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REGULATION BY GROWTH FACTORS OF LEYDIG CELL DIFFERENTIATED FUNCTIONS

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Summary—In this paper the effects of growth factors on the differentiated function of pig Leydig cells and other steroidogenic cells are reviewed. Two types of action have been observed, i.e. positive or negative acute effects on testosterone secretion, and long-term trophic effects on hCG receptor and responsiveness to hCG. Among the growth factors, insulin-like growth factor I (IGF-I) and transforming growth factor β (TGFβ-1) are of particular interest. IGF-I is required for the maintenance and probably the expression of differentiated functions of several steroidogenic cells, including the Leydig cells. TGFβ-1 has effects opposite to IGF-I on Leydig cell functions. When considering effects of growth factors on Leydig cells, caution should be taken in extrapolating results obtained in one species to another.

INTRODUCTION

Steroid production by Leydig cells is mainly under the control of gonadotropins. The interaction of LH/hCG with its receptors, which contain the seven transmembrane domains [1, 2], typical of membrane receptors coupled to guanine triphosphate (GTP) binding proteins, initiates a sequence of events at the membrane level, followed by intracellular modifications which are involved in the steroidogenic and trophic effects of the hormone [3]. Among the processes in the plasma membrane which follow the binding of the hormones, are hormone induced guanyl nucleotide binding and adenylate cyclase activation [4]. In addition to this well established transducing system, recent data suggest that physiological levels of LH might also activate a transducing system that does not involve cAMP, and lead to mobilization of Ca^{2+} from intracellular stores and/or opening of the plasma membrane Ca^{2+} channels [5]. However, the physiological role of these pathways on Leydig cells to agents which activate protein kinase C and/or increase intracellular Ca^{2+}, is very small compared to the responses induced by LH/hCG or cAMP derivatives [6].

Many *in vivo* and *in vitro* studies have demonstrated that the steroidogenic responsiveness of Leydig cells to acute stimulation by LH/hCG can be modulated not only by this hormone [3] but also by the long-term action of many other peptide and non-peptide hormones, including FSH [7, 8], prolactin and growth hormone [9, 10], insulin [11, 12], insulin-like growth factor I (IGF-I) [12–14], transforming growth factor β (TGFβ) [15, 16], epidermal growth factor (EGF) [17, 18], interleukin-1 [19], fibroblast growth factor [20], glucocorticoids [21], estrogens [22] and androgens [23]. However, the interpretation of the above results is sometimes difficult for two reasons: firstly, some of these results were obtained *in vivo*, but given the multiple cell-to-cell interactions in the testis [24–27], it is not clear whether the increased or decreased steroidogenic capacity observed following the administration of any of these hormones is due to a direct effect on Leydig cells or whether the effect is mediated by another cell type; secondly, most of the data concerning the regulation of Leydig cell function have been observed in the rat, but some of the results obtained in this model were not confirmed in other mammals [28].

To study the acute and chronic effects of any factor on Leydig cell steroidogenesis and in order to overcome the difficulties involved in interpreting the *in vivo* data, it would seem necessary to work with purified preparations of Leydig cells cultured in a chemically defined medium. The MA-10 cell line established from a mouse Leydig cell tumor [29] has several characteristics of Leydig cells and has been of great value for the study of several membrane-bound receptors and their coupling to intracellular effectors, as well as for the investigation of the long-term effects of some factors, including EGF [17, 30, 31]. However, the main steroid

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secreted by this cell line is progesterone rather than testosterone and therefore some of the results derived from this cell line can not be extrapolated to normal Leydig cells. Primary culture of rat or mouse Leydig cells have been used by many groups [32–36]. Although these models have been useful to study the acute and short-term response of Leydig cells to several factors, the long-term effects are more difficult to investigate since the cells lose most of their hCG receptors and some of the enzymes involved in steroidogenesis within the first days of culture. In contrast, immature pig Leydig cell cultures in a chemically defined medium keep most of their specific function for several days [28, 37].

In this review, we will concentrate mainly on the effects of several growth factors on the maintenance of pig Leydig cell differentiated functions, in particular on the ability of these cells to respond to acute stimulation with LH/hCG. We will also briefly review the effects of these and other growth factors on the differentiated function of other steroidogenic cells.

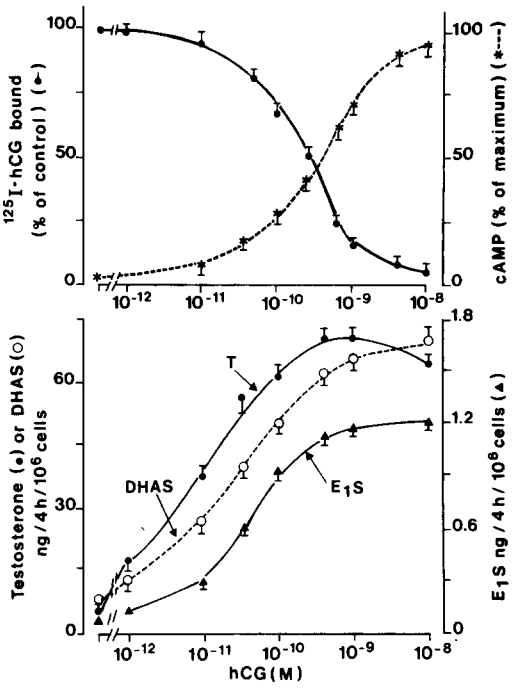

Fig. 1. Acute effects of hCG on Leydig cells. Top: Displacement of ^{125}I-hCG bound by hCG (●) and hCG-induced cAMP production (*). Bottom: hCG-induced testosterone (●); E_1S (▲) and DHAS (○) production.

EFFECTS OF IGF-I

Immature pig Leydig cells cultured in F12/DME medium supplemented with insulin (5 μg/ml) and transferrin (10 μg/ml) maintain for several days their specific functions. After 4 days in culture they contain about 50,000 sites/cell of high affinity hCG binding sites ($k_d \simeq 3 \pm 0.8 \times 10^{-10}$ M). Acute stimulation with this hormone produces in a dose-dependent manner an increased cAMP production with an $ED_{50} \simeq 7 \times 10^{-10}$ M, suggesting that most hCG binding sites need to be occupied to induce maximal cAMP production (Fig. 1). In contrast, the ED_{50} for the steroidogenic response was about 1 order of magnitude lower. Adult pig Leydig cells produce high amounts of several steroids, including testosterone, dehydroepiandrosterone sulfate (DHAS), 5α-androsterone and estrogens, mainly as estrone sulfate (E_1S) [38]. Immature cultured pig Leydig cells produce equivalent amounts of testosterone and DHAS but lower amounts of E_1S (Fig. 1). It must be pointed out that in both adult [39] and immature pig testes [20, 40], most of the aromatase activity of the testis is located in Leydig cells, and that in both hCG markedly enhanced this activity.

When immature pig Leydig cells were cultured in the same medium without insulin, they lost within 3 to 4 days hCG receptors and hCG responsiveness (Table 1). However, at these high concentrations, insulin might exert its effects through its own receptors and/or through IGF-I receptors, since pig Leydig cells contain both [13, 41] and since IGF-I receptors are known to bind and respond to high insulin concentrations [42, 43]. As shown in Table 1, treatment of insulin-deprived cells with either insulin at micromolar concentrations or IGF-I at nanomolar concentrations, restored both hCG receptors and the cAMP and testosterone response to this hormone. Insulin at nanomolar concentrations was less active. Moreover, no additive effect was observed when cells were treated with both insulin (5 μg/ml) and IGF-I (50 ng/ml). It must be emphasized that none of these peptides has any effect on the basal production of cAMP or testosterone.

Treatment of pig Leydig cells with increasing concentrations of IGF-I produced two opposite effects: down-regulation of its own receptors and up-regulation of hCG receptors (Fig. 2, top). Half-maximal and maximal effects were observed at about 10^{-9} M and 10^{-8} M, respectively. At maximal concentrations, there was a 50% decrease of its own receptors and a 3-fold increase of hCG receptors. It is interesting to note that hCG regulates positively the IGF-I

Table 1. Effects of insulin and IGF-I on Leydig cell function

Pretreatment	^{125}I-hCG bound (cpm × 10^3/10^6 cells)	cAMP (pmol/1 h/10^6 cells)		Testosterone (ng/4 h/10^6 cells)	
		Basal	hCG	Basal	hCG
Control	18 ± 1	0.4 ± 0.02	14 ± 0.9	0.6 ± 0.04	11 ± 2
+ insulin (50 ng/ml)	44 ± 3[a]	0.5 ± 0.03	44 ± 4[a]	0.8 ± 0.06	35 ± 3[a]
+ insulin (5 μg/ml)	112 ± 8[a]	0.5 ± 0.04	102 ± 6[a]	0.9 ± 0.04	98 ± 6[a]
+ IGF-I (50 ng/ml)	132 ± 4[a]	0.4 ± 0.03	99 ± 8[a]	1.1 ± 0.07	108 ± 8[a]
+ insulin (5 μg/ml) + IGF-I (50 ng)	134 ± 8[a]	0.5 ± 0.03	102 ± 7[a]	1.1 ± 0.08	112 ± 8[a]
Insulin continuously	129 ± 7[a]	0.6 ± 0.04	100 ± 8[a]	1.0 ± 0.07	109 ± 7[a]

Leydig cells were cultured for two days in medium without insulin. Then the medium was replaced by fresh medium without (control) or with the indicated concentrations of insulin or IGF-I. The peptides were added daily and the incubation continued for 3 additional days. "Insulin continuously" refers to cells cultured all the time with insulin (5 μg/ml). At the end of the experiment, the medium was removed and the binding of ^{125}I-hCG and the production of cAMP and testosterone in the absence (basal) or presence of hCG (10^{-9} M) were measured. Results are the mean ± SD of 4 cultures.

[a] $P < 0.01$ compared to control.

receptors in both pig [13] and rat [44] Leydig cells. Moreover, IGF-I pretreatment enhanced the cAMP and testosterone responses to hCG (Fig. 2, bottom). Similar effects of IGF-I on the steroidogenic capacity of cultured rat Leydig cells have been reported [14, 45].

The enhanced steroidogenic response to hCG of IGF-I pretreated Leydig cells was not only due to an increase of hCG receptor number but also to an improvement of several steps of the steroidogenic pathway. First, testosterone response, but not cAMP response, to forskolin was higher in IGF-I treated cells than in control cells (Fig. 3). Second, the amount of pregnenolone produced by IGF-I pretreated cells in the presence of hCG and inhibitors of pregnenolone metabolism was higher than in control cells. Third, the conversion of exogenous pregnenolone (Fig. 3) or 22(R)-hydroxycholesterol to testosterone but not to E_1S (Table 2) was enhanced by IGF-I pretreatment. These results suggest that IGF-I enhanced the availability of cholesterol for steroidogenesis and/or the activity of the cholesterol side-chain cleavage and also the activity of some enzyme(s) involved in the conversion of pregnenolone to testosterone, but has no effect on Leydig cell aromatase

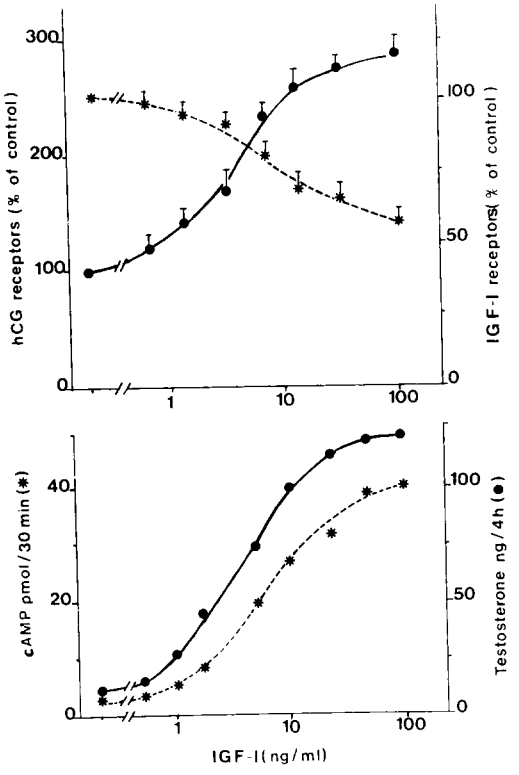

Fig. 2. Effects of IGF-I on Leydig cells. Cells were treated with the indicated concentration of IGF-I for 2 days. At the end of the experiment the number of hCG (●) and IGF-I (*) receptors (top) and the acute cAMP (*) and testosterone (●) responses (bottom) to hCG (10^{-8} M) were measured.

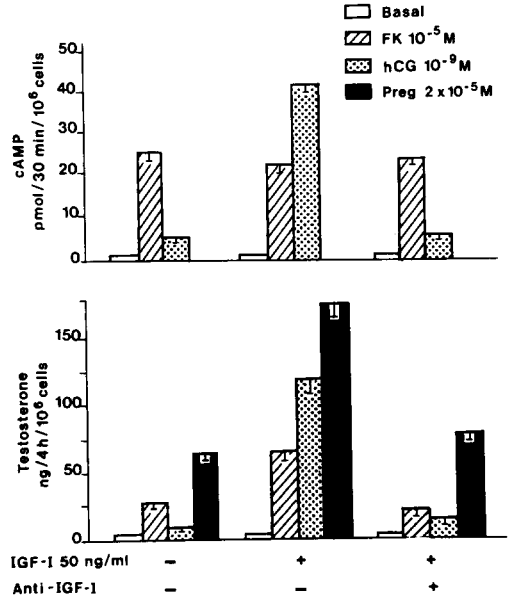

Fig. 3. Inhibition of IGF-I effects on Leydig cells by anti-IGF-I antibodies. Cells were cultured for 2 days in the presence of IGF-I and/or anti-IGF-I antibodies. Then the medium was removed and the cAMP and testosterone responses to forskolin and hCG as well as the conversion of pregnenolone to testosterone were measured.

Table 2

	Testosterone (ng/h/10^6 cells)		E_1S (ng/4 h/10^6 cells)	
	hCG	22(R)OH-cholesterol	hCG	22(R)OH-cholesterol
Control	48 ± 4	401 ± 20	0.42 ± 0.01	0.57 ± 0.01
IGF-I	154 ± 11	840 ± 34	0.62 ± 0.02	0.88 ± 0.03

Cells were incubated for 2 days in the absence or presence of IGF-I (50 ng/ml). Then the cells were incubated for 4 h in the presence of hCG (10^{-9} M) or 22(R)-hydroxycholesterol (5×10^{-5} M) and the amounts of testosterone and E_1S measured.

activity. The specificity of the differentiating effects of IGF-I on Leydig cell functions was proven by the fact that their effects were blunted when Leydig cells were treated simultaneously with IGF-I and an anti-IGF-I antibody (Fig. 3).

The interest of the effects of IGF-I on Leydig cell differentiated functions is strengthened by the fact that Sertoli and Leydig cells from both the rat [46–48] and pig [49, 50] produced IGF-I and that this production was stimulated by FSH and hCG, respectively, but not by hGH (Table 3). In addition, the production of IGF-I by the coculture of Leydig and Sertoli cells in the presence of FGF, hCG and/or FSH was higher than the addition of the IGF-I secreted by each cell type alone (Table 3).

The trophic differentiating effects of IGF-I have also been observed with other steroidogenic cells: granulosa and adrenal cells. Granulosa cells from both swine and rat contain specific IGF-I receptors [51, 52] and this peptide at nanomolar concentrations exerts potent and specific differentiating effects on granulosa cell steroidogenesis [52, 53] and potentiates the effect of FSH on rat granulosa LH receptors and steroidogenic responsiveness to this hormone [51, 54, 55]. Similarly, bovine adrenal cells also contain specific IGF-I receptors [56, 57] and this peptide at nanomolar concentrations increases the number of angiotensin II- and ACTH-receptors [56, 58] and the steroidogenic capacity of both bovine [56] and ovine [59] adrenocortical cells. Moreover, both granulosa [60, 61a, 61b] and bovine adrenal [62] cells produce IGF-I and this production is stimulated by the specific hormones for each cell, but not by GH.

Table 3. Effect of hGH, bFGF, hCG and/or FSH on IGF-I secretion by Leydig cells and Sertoli cells cultured alone or together

	IGF-I (ng/48 h/well)		
	Leydig	Sertoli	Leydig + Sertoli
Control	7.5 ± 1.2	10.1 ± 1.4	16.8 ± 2.5
hGH (5 μg/ml)	7.8 ± 1.1	9.8 ± 1.1	17.2 ± 2.1
bFGF (20 ng/ml)	14.2 ± 1.2[a]	22.3 ± 1.9[a]	47.8 ± 3.2[a,b]
hCG (10^{-9} M)	18.4 ± 2.1[a]	9.4 ± 1.2	45.8 ± 3.1[a,b]
hFSH (200 ng/ml)	7.2 ± 1.3	18.5 ± 2.2[a]	33.4 ± 2.7[a,b]
hCG + hFSH	17.1 ± 2.4[a]	16.8 ± 1.2[a]	53.8 ± 3.4[a,b]

Leydig cells (0.38–0.41 × 10^6 cells) and Sertoli cells (0.78–0.81 × 10^6 cells) were cultured alone or together in complete medium for 2 days. The medium was removed and replaced by fresh medium in the absence or presence of the indicated factors and cultured for 2 additional days. Then the medium was removed and its IGF-I content measured. The results are the mean ± SEM of 3–4 experiments, each done in triplicate.

[a] $P < 0.05$ vs control of the same column.

[b] $P < 0.05$ vs the addition of Leydig and Sertoli cells.

Table 4. Effects of hGH and IGF-I treatments of Snell dwarf mice (dw/dw) on plasma testosterone and testicular hCG receptors (m ± SEM)

	dw/dw		
	Saline	GH	IGF-I
Plasma testosterone, basal (ng/ml)	0.21, 0.17[a]	0.19, 0.18[a]	0.18, 0.19[a]
Plasma testosterone hCG (ng/ml)	7.9 ± 2.1 ($n = 13$)	32.1 ± 2.1[b] ($n = 10$)	25.2 ± 2.5[b] ($n = 11$)
hCG bound (fmol/testes)	2.5 ± 0.2	6.6 ± 0.4[b]	5.6 ± 0.3[b]
(fmol/g testis)	89 ± 7 ($n = 10$)	120 ± 7[b] ($n = 8$)	133 ± 7[b] ($n = 8$)

Snell dwarf mice 6 to 7-weeks-old were treated 3 times daily for 6 days with either saline, hGH (1.5 μg/g of body wt) or human IGF-I (1 μg/g of body wt). At the end of the experiment some animals received saline whereas others received 10 IU of hCG and the animals were sacrificed 2 h later.

[a] Values of 2 pools of 2 animals each.

[b] $P < 0.001$ compared to saline treated animals.

Further evidence of the crucial role of IGF-I on Leydig cell maturation and steroidogenic capacity was obtained by recent *in vivo* studies showing that a 7 day treatment of growth hormone-deficient Snell dwarf mice with hGH or IGF-I, not only increased body weight [63] but also produced a marked increase in hCG receptors and in the steroidogenic responsiveness to this hormone (Table 4). Since the stimulatory effects of IGF-I on Leydig cell maturation were similar to those produced by hGH, it is very likely that the effects of hGH on Leydig cell maturation were mediated by IGF-I.

Taken together, the above results indicate that IGF-I is required for the maintenance and probably for the expression of several specific functions of steroidogenic cells. Thus, in addition to its endocrine GH-dependent action, IGF-I might play an important paracrine/autocrine role in these processes. The results also suggest the key role of IGF-I in the expression and maintenance of cell differentiation, in addition to its well-known role in cell growth.

TRANSFORMING GROWTH FACTOR β (TGFβ)

TGFβ, a peptide first identified by its ability to cause phenotype transformation of rat fibroblasts [64], has been shown to have multifunctional properties since it can either stimulate or inhibit differentiation of many cells [65]. The potential role of TGFβ on gonadal function as well as its secretion by the gonads has been suspected on account of the striking similarities

in the sequence and the subunit organization between TGFβ [66] and the other peptides secreted by granulosa cells and Sertoli cells, i.e. inhibin [67, 68], activin [69] and anti-müllerian hormone [70, 71].

In contrast to IGF-I, TGFβ is a potent inhibitor of differentiated functions of pig [15], rat [16] and mouse [72] Leydig cells. Pig Leydig cells contain specific TGFβ receptors of high affinity ($k_d \simeq 6 \pm 0.2 \times 10^{-11}$ M) and low capacity (Fig. 4). When added to primary cultures of pig Leydig cells, TGFβ reduced the number of hCG receptors, without affecting the binding affinity and decreased the hCG-induced cAMP and testosterone production [15]. The effects were dose-dependent with an $ED_{50} \simeq 4 \times 10^{12}$ M (Fig. 4). The decreased responsiveness to hCG of TGFβ pretreated cells was due to a decrease of the maximal response without modification of the ED_{50}. In both pig [15] and rat [16] Leydig cells, TGFβ pretreatment also reduced the steroidogenic response to cAMP derivatives. Moreover, TGFβ decreased the expected forskolin-induced cAMP and testosterone production, in both the presence and absence of hCG, and actually enhanced the conversion of pregnenolone to testosterone. This peptide, therefore, appeared to be acting at multiple points in the steroidogenic pathway of Leydig cells. Similarly, TGFβ is a potent inhibitor of both bovine [73–76] and ovine [77–79] adrenal fasciculata cells. Both cell types contain TGFβ receptors [76, 77] which, at least in bovine, are regulated by ACTH. TGFβ treatment of adrenal cells reduced angiotensin [74] and ACTH [78] receptors and the steroidogenic responsiveness to both hormones. The reduced steroidogenic responsiveness of TGFβ treated cells was related not only to a modification of membrane receptors, but also to an impairment of several steps of the steroidogenic pathway, in particular a reduction in the activity of the 17α-hydroxylase [73, 77], as well as in P_{450} 17α enzyme content and mRNA expression [77, 79].

In contrast to these inhibitory effects of TGFβ on Leydig and adrenocortical cells, this peptide has a positive effect on granulosa cell differentiation [80–84]. Although TGFβ alone has no effect, it potentiates FSH-stimulated LH receptor induction [81, 82], progesterone and inhibin secretion [82, 84] and aromatase activity [80, 85].

A potential paracrine/autocrine role of TGFβ on the regulation of the steroidogenesis of the three tissues, adrenal, testis and ovary, is inferred by the fact that this peptide is produced by the three tissues. Pig Sertoli cells contain TGFβ mRNA [86] which is negatively regulated by FSH (Fig. 5). Moreover, these cells secrete a TGFβ-like peptide, most of it in a latent form, and this secretion is decreased by FSH ([86] and unpublished data). Similarly, pig Leydig cells also contain TGFβ mRNA, which is decreased by hCG treatment (Fig. 5). Moreover, in the rat,

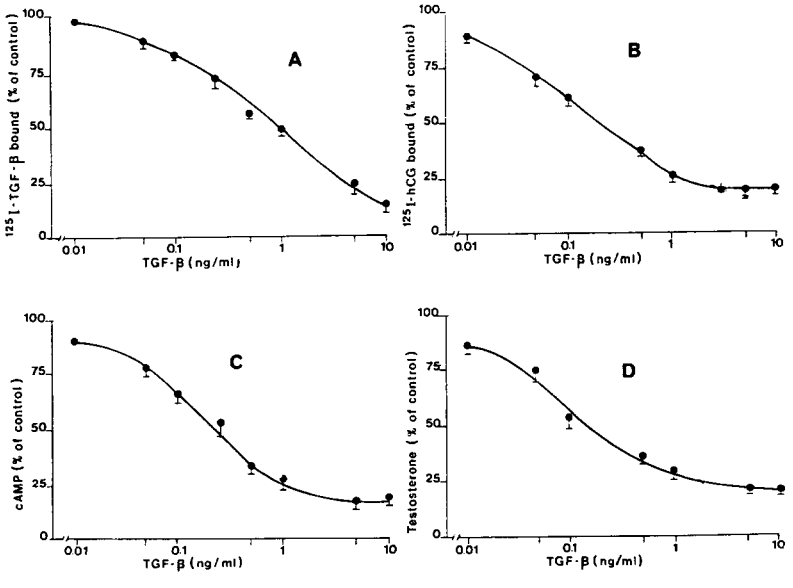

Fig. 4. Effects of TGFβ on pig Leydig cells: (A) Displacement of bound ^{125}I-TGFβ by increasing concentrations of unlabelled peptide. Effects of 2 days treatment of Leydig cells with increasing concentrations of TGFβ on the hCG receptor (B) and the cAMP (C) and testosterone (D) response to acute hCG (10^{-8} M) stimulation.

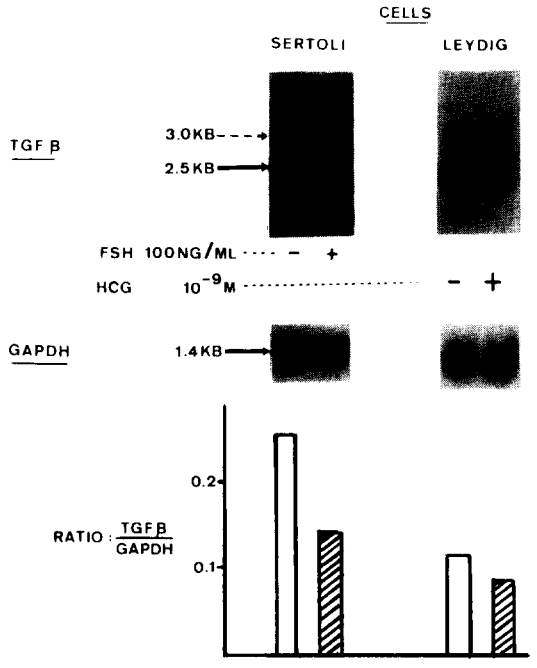

Fig. 5. Upper panel: Northern blot analysis of total RNA isolated from pig Sertoli cells and Leydig cells cultured in the absence (−) or presence (+) of FSH or hCG. The blots were hybridized with TGFβ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes. Lower panel: Graphic representation of the ratios TGFβ/GADPH of the data.

both Sertoli cells and peritubular cells contain TGFβ mRNA and secrete a TGFβ-like peptide [87]. Secretion of a TGFβ-like peptide by rat thecal and granulosa cells [88, 89] as well as by bovine thecal cells [88] has also been reported. Again, most of it is secreted in a latent form. Finally, bovine fasciculata cells contain TGFβ mRNA (unpublished data), but as in the ovary no study has been performed to study its regulation. Despite the fact that the above results demonstrate that TGFβ is produced by these steroidogenic tissues, further studies are required before any clear-cut conclusion can be drawn concerning its paracrine/autocrine role, in particular we need to know not only to determine how this secretion is regulated, but also how the latent form can be activated.

EFFECTS OF EGF ON LEYDIG CELLS

The effects of EGF on Leydig cell function have been studied using several models: a mouse Leydig tumor cell line [17, 29–31], fresh isolated rat and mouse Leydig cells [18] and cultured rat interstitial cells [18, 90, 91]. In the mouse Leydig tumor cells, EGF acutely stimulates progesterone production by about 10-fold, without affecting cAMP levels, and potentiates the steroidogenic effects of submaximal concentrations of hCG and cAMP analogues [17]. However, the steroidogenic effect of EGF was about 100-fold lower than that produced by maximal concentrations of hCG. After 2 days treatment, EGF produced a dose-dependent diminution of hCG receptor and hCG-induced steroidogenic responsiveness which is related mainly to the receptor loss [30]. In freshly prepared rat Leydig cells, EGF alone produced a 2- to 3-fold increase in C19- and C21-steroid production which was several times lower than that produced by maximal concentrations of LH [18]. Moreover, stimulation of cells with both LH and EGF produced a small increase in C21-steroid output, but had no effect on C19-steroid secretion. In cultured interstitial cells, EGF alone produced a slight stimulation of C19- and C21-steroid secretion [18], but it inhibited the stimulatory effect of hCG [18, 90, 91]. Under any one of these experimental conditions, EGF modified cAMP production [18].

The acute and the long-term effects of EGF on pig Leydig cells are summarized in Table 5. EGF had no effect on cAMP production but stimulated testosterone production by about 2-fold. However, this stimulation was about 10 times lower than that produced by hCG. Treatment of cells for 2 days with EGF produced a dose-dependent decrease of hCG receptor number and a decreased cAMP response to maximal concentrations of hCG. Nevertheless, the effects on hCG-induced testosterone and E_1S production were negligible. Moreover, EGF was unable to block the stimulatory effects of hCG on Leydig cell aromatase activity (data not shown).

Taken together, the above results indicate that EGF has a small acute steroidogenic action on Leydig cells, which is exerted through a cAMP-independent pathway, and a long-term inhibitory effect on hCG receptors, which in the mouse Leydig tumor cells [30] but not in pig Leydig cells, was associated with a decreased steroidogenic response to this hormone. In the rat, the impaired testosterone output of interstitial cells pretreated with EGF seems to be mainly related to a decreased activity of the 17α-hydroxylase/17-20-lyase [18, 90, 91]. How these small effects of EGF on Leydig cell function can be related to its *in vivo* stimulatory effects on the meiotic phase of spermatogenesis [92] is unknown. The recent demonstration that rat Sertoli cells and peritubular

Table 5. Effects of EGF on Leydig cell function

	Acute effects		Late effects			
			^{125}I-hCG binding	hCG (10^{-8} M)		
EGF ng/ml	cAMP	Testosterone		cAMP	Testosterone	E_1S
0	100	100	100	100	100	100
1	98 ± 5	134 ± 8	88 ± 6	93 ± 2	108 ± 8	103 ± 2
5	101 ± 4	187 ± 15	64 ± 3	80 ± 4	112 ± 9	109 ± 41
10	104 ± 6	197 ± 24	56 ± 4	72 ± 2	124 ± 8	103 ± 6
50	95 ± 8	254 ± 18	49 ± 3	65 ± 5	113 ± 6	96 ± 6
hCG 10^{-9} M	2300 ± 210	1550 ± 140	19 ± 1	24 ± 7	41 ± 2	980 ± 40

Leydig cells were incubated with the indicated concentrations of EGF and the acute effects on cAMP and testosterone production measured. The incubation was continued for 24 h and the binding of ^{125}I-hCG and the cAMP (30 min), testosterone and E_1S (4 h) responses to this hormone (late effects) were measured. The results, expressed as percent of control cells, are the mean ± SEM of 3–5 experiments.

cells secrete TGFα but not EGF [93] might indicate a paracrine role of TGFα acting through EGF receptors.

EFFECTS OF OTHER GROWTH FACTORS

Basic fibroblast growth factor (bFGF) is a potent mitogen for a wide variety of mesoderm- and neuroectoderm-derived cells [94]. In addition, bFGF has now been repeatedly shown to exert profound regulatory effects on cell differentiation [94]. The potential role of bFGF on testicular cells has been studied using Sertoli and Leydig cells prepared from immature pig testis. FGF treatment of pig Sertoli cells not only stimulates cell proliferation but also the specific function of these cells, i.e. FSH receptor number and the responsiveness to this hormone [95]. In addition, bFGF is a potent stimulator of Sertoli cell IGF-I secretion (Table 3). The main effect of bFGF on pig Leydig cell steroidogenesis is to induce aromatase activity [20]. In addition, bFGF alone produced a small increase in testosterone production (Table 6), but after 48 h treatment reduced hCG receptor number and the steroidogenic response to this hormone ([20] and Table 5). These inhibitory effects of bFGF on hCG-induced androgen production have also been observed in cultured rat ovarian cells [96]. Moreover, bFGF is able to stimulate the secretion of IGF-I by pig Leydig cells (Table 3). Since bFGF has been isolated from bovine testis [97] and since both Sertoli and Leydig cells contain bFGF mRNA (unpublished data) a potential role of this factor in testicular function can be supposed. However, due to the lack of a signal peptide, bFGF cannot be secreted in a soluble form, but only in association with some components of the extracellular matrix. Therefore, if bFGF has a paracrine/autocrine role it should be released from the extracellular matrix that, in the testis, is mainly formed by the secretory products of Sertoli and peritubular myoid cells [98].

Another factor which is able to modify Leydig cell steroidogenesis is interleukin-1β [19, 99]. This factor, either alone or in the presence of low concentrations of LH, stimulates the production of C19- and C21-steroids by cultured rat Leydig cells but, in the presence of maximally effective doses of LH, interleukin-1β inhibits C19-steroid production by inhibition of the 17,20-desmolase [19]. However, the potential role of this factor *in vivo* is unlikely, since the testis contains only interleukin-1α [100] and this factor has no effect on Leydig cells [19].

Many other peptide factors have been reported to modulate positively or negatively the steroidogenic responsiveness of rat interstitial cells. Among them, are vasopressin [101], corticotropin-releasing factor [102], atrial natriuretic factor [103] and angiotensin [104]. In addition to their potential endocrine role, some of the above factors could play a paracrine role, since some of them are produced locally in the testis. The acute and long-term effects of LHRH

Table 6. Effects of FGF on Leydig cells

	(ng/10^6 cells/48 h)		hCG stimulation (ng/10^6 cells/4 h)	
Pretreatment	Testosterone	E_1S	Testosterone	E_1S
Control	3 ± 0.2	5 ± 0.3	60 ± 3	1.3 ± 0.2
FGF (10 ng/ml)	6 ± 0.3	14 ± 0.8	42 ± 4	15 ± 2
hCG (10^{-10} M)	120 ± 10	50 ± 4	48 ± 4	13 ± 2

Leydig cells were cultured for 48 h in the absence or presence of FGF or hCG. At the end of the incubation, the amounts of testosterone and E_1S were measured. Then the cells were incubated in fresh medium containing hCG (10^{-8} M) and the amounts of testosterone and E_1S were measured.

on rat Leydig cells have been extensively analysed [105]. However, this peptide is devoid of effects on Leydig cells of other species. Species specificity has also been found concerning the acute steroidogenic effects of ACTH on perfused testis: rabbit and guinea pig, but not rat, hamster or dog testes secrete testosterone in response to ACTH [106]. Conflicting results have been reported concerning the effects of inhibin and activin on rat Leydig cells: one group [107] reported that LH-induced testosterone production by cultured interstitial cells was potentiated by inhibin and inhibited by activin, whereas another group [108] did not observe any effect of these peptides on basal or hCG-stimulated testosterone secretion.

CONCLUDING REMARKS

This paper has reviewed the data concerning the effects of several growth factors on Leydig cell functions. Two types of action have been observed (1) Acute effects on testosterone secretion, which can be either positive or negative. Although the stimulatory effects reach the level of significance, they are many times lower than those induced by LH/hCG, and in most cases the effects of the factors are not additive to those of LH/hCG. The acute inhibitory action is more pronounced in the absence than in the presence of LH/hCG. (2) Long-term trophic effects on hCG receptor and hCG responsiveness. Again, these effects can be positive or negative. In general, these trophic actions which involve the expression and/or the maintenance of several specific functions of Leydig cells, and therefore the sensitivity and the capacity to respond to LH/hCG, are more clear-cut than the acute effects. Among the growth factors, two appear to be more relevant. IGF-I is required for the maintenance and probably the expression of differentiated function of several steroidogenic cells, including Leydig cells. Both experimental and clinical data suggest that GH is required for normal Leydig cell function. Thus, in humans, isolated GH deficiency [109, 110] or GH resistance, as in the case of Laron dwarfism [111], are associated with delayed puberty and poor response to exogenous hCG [110–113] which, in the case of GH deficiency, is very often improved following treatment with GH [109, 110, 112]. Since exogenous IGF-I as well as GH are able to improve the steroidogenic response of Leydig cells to hCG in GH-deficient Snell dwarf mice [63], it is very likely that the effects of GH are mediated through IGF-I. Thus, under physiological conditions, the GH-dependent IGF-I endocrine secretion adds its effects to speed up the testicular maturation process and participates in the normal timing of puberty. In contrast, in situations of persistent GH deficiency, as in Laron dwarfism due to an abnormal GH receptor [114] or in Snell dwarf mice with genetic GH deficiency [115], the local production of IGF-I regulated by gonadotropins, but not by GH, might be sufficient to induce an almost complete, although very delayed, development of testicular growth and function. The second factor is TGFβ-1 which has effects opposite to IGF-I on Leydig cell functions. Although the regulation of the secretion of this factor by testicular cells remains to be clarified, it appears that the TGFβ-1 mRNA content of Leydig and Sertoli cells is regulated negatively by LH-hCG and FSH, respectively. Thus, in the absence of gonadotropins the secretion of IGF-I is decreased whereas the secretion of TGFβ-1 is probably increased, and therefore the expression of Leydig cell differentiated function is blocked. The contrary happens in the presence of gonadotropins.

For the other growth factors, the review has highlighted the species specificity, in particular some of the effects observed in the rat model are not observed in other species. Thus, some caution should be taken before the results observed in one species are extrapolated to other mammals. This is not only true for testis, but also for many other tissues, as has been discussed recently [116].

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