Local control mechanisms in the testis

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ABSTRACT. The gonads are unique organs in that they harbor the cells of the germline and consequently provide the local environment necessary for the normal development and differentiation of gametes. Since the local requirements for germ differentiation differ considerably from those of somatic cells the structural and physiological organization of the gonad is complex and compartmentalized. An elaborate network of local paracrine interactions between the somatic and gametogenic elements appears to be essential for normal germ cell development in mammals. This is especially true for the testis where meiosis is continuous throughout adulthood and where the spermatogenic cycle of the seminiferous epithelium is strictly controlled in time and space. The present paper reviews briefly the rapidly expanding field of testicular paracrinology. Special emphasis is given to the role of intra- and intercompartmental paracrine communication in the development of the male gamete.

KEY WORDS: *Paracrine control, testis, gamete differentiation, androgen synthesis*

Introduction

Recent studies on the control of reproduction have added multiple new ideas to the pre-existing hypothalamo-pituitary centered concept which had emerged by the 1960's and early 70's.

It is still accepted that the functional state of the gonad is determined to a large extent by the hormonal dialogue between the target organ and the hypothalamo-pituitary system. Novel findings indicate however, that the coordination and integration of target tissue function, especially in organs like the gonads which manifest spatio-temporal functional heterogeneity, is achieved through local regulatory interactions. The regulation of gonadal function is evidently a far more complicated process than was initially envisaged. Cells apparently receive chemical input not only from other organs via the blood but also from different cell types within the target organ itself (Parvinen *et al.*, 1986; Sharpe, 1986; Tähkä, 1986; Saez *et al.*, 1987). Local control systems enable the functional response of the individual cell to be monitored with greater fidelity than would be possible through pituitary regulation alone. Thus a given cell population belonging to the same cell type (i.e. Leydig cells, Sertoli cells) is not necessarily functionally homogeneous. The functional state of a specific cell is ultimately determined by its relations to adjacent tissue elements which exert local regulatory influences on its function.

The focus of research seems to be shifting from endocrinology to paracrinology, i.e. from the study of hormonal interactions between different organs to that of local communication between different cell types within an organ. These new studies have opened interesting vistas concerning basic mechanisms of chemical communication as well as the evolution of hormonal regulation in eucaryotes. This paper reviews some of the novel findings concerning the paracrine regulation of testicular function.

Paracrine regulation of gametogenesis

The seminiferous tubules of the mature mammalian testis are involved in the continuous exocrine production of spermatozoa. Spermatogenesis, i.e. the maturation and differentiation of diploid spermatogonia into fully developed haploid germ cells is a complicated process involving numerous mitoses and a meiosis of germ cell precursors as well as their controlled dislocation from the basal lamina across the blood-testis barrier to the tubule lumen during their differentiation (Parvinen, 1982; Sharpe, 1986). A group of spermatogonia are induced to commence development at the same time and these cells also proceed through spermatogenesis in synchrony. Throughout their development (42 days in the rat) the germ cells are in close association with adjacent Sertoli cells, which provide essential nutritional and physical support for gametogenesis. Since the time between the initiation of a new cohort of germ cells and commencement of their development is considerably shorter than what is needed for the completion of spermatogenesis, each Sertoli cell has to cope with the task of simultaneously meeting the nutritional and metabolic requirements of several germ cell generations (4 to 5) in different phases of development. The temporal relationships between the different developing germ cell generations are remarkably constant. Thus, specific developmental phases are always associated with each other giving rise to the spermatogenic stages (Leblond and Clermont, 1952). This strict kinetic developmental control of successive germ cell generations in relation to each other is probably necessary in order to enable the Sertoli cell to satisfy simultaneously the nutritional and metabolic needs of different germ cell stages. Spermatogenesis is not synchronous throughout the seminiferous tubules. Distinct, regularly reoccurring differences in the phase of the spermatogenic cycle are noted between different sections of the seminiferous tubules (the wave of the seminiferous epithelium) (Leblond and Clermont, 1952). Such kinetic control is probably the result of a rather sophisticated paracrine dialogue between the Sertoli cells and the germ cells (Mali *et al.*, 1985; Saez *et al.*, 1985; Le Maguer-esse *et al.*, 1986; Parvinen *et al.*, 1986; Vihko et al ., 1987 a, b).

The induction and maintenance of testicular gametogenesis is dependent on FSH and androgens (Steinberger, 1971;Parvinen, 1982; Tähkä *et al.*, 1983 a, b). Although there exists some conflicting data (Isomaa *et al.*, 1985), it is generally believed that the Sertoli cells, but not the developing germ cells, are dir-

ectly responsive to these hormones (Lyon, 1975; Ritzen *et al.*, 1981). Therefore, the hormonal control of spermatogenesis appears to be almost entirely dependent on normal Sertoli cell function. The aforementioned finding implies that, in addition to the well-documented hormonal interactions between the pituitary and the testis, additional paracrine control mechanisms are imperative in order to satisfy local requirements in the seminiferous epithelium. Thus it would appear that local communication between the Sertoli cells and the developing germ cells plays an important role in the control of spermatogenesis. Indeed recent studies strongly suggest that this is the case (Jutte *et al.*, 1982; Parvinen *et al.*, 1986; Huggenvik *et al.*, 1987; Swift and Dias, 1987; Vihko et al ., 1987 a, b).

The cycle of the Sertoli Cell

Numerous findings indicate that Sertoli cell volume and ultrastructure as well as many aspects of its function vary in accordance with the phase of the spermatogenic cycle (Russell, 1979; 1980; Clermont, *et al.*, 1980; Bugge and Plöen, 1986; Parvinen *et al.*, 1986; Sharpe, 1986). Though the majority of the Sertoli cell-secreted proteins remain to be identified (Cheng *et al.*, 1986), some specific proteins like ABP, cyclic protein, transferrin and ceruloplasmin are secreted cyclically during the spermatogenic cycle. Also the total secretion, but not the synthesis, of protein in the rat is highest at stages VI and XII (Parvinen, 1982). Cyclical changes in Sertoli cell cytoskeletal organization, calmodulin concentrations, FSH and androgen receptors, lipids and in the activity of several tubular enzymes as well as in the secretion of MIS have also been encountered (Fig. 1) (Parvinen, 1982; Mali *et al.*, 1985; Parvinen *et al.*, 1986; Mali *et al.*, 1987; Nikula *et al.*, 1987). Though the functional significance of many of these cyclical processes remains unknown for the present, in certain cases it has been possible to demonstrate that they reflect the phase-specific needs of the developing germ cells. For instance in the rat at stages VII and VIII when spermatogenesis is androgen dependent, the secretion of ABP is the highest (Ritzen *et al.*, 1982; Parvinen *et al.*, 1986).

Also, the secretion of plasminogen activator and MIS is highest at stages VII-VIII, respectively, when the onset of meiosis occurs and the translocation of preleptotene spermatocytes through the tight Sertoli cell junctions commences (Lacroix *et al.*, 1981;Parvinen, 1982). There exist two types of plasminogen activators (PAs) in the testis, i.e. tissue type and urokinase type. It is the urokinase type which is secreted preferentially at stages VII and VIII (Vihko *et al.*, 1987a). PA secretion is under the control of FSH and retinoic acid (RA) (Vihko *et al.*, 1987b). It would appear that the urokinase type PA is involved in the opening of Sertoli cell junctions allowing the transduction of preleptotene spermatocytes to the adluminal compartment (Fritz and Karmally, 1983; Vihko *et al.*, 1987a). Preleptotene spermatocytes may locally stimulate the production of PA (Vihko *et al.*, 1987b). Since germ cells, but not Sertoli cells, possess most of cellular retinoic acid binding protein (Porter *et al.*, 1985), it is possible that the RA-induced transcription of urokinase type PA in Sertoli cells is controlled indirectly via germ cells.

The cyclicity in Sertoli cell function may be governed to a large extent by the different germ cell stages, since their depletion from the seminiferous epithelium modulates Sertoli cell function, e.g. the secretion of ABP and inhibin (Galdieri *et al.*, 1981; Jegou *et al.*, 1984). The selective destruction of preleptotene spermatocytes, but not of other developing germ cells, abolishes the phase - specific increase in plasminogen activator

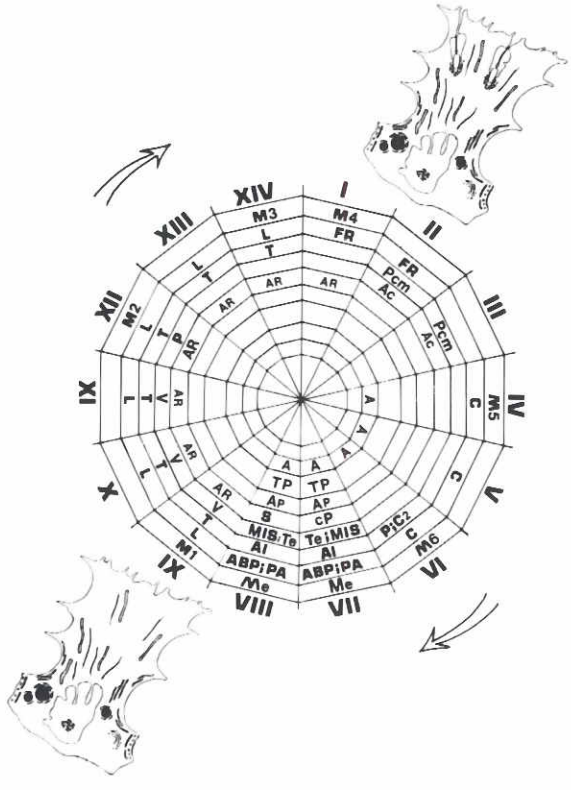

Fig. 1. *Cyclical changes in tubular function during the spermatogenic cycle. L = increased lipid in Sertoli cells; V = highest volume density of Sertoli cells; P = peak in total protein secretion; T = maximal secretion of transferrin; FR = highest number of FSH receptors; AR = highest number of nuclear androgen receptors; MIS = highest relative activity of meiosis inducing substance; ABP = maximum secretion of androgen binding protein; Te = highest concentration of testosterone; AI = peak secretion of aromatase inhibitor; PA = maximal secretion of urokinase type plasminogen activator; CP = highest secretion of ceruloplasmin; S = peak secretion of somatomedin-like factor; C2 = peak secretion of cyclic protein 2; Ac = highest FSH stimulated activity of Sertoli cell Mg^{++} dependent adenylyl cyclase; PCM = highest protein caboxyl-methylase activity; A = maximal activity of aminopeptidase III; Ap = peak activity of acidphosphatase; TP = highest activity of thiamine pyrophosphatase.*

Some stage-specific events of spermatogenensis: occurrence of spermatogonial mitoses (M_1-M_6, stages IX, XII, XIV, I, IV, VI); meiosis commences (Me, stages VII, VII); translocation of germ cells across the blood-testis barrier and spermiation (stages VIII, IX); occurrence of the first and the second meiotic division (stage XIV).

secretion at stages VII-VIII (Vihko *et al.*, 1984). Recent co-culture studies indicate that different germ cell classes have divergent effects on the function of Sertoli cells (Galdieri *et al.*, 1984; Saez *et al.*, 1985; Le Margueresse *et al.*, 1986).

It would seem that Sertoli cell-germ cell interactions involve modes of cellular communication requiring cell-to-cell contact as well as diffusible factors (Russell, 1980; Saez *et al.*, 1985).

Paracrine regulation within the interstitium; control of Leydig cell responsiveness, steroidogenesis and blood flow

The intertubular compartment of the testis consists of an extensive vascular system meeting the high energy and oxygen

demands of spermatogenesis, as well as the androgen synthesizing Leydig cells and other cell types such as fibroblasts, macrophages, mast cells and lymphocytes.

At first sight, it would seem that there should be relatively little need for paracrine regulatory mechanisms in the endocrine compartment of the testis since the androgen synthesizing Leydig cells are themselves directly responsive to pituitary hormones. Also the process of secretion and synthesis of androgens in itself, unlike spermatogenesis, is not the result of a complex interplay between different cell types. However, it would appear that the main need for the paracrine modulation of Leydig cell function lies in the absolute androgen dependence of specific spermatogenic phases (Steinberger, 1971; Sharpe, 1986). Paracrine mechanisms may be needed to ensure sufficiently high local testosterone concentrations in the vicinity of those tubular localities manifesting androgen dependent phases in spermatogenesis. Therefore testosterone has a dual role, i.e. it is a paracrine modulator of spermatogenesis as well as being an endocrine hormone regulating other organs via the systemic circulation. It is the former role of the Leydig cell that makes paracrine control systems in the interstitium necessary.

The Leydig cell and gametogenesis

The coordination of peritubular Leydig cell function with the spermatogenic cycle of the adjacent seminiferous tubule requires local chemical communication between the testicular compartments. Recently a considerable body of data has accumulated in favor of the existence of intercompartmental communication in the mammalian testis. Disruption of seminiferous tubule function has been shown to induce changes in Leydig cell morphology, LH receptors and steroidogenesis (Aoki and Fawcett, 1978; Huhtaniemi *et al.*, 1984; Tähkä and Rajaniemi, 1985; Kerr and Donachie, 1986). Also the total volume of peritubular Leydig cells in the rat seems to change cyclically in accordance with the spermatogenic cycle. Leydig cell volume appears to be largest when the adjacent seminiferous tubules manifest the androgen dependent phases (VII-VIII) in steroidogenesis (Bergh, 1983). These volumetric changes can be abolished by experimentally-induced spermatogenic distribution or unilateral cryptorchidism (Bergh, 1983; Bergh and Damber, 1984). Moreover, *in vitro* studies with dissected tubules at specific stages indicate that the androgen dependent phases have a significant stimulatory effect on the testosterone secretion of purified but not crude Leydig cell preparations (Parvinen *et al.*, 1984; Syed *et al.*, 1985).

The source of these tubular factors is probably the Sertoli cell since both spent media from Sertoli cell cultures and coculturing these cells with Leydig cells have been observed to increase Leydig cell testosterone production and LH receptor numbers (Tabone *et al.*, 1984; Saez *et al.*, 1985; Verhoeven and Cailleau, 1985, 1987; Carreau *et al.*, 1988).

The chemical identity of these paracrine substances remains obscure for the present. A «LHRH-like factor» presumably of Sertoli cell origin has been characterized from the rat testis (Hsueh, 1982; Sharpe *et al.*, 1982; Hedger *et al.*, 1985). LHRH agonists have been observed to have direct receptor-mediated effects on Leydig cell function, i.e. short-term administration stimulates testosterone secretion whereas long-term administration (3 days or more) has inhibitory effects on steroidogenesis and decreases LH receptor numbers (Hsueh, 1982; Sharpe, 1986). The effects of this ligand on Leydig cell function seem to be mediated by proteinkinase C (Nikula and Huhtaniemi, 1988). The LHRH-like factor may also act as a paracrine modulator of testicular microcirculation (Sharpe *et al.*, 1983; Damber *et al.*, 1985). Hedger *et al.*, (1986) have recently provided additional evidence for the existence of a testicular LHRH-peptidase presumably of Sertoli cell origin in the rat testis. These studies suggest a physiological role for the LHRH-like factor in the testes of this species.

Considerable difficulties have arisen, however, in the purification of this factor. Moreover, attempts to find LHRH receptors in the testes of other species as well as attempts to modify Leydig cell function by blocking testicular LHRH receptors with antagonist have failed. For the present the role of this substance as a modulator of Leydig cell function and testicular microcirculation under normal physiological conditions remains obscure (Clayton *et al.*, 1985; Rommerts and Themmen, 1986; Huhtaniemi *et al.*, 1987).

The seminiferous tubules produce local regulators of Leydig cell function other than the LHRH-like factor which are primarily stimulatory in nature (Parvinen *et al.*, 1984; Sharpe and Bart-

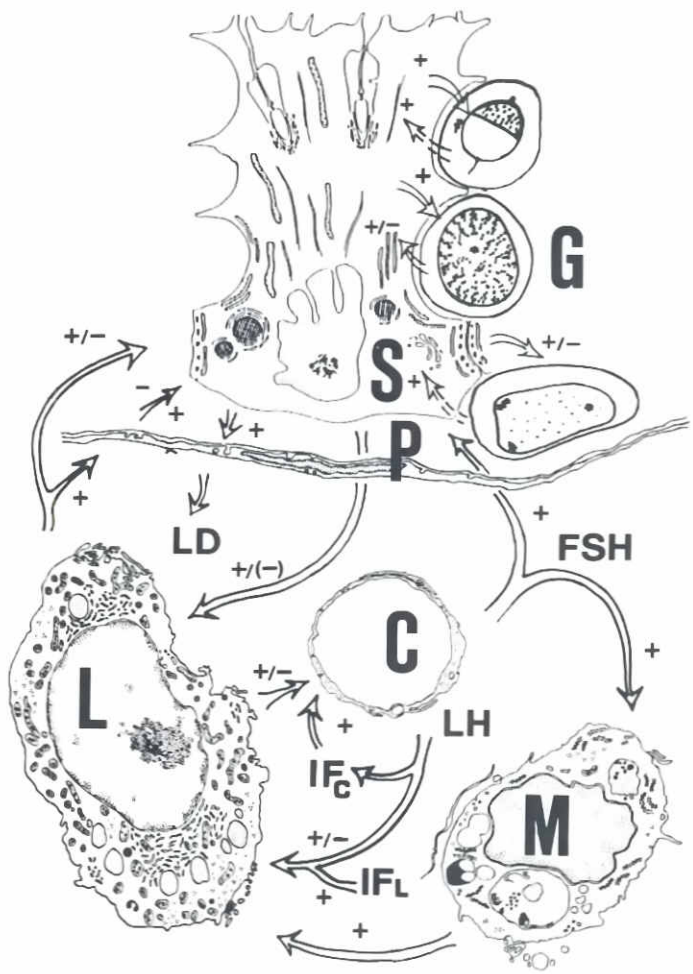

Fig. 2. *Paracrine regulatory interactions in the testis. G = germ cell; S = Sertoli cell; P = peritubular cell; L = Leydig cell; M = macrophage; C = capillary; LD = Leydig cell differentiation; IFc = factor(s) in the interstitial fluid which regulate capillary permeability; IFL = interstitial fluid factor(s) modulating Leydig cell function. + = stimulatory interaction; − = inhibition.*

lett, 1985; Verhoeven and Cailleau, 1985). There is also evidence for the existence of an inhibitory factor regulating aromatization in the rat testis which is secreted specifically at the androgen dependent stages (VII-VIII) of the spermatogenic cycle (Boitani *et al.*, 1981). Novel studies indicate that Sertoli cells secrete several factors which regulate different steps of the steroidogenic response of Leydig cells to LH stimulation. There exists a Sertoli cell-secreted protein which regulates Leydig cell adenylate cyclase, and one or two additional factors which stimulate testosterone and estrogen synthesis (Papadopoulos *et al.*, 1987; Carreau *et al.*, 1988).

The presence of luteinizing hormone receptor binding inhibitor, renin, POMC, prodynorphin and CRF in the testis (Kalla and Zarabi, 1982; Pandey *et al.*, 1984; Bardin *et al.*, 1987; Cox *et al.*, 1987; Yoon *et al.*, 1988), as well as the fact that Leydig cells possess specific receptors for hitherto unmentioned chemical effectors of which many modulate LH receptors and steroidogenesis (e.g. prolactin, glucocorticoids, insulin, benzodiazepine, epidermal growth factor and catecholamines) (Tähkä, 1986), indicates that the regulatory interactions modulating Leydig cell function in the mammalian testis are extremely complex (Fig. 2).

Scavengers in the interstitium

The interstitium contains a relatively stationary population of macrophages, which in some species such as the rat may constitute as much as 25% of the cells in this compartment (Miller *et al.*, 1983; Niemi *et al.*, 1986). Ultrastructural and histological studies suggest that there is functional coupling between macrophages and Leydig cells (Miller *et al.*, 1983). Macrophages have been observed to stimulate steroidogenesis in ovarian lutein cells as well as to increase Leydig cell testosterone secretion and LH receptor numbers *in vitro* (Kirsch *et al.*, 1981; Yee and Hutson, 1985). Also the selective destruction of these cells with silica induces a significant decrease in testosterone, LH, FSH and PRL receptor contents and concentrations in the rat testis (Hovatta *et al.*, 1986b). Macrophages possess FSH receptors and the paracrine effects of these cells on Leydig cell function is controlled by FSH (Yee and Hutson, 1985; Hovatta *et al.*, 1986a).

In addition to macrophages, leukocytes may also modulate testicular steroidogenesis. Peripheral leukocytes are known to secrete ACTH and other POMC-derived peptides (e.g. β-endorphin) when stimulated with corticotrophin releasing factor-41 (CRF-41), arginine vasopressin or pathogens (Smith *et al.*, 1986). Activated leukocytes in turn stimulated adrenal steroidogenesis both *in vitro* and *in vivo* (Smith *et al.*, 1982; 1986). The physiological mechanism modulating ACTH secretion is quite similar to mechanisms operating at the pituitary level (Smith *et al.*, 1986). Since AVP and CRF are present in the testis (Kasson and Hsueh, 1986; Yoon *et al.*, 1988) and since ACTH as well as POMC-derived peptides are known to modulate Leydig cell function (Bardin *et al.*, 1987; Juniewics *et al.*, 1988), interstitial leukocytes may participate in the paracrine regulation of testosterone synthesis.

There appears to exist a further link between the immunological system and testicular function. Human and rat gonads contain high levels of interleukin-1-like factor(s) (Arver and Söder, 1986; Khan *et al.*, 1987). In the testis their likely source are the Sertoli and/or the germ cells. The endogenous production and the high intratesticular levels of interleukin-1-like factor(s) imply a physiological role for this substance in the testis. The potential physiological functions of this factor(s) are diverse. They may participate in the control of germ cell proliferation and in maintaining the privileged immunological status of the testis. They have also been implicated in the control of prostaglandin and plasminogen activator secretion as well as in the modulation of testicular microcirculation and testosterone synthesis (Verhoeven *et al.*, 1988).

Local control of blood flow and vascular permeability

All the paracrine interactions involving Leydig cells as well as the transport of nutrients and oxygen to the intensively proliferating seminiferous epithelium are mediated by the interstitial fluid or lymph. Thus, the capacity to modulate the chemical composition and total volume of interstitial fluid may be of considerable regulatory importance. Indeed, it would appear that testicular microcirculation is an important target for paracrine regulation in the interstitium.

LHRH and hCG modulate testicular blood flow and capillary permeability (Setchell and Sharpe, 1981; Sharpe *et al.*, 1983; Damber *et al.*, 1985). hCG treatment modifies capillary blood flow from a pulsatile to a continuous pattern, increases vascular permeability and transvascular leakage of macromolecules from venules and increases IF volume (Bergh *et al.*, 1986; Damber *et al.*, 1987). The actual mechanisms which bring about these changes remain obscure for the present. It has been proposed that these effects are caused by Leydig cells, which would secrete vasoactive substances (e.g. estradiol and protaglandins) as a response to LHRH or LH stimulation (Sharpe, 1986).

Changes in testicular blood flow and vascular permeability are possibly mediated by different mechanisms (Veijola and Rajaniemi, 1986). There is also some evidence indicating that LH directly activates some latent form of a vasoactive substance (possibly an enzyme) in the interstitial fluid which would increase vascular permeability (Veijola and Rajaniemi, 1985, 1986).

Interestingly, recent studies suggest that leukocytes are involved in the control of the hCG-induced vascular permeability change (Bergh *et al.*, 1986). hCG treatment in doses eliciting a maximal testosterone response induces an accumulation and adherence of polymorphonuclear leukocytes to the endothelium of testicular blood vessels and their translocation into the interstitial space. These events are associated with the hCG-induced increase in interstitial fluid volume (Bergh *et al.*, 1986).

It is suggested that the hCG stimulates Leydig cells to secrete leukotactic factor(s), which cause the accumulation and translocation of leukocytes and consequently an increase in vascular permeability and IF volume. The fact that the vascular permeability change is not encountered in leukopenic rats nor in EDS-treated ones suggests that leukocytes as well as Leydig cells participate in the local modulation of IF volume (Damber *et al.*, 1987).

The Sertoli and peritubular cells as coordinators of intercompartmental communication

The endocrine and the exocrine functions of the testes are strikingly compartamentalized in mammals. Moreover, the tubular and the interstitial compartments are under the control of different pituitary hormones. Despite the apparent independence of testicular androgen synthesis and gametogenesis, it is becoming increasingly evident that considerable interdependence exists between these two processes. Since all the deve-

loping germ cells beyond the preleptotene stage are situated on the luminal side of the blood-testis barrier, all the paracrine communication between the interstitial elements and the more advanced germ cell stages has to transverse the Sertoli cell. Moreover, since there is little evidence that germ cells possess any specific receptor sites for effectors produced by Leydig cells and vice-versa, it would seem that these cells communicate primarily by modifying Sertoli cell function. Therefore the Sertoli-cell appears to have a central role in the coordination of inter-compartmental communication in the mammalian testis.

Leydig cell -Sertoli cell interactions revisited

The functional interactions between germ cells and Sertoli cells and between the Sertoli cells and the Leydig cells have already been discussed to some extent in previous sections. However, the paracrine control exerted by Leydig cells on seminiferous tubules warrants some further consideration. In addition to the well-documented local effects of testosterone on the seminiferous epithelium, recent studies indicate that Leydig cells also secrete other substances such as estrogen, prostaglandins, angiotensin and oxytocin which may act as paracrine modulators of tubular function (Guldenaar and Pickering, 1985; Sharpe, 1986; Tähkä, 1986).

Interestingly, genes for the opioid-peptide precursors pro-encephalin, prodynorphin and pro-opiomelanocortin (POMC) are all expressed in the rat testis (Kilpatrick *et al.*, 1985; Gerendai *et al.*, 1986; Bardin *et al.*, 1987). Pro-opiomelanocortin is synthesized in Leydig cells and recent studies suggest that opioid peptides modulate Sertoli cell function (Boitani *et al.*, 1986; Gerendai *et al.*, 1986; Bardin *et al.*, 1987).

The effects of POMC-derived peptides on testicular function have been studied most extensively (Bardin *et al.*, 1987). It would appear that at least two of these peptides, β-endorphin and des-acetyl α MHS, are secreted by Leydig cells and act as autocrine and paracrine regulators of testicular function. The main target cell appears to be the Sertoli cell and evidence has accumulated indicating that these peptides, derived from different parts of the POMC molecule, have opposite effects on Sertoli cell function. MHS and ACTH-like peptides increase cAMP accumulation and consequently also the sensitivity of Sertoli cells to FHS stimulation, whereas β-endorphin inhibits FHS-induced protein secretion and mitotic activity in Sertoli cells (Boitani *et al.*, 1986; Bardin *et al.*, 1987). The secretion of β-endorphin appears to be LH-dependent and under the autocrine regulation of androgens and estrogens produced by the Leydig cell (Frabbri *et al.*, 1988).

The low levels of testicular POMC- and prodynorphin-derived peptides in comparison to pituitary concentrations are at least partly attributable to the faster degradation and limited storage capacity of these substances in the testis and is in accordance with the proposed paracrine role of these peptides in this organ (Fabbri *et al.*, 1988).

It would appear that there exists a paracrine dialogue between the Sertoli cells and Leydig cells and that this dialogue involves several chemical messengers with divergent effects on their target cells.

The intercompartmental interface

The intercompartmental interface, that is, the peritubular cells, have been shown to modify Sertoli cell structure and function (Skinner, 1987). These cells also possess androgen receptors and secrete as a response to testosterone 55-59 kDA nonmitogenic paracrine factors P-Mod S-A and -B, which stimulate transferrin and ABP production as well as induces the synthesis of several unidentified proteins (including a lactalbumin-like protein) in Sertoli cells (Skinner and Fritz, 1986; Skinner, 1987). Peritubular cells also secrete a PA inhibitor, which may induce the stage-specific differences in PA secretion (Hettle *et al.*, 1988).

Further evidence in favor of the participation of these cells in the local regulation of testicular function comes from studies which suggest that peritubular cells produce somatomedin-C. This growth factor is known to modulate both Sertoli cell and Leydig cell function (Skinner and Fritz, 1986; Tres *et al.*, 1986; Jaillard *et al.*, 1987; Perrard-Sappori *et al.* , 1987).

The origin of the Leydig cell

It has been proposed that peritubular cells may participate in the differentiation of the seminiferous epithelium by producing mesenchymal inducer substance(s) (Skinner, 1987). Moreover, recent data indicates that the peritubular cells are likely precursors of Leydig cells (Kerr and Donachie, 1986). These cells may thus contribute to the differentiation of both major somatic cell types of the testis, i.e. Sertoli cells and Leydig cells.

The studies of Kerr and his associates suggest that the differentiation of Leydig cells from peritubular cells is predominantly under the control of Sertoli cells and pituitary FSH (Kerr and Sharpe, 1985, 1986; Kerr and Donachie, 1986).

These concepts are in accordance with the ideas of Pudney and his co-workers (Pudney *et al.*, 1983; Pudney and Callard, 1984; Callard *et al.*, 1985), i.e. that in some vertebrates the paracrine regulation of spermatogenesis was the original role of testicular androgens and that their hormonal secretion is a later evolutionary innovation. As the hormonal secretion of testicular androgens gained physiological momentum during evolution, the production of these steroids may have shifted from the intratubular Sertoli cells to the more perivascularly-oriented Leydig cells. Thus many of the paracrine mechanisms involved in intercompartmental communication may have arisen rather late in evolution in order to enable the Leydig cell to cope with its newly-evolved dual role as a paracrine modulator of spermatogenesis, and as a hormonal regulator of androgen dependent target tissues.

Conclusions and future perspectives

By virtue of its extensive compartmentalization as well as its spatio-temporal functional heterogeneity (i.e. the wave of the seminiferous epithelium) the testis is an excellent model for studying paracrine communication in general. Recent studies have revealed a hoard of potential signal molecules as well as a variety of signal transduction mechanisms involved in local communication in this organ.

Paracrine communication appears to be essential for normal spermatogenesis and for the local control of androgen synthesis, testicular microcirculation, capillary permeability and Leydig cell differentiation. Novel studies also imply paracrine interactions between the endocrine and the immunological elements of the testis. Growth factors as well as agents similar to hormonally active peptides previously described in the central nervous system appear to act as signal molecules in the local coordination of testicular function.

Current testicular paracrinology is in a descriptive phase. New potential paracrine effector substances are being discovered at a bewildering rate. Since the physiological concentrations of these signal substances are quite low in comparison to hormonal ones, effective analytical techniques are needed for their chemical characterization. High performance liquid chromatography will undoubtedly be of considerable value in this respect. The technique of Morales and Griswold 1987, by which spermatogenesis can be synchronized in the whole testis, may prove useful for providing sufficient material for characterizing effectors engaged in intratubular and intercompartmental communication. Current methods in molecular biology *(in situ* hydridization, immunohistochemistry, blotting techniques, etc.) are making possible the investigation of gene expression and its topographical distribution within the testis.

In order to assess the physiological significance of novel potential paracrine effectors, an array of different experimental *in vitro* techniques must be devised representing varying degrees of complexity (i.e. from very simple systems with purified cell types to complex systems simulating the actual tissue organization of the testis). The bicameral culture system (Dym *et al.*, 1987) together with new perfusion techniques (Jakubowiak *et al.*, 1987) are good examples of the type of novel designs which are needed to bridge the gap between the conditions existing in a simple routine *in vitro* study and those prevailing under actual *in vivo* conditions.

Along with the rapidly accumulating experimental data there is also a great need for theoretical studies (Moyle *et al.*, 1985) seeking general principles of chemical communication in eucaryotes.

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