

The role of calcium in signal transduction processes in Sertoli cells

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SUMMARY

Sertoli cells play a pivotal role in regulation and maintenance of spermatogenesis. They are hormonally regulated predominantly by follicle-stimulating hormone (FSH) and testosterone (T). Although FSH and T have distinct mechanisms of action they act synergistically in promoting spermatogenesis. Stimulation of freshly isolated Sertoli cells with FSH evokes a prompt rise in cytosolic calcium which is quantitatively reproduced by cAMP. The cytosolic calcium response to FSH in Sertoli cells is mainly attributable to serial signaling after the generation of endogenous cAMP. Calcium homeostasis of Sertoli cells may also be regulated by cAMP-independent metabolism. Vasoactive testicular paracrine hormones such as angiotensin II (AII) and vasopressin acting via inositol triphosphate generation induce cytosolic calcium rise primarily derived from the thapsigargin-sensitive endoplasmic reticulum. Investigations involving androgens action on cytosolic calcium reveal a common mechanism of action between the peptide and steroid regulators of Sertoli cell function, indicating that cytosolic calcium

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ions may represent a unifying biochemical mechanism that could explain the synergism of FSH and T. Androgens rapidly and specifically increase cytosolic calcium, consistent with a plasma membrane site of action. This argues for the possible existence of a short term non-genomic signaling pathway in hormonal regulation of Sertoli cell function in addition to the classical longer term, slower genomic response. *Reproductive Biology* 2004 4(3): 219-241.

Key words: Sertoli cells, calcium, follicle-stimulating hormone, testosterone, channels, pH, signal transduction, non-genomic testosterone signaling

INTRODUCTION

Sertoli cells are the site of action of all hormonal influences governing spermatogenesis, supporting and nourishing the germ cells [82]. They are the key cells involved in development and maintenance of stem cell spermatogonia as well as secretion of a chloride- and potassium-rich fluid into the lumen of seminiferous tubules [7]. Their hormonal regulation by FSH and T depends upon cognate receptors expressed specifically by Sertoli cells, but not by germinal cells [103]. In promoting spermatogenesis, FSH and T act synergistically on Sertoli cells despite their distinct molecular mechanisms of action as peptide and steroid hormones. FSH acts upon a cell surface FSH receptor, which is a member of the heptahelical, G-protein linked family of hormone receptors [94], located on the basolateral surface of Sertoli cell contiguous with the extra-luminal or basal compartment of the seminiferous tubules [8]. In contrast, T binds to intracellular androgen receptor, a member of the steroid receptor supergene family of ligand-activated transcription factors, which reside predominantly in the nucleus and have a genomic site of action [87]. Consequently, until 1995 it has been considered unlikely that in Sertoli cells T may exert a membrane-based, short term signaling pathway, distinct from the classical longer term genomic activation, and that cytosolic calcium may represent a common biochemical pathway for synergism between the actions of FSH and T in maintaining spermatogenesis.

The rise in intracellular cAMP has long been believed to be the sole or principal FSH signal transduction system in Sertoli cells [61]. The involvement of other signal transduction systems such as cytosolic calcium in the FSH signal transduction mechanisms although implicated indirectly by measuring transmembrane radiocalcium flux [38] has not been verified in a direct recording until 1991 [32]. Modified by Gorzynska and Handelsman a single-stage cell isolation technique [32] originally developed by Dr J. A. Grootegoed from Erasmus University in Rotherdam (J. A. Grootegoed, unpublished) provided a more rapid and higher yield (98%) of freshly isolated rat Sertoli cells than a two-stage method. Compared to the cultured Sertoli cells the freshly isolated cells are more responsive to FSH stimulation linked to cytosolic calcium increase (Gorzynska, unpublished). Using a spectrofluorimetry method with intracellular fluorescent dye Fura-2AM [42] allowed for the first time to directly record the basal cytosolic calcium level and the effects of FSH and T stimulation on cytosolic calcium concentrations in Sertoli cells [32, 34]. Many studies have since focused on the relationship between cAMP and calcium ions and also the presence of cAMP-independent pathway in Sertoli cells, using microfluorimetry methods on cells suspension or a single-cell technique [35, 36, 49, 63].

THE ROLE OF CALCIUM IN FOLLICLE-STIMULATING HORMONE SIGNAL TRANSDUCTION IN SERTOLI CELLS

FSH, the major trophic hormone for Sertoli cells stimulates a prompt rise in cytosolic calcium from basal level of approximately 90 nM - 100 nM, a value that falls within the range found in most eukaryotic cells, to 190 nM [32]. The FSH-induced rise in cytosolic calcium in freshly isolated Sertoli cells is highly specific as indicated by the failure of other pituitary hormones such as growth hormone, prolactin, adrenocorticotropin or human chorionic gonadotropin to modify cytosolic calcium concentration in Sertoli cells. Stimulation with FSH on cytosolic calcium increase is time-dependent, with 1 mg/l inducing rise in cytosolic calcium within 20s, which sustains for at least 10 min. Sertoli cells exposed to increasing doses of FSH demonstrate a dose-dependent increase in cytosolic calcium concentration.

The median effective dose is 5 ng/ml and a maximally effective dose of 20 ng/ml increases cytosolic calcium by about 100 nM, consistent with a physiological effect [32]. The observations indicate that the activation of calcium flux in freshly isolated Sertoli cells through one or more types of calcium channels is intimately involved in biochemical expression of FSH action. The abolition of the FSH-induced rise of cytosolic calcium by the removal of extracellular calcium and by blockers of both voltage-gated and voltage-independent calcium channels suggests that more than one class of calcium channels is involved in the FSH-induced rise of cytosolic calcium [32]. This concept is further supported by the finding that a nonselective ionophore, ionomycin, has far greater effect on cytosolic calcium than an ionophore selective for voltage-gated channels (Bay K8644) [32]. Moreover, calcium channels blockers selective for voltage-gated channels (nicardipine, verapamil) as well as for voltage-independent channels (ruthenium red) lower the basal cytosolic calcium levels and prevent the FSH-induced rise [32]. The presence of voltage-gated Ca^{2+} channels sensitive to nicardipine, nifedipine and omega-conotoxin has also been elucidated in Sertoli cells monolayers in culture [19].

Despite the consistent observations on the involvement of voltage-gated calcium channels, surprisingly diltiazem, a benzothiazepine blocker of voltage-gated calcium channels, does not alter the FSH-induced rise in cytosolic calcium at doses exceeding those effective in other cells [31, 47]. The selective failure of diltiazem to block FSH-induced rise of cytosolic calcium in rat Sertoli cells has not been expected because all three classes of calcium channel blockers bind to the same α -1 subunit of the calcium channel proteins [47]. A most likely explanation would be heterogeneity in tissue sensitivity within a species to diltiazem in blocking voltage-gated calcium channels [53], or it might be attributable to tissue-specific variants in the α -1 subunit of the L-type calcium channel protein [24, 65].

The presence of calcium channels both L- and N-type has been confirmed in cultured rat Sertoli cells [98]. Using a whole-cell patch-clamp configuration the voltage-gated calcium channels with properties of T-type current have also been identified in the membrane of Sertoli cells [50]. These channels do not appear to be directly sensitive to FSH and therefore

their involvement in calcium movement remains unclear [50]. A strong evidence of a physiological role of voltage-operated Ca^{2+} channels in plasma membrane of Sertoli cells has been provided in studies demonstrating that a major fraction of secreted proteins from cultured Sertoli cells is Ca^{2+} -dependent [98] and also in experiments revealing that Sertoli cells modulate the methoxyacetic acid-induced apoptosis of germ cells through voltage-operated Ca^{2+} channels [9]. The Ca^{2+} -dependent Cl^- channels, which are inhibited by cAMP-dependent protein kinase have also been recorded in cultured Sertoli cells [48].

The importance of voltage-gated channels is reinforced by the observation that exposure of the Sertoli cells to an extracellular environment reversing potassium and sodium ion concentrations (high K^+ , low Na^+) causes an elevation in cytosolic calcium similar to that induced by FSH. This suggests that depolarizing the Sertoli cell plasma membrane activates the voltage-sensitive calcium channels. The lowering of basal cytosolic calcium and blockade of the FSH-induced increase in cytosolic calcium by ruthenium red or cobalt as well as the prominent effect of ionomycin to increase cytosolic calcium indicates that non-voltage-gated calcium channels are also important in FSH signal transduction system in Sertoli cells [32, 49]. The decrease of resting calcium concentration and blockade of the FSH-induced rise when Sertoli cells are incubated in calcium-free medium is consistent with the requirement for external calcium in resting conditions as well as in FSH signal transduction mechanism. Since FSH binding to its receptor is partially dependent upon calcium [37] it is possible that the blockade of the FSH-induced rise may be at least partly due to blockade of FSH binding to its receptor.

An important question was whether the source of calcium, which is involved in the FSH-induced flux, is external or internal in the Sertoli cell. Studies utilizing radioactive calcium [39] demonstrate that transmembrane calcium flux from extracellular calcium sources is activated by FSH. The requirement for external calcium sources and the involvement of plasma membrane calcium channels indicated by ionophore and blocker experiments in the rise of cytosolic calcium due to FSH argue that an external calcium source is involved [32]. The sustained elevation of cytosolic calcium due to

FSH observed on the suspension of Sertoli cells is not consistent with the fast transient rise in cytosolic calcium observed when inositol triphosphate (IP_3) generation is stimulated by a receptor-mediated mechanism [5, 83]. Furthermore, FSH has been shown to inhibit rather than stimulate phosphoinositide turnover in Sertoli cells [67, 77]. Studies performed on a single Sertoli cell indicate however a biphasic rise in cytosolic calcium and imply that FSH and cAMP promote slow redistribution of intracellular calcium from exchangeable pool to the bound non-exchangeable pools [49].

REQUIREMENT FOR TRANSMEMBRANE SODIUM FLUX IN MAINTENANCE OF CYTOSOLIC CALCIUM IN SERTOLI CELLS

In secretory cells calcium homeostasis is affected by changes in the concentration of extracellular [22, 75] as well as intracellular sodium [4]. A sodium selective ionophore, monensin, increases cytoplasmic sodium levels by exchange of extracellular sodium for intracellular potassium [14]. Monensin administration promotes an increase in cytosolic calcium in freshly isolated Sertoli cells to approximately 150 nM, with half of this response occurring in the absence of extracellular calcium, indicating that intracellular calcium sources are mobilized by increases in intracellular sodium [33]. Removal of sodium ions from the extracellular medium depressed FSH-induced increase in cytosolic calcium. Similarly, basal cytosolic calcium levels also depend on sodium flux because the absence of extracellular sodium slightly lowers basal cytosolic calcium in unstimulated Sertoli cells [33]. These findings indicate that calcium fluxes in Sertoli cells are linked to transmembrane sodium fluxes under FSH-stimulated and possibly under basal conditions. Studies with TTX (tetrodotoxin) suggest that TTX-sensitive voltage-gated sodium channels might not be involved in FSH signal transduction in Sertoli cells [33], supporting the hypothesis that Na^+ - Ca^{2+} exchanger rather than the specific sodium channels might be involved in the transmembrane sodium ion flux in FSH-stimulated Sertoli cells. This interpretation is consistent with studies on other cells in which increased concentration of cytosolic sodium due to monensin provokes calcium release from the intracellular stores such as mitochondria via Na^+ - Ca^{2+} exchange pathway [14, 86]. It

might be speculated that in Sertoli cells, similarly to pancreatic β -cells [58], increases in intracellular sodium act as a physiological mechanism to trigger calcium release from intracellular stores. The susceptibility of monensin-induced elevation in intracellular calcium to the absence of extracellular sodium favors the possibility of the Na^+ - Ca^{2+} exchange involvement in calcium translocation across the plasma membrane of Sertoli cells. The small residual effect of monensin to raise cytosolic calcium in Sertoli cells even in the absence of extracellular sodium might be attributed to non-ionophore actions of monensin such as catalyzing Na^+ - H^+ exchange, which increases intracellular pH, in turn causing an increase in cytosolic calcium similarly to that observed in rat thymocytes [41] and sea urchin sperm [86].

An increase in Sertoli cell cytosolic calcium concentration was also observed after administration of veratridine, a sodium channel agonist, which is much less potent than monensin [33]. Similar experiments with rat FRTL-5 thyroid cells are particularly relevant to studies with Sertoli cells in view of the analogy in structure and function between Sertoli cells and thyroid cells [72] both being epithelial cells with diverse secretory and regulatory functions and regulation by the closely related pituitary glycoproteins FSH and TSH (thyroid stimulating hormone), respectively, acting on evolutionarily preserved membrane receptor. The similar pattern of regulation of sodium and calcium ion fluxes in these two cells does suggest that these patterns of hormonal regulation may be evolutionarily conserved and therefore quite fundamental physiologically.

THE RELATIONSHIP BETWEEN cAMP AND CALCIUM IN MEDIATING FSH SIGNAL TRANSDUCTION IN SERTOLI CELLS

The observations indicate that FSH is one of a variety of hormones, which utilize increases in cytosolic calcium as a signal transduction system [20]. Increased cytosolic calcium levels due to FSH in freshly isolated Sertoli cells could be quantitatively reproduced by replicating intracellular cAMP effects through using either a membrane-permeable analog [$(\text{Bu})_2\text{cAMP}$] or by direct nonreceptor-mediated activation of adenylate cyclase (forskolin or cholera toxin) [32]. In contrary to experiments performed with micro-

fluorimetry method on suspension of freshly isolated Sertoli cells where sustained elevation of cytosolic calcium due to FSH or cAMP is observed [32] studies on a single-cell microfluorimetry in individual cultured Sertoli cells revealed a biphasic increase in cytosolic calcium due to FSH, cAMP or ATP stimulation [49]. The involvement of calcium in FSH signal transduction appears to be analogous to its role in LH signal transduction in which both cAMP and calcium fluxes are stimulated [17]. LH and FSH are closely related, dimeric pituitary glycoprotein hormones sharing an identical α subunit, highly homologous β subunits [74], and cell surface receptors, which are members of the G-protein-linked membrane receptor family [60, 94], supporting the notion that both hormones are derived from common ancestral genes [94].

In sequence of events involving cAMP and calcium in FSH action on Sertoli cells it has been unclear whether FSH-induced calcium fluxes were an alternative primary signal transduction mechanism or signal amplification or modulating mechanism in Sertoli cells. In closely related glycoprotein hormones and receptor systems of other endocrine cells most evidence pointed toward dual or parallel signaling functions between cAMP and calcium in FSH signal transduction. For example, transfection studies with both the TSH [84] and LH [43] receptors into host cells lacking these receptors produced unequivocal evidence of dissociation of cAMP and calcium signals after membrane receptor-mediated signal transduction by the appropriate ligand. Furthermore, FSH stimulated dissociable increases in cAMP and calcium in swine granulosa cells, the ovarian homolog of the testicular Sertoli cells [27, 28]. Since the FSH receptor contains a classical G-protein-linked domain [80, 94] activation of the FSH receptor could induce calcium channel activation by phosphorylation of channel proteins through the activity of the catalytic subunit of cAMP-dependent protein kinases A [68]. This line of thought promoted the hypothesis that FSH caused an increase in cytosolic calcium levels through cAMP-mediated effects in activating membrane calcium channels [36] possibly via the activation of cAMP-dependent protein kinase, which phosphorylates proteins constituting membrane calcium channel pores or their regulators [6]. Alternative explanations included the possibilities that cAMP and calcium are dual independent

signals [28] or that the FSH receptor could be itself the calcium channel [6, 27]. The latter hypotheses has been disproved by transfection of functional human FSH receptors into human embryonic kidney cells to create a stable transformant line [293(wt1) cells], which exhibit high affinity, specific FSH binding and cAMP responses to FSH but no inward calcium currents under basal or FSH-stimulated conditions [91]. Studies have demonstrated that different inhibitors of the endogenous cAMP pathway block the cytosolic calcium response to FSH [36], and indicate that endogenous cAMP generated after the interaction of FSH with its receptor is most probably involved in mediating the increased cytosolic calcium levels. The almost complete (more than 90%) blockade by an adenylate cyclase inhibitor, MDL 12330A [36] of the cytosolic calcium response to FSH or forskolin provides evidence for the involvement of an endogenous cAMP generating mechanism in the rise of cytosolic calcium due to stimulation by FSH or cAMP analogs or agonists. Furthermore, in the presence of effective adenylate cyclase inhibition (Bu)₂cAMP still is able to increase cytosolic calcium levels, indicating that the abolition of a cytosolic calcium response to FSH could be attributed specifically to the block in generation of intracellular cAMP [78]. This mode of action appears to be consistent with the inference that MDL 12330A interacts directly with the catalytic unit rather than the nucleotide binding site of the adenylate cyclase enzyme [89].

Further studies aimed to examine whether the prevailing endogenous cAMP levels were sufficient to induce the rise of cytosolic calcium following stimulation with FSH, exogenous cAMP agonists or after the activation of adenylate cyclase. Using the membrane-permeable cAMP antagonist, R_p-cAMP, at doses which blocked cAMP-dependent systems in other secretory cells [1, 52, 73, 96] inhibited by 75% the FSH-induced rise in cytosolic calcium in Sertoli cells [50]. This was consistent with the assumption that endogenous cAMP concentration achieved by FSH or cAMP analogs or agonists are sufficient to create the changes observed in cytosolic calcium concentrations in Sertoli cells. The failure to fully neutralize the FSH-induced increase in cytosolic calcium concentration by R_p-cAMP left the possibility of non-cAMP-mediated effects of FSH on calcium. Similar findings of the incomplete neutralization of the FSH-induced effects by

R_p -cAMP in granulosa cells [1] are consistent with non-cAMP second messenger(s) participating in signaling of these cells. Further experiments on the mechanism of action of cAMP in mediating the FSH-induced rise in cytosolic calcium involved a protein kinase blocker, staurosporine [51, 97], which blocks both protein kinase A and C. The inhibition by 67% of the FSH-induced rise in cytosolic calcium caused by staurosporine appears to be unlikely due to involvement of protein kinase C, as FSH has been shown to inhibit rather than to activate the generation of inositol triphosphate [67, 77]. Moreover, removal of extracellular calcium completely abolished FSH-induced calcium increase in freshly isolated Sertoli cells [32], which does not appear to be consistent with the activation of protein kinase C leading to calcium release from the intracellular stores. The inhibitory effect of staurosporine may favor the hypothesis that cAMP acts indirectly through presumably cAMP-dependent protein kinase, which mediates protein phosphorylation to activate calcium channels in response to stimulation with FSH or cAMP. Studies on regulation of gamma-glutamyl transpeptidase activity by calcium and protein kinase C-dependent pathways indicate however that a protein kinase C-dependent pathway can interact with the FSH-stimulated cAMP-dependent pathway in Sertoli cells [63].

Whereas the FSH-mediated increase in cytosolic calcium in freshly isolated Sertoli cells is predominantly dependent upon the availability of extracellular calcium [32, 38], the cAMP-evoked rise involves calcium from both extracellular and intracellular calcium sources [36]. An incomplete blockade of cAMP-induced cytosolic calcium rise has been observed in the absence of extracellular calcium or in the presence of verapamil. Withdrawal of extracellular calcium or the presence of verapamil effectively abolish the FSH-induced calcium response in cultured [38] or freshly isolated Sertoli cells [32]. Similar diversities of pathways involved in FSH- and cAMP-evoked increases in cytosolic calcium have been observed in granulosa cells [28]. Studies performed on a single Sertoli cell using microfluorimetry technique with the calcium probe indo-1 confirmed that voltage-gated calcium channels blockers failed to abolish calcium response to either cAMP or ATP, and revealed the involvement of thapsigargin-sensitive calcium stores in response to stimulation with cAMP or ATP [49]. A possible role

of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) pathway in regulation of Sertoli cells function by FSH has also been recently examined using PI3K inhibitors such as wortmannin and Ly 294002 [62]. These studies indicate that FSH increases levels of phosphorylated PKB in PI3K-dependent and protein kinase A (PKA)-independent manner in cultured rat Sertoli cells, suggesting the role of the PI3K and PKB in the mechanism of FSH action [62].

Studies on freshly isolated Sertoli cells indicate the crucial role of cAMP in raising cytosolic calcium following FSH stimulation of Sertoli cells and strengthen the premise that the cytosolic calcium response to FSH in Sertoli cells is predominantly attributable to serial signaling after the generation of endogenous cAMP. The generation of cAMP after interaction of FSH with its receptor involves the linkage of the FSH receptor to membrane G proteins, which act as transducers, coupling ligand-induced action on membrane-bound receptors to the post-receptor generation of intracellular effectors or messengers. Experiments with pertussis toxin used to dissociate FSH interaction with its receptor from post-receptor adenylate cyclase activation further reinforce the view that generation of endogenous cAMP might be an obligatory step in the generation of the FSH-induced rise in cytosolic calcium. Observation that pertussis toxin blocks the FSH-induced rise in cytosolic calcium may indicate that in addition to the generally agreed notion that FSH stimulates adenylate cyclase via coupling to a G_s protein [23, 57], the FSH receptor may also be linked to a G_i protein. This hypothesis might be supported by observations consistent with G_i proteins being operative in Sertoli cells, such as finding that pertussis toxin augments FSH-induced aromatase [39] and abolishes the effect of β -endorphin on FSH responsiveness in neonatal Sertoli cells [70].

THE ROLE OF CALCIUM IN SYNERGISTIC ACTION OF FSH AND TESTOSTERONE IN SERTOLI CELLS

The hormonal regulation of spermatogenesis is manifest primarily by the action of FSH and T on Sertoli cells, with subsequent fine modulation by myriad paracrine factors [82, 103]. In the rat, the requirement for FSH in

maintenance of spermatogenesis can be replaced by T [10, 85] or DHT (dihydrotestosterone) [2]. Although FSH is considered critical to the initiation of spermatogenesis [3] complete initiation of spermatogenesis requires both FSH and T [10, 79], which have synergistic effects [21, 46, 81]. A possible synergistic mechanism linking FSH and T action might be through common effects on cytosolic calcium. Cyclic AMP being a classical second messenger for FSH most likely activates protein kinase-A pathway leading to phosphorylation of specific proteins and nuclear binding of associated protein complexes to cAMP response elements in regulatory regions of genes to act as DNA transcription factors [30, 93]. T does not influence Sertoli cell cAMP, consistent with the restriction of steroid receptor action within the nucleus as DNA transcription factors. Apart from cAMP cytosolic calcium constitutes an additional messenger system for FSH [32, 36], which predominantly but not exclusively depends upon generation of cAMP [36]. This additional messenger system may explain the effects of FSH on genes that lack all cAMP response elements [105]. In contrary, T and its metabolites, DHT and estradiol (converted by 5α -reductase and aromatase, respectively), are believed to diffuse freely across membranes into cells and bind to steroid receptors, which act as ligand-activated DNA transcription factors. The importance of non-genomic signaling as a complementary route for cell regulation has recently become evident as the rapid mechanisms are utilized by steroids, apart from rapid actions exerted by peptide hormones. Studies on androgens action on cytosolic calcium in Sertoli cells reveal a common mechanism of action between the peptide and steroid regulators of Sertoli cell function, indicating that cytosolic calcium ions may represent a unifying biochemical mechanism that could explain the synergism of FSH and T [34]. In freshly isolated Sertoli cells testosterone-evoked cytosolic calcium increase to approximately 150 nM is characterized by (i) rapid, within 20s - 40s onset, (ii) plasma membrane site of T action, (iii) extracellular source of calcium and (iv) T acting via androgen receptors and at least partially after 5α -reduction to DHT [34]. The effects of T have been reproduced by DHT, its 5α -reduced active metabolite, whereas blockade of 5α -reductase by finasteride significantly reduced, but did not eliminate the effects of T on cytosolic calcium in Sertoli cells. The interaction between the effects of T

and FSH was non-additive, which might imply that both hormones influence Sertoli cell cytosolic calcium by initiating transmembrane calcium influx through similar types of membrane calcium channels [34].

Direct evidence for a membrane site of action has been provided by demonstrating that the rate, magnitude, and dependence of 5α -reduction of the effects of T on cytosolic calcium have been replicated by T immobilized to BSA (bovine serum albumin) in a conjugate (T:BSA conjugate), thus preventing rapid cellular uptake of T. For the first time these experiments broaden the concept that in Sertoli cells T may exert a membrane-based, short term signaling pathway distinct from the classical longer term genomic effect as a transcription factor [34]. Recent observations using the conventional intracellular microelectrode technique have confirmed the rapidity of testosterone action on Sertoli cell, indicating a significant dose-dependent membrane depolarization within 30 s of stimulation with testosterone [100]. These studies also suggest that the immediate action of testosterone is associated with the closing of K^+ -ATP channels, thereby depolarizing the membrane.

CYCLIC AMP-INDEPENDENT REGULATION OF CYTOSOLIC CALCIUM IN SERTOLI CELLS

In the relationship between cAMP and calcium following FSH stimulation a divergence in calcium origin in the FSH- and cAMP-induced cytosolic calcium increase has been noticed. Whereas the FSH-evoked cytosolic calcium rise in freshly isolated Sertoli cells is dependent on the availability of extracellular calcium [32], a large proportion of calcium participating in the cAMP-induced calcium rise is derived from the intracellular calcium sources [36] indicating that cAMP-independent pathway of cytosolic calcium increase might be operative in Sertoli cells. Similar results were observed in cultured Sertoli cells [90] and in granulosa cells [28]. Other glycoprotein hormones, such as LH [43] and TSH [99, 102] have also been reported to activate the cAMP-independent pathway of the intracellular calcium increase in addition to classical cAMP-dependent cascade. Vasoactive testicular paracrine hormones such as AII and vasopressin have a mode of

action bypassing the involvement of cAMP [55, 92], leading to the generation of inositol 1,4,5-triphosphate, which in turn releases calcium from the intracellular calcium stores [13, 56]. A number of gonadal factors, such as angiotensin, vasopressin, ANP (atrial natriuretic peptide) and prostaglandin $\text{PGF}_{2\alpha}$ are locally synthesized in various testicular cells, with their specific receptors identified within the testis [11, 54, 71, 95, 104]. Whereas both AII or vasopressin evoke a rise in cytosolic calcium to approximately 140 nM and 150 nM, respectively, $\text{PGF}_{2\alpha}$ has only a minimal effect and ANP no effect on cytosolic calcium concentration in freshly isolated Sertoli cells [35]. In contrast to findings in many other cells in which transient calcium increases are observed after stimulation with AII or vasopressin [15, 40, 45, 88], in freshly isolated Sertoli cells both AII and vasopressin evoke monotonic calcium increase [35]. This rather slow calcium response occurring within 20-40s or at 20s of stimulation with AII or vasopressin, respectively, appears to be in agreement with the findings of studies performed on ovarian cells [29, 101]. In porcine granulosa cells the calcium response occurs within 60-80s of angiotensin administration [29] whereas in rat granulosa cells it is within 20-40s [18] or within 60-140s [101]. Removal of extracellular calcium or blockade of calcium channels does not inhibit the cytosolic calcium increase due to AII or vasopressin, which is predominantly derived from the thapsigargin-sensitive endoplasmic reticulum [35]. These experiments confirm the premise that the action of angiotensin or vasopressin most likely depends upon the intracellular calcium sources [12, 45, 64, 69]. Furthermore, they indicate that both angiotensin and vasopressin may serve as important paracrine regulators of Sertoli cell metabolism.

Functional heterogeneity of the AII receptors and vasopressin receptors on Sertoli cell membranes has been evaluated using selective AT_1 and AT_2 subtypes of AII receptors antagonists and V_1 and V_2 vasopressin receptor antagonists. The effect of AII on calcium has been blocked by the selective AT_2 but not by AT_1 whereas the vasopressin-induced calcium response has been inhibited by vasopressin V_1 but not by V_2 receptor antagonist. These selective failures of AT_1 angiotensin receptor antagonist and V_2 receptor antagonist to block the angiotensin- or vasopressin-evoked calcium increase indicate that the action of angiotensin was mediated by AT_2 angiotensin

receptor and by V_1 vasopressin receptor mechanisms, respectively [35]. The observation of AII-induced cytosolic calcium rise via AT_2 in Sertoli cells is consistent with studies in rat ovarian granulosa cell, which also contain exclusively the AT_2 receptor subtype [76]. These findings have provided evidence for the existence of AT_2 angiotensin and V_1 vasopressin receptors in Sertoli cells [35]. Investigations of angiotensin receptors in male gonads established their location also in the Leydig cells [66]. It has been therefore suggested that factors such as vasopressin produced within the testis [26] might be involved in cell-cell interactions to provide a highly specialized microenvironment that is prerequisite for germ cell differentiation [35]. These arguments support a notion that angiotensin and vasopressin act as intra-testicular regulators, consistent with their paracrine and autocrine roles in governing testicular function. Observations on Sertoli cell cultures indicate that stimulation with purinergic agonist resulted in an increase in the activity of phosphoinositide turnover and cytosolic calcium mobilization, accompanied by simultaneously occurring inhibition of the FSH-dependent pathway [25, 67]. This could indicate that stimuli coupled to phosphoinositide turnover may induce profound biological effects in terms of responsiveness of Sertoli cells to FSH. Therefore, the physiological responses of Sertoli cells to hormones might be dependent on continuous cross-talking of signal transduction pathways, both cAMP-dependent and cAMP-independent.

REGULATION OF ACID-BASE TRANSPORT IN SERTOLI CELLS

The intracellular pH regulation appears to play an important role in secretory cells responses to hormones, particularly those that act via cytosolic calcium signalling [16]. Since FSH or T action on Sertoli cells provokes a rapid increase in cytosolic calcium concentrations [32, 34], it therefore appears possible that alterations in their pH_i may be of significance in coordination of metabolic processes and their responsiveness to hormonal stimulation. Studies on functional characterisation of acid-base transport mechanisms under basal conditions in Sertoli cells, using the microspectrofluorimetry method with the pH-sensitive dye, BCECF/AM, have revealed that Na^+/H^+ -

exchange, Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ -exchange and $\text{Na}^+/\text{HCO}_3^-$ co-transport actively participate in the regulation of Sertoli cells intracellular pH homeostasis (Gorczyńska, unpublished). FSH provokes a transient increase in pH_i when cells are exposed to CO_2 in HCO_3^- -buffered solution, indicating the involvement of the HCO_3^- -dependent transport rather than Na^+/H^+ exchange mechanism. The $\text{Na}^+/\text{HCO}_3^-$ co-transport in freshly isolated Sertoli cells is H_2DIDS (disulphonic stilbene)-sensitive and Cl^- -independent. Complete inhibition of pH_i recovery by blocking the Na^+/H^+ exchange, $\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{HCO}_3^-$ co-transport with HOE694 (benzoylguanidine derivative) or H_2DIDS , respectively, suggests that Na^+/H^+ exchangers in conjunction with HCO_3^- -transport pumps constitute the main mechanisms responsible for the recovery from intracellular acidification in Sertoli cells. Further experiments have identified the existence of the isoforms NHE1 and NHE2 of Na^+/H^+ exchanger, and indicated the presence of a small fraction of NHE3 Na^+/H^+ antiport isoform (Gorczyńska, unpublished).

Since Sertoli cells actively modulate and secrete a Cl^- and K^+ -rich testicular fluid into the lumen of seminiferous tubule [44] with K^+ reaching up to 50 mM, and given the significance of high K^+ concentration in provoking an increase in cytosolic calcium [32] it has become important to examine whether the pH_i recovery mechanism via Na^+/H^+ exchanger is associated with the extracellular K^+ concentration. No indication of a close relationship has been noticed between changes in the extracellular potassium concentration and the rate of pH_i recovery, after NH_3 -induced acidification (Gorczyńska, unpublished). The different responses of Na^+/H^+ exchange activation to high K^+ concentrations in various secretory cell types might partly result from the different, if any, K^+ requirements for intracellular proton activation of the antiporter in various cells. Although in Sertoli cells the ion transporters are most likely to be primarily involved in regulation of intracellular pH, the possibility that they could also take a part in controlling cellular osmolarity and volume, as it has been evidenced in alveolar epithelial cells [59], cannot be eliminated. Recently identified with a whole-cell patch clamp recording in cultured rat Sertoli cells a novel Cl^- current insensitive to intracellular or extracellular Ca^{2+} variations is activated only in the presence of an extracellular acidic pH [7]. Alterations in pH_i may be

therefore an important early signal in the hormonal action and contribute to the hormonal signal transduction processes.

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