of FGF on plasminogen activator activity secretion by bovine capillary endothelial cells has been reported (Moscatelli et al., 1986).

Although the isolated Sertoli cell aggregates were contaminated with myoid cells, most of the effects of the factors investigated, except those induced by FCS, are probably related to modifications in the cell number and/or biochemical properties of Sertoli cells. The main argument is the fact that at the end of the experimental period, the contaminant cells were even lower than in the fresh isolated cells (except when cells were treated with FCS). The present study does not exclude, however, the possibility that some of the factors might influence Sertoli cells indirectly by acting first in myoid cells and inducing the secretion of some factors, which, in turn, regulate Sertoli cell multiplication and function. Such a paracrine regulation of Sertoli cells by diffusible factors secreted by myoid cells has been reported (Skinner and Fritz, 1985a,b; Skinner et al., 1985).

In vivo studies in the rat have shown that proliferation of Sertoli cells is maximal before birth and falls steadily after parturition (Orth, 1982), with complete cessation of mitotic activity beyond the second week of postnatal life (Steinberger and Steinberger, 1971; Orth, 1982) at the time of initiation of spermatogenesis. FSH seems to be one of the factors involved in Sertoli cell multiplication during the pre- and postnatal periods, since the hormone is able, both in vivo (Orth, 1984) and in vitro (Griswold et al., 1976, 1977), to stimulate DNA synthesis. However, the mitogenic activity of FSH (Griswold et al., 1977) and other growth factors (Rich et al., 1983) in Sertoli cells becomes progressively smaller in preparations from testis of rats over 20 days of age, and the responsiveness appears to be determined by the total age (animal and culture age) of Sertoli cells (Rich et al., 1983). The pig Sertoli cells used in the present study (2- to 3-wk-old pigs) are very immature, since puberty in this animal occurs at about 25 wk of age (Van Straaten and Wensing, 1977; Chevalier, 1978). Therefore, the mitogenic responsiveness to several growth factors, including FSH, is in agreement with the results reported in the rat. However, the responsiveness to FGF of rat Sertoli cells prepared from 15- and 29-day-old animals (total age at the beginning of in vitro stimulation: 20 and 34 days) was similar, suggesting that FGF might overcome the apparent inability of mature Sertoli cells to divide. Clearly, further studies are required to confirm this hypothesis.

The synergistic effects of Sm-C and FGF on Sertoli cell growth and function are of great interest. Recently, it has been shown that Sm-C is secreted by both rat (Tres et al., 1986) and pig (Chatelain et al., 1986) Sertoli cells, and that at least in the pig model FGF, but not FSH, is able to stimulate Sm-C secretion (Chatelain et al., 1986). In addition, Sm-C enhances human chorionic gonadotropin (hCG) receptor number and hCG steroidogenic responsiveness of both pig (Bernier et al., 1986b; Perrard-Sapori et al., 1987b) and rat (Lin et al., 1986a,b) Leydig cells. Hence, Sm-C might play an autocrine and paracrine role in the growth and differentiation of both the testis and the ovary (Adashi et al., 1985). Whether FGF is secreted by Sertoli cells is unknown at the present time, but it should not be forgotten that basic FGF is synthesized by many tissues, including

adrenal and granulosa cells (Gospodarowicz et al., 1986).

In summary, the present data indicate that FGF is the most potent mitogen of the factors studied for Sertoli cells. I In addition, FGF enhances the phenotypic expression of some functions of cultured Sertoli cells. The physiological significance of these results is strengthened by recent data (Ueno et al., 1987) showing that basic FGF is present in bovine testis. Research in this laboratory is underway to learn whether FGF is secreted by testicular cells and to determine the effects of this factor in Leydig and germinal cells.

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