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Possible role of arachidonic acid in the regulation of lactate production in rat Sertoli cells

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Summary

The aim of the study was to determine whether arachidonic acid (AA) is involved in the regulation of Sertoli cell lactate production and if this fatty acid participates in follicle-stimulating hormone (FSH) regulation of Sertoli cell function. In a first set of experiments the effect of AA and porcine pancreas phospholipase A_2 (PLA_2) on lactate production, glucose uptake, lactate dehydrogenase (LDH) activity and LDH A mRNA levels in Sertoli cell cultures obtained from 20-day-old rats was evaluated. In a second set of experiments the effect of two PLA_2 inhibitors – quinacrine (Q) and AACOCF3 – on FSH stimulation of the above-mentioned parameters of Sertoli cell function was investigated. Treatment with PLA_2 and AA increased Sertoli cell lactate production. The observed action of exogenously added PLA_2 involved its catalytic properties responsible for AA release. PLA_2 and AA treatments also stimulated Sertoli cell glucose uptake, LDH activity and LDH A mRNA levels. In order to determine whether AA participates in FSH regulation of Sertoli cell lactate production cells were incubated with FSH in the absence or presence of the PLA_2 inhibitors Q and AACOCF3. Both drugs partially inhibited the ability of FSH to stimulate lactate production, glucose uptake and LDH activity. The present investigation suggests that AA is involved in the regulation of lactate production, glucose transport, LDH activity and LDH A mRNA levels. In addition, these results suggest that cytosolic PLA_2 and AA may participate in FSH-regulation of Sertoli cell energetic metabolism.

Keywords: arachidonic acid, follicle-stimulating hormone, lactate, phospholipase A_2, Sertoli cells, testis

Introduction

Spermatogenesis is an intricate process highly dependent on Sertoli cell function that is under follicle-stimulating hormone (FSH) and androgen control. FSH produces its effects on Sertoli cells by binding to specific plasma membrane receptors and by stimulating adenylyl cyclase. The subsequent increase in cAMP leads to the activation of protein kinase A and protein phosphorylation. As for the mechanism of action of FSH in Sertoli cells, other signalling events have been demonstrated, namely, increased intracellular Ca^{2+} levels (Grasso & Reichert, 1989; Gorczynska & Handelsman, 1991), NF-κB translocation (Delfino & Walker, 1998) and protein kinase B activation (Meroni *et al.*, 2002). In addition, it has been observed that FSH induces phospholipase A_2 (PLA_2) activation with the consequent release of arachidonic acid (AA) (Jannini *et al.*, 1994).

Metabolic responses to AA are variable and depend on the cell type. For example, AA regulates superoxide

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production and degranulation in neutrophil (Badwey *et al.*, 1981; Smith *et al.*, 1987), insulin secretion by isolated rat islet of Langerghans (Band *et al.*, 1992) and differentiation of HL60 cells (Finstad *et al.*, 1994). In addition, AA inhibits cell–cell communication via gap junction in Chinese hamster V79 cells and rat liver epithelial WB cells (Aylsworth *et al.*, 1986; Hii *et al.*, 1995) as well as cytokine-induced adhesion molecule expression in endothelial cells (Stuhlmeier *et al.*, 1996). These results seem to point out the need for further studies on its biological significance in every type of cell.

To our knowledge, the participation of AA in the regulation of Sertoli cell function has not been explored. Studies on the metabolism of glucose have shown that Sertoli cells actively metabolize glucose but the majority of it is converted to lactate and is not oxidized via the citric acid cycle (Robinson & Fritz, 1981; Grootegoed *et al.*, 1986a). In addition, germ cells (particularly post-meiotic germ cells) are unable to use glucose for their energetic metabolism and they do prefer lactate as an energy source (Jutte *et al.*, 1981; Mita & Hall, 1982; Grootegoed *et al.*, 1986b). In this way, among Sertoli cell functions that might be of interest to germ cell development is the provision of adequate levels of lactate. The importance of lactate for normal spermatogenesis was highlighted in a recent report showing that spermatogenesis in adult cryptorchid rat testis is improved by intratesticular infusion of this carbohydrate (Courtens & Plöen, 1999). We have recently shown that increments in glucose uptake, lactate dehydrogenase (LDH) activity and LDH A mRNA levels are part of the mechanisms utilized by FSH to increase lactate production in rat Sertoli cells (Riera *et al.*, 2001). The aim of this study is to determine whether AA is involved in the regulation of Sertoli cell lactate production and if this fatty acid participates in FSH regulation of Sertoli cell function.

Materials and methods

Materials

Ovine FSH (NIH-oFSH-S-16) was obtained from the National Hormone and Pituitary Program, National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) (Bethesda, MD, USA). [2,6-^{3}H]-2-deoxy-D-glucose ([2,6-^{3}H]-2-DOG) was purchased from NEN (Boston, MA, USA). Arachidonyltrifluoromethyl ketone (AACOCF3) was obtained from BIOMOL (Plymouth Meeting, PA, USA). Phospholipase A_2 from porcine pancreas (14 kDa protein) as well as all other drugs and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

Sertoli cell isolation and culture

Sertoli cells from 20-day-old Sprague–Dawley rats were isolated as previously described (Meroni *et al.*, 1999). Briefly, decapsulated testes were digested with 0.1% collagenase and 0.006% soya bean trypsin inhibitor in Hanks' balanced salt solution for 5 min at room temperature. Seminiferous tubules were saved, cut and submitted to 1 M glycine–2 mM EDTA (pH 7.4) treatment to remove peritubular cells. The washed tubular pellet was then digested again with collagenase for 10 min at room temperature to remove germinal cells. The Sertoli cell suspension, collected by sedimentation, was resuspended in culture medium which consists in a 1 : 1 mixture of Ham's F-12 and Dulbecco's modified Eagle medium, supplemented with 20 mM HEPES, 100 IU/mL penicillin, 2.5 μg/mL amphotericin B, 1.2 mg/mL sodium bicarbonate, 10 μg/mL transferrin, 5 μg/mL insulin, 5 μg/mL vitamin E and 4 ng/mL hydrocortisone. Sertoli cells were cultured in 24 multiwell plates or 25 cm^2 tissue culture flasks (5 μg DNA/cm^2) at 34 °C in a mixture of 5% CO_2 : 95% air. Purity of Sertoli cells reached 95% after 6 days in culture as examined by phase contrast microscopy. No myoid cell contamination was revealed when an immunoperoxidase technique was applied to Sertoli cell cultures using a specific antiserum to alpha-smooth muscle actin. Remaining cell contaminants were of germ cell origin.

Culture conditions

Sertoli cells were allowed to attach for 48 h and medium was replaced at this time with fresh medium without insulin. Cells were stimulated with either 100 ng/mL FSH or variable doses of PLA_2 and AA on day 3. PLA_2 and FSH treatments were also performed in the presence of two PLA_2 inhibitors – quinacrine and AACOCF3. The 72-h conditioned media obtained on day 6 was used to evaluate lactate levels. Cells harvested on day 6 were used to determine LDH activity. For glucose uptake studies, cells were stimulated for 48 h and assayed on day 5. To determine mRNA levels for LDH A, cells stimulated for 12 h and collected on day 6 were utilized.

Cell viability at the end of the culture period was evaluated by a trypan-blue exclusion test. Under all experimental conditions tested, cell viability was higher than 95%.

Lactate determination

Lactate was measured by a method involving conversion of NAD^+ to NADH determined as the rate of increase of absorbance at 340 nm. A commercial kit from Sigma Chemical Co. (St Louis, MO, USA) was used.

Measurement of 2-deoxy-D-glucose uptake

Glucose transport was studied using the uptake of the labelled non-metabolizable glucose analogue 2-deoxy-D-glucose (2-DOG). After treatment, culture medium was discarded and cells were washed three times with glucose-free phosphate-buffered saline (PBS) at room temperature. Then, Sertoli cells were incubated at 34 °C in 0.5 mL glucose-free PBS containing [2,6-^{3}H]-2-DOG (0.5 μCi/mL) for 30 min. Unspecific uptake was determined in incubations performed in the presence of a 10 000-fold higher concentration of unlabelled 2-DOG. At the end of the incubation period,

dishes were placed on ice and extensively washed with ice-cold PBS until no radioactivity was present in the washings. Cells were then dissolved with 0.5 N sodium hydroxide, 0.4% w/v sodium deoxycholate and counted in a liquid scintillation spectrophotometer. Parallel cultures receiving identical treatments to those performed before the glucose uptake assay were destined to DNA determinations. Results were expressed on a per μg DNA basis.

LDH activity measurement

After incubation of Sertoli cells in the absence or presence of the different stimuli, culture medium was saved and cells were disrupted by ultrasonic irradiation in NaCl 0.9% w/v and centrifuged (15 800 ***g***, 10 min). The supernatant was used to measure total LDH activity that was determined by a routinely used spectrophotometric method (Randox Laboratories, Crumlin, UK).

Analysis of LDH A mRNA levels

Total RNA was isolated from Sertoli cells cultured in 25 cm^2 tissue culture flasks with TRI Reagent (Sigma Chemical Co.), a monophasic solution of phenol and guanidine isothiocyanate (Chomczynski & Sacchi, 1987). About 20 μg total RNA were electrophoresed on a 1% agarose–10% formaldehyde gel. After migration, RNAs were transferred to Hybond-N nylon membrane by capillary transfer and fixed with UV complementary DNA (cDNA) probes [rat LDH-A 3′UTR 0.4-kilobase insert, Pst I-Bgl II generously provided by Dr R Jungmann (Northwestern University Medical School, Chicago, IL, USA) and a 28S oligonucleotide] were labelled with [α-^{32}P] deoxy-CTP using a random-primed labelling kit. Blots were pre-hybridized for 5 h at 42 °C in 50% formamide, NaCl/Pi/EDTA (0.75 M NaCl, 20 mM sodium phosphate pH 7.5 and 1 mM EDTA), 5× Denhardt's solution, 10% dextran sulphate, 0.5% SDS, and 100 μg/mL herring sperm DNA. Hybridization was then performed overnight at 42 °C in the same hybridization buffer containing 1–4 × 10^6 cpm/mL ^{32}P-labelled probe. Membranes were washed twice in 2× SSC–0.5% SDS (20 min, room temperature) followed by two washes in 1× SSC–0.1% SDS (30 min, 65 °C). Membranes were exposed to Kodak X-Omat S films (Eastman Kodak Company, Rochester, NY, USA) for 1–2 days at −70 °C. The intensities of the autoradiographic bands were estimated by densitometric scanning using NIH Image (Scion Corporation, Frederick, MD, USA) software.

Other assays

DNA was determined by the method of Labarca & Paigen (1980).

Statistical analysis

One-way analysis of variance (ANOVA) followed by the Tukey–Kramer test for multiple comparisons using the GB-STAT version 4.0 statistical program (Dynamic Microsystems Inc., Silver Spring, MD, USA) was performed. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of exogenously added PLA_2 and AA on Sertoli cell lactate production

The first set of experiments explored a possible role of AA on Sertoli cell lactate production. To achieve this goal, Sertoli cells were exposed to different doses of PLA_2 and AA for 3 days. Figure 1 shows that both treatments increased lactate production. The lowest doses that produced a statistically significant stimulation were 1 IU/mL PLA_2 and 10 μM AA. In order to determine whether the observed action of exogenously added PLA_2 involved its catalytic properties or not, two inhibitors – quinacrine and AACOCF3 – were used. Quinacrine (30 μM) inhibited the stimulatory effect of exogenously added PLA_2 whereas AACOCF3 (10 μM) did not (Table 1). No effect of these inhibitors on basal lactate production was observed (data not shown).

Effect of exogenously added PLA_2 and AA on glucose uptake, LDH activity and LDH A mRNA levels

The next set of experiments was undertaken to evaluate whether the effects of PLA_2 and AA involved modifications in Sertoli cell glucose uptake and LDH activity. PLA_2 (10 IU/mL) and AA (10 μM) stimulated 2-DOG uptake (Fig. 2a), LDH activity (Fig. 2b) and LDH A mRNA levels (Fig. 3) in rat Sertoli cells.

Effect of PLA_2 inhibitors on FSH stimulation of lactate production

The above-mentioned experiments suggested a role of AA on the regulation of lactate production in rat Sertoli cells

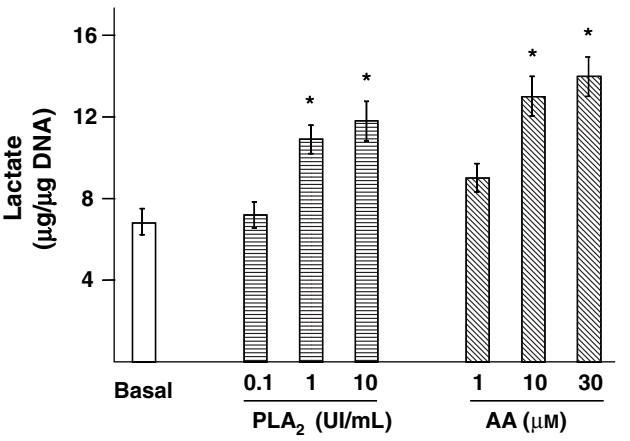

Figure 1. Effect of exogenously added 14 kDa phospholipase A_2 (PLA_2) and arachidonic acid (AA) on Sertoli cell lactate production. Sertoli cells were stimulated for 72 h (day 3 to day 6) with variable doses of PLA_2 or AA. Lactate was determined in the 72-h conditioned media recovered on day 6. Results represent mean ± SD of triplicate incubations in one representative experiment out of three (*$p < 0.01$).

Table 1. Effect of quinacrine and AACOCF3 on PLA_2 stimulation of lactate production

	Lactate production (μg/μg DNA)
Basal	8.7 ± 0.9[a]
PLA_2	17.8 ± 1.9[b]
PLA_2 + Q	12.9 ± 1.1[c]
PLA_2 + AACO	18.2 ± 1.6[b]

Sertoli cells were stimulated for 72 h (day 3 to day 6) with 10 IU/mL PLA_2 in the absence or presence of quinacrine (Q, 30 μM) or AACOCF3 (AACO, 10 μM). Lactate was determined in the 72-h conditioned media recovered on day 6. Results represent mean ± SD of triplicate incubations in one representative experiment out of three. Different superscript letters indicate statistically significant differences ($p < 0.01$).

through modifications in glucose uptake and LDH activity. This last set of experiments was designed to determine whether AA might participate in FSH regulation of Sertoli cell lactate production. For this purpose, Sertoli cells were incubated with FSH (100 ng/mL) in the absence or presence of the PLA_2 inhibitors quinacrine and AACOCF3. Figure 4 shows that both drugs partially inhibited the ability of FSH to stimulate lactate production.

Quinacrine (30 μM) and AACOCF3 (10 μM) inhibited FSH stimulation of glucose uptake (Fig. 5a) and LDH activity (Fig. 5b). Finally, Fig. 6 shows that quinacrine partially inhibited FSH stimulation of LDH A mRNA levels. No effect of the inhibitors on basal glucose uptake or LDH activity was observed (data not shown).

Discussion

Phospholipase A_2 represents a growing family of enzymes that catalyses the hydrolysis of glycerophopholipids at the *sn*-2 position liberating free fatty acids including AA (Dennis, 1997). In the last years, purification and molecular cloning of PLA_2 has allowed the characterization of two groups of enzymes displaying significant differences in both structural and functional properties. On the one hand, secretory PLA_2 (14 kDa protein) with broad specificity for the carboxylic acid present in position 2 and potentially involved in AA release in cells and tissues (Tischfield, 1997). On the other hand, cytosolic PLA_2 (85 kDa protein) with a central role in the release of AA and triggered by growth factors, neurotransmitters (Clark *et al.*, 1995) and by secretory

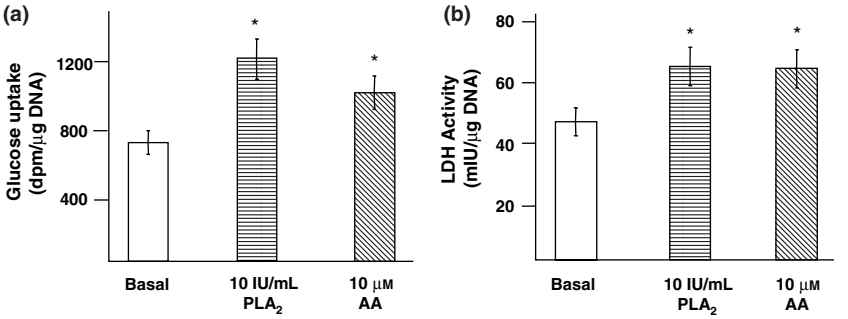

Figure 2. Effect of exogenously added PLA_2 and AA on Sertoli cell glucose uptake and lactate dehydrogenase (LDH) activity. (a) On day 5, Sertoli cells were maintained for 48 h under basal, 10 IU/mL PLA_2 or 10 μM AA stimulated conditions. Glucose uptake assay was performed after this incubation period. (b) Sertoli cells were stimulated for 72 h (day 3 to day 6) with 10 IU/mL PLA_2 or 10 μM AA. LDH activity was determined on cells harvested on day 6. Results represent mean ± SD of triplicate incubations in one representative experiment out of three (*$p < 0.01$).

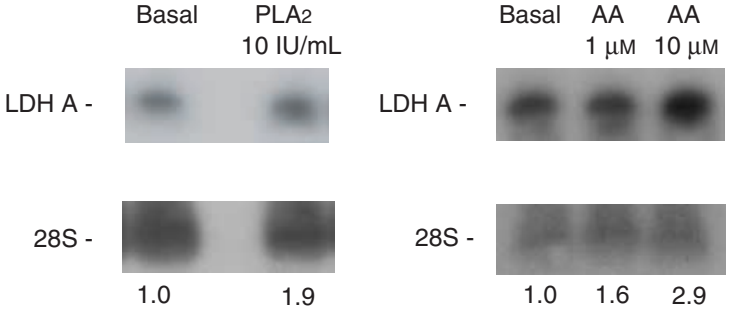

Figure 3. Effect of exogenously added PLA_2 and AA on LDH A mRNA levels in rat Sertoli cells. Total RNAs were extracted from Sertoli cells harvested on day 6 pre-incubated for 12 h with 10 IU/mL PLA_2 or 1 and 10 μM AA. Representative Northern Blots for LDH A are shown. Numbers at the bottom of each lane indicate the fold increases in LDH A mRNA levels (ratio of LDH A to 28S in each sample) relative to basal. Blots correspond to a representative experiment out of two.

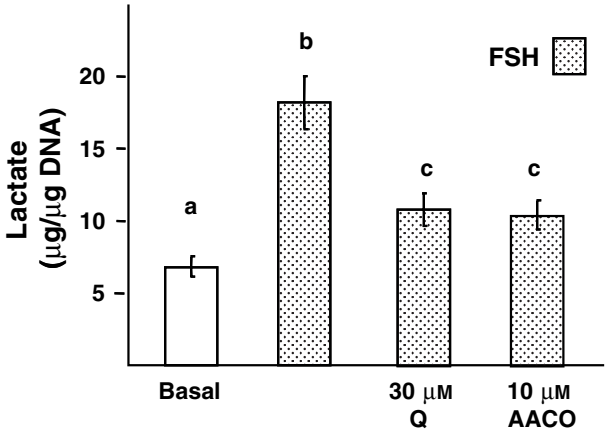

Figure 4. Effects of PLA_2 catalytic activity inhibitors in follicle-stimulating hormone (FSH)-stimulated lactate production. Sertoli cells were stimulated for 72 h (day 3 to day 6) with 100 ng/mL FSH in the absence or presence of quinacrine (Q, 30 μM) or AACOCF3 (AACO, 10 μM). Lactate was determined in the 72-h conditioned media recovered on day 6. Results represent mean ± SD of triplicate incubations in one representative experiment out of three. Different letters indicate statistically significant differences ($p < 0.01$).

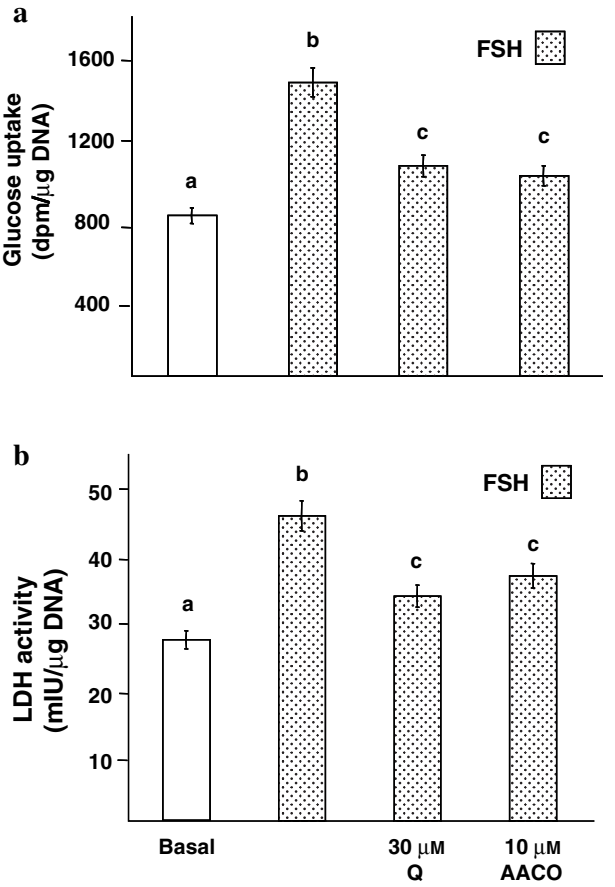

Figure 5. Effects of PLA_2 inhibitors in FSH-stimulated glucose transport and LDH activity. (a) Sertoli cells were cultured for 48 h under basal or FSH-stimulated (100 ng/mL) conditions in the absence or presence of quinacrine (Q, 30 μM) or AACOCF3 (AACO, 10 μM). Glucose uptake assay was performed after this incubation period (day 5). (b) Sertoli cells were stimulated for 72 h (day 3 to day 6) with 100 ng/mL FSH in the absence or presence of quinacrine (Q, 30 μM) or AACOCF3 (AACO, 10 μM). LDH activity was determined on cells harvested on day 6. Results represent mean ± SD of triplicate incubations in a representative experiment out of three. Different letters indicate statistically significant differences ($p < 0.01$).

PLA_2 itself through binding to cell surface receptors (Hernández *et al.*, 1998).

As mentioned in the introduction, AA can exert stimulatory or inhibitory effects on cellular responses. The contribution of an AA pathway to the regulation of Sertoli cell metabolic responses has not been analysed in detail. In the present investigation, two strategies were utilized to evaluate a possible role of AA on Sertoli cell metabolism: (a) cells were exposed to PLA_2 from bovine pancreas and (b) cells were incubated with AA itself.

Results presented herein show that exogenously added PLA_2 from porcine pancreas and AA are able to stimulate lactate production in rat Sertoli cells. In addition, this investigation shows that both treatments stimulate glucose uptake in these cells. Noteworthy, the ability of AA to stimulate glucose uptake was previously observed in Swiss 3T3 fibroblasts (Takuwa *et al.*, 1988; Magistretti *et al.*, 1991), cerebral cortical astrocytes (Yu *et al.*, 1993), and 3T3-L1 adipocytes, where it was associated with an increment in the number of GLUT1 transporters (Fong *et al.*, 1996). We had previously observed that increments in glucose uptake in Sertoli cell long-term incubations were associated with increased GLUT1 mRNA levels (Riera *et al.*, 2002). Although no attempts were made in the present investigation to determine the number of GLUT1 transporter molecules, it is tempting to speculate that an increase in the number of transporters at the plasma membrane is responsible for the observed effects of AA on glucose uptake.

Moreover, results shown in the present investigation evidence that PLA_2 and AA stimulate LDH activity and LDH A mRNA levels. Several reports have shown that Sertoli cell stimulation by growth factors and cytokines results in increments in LDH activity that are accompanied by increments in LDH A mRNA levels (Boussouar & Benahmed, 1999; Grataroli *et al.*, 2000). These reports also showed that different second messengers such as sphingosine, protein kinase C, protein kinase A and tyrosine kinase might regulate LDH A mRNA levels. Our results suggest that AA might also be considered a signalling molecule that participates in the stimulation of LDH activity and LDH A mRNA levels.

At least two mechanisms may be responsible for an increase in intracellular AA levels upon cell stimulation with exogenously added PLA_2: (a) activation of cytosolic PLA_2 as a consequence of PLA_2 binding to receptors at the plasma membrane (Murakami *et al.*, 1998) and/or (b) release of AA from plasma membrane lipids because of the catalytic properties of exogenously added PLA_2. In order to analyse the likelihood of this mechanism/s we have studied the effect

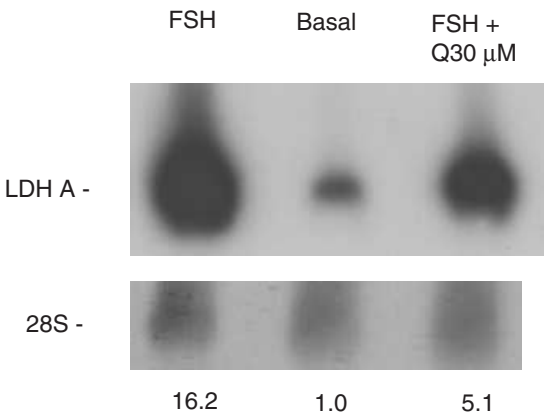

Figure 6. Effect of quinacrine on FSH-stimulated LDH A mRNA levels in rat Sertoli cells. Total RNAs were extracted from Sertoli cells harvested on day 6 pre-incubated for 12 h with 100 ng/mL FSH in the absence or presence of quinacrine (Q, 30 μM). Representative Northern Blots for LDH A are shown. Numbers at the bottom of each lane indicate the fold increases in LDH A mRNA levels (ratio of LDH A to 28S in each sample) relative to basal. Blots correspond to a representative experiment out of two.

of PLA_2 from porcine pancreas in combination with two different enzyme inhibitors – quinacrine and AACOCF3. Quinacrine is a widely used inhibitor of PLA_2 activity that inhibits both groups of PLA_2 – secretory and cytosolic (Mustonen *et al.*, 1998). AACOCF3, the other PLA_2 inhibitor used in this study, inhibits specifically cytosolic PLA_2 (Street *et al.*, 1993). The effect of exogenously added PLA_2 on rat Sertoli cell function is inhibited in the presence of quinacrine and is not modified in the presence of AACOCF3. These results suggest that exogenously added PLA_2 action does not require cytosolic PLA_2 activation. Taking together the results mentioned before and the fact that AA mimics the effects of exogenously added PLA_2, it may be suggested that the release of AA from plasma membrane lipids because of the catalytic properties of exogenously added PLA_2 is responsible for the observed effects of the enzyme on Sertoli cell metabolic function.

As for the mechanism/s that AA may utilize to regulate Sertoli cell functions little is known. However, it is worth mentioning that several signal transduction pathways may be activated in response to increments in AA levels. Among them, the one involving neutral sphingomyelinase, which participates in the release of ceramide, has been shown (Robinson *et al.*, 1997). We have previously shown that a ceramide-dependent pathway participates in the regulation of lactate production in Sertoli cells (Meroni *et al.*, 1999). In this context, the possibility exists that stimulation of a ceramide-dependent pathway by AA may be responsible for the increase in lactate production observed in rat Sertoli cells.

Phospholipase A_2 activity from the sperm of humans (Thakkar *et al.*, 1984; Fry *et al.*, 1992), mice (Thakkar *et al.*, 1983) and hamsters (Llanos *et al.*, 1982), as well as from the seminal plasma of several different animals and man (Kunze *et al.*, 1974; Wurl & Kunze, 1985) has been investigated. Secretory PLA_2 are believed to play a key role in the sperm acrosome reaction (Thakkar *et al.*, 1983, 1984), the fusion of sperm and oocyte plasma membranes (Fry *et al.*, 1992) and the production of free fatty acids for sperm germ energy metabolism (Ellis *et al.*, 1981). Noticeably, a novel member of group II secretory PLA_2, PLA_2 IIc, has been recently found exclusively in the testis. A high expression of PLA_2 IIc starting 2 weeks postnatally was observed (Chen *et al.*, 1994). This expression is mainly localized in pachytene spermatocytes, secondary spermatocytes and round spermatides but not in spermatogonia, elongating spermatids or Sertoli cells (Chen *et al.*, 1997).

All secretory PLA_2 bind to receptors at the plasma membrane through a highly conserved Ca^{2+}-binding domain (Lambeau *et al.*, 1995). Although no reports for the presence of PLA_2 receptors in Sertoli cells are available, it is tempting to speculate that secretory PLA_2 from germ cell origin – probably through the elevation of intracellular AA levels – may be considered a messenger that can accommodate Sertoli cell metabolism to the increasing germ cell number that occurs at the time of sexual development.

The importance of PLA_2 and AA to Sertoli cell metabolism in relation to FSH mechanism of action was first pointed out by Jannini *et al.* (1994). With the exception of the above-mentioned study the contribution of AA to the regulation of Sertoli cell metabolic responses to FSH has not been analysed in detail. In the present study, we show that FSH stimulation of lactate production is partially inhibited when hormone treatment is combined with quinacrine and AACOCF3. The latter results suggest that PLA_2 may be involved in the regulation of the levels of this energetic metabolite by FSH. As mentioned before, FSH stimulation of lactate production can be accounted for, at least partly, by increments in glucose uptake and LDH activity that are accompanied by increments in LDH A mRNA levels (Riera *et al.*, 2001). Experiments performed to explore the involvement of PLA_2 activity in these FSH metabolic responses showed that inhibition of PLA_2 activity partially decreased FSH action. It is worth mentioning that in the work done by Jannini *et al.* (1994) to show PLA_2 activation in response to FSH, quinacrine was used to inhibit enzyme activity. In this way, no distinction between secretory and cytosolic PLA_2 activation was performed. Our results show that FSH action in rat Sertoli cells involves a PLA_2 activity that can be inhibited by the specific cytosolic PLA_2 inhibitor AACOCF3 and suggest that FSH action may involve the activation of cytosolic PLA_2. In this respect, it has been demonstrated that activation of cytosolic PLA_2 is regulated mainly by MAPK-dependent phosphorylation and by intracellular calcium levels (Leslie, 1997). FSH does not stimulate MAPK kinase activity in 20-day-old Sertoli cells (Crepieux *et al.*, 2001). However, it is well known that FSH increases intracellular Ca^{2+} levels (Grasso & Reichert, 1989;

Gorczynska & Handelsman, 1991). Elevations in intracellular Ca^{2+} levels may then be responsible for FSH stimulation of cytosolic PLA_2 activity in rat Sertoli cells.

The present investigation shows that AA is involved in the regulation of lactate production, glucose transport, LDH activity and LDH A mRNA levels. Elevations of intracellular AA levels may arise from the action of secretory PLA_2 from germ cell origin and from the activation of cytosolic PLA_2 as part of the mechanism of action of FSH. Altogether, these results point to a central role of AA in the regulation of Sertoli cell energetic metabolism.

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