Current aspects of Leydig cell function and its regulation

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Summary. Recent findings on Leydig cell function and its regulation are discussed. Regulatory mechanisms at different organizational levels, i.e. at the level of the pituitary, the testis and the intracellular elements, are briefly reviewed as well as the transmission and the modulation of the hormonal signal at the Leydig cell membrane. Special emphasis is given to local paracrine and autocrine regulatory interactions operating at the level of the testis.

Introduction

Ever since Franz Leydig in 1850 described for the first time the interstitial cells of the mammalian testis there has been a steady increase in information on the function and hormonal regulation of these testicular elements. In 1903, Bouin & Ancel proposed an endocrine role for the Leydig cell. By the 1930s it was evident that the male hormone was testosterone and that testicular function was under the hormonal control of the pituitary. However, it was not until the early 1960s that it was conclusively demonstrated that the Leydig cell was the main source of testicular androgens. By the late 1960s and early 1970s the main hormonal interactions between the hypothalamus, pituitary and the testis as well as the principal biosynthetic and metabolic pathways for steroids in the Leydig cell had been delineated (Eik-Nes, 1975). The next decade would witness important new findings concerning the local control of testicular function and the mode of pituitary gonadotrophin secretion as well as the effect of these hormones on their target cells. Perhaps one of the most exciting new topics that has evolved are the paracrine and autocrine regulatory interactions through which the fine tuning of testicular function is achieved. In this article some of the novel findings concerning Leydig cell function and its regulation are briefly discussed. The studies reviewed deal predominately with rodents, the rat in particular. No systematic attempt is made to discuss the comparative aspects of Leydig cell function.

Pituitary control: gland-to-gland communication

According to current knowledge, gonadotrophins are secreted from the pituitary in pulses of high biological activity (Ellis & Desjardins, 1982; Kimura & Kawakami, 1982; Ellis, Desjardins & Fraser, 1983; Ellis & Desjardins, 1984b). Changes in reproductive state are usually associated with changes in the amplitude as well as frequency of these secretory pulses (Lincoln, 1981; Karsch *et al.*, 1984; Ellis & Desjardins, 1984a). Qualitative changes in the chemical composition of LH have also been noted during illness and different developmental stages as well as during pulsatile secretion (Conn, Cooper, McNamara, Rogers & Shoenhardt, 1980; Reiter, Beitins, Ostrea & Gutai, 1982; Warner, Dufau & Santen, 1984).

Considerable inter- and intra-individual variation in the secretory patterns of these hormones seem to exist and so the functional significance of this episodic mode of secretion remains obscure (Ellis & Desjardins, 1982). However, some relatively recent studies concerning the effect of different

doses of hCG as well as the effect of different modes of hormone administration on Leydig cell function shed some light on the significance of episodic hormonal signalling. The effect of LH/hCG on Leydig cell function in the mature rat is dose-dependent. While small doses of LH/hCG increase the number of receptors as well as stimulate testosterone synthesis, large doses have opposite effects (Dufau *et al.*, 1984).

The recovery of the LH receptors and the steroidogenic elements from the stimulatory and inhibitory effects of LH/hCG follow a distinct temporal pattern (Dufau *et al.*, 1984; Huhtaniemi, Warren & Catt, 1984b). The steroidogenic response of the Leydig cell to a pulse of LH seems to be determined by the pulse amplitude as well as by the previous history of LH pulses to which it has been subjected (Barr & Pomerantz, 1984).

Despite intensive studies no consensus has emerged on the temporal distribution of LH pulses in normal male rats. In fact, a hormonal profile acquired from one individual during a '12-h' period does not allow reliable predictions to be made concerning subsequent secretory patterns even in the same specimen (Ellis & Desjardins, 1982). Some of the inconsistencies may be due to discrepancies in blood sampling protocols (Ellis & Desjardins, 1984b) as well as to failure to measure changes in the ratio of biologically and immunologically active LH that is secreted (Conn *et al.*, 1980; Warner *et al.*, 1984).

Paracrine regulation: intratesticular communication

Although firm evidence is still lacking, it would seem that a prerequisite for the normal functioning of the spermatogenic cycle is the spatial and temporal coordination of Leydig cell function in accordance to the needs of the spermatogenic phase of the seminiferous tubules (Aoki & Fawcett, 1978; Parvinen, 1982; Bergh, 1982, 1983; Sharpe, Doogan & Cooper, 1983; Parvinen, Nikula & Huhtaniemi, 1984; Syed, Khan & Ritzen, 1985). If this were the case, peritubular Leydig cells would form a heterogeneous group of cells in which the functional cycles of different cell populations are out of phase with one another (Callard, Pudney, Mak & Canik, 1985). It is hard to understand how such functional divergence could be achieved through gland-to-gland communication, that is pituitary control alone. Most certainly paracrine regulation, i.e. the reciprocal transfer of information between the Leydig cells and the seminiferous tubules, would also be needed (Wilton & de Kretser, 1984).

It has been known for some time that androgens are essential for normal Sertoli cell functioning as well as spermatogenesis in mammals (Steinberger, 1971; Ritzen, Hansson & French, 1981; Sanborn, Wagle & Steinberger, 1984). Until recently the synthesis of testosterone was considered to be the sole function of Leydig cells. At present, however, it seems evident that the Leydig cell synthesizes other bioactive substances which are mainly involved in the paracrine and autocrine regulation of testicular function. Pro-opiomelanocortin-derived (POMC) peptides have been localized in Leydig cells (Shu-Dong, Phillips, Halmi, Krieger & Bardin, 1982; Margioris, Liotta, Vaudry, Bardin & Krieger, 1983). The synthesis of testicular POMC and its cleavage products seem to be under the control of pituitary LH. Specific receptors for β -endorphin have been detected in Sertoli cells and the paracrine signal seems to be mediated through an adenylate cyclase-dependent mechanism (Dave, Rubinstein & Eskay, 1985; Fabbri, Tsai-Morris, Luna, Fraioli & Dufau, 1985). The studies of Bardin *et al.* (1984) suggest that different POMC-derived peptides have opposing effects on Sertoli cell function. Renin-like activity has also been noted in Leydig cells (Parmentier, Inagami, Pochet & Desclin, 1983; Pandey, Melner, Parmentier & Inagami, 1984).

Inverse regulatory interactions seem to exist between Leydig cells and seminiferous tubules. Initially it was believed that oestrogen produced by the Sertoli cells was the regulatory link between the tubules and the Leydig cells (di Zerega & Sherins, 1981). However, studies with the rat indicated that aromatase activity in Sertoli cells is restricted to the period of immaturity and that the Leydig cell is the main source of testicular oestrogen after sexual maturation (Payne & Valladares, 1980).

It was not until recently that significant new information was obtained on the tubular control of testicular function. Rat Leydig cells possess stereospecific receptors for LHRH-like peptides; putative candidates for these peptides have been isolated from the testis, and LHRH and its agonists have been shown to have direct stimulatory and inhibitory effects on testicular microcirculation, LH receptors and steroidogenesis in rats (Hsueh & Jones, 1981; Bourne, Dockrill, Regiani, Marshall & Payne, 1982; Sharpe, Fraser, Cooper & Rommerts, 1982; Sharpe *et al.*, 1983; Sharpe & Cooper, 1983; Browning, D'agata, Steinberger, Grotjan & Steinberger, 1983; Damber, Widmark & Bergh, 1985). However, the chemical identity and the physiological role of these putative peptides presumably secreted by the Sertoli cells is far from resolved (Hedger, Robertson, Browne & de Kretser, 1984; Huhtaniemi & Catt, 1985; Melson, Brown, Schoenemann, Tarnavsky & Reeves, 1986). Considerable variation in the reported molecular weights of these substances seems to exist (Arimura & Turkelson, 1984).

Interstitial macrophages are closely associated with Leydig cells and their functional state is related to that of interstitial cells. Macrophages are also steroidogenic and possess common surface antigens with Leydig cells (Milewich *et al.*, 1982; Molenaar, Rommerts & van der Molen, 1984; Bergh, 1985). It has even been suggested that these cells are precursors for Leydig cells (Clegg & Macmillan, 1965; Wartenberg, 1981). Some studies suggest that macrophages possess FSH receptors (Hovatta, Nikula, Wahlström & Huhtaniemi, 1985; Yee & Hutson, 1985). It has been known for some time that FSH also modulates Leydig cell function, i.e. induces LH receptors and increases the steroidogenic capacity of the testis, and that FSH and LH seem to have synergistic

Fig. 1. Main features in the hormonal and paracrine control of testicular function. **H** = hypothalamus; **A** = hypophysis; **S** = seminiferous tubule; **L** = Leydig cell; **RF** = releasing factors **PRL** = prolactin; **T** = testosterone; **E** = oestradiol; **I** = inhibin; + = stimulation; - = inhibition.

Fig. 2. Paracrine regulation in the testis. Solid arrows represent established regulatory interactions whereas the broken ones indicate emerging concepts of paracrine control. S = Sertoli cell; L = Leydig cell; M = macrophage; C = capillary; P = pro-opiomelanocortin derived peptides; Fs = unknown factors of Sertoli cell origin; LHRHF = LHRH-like factor(s); IFL = factor(s) in the interstitial fluid which modulate Leydig cell function; IFC = factor(s) in the interstitial fluid which regulate capillary permeability when activated by LH; MF = factor(s) secreted by macrophages which modulate Leydig cell function. * = synthesis possibly restricted to the prepubertal period; + = stimulation, or increased capillary permeability or vascular resistance; - = inhibition, or decreased capillary permeability or vascular resistance.

effects on testicular function (Johnson & Ewing, 1971; Chen, Shaw & Payne, 1977; Shaw, Georgopoulos & Payne, 1979; Moger & Murphy, 1982; Benahmed, Reventos, Tabone & Saez, 1984; Kerr & Sharpe, 1985). It is therefore possible that the effects of FSH are not merely conveyed through functional changes in Sertoli cells (Benahmed, Morera, Sanchez, Berthelon & Grenot, 1985) but that also 'FSH-induced' changes in macrophage function are involved (Yee & Hutson, 1985).

Since all paracrine interactions involving the Leydig cell take place in the interstitial fluid, local changes in its volume and chemical composition is of primary regulatory importance. Indeed LHRH and hCG have been shown to induce changes in testicular vascular resistance and capillary permeability (Damber *et al.*, 1985; Veijola & Rajaniemi, 1985). Moreover, recent studies suggest that the interstitial fluid contains factors which mediate and potentiate the effects of LH on

capillary permeability and steroidogenesis (Veijola & Rajaniemi, 1985; Rommerts, Hoogerbrugge & van der Molen, 1985; Sharpe & Bartlett, 1985).

The functional state of the Leydig cell is predominantly determined by the pituitary secretion of gonadotrophins, prolactin (Fig. 1) and possibly also ACTH (Bartke, 1971; Liptrap & Raeside, 1975; Juniewicz & Johnson, 1981, 1983; Bartke, Klemcke & Matt, 1985b). However, the integration and coordination of Leydig cell and seminiferous tubule function is achieved through relatively sophisticated paracrine and autocrine regulatory interactions (Fig. 2) involving numerous bioactive compounds of which many share similarities with hormonally active peptides previously described in the central nervous system.

The membrane: transmission and modulation of the hormonal signal

Signal transmission

The hormonal message conveyed by gonadotrophins is transmitted to the target cells through a receptor-mediated process on the cell membrane. The LH receptors, as well as the cascade of events within the membrane triggered by the hormone–receptor interaction, are of primary importance in mediating the LH signal to the intracellular compartment of the Leydig cell. The binding of LH to its respective receptors, which are dimeric transmembrane glycoproteins (M_r 190 000), triggers a series of reactions within the membrane leading eventually to the activation of adenylate cyclase. Although causal relationships are hard to establish it would seem that the hormone–receptor interaction activates a large number of nucleotide regulatory units resulting in an increased binding of GTP to the membrane and a subsequent phosphorylation of specific membrane protein(s) and the activation of adenylate cyclase (Dufau, Baukal Catt, 1980; Dufau *et al.*, 1984). In addition to guanyl nucleotides and the phosphorylation of membrane proteins, methylation processes and cytoskeletal elements seem to be involved in the activation of adenylate cyclase as well as in subsequent cytosolic events (Carreau, Papadopoulos & Drosdowsky, 1985). Ca^{2+} also seems to have a regulatory role in the control of Leydig cell steroidogenesis (Hall, Osawa & Mrotek, 1981; Cooke & Sullivan, 1985). Some studies suggest that calmodulin is involved in the transport of cholesterol to the mitochondria and that, in addition to the classical cAMP-dependent protein kinase pathway, Ca^{2+} /phospholipid-dependent protein kinase may also be involved in the control of Leydig cell steroidogenesis (Fig. 3) (Hall *et al.*, 1981; Welsh, Jones & Hsueh, 1984; Lin, 1985; Themmen, Hoogerbrugge, Rommerts & van der Molen, 1985b; Cooke & Sullivan, 1985). In fact the studies of Themmen, Hoogerbrugge, Rommerts & van der Molen (1985a) and Themmen *et al.* (1985b) suggest that cAMP is not obligatory for the stimulation of Leydig cell steroidogenesis.

Although LH is the primary hormonal factor regulating Leydig cell function, these cells receive other hormonal and paracrine input. Stereospecific receptors for prolactin, AVT- and LHRH-like substances, insulin-like growth factor, epidermal growth factor, catecholamines and benzodiazepine have been localized on the rat Leydig cell membrane (Fig. 3) (Ascoli, 1981; Huhtaniemi & Catt, 1981; Welsh & Hsueh, 1982; Bourne *et al.*, 1982; Meidan & Hsueh, 1985; De Souza, Anholt, Murphy, Snyder & Kuhar, 1985; Handelsman, Spaliviero, Scott & Baxter, 1985; Anakwe, Moger & Nance, 1985).

Some of these endocrine and paracrine factors seem to use second messengers other than cAMP. The testicular LHRH-like factor induces changes in phosphatidyl inositol turnover, Ca^{2+} permeability and prostaglandin E production, whereas the effects of prolactin are possibly conveyed by a peptide cleaved from the receptor itself (Kelly *et al.*, 1984; Cooke, Dix, Habberfield & Sullivan, 1984; Molcho, Zakut & Naor, 1984). However, AVT seems to exert its influence via the inhibitory membrane protein Ni, the universal coupler of inhibitory receptors to adenylate cyclase (Adashi, Resnik, Cronin & Hewlett, 1984). How are these multiple incoming signals integrated at the level of the Leydig cell membrane? Although many aspects of these mechanisms remain obscure, it would seem that the integration of adenylate cyclase-dependent inhibitory and

Fig. 3. Some aspects of Leydig cell function and its regulation. White arrows indicate established key enzymatic reactions and hormones involved in the control of Leydig cell LH receptors and steroidogenesis. The physiological significance and the general relevance of many of the other regulatory mechanisms presented remain obscure. **POMCp** = pro-opiomelanocortin-derived peptides; **LHRHF** = LHRH-like factor(s); **AVT** = arginine vasotocin-like factor(s); **GC** = glucocorticoids; **CA** = catecholamines; **PrK** = cAMP-dependent protein kinase; **PrKC** = Ca^{2+} /phospholipid-dependent protein kinase; **Me** = mevalonate; **C** = cholesterol; **CI** = calmodulin; $\Delta^5\text{P}$ = pregnenolone; $\Delta^4\text{P}$ = progesterone; $17\Delta^5\text{P}$ = 17α -hydroxypregnenolone; $17\Delta^4\text{P}$ = 17α -hydroxyprogesterone; **DHA** = dehydroepiandrosterone; $\Delta^4\text{A}$ = androstenedione; $5\alpha\text{A}$ = 5α -androstane-3 α -diol; **Pr** = protein; **Adiol** = androstenediol; ? = chemically undefined factors; + = stimulation of androgen synthesis (in association with arrows pointing towards the smooth endoplasmic reticulum) or up-regulation of LH receptors (in association with arrows pointing towards the cell membrane); - = inhibition of androgen synthesis or down-regulation of LH receptors.

excitatory input is achieved by coupler proteins within the membrane. Functional interactions between Ca^{2+} and cAMP-mediated control mechanisms also seem to exist (Rasmussen, 1980; Tsang & Carnegie, 1983; Cooke *et al.*, 1984; Peake & Smoake, 1985).

Signal modulation

Since the chemical signals have to traverse the plasma membrane to control the cytoplasmic and genomic events within the target cells, the membrane comprises an important regulatory site for modulating incoming hormonal input. Indeed the transmission of the hormonal signal is not a rigidly constant process for a given chemical effector but a flexible and adaptive one, which is dependent on the dose of the hormone as well as the developmental stage of the target cells

(Warren, Dufau & Catt, 1982; Huhtaniemi, Bergh, Nikula & Damber, 1984a; Huhtaniemi *et al.*, 1984b; Irby, Kerr, Risbridger & de Kretser, 1984; Bartke *et al.*, 1985a; Moyle, Kuczek & Bailey, 1985).

Receptor regulation is of primary importance in modulating target cell responsiveness to hormonal stimulation. The total number of LH receptors per Leydig cell is regulated by the homologous hormone itself (autoregulation) as well as by other chemical effectors such as prolactin, FSH, oestrogen, AVT- and LHRH-like factors and by chemically undefined substances of Sertoli cell origin (heterologous regulation) (Chen *et al.*, 1977; Bartke, Klemcke, Amador & Van Sickle, 1982; Sharpe *et al.*, 1982; Sharpe & Cooper, 1983; Adashi & Resnik, 1984; Benahmed *et al.*, 1985; Stallings *et al.*, 1985). In rodents, at least, prolactin and FSH seem to have a central role in the heterologous regulation of LH receptors, especially in receptor induction during the pubertal period (Huhtaniemi & Catt, 1981; Klemcke, Bartke & Borer, 1984; Stallings *et al.*, 1985; Tähkä & Rajaniemi, 1985).

In the mature rat LH modulates the sensitivity of the Leydig cells by regulating the number of its receptors on the membrane. A single physiological dose of LH induces a rather slow and sustained up-regulation of homologous receptors (evident after 1–2 days), whereas a single supraphysiological dose results in a more rapid (3–6 h after administration) receptor increase. The former seems to be due to the trophic actions of LH on receptor induction and maintenance whereas the latter is evidently the result of the ‘unmasking’ of cryptic receptors and/or the incorporation of presynthesized receptors into the membrane (Huhtaniemi, Katikineni, Chan & Catt, 1981a; Dufau *et al.*, 1984). After the initial transient up-regulation, high doses of LH induce a prominent reduction (down-regulation) in LH receptor numbers and an impaired responsiveness of the Leydig cell to subsequent hormone stimulation (desensitization) (Nozu, Matsuura, Catt & Dufau, 1981a; Nozu, Dehejia, Zawistowich, Catt & Dufau, 1981b, 1982). Desensitization is due to the combined effects of LH-induced receptor loss as well as to specific steroidogenic lesions in the target cells (Dufau *et al.*, 1979). At the membrane level there seems to be an uncoupling of the hormone–receptor complex from adenylate cyclase, its subsequent proteolysis and internalization (homologous desensitization) (Rajaniemi, Manninen & Huhtaniemi, 1979; Kirchick, Iyengar & Birnbaumer, 1983; Kellokumpu & Rajaniemi, 1985) as well as a decrease in the activity of the nucleotide-binding regulatory component (heterologous desensitization) (Kirchick *et al.*, 1983; Dufau *et al.*, 1984). In heterologous desensitization, therefore, responsiveness of the adenylate cyclase is lost not only to the homologous hormone but also to other cAMP dependent stimulatory agents. It has been suggested that these membrane events are due to the specific phosphorylation of receptors as well as the N terminal component (Kirchick *et al.*, 1983). High doses of LH have also been observed to induce up- and subsequent down-regulation of Leydig cell prolactin receptors (heterologous regulation) (Davies *et al.*, 1979; Huhtaniemi, Warren & Catt, 1983).

Desensitization seems to be specific for the mature Leydig cells. The interstitial cells of fetal, neonatal, prepubertal and seasonally quiescent animals respond to desensitizing doses of hCG with an increase in testosterone synthesis and LH receptor numbers (Warren *et al.*, 1982; Huhtaniemi, Katikineni & Catt, 1981b; Huhtaniemi, Nozu, Warren, Dufau & Catt, 1982; Huhtaniemi *et al.*, 1984b; Amador, Bartke, Klemcke, Siler-Khodr & Stallings, 1985; Amsterdam, Venter & Huhtaniemi, 1985). It has been proposed that the lack of heterologous and homologous down-regulation in the Leydig cells of neonatal and fetal animals is an adaptation to high levels of placental hCG and reflects the need for high androgen concentrations during differentiation of the genitalia and the masculinization of hypothalamic structures in the male fetus (Huhtaniemi *et al.*, 1984b).

Intracellular events: regulation of steroidogenic elements

The Leydig cell is a highly differentiated cell specialized in the synthesis and secretion of androgens. The array of enzymes participating in steroidogenesis are strictly compartmentalized between the

mitochondria, SER and the cytoplasm (Ewing & Zirkin, 1983). The differentiated state of these Leydig cell organelles as well as the induction and maintenance of the steroidogenic enzymes is under the trophic control of pituitary LH and prolactin (Fig. 3) (Dufau, Hsueh, Cigorraga, Baukal & Catt, 1978; Bartke *et al.*, 1985b). In addition to trophic actions, LH has also acute effects on Leydig cell steroidogenesis (Purvis, Cusan & Hansson, 1981). However, the steroidogenic response of the Leydig cell to an LH signal is not stereotypic but dependent on the LH dose as well as the developmental stage of the gonads (Ficher & Steinberger, 1971; Huhtaniemi *et al.*, 1982, 1984a,b; Tähkä, Teräväinen & Wallgren, 1982, 1983b; Tähkä, Ruokonen, Wallgren & Teräväinen, 1983a; Teräväinen & Tähkä, 1985; Bartke *et al.*, 1985a; Moyle *et al.*, 1985). It seems that the dose- as well as the age-dependent differences in the steroidogenic response of Leydig cells are not merely quantitative in nature (Ficher & Steinberger, 1971; Lacroix, Eecheute & Leusen, 1977; Eecheute, Lacroix & Leusen, 1980). In many rodents, during the prepubertal period there is a qualitative shift from the production of hormonally active C_{19} steroids to that of 5α - and 5β -reduced androgens (Ficher & Steinberger, 1971; Terada, Sato & Matsumoto, 1980; Tsuji *et al.*, 1984).

Physiological doses of LH induce an acute stimulation of steroid production, mainly by modulating the hydrolysis of cholesterol esters, the permeability of the mitochondrial membrane to cholesterol and the activity of the enzymes catalysing cholesterol side-chain cleavage (Hall, Charponnier, Nakamura & Gabbiana, 1979; Mori & Marsh, 1982).

It has been established that the stimulatory effects of LH on androgen synthesis are mainly brought about by changes in the production of intracellular cAMP, the subsequent activation of protein kinase and the phosphorylation of specific proteins and the eventual increase in the activity of steroidogenic enzymes (Purvis *et al.*, 1981; Dufau *et al.*, 1984). Some recent data suggest, however, that calmodulin- and Ca^{2+} -activated phospholipid-dependent protein kinase are involved in the regulation of Leydig cell steroidogenesis (Fig. 3) Hall *et al.*, 1981; Welsh *et al.*, 1984; Kimura, Katoh, Sakurada & Kubo, 1984; Lin, 1985; Nikula, Parvinen & Huhtaniemi, 1985; Themmen *et al.*, 1985b).

In the mature rat, Leydig cell desensitization is not merely associated with a modification of membrane events but also by functional changes in the intracellular androgen synthesizing elements. Desensitization at the intracellular level is associated with several specific steroidogenic lesions in testosterone biosynthesis, which seem to be related but not causally linked to the degree of down-regulation (receptor loss) induced by hCG. Lower doses of hCG (resulting in 50% receptor loss) induce a 'late' steroidogenic lesion that results from a decrease in the activity of the microsomal enzymes 17α -hydroxylase and C_{17-20} lyase which catalyse the conversion of C_{21} steroids to androgens (Dufau *et al.*, 1979; Nozu *et al.*, 1981a). This 'late' lesion is thought to be the result of an ultrashort negative feedback action of oestrogen on Leydig cell steroidogenesis (Fig. 3) (autocrine regulation). Other intracellularly produced steroids also seem to exert regulatory effects on testosterone synthesis. These regulatory interactions are either receptor mediated, involving transcriptional events, or based on the direct inhibition of steroidogenic enzymes (Fan, Oshima, Troen & Troen, 1974; Rosness, Sunde & Eik-Nes, 1977; Hosaka, Oshima & Troen, 1980; Galarreta, Fanjul, Adashi & Hsueh, 1984; Kuhn-Velten & Staib, 1984b). It has been suggested that the 'late' steroidogenic lesion is brought about by an hCG/LH-induced increase in Leydig cell oestrogen synthesis which in turn, through a receptor-mediated process, inhibits the activity of the cytochrome P450-dependent microsomal enzymes (Nozu *et al.*, 1981b, 1982; Tsai-Morris, Aquilano & Dufau, 1985). Several other effectors, such as glucocorticoids, epidermal growth factor and LHRH- and AVT-like factors as well as proteins of Sertoli cell origin, which have been proposed as participants in the endocrine and paracrine control of testicular function, modulate the activity of 17α -hydroxylase and C_{17-20} lyase (Sharpe, 1982; Hsueh, 1982; Welsh, Bambino & Hsueh, 1982; Welsh & Hsueh, 1982; Adashi & Hsueh, 1982; Hsueh, Bambino, Zhuang, Welsh & Ling, 1983; Verhoeven & Cailleau, 1985; Kasson & Hsueh, 1986).

The absence of steroidogenic lesions in the fetal and neonatal rat Leydig cells exposed to desensitizing doses of hCG is partly attributed to the lack of oestrogen receptors in these cells

(Huhtaniemi *et al.*, 1984b). However, the role of oestrogen in the induction of the 'late' steroidogenic lesion is still controversial (Kuhn-Velten & Staib, 1984a; Quinn & Payne, 1985).

An 'early' steroidogenic lesion before pregnenolone formation can be detected in Leydig cells exposed to high desensitizing doses of hCG (resulting in 70% receptor loss). This early lesion is thought to be caused by a decrease in the activity of 3-hydroxy-3-methylglutaryl Coenzyme A reductase as well as by a hCG-induced activation or increase in an intramitochondrial protein which inhibits cholesterol side-chain cleavage activity (Charreau *et al.*, 1981; Hattori, Aquilano & Dufau, 1984; Aquilano, Tsai-Morris, Hattori & Dufau, 1985).

In addition to these steroidogenic lesions, chronic hCG and LHRH treatment seems to cause, in the mature rat, a qualitative shift from the normal dominating 7 α -hydroxylation pathways to 5 α -reduction pathways (Eechaute *et al.*, 1980; Carmichael *et al.*, 1980; Lacroix *et al.*, 1977, 1984). There is therefore a shift from a adult type of steroidogenic pattern to one similar to that observed in immature testes (Ficher & Steinberger, 1971; Rosness *et al.*, 1977; Terada *et al.*, 1980; Tsuji *et al.*, 1984). The functional significance of such multistep control is unknown but there may be some physiological significance in the accumulation of specific steroid metabolites in testes exposed to different doses of hCG/LH.

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