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Exploring the cyclooxygenase 2 (COX2)/15d-$\Delta^{12,14}PGJ_2$ system in hamster Sertoli cells: Regulation by FSH/testosterone and relevance to glucose uptake

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ABSTRACT

We have previously described a stimulatory effect of testosterone on cyclooxygenase 2 (COX2) expression and prostaglandin (PG) synthesis, and the involvement of PGs in the modulation of testosterone production in Leydig cells of the seasonal breeder Syrian hamster. In this study, we investigated the existence of a COX2/PGs system in hamster Sertoli cells, its regulation by testosterone and FSH, and its effect on glucose uptake. COX2 expression was observed in Sertoli cells of both reproductively active and inactive adult hamsters. Testosterone and the plasma membrane-impermeable testosterone-BSA significantly induced COX2 expression, mitogen activated protein kinases 1/2 (MAPK1/2) phosphorylation and 15d-$\Delta^{12,14}PGJ_2$ production in Sertoli cells purified from photoperiodically regressed hamsters. These actions were abolished by the antiandrogen bicalutamide and by the inhibitor of MAPK kinase (MEK1/2) U0126, suggesting that testosterone exerts its stimulatory effect on COX2/PGs through a non-classical mechanism that involves the presence of androgen receptors and MAPK1/2 activation. FSH also stimulated COX2/PGs via MAPK1/2 phosphorylation.

FSH and testosterone stimulate, whereas 15d-$\Delta^{12,14}PGJ_2$ via PPARγ inhibits, [2,6-^{3}H]-2-deoxy-D-glucose ([^{3}H]-2-DOG) uptake. Meloxicam, a selective COX2 inhibitor, further increases [^{3}H]-2-DOG uptake in the presence of FSH or testosterone. Thus, in addition to their positive effect, FSH and testosterone may also exert an indirect negative regulation on glucose uptake which involves the COX2/15d-$\Delta^{12,14}PGJ_2$/PPARγ system.

Overall, these results demonstrate the presence of a COX2/PG system in hamster Sertoli cells which might act as a local modulator of FSH and testosterone actions.

1. Introduction

The possible actions of prostaglandins (PGs) on testicular activity are not yet well understood. However, recent studies have shown that cyclooxygenase 2 (COX2), the key enzyme in the PG biosynthetic pathway, might play a role in the regulation of testicular function mainly in cancer, aging, and fertility disorders [22,29,90]. In this context, COX2 has been found to be up-regulated in testicular cancer [29] and 15d-$\Delta^{12,14}PGJ_2$ seems to participate in the development of human testicular fibrosis [22]. COX2 has also been associated to the age-related decline in testosterone biosynthesis [90].

We have previously found that, while COX2 is not detected by immunohistochemistry in human testicular biopsies with no evident morphological alterations or abnormalities, it is expressed in Leydig cells and macrophages in testes of men suffering from idiopathic infertility [19,22,51]. Recently, Welter et al. [91] have also detected COX2 expression in Leydig cells and mast cells in human testicular biopsies from patients with mixed atrophy.

In addition to their participation in the control of testicular activity under pathological conditions and aging, COX2 and PGs might play a critical role in the physiological regulation of testicular function in seasonal breeding mammals. We have recently found COX2 immunostaining in testes of the reproductively active seasonal breeder Syrian hamster (*Mesocricetus auratus*), and established the participation of testosterone in the regulation of testicular COX2 expression and, consequently, of PG synthesis [21,50]. In contrast, COX1 expression was not detected in hamster testes [21]. Furthermore, it has been shown that PGD_2 and $PGF_{2\alpha}$ contribute to the fine modulation of the steroidogenic process in hamster

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Leydig cells [21,79]. Previous reports from other authors also support a role played by some PGs (i.e. PGD_2, PGE_2, $PGF_{2\alpha}$) in the regulation of testicular testosterone production [13,27,39,66,70,76].

PGs and the arachidonic acid (AA), precursor in PG synthesis, have also been postulated to participate in the autocrine/paracrine control of Sertoli cells functions. Sertoli cells produce PGs such as $PGF_{2\alpha}$, PGE_2 and PGI [34] and express prostanoid receptors [32]. Nevertheless, few reports on the biological effects of PGs on Sertoli cells are available [34,53].

Male fertility and the process of spermatogenesis are dependent upon an adequate Sertoli cell function [26,28,81]. FSH and testosterone are the two major endocrine signals that act in the testis to regulate spermatogenesis [69]. *In situ* hybridization studies have confirmed that Sertoli cells are the only cell type expressing the FSH receptor in the rat testis [41,56,62]. Furthermore, Sertoli cells express androgen receptors [2,5,40,50,73,88,94]. Therefore, in the testis, Sertoli cells transduce signals from both testosterone and FSH into the synthesis of factors that are required for spermatogenesis. Furthermore, Sertoli cells are under the control of a plethora of locally produced factors that contribute to the normal functioning of the testis [84,86]. In consequence, Sertoli cell support of germ cell development is dependent on a complex interplay of endocrine and paracrine inputs. Among Sertoli cell functions that may be important to germ cell development is the provision of adequate levels of energy substrates such as lactate. Sertoli cell lactate production is exquisitely regulated by hormones and locally produced factors and several biochemical mechanisms may contribute to an increase in lactate secretion. In this context, the transport of glucose through the plasma membrane is the rate-limiting step in glucose metabolism and, consequently, in lactate production [63–65].

Because of germ cell contamination, studies in Sertoli cells purified from adult rodent testes have been scarce. Either under natural winter months or rigorously controlled laboratory conditions of less than 12.5 h light per day, adult Syrian hamsters experience a drastic physiological testicular regression with profound morphological changes [3]. Only Sertoli cells, spermatogonia and a few primary spermatocytes are observed in the tubular compartment [83]. Thus, the Syrian hamster becomes a useful and available experimental model that allows for isolation of Sertoli cells from testes of adult animals without germ cell contamination [3,20,83].

The aim of this study was to investigate the existence of a COX2/PG system in Sertoli cells of adult Syrian hamster, the possible mechanisms that regulate this system and its involvement in glucose uptake.

2. Materials and methods

2.1. Animals

Male Syrian hamsters (*Mesocricetus auratus)* were raised in our animal care facility [Charles River descendants, Animal Care Lab., Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires] and kept from birth to adulthood in rooms at 23 ± 2 °C under a long-day (LD) photoperiod (14 h light, 10 h darkness; lights on 0700–2100 h). Animals had free access to water and Purina formula chow.

Young adult hamsters aged 90 days were transferred to a short-day (SD) photoperiod (6 h light, 18 h darkness; lights on 0900–1500 h) for 16 weeks. It is worth noting that hamsters from our colony reach maximum testicular regression after 16 weeks in a SD photoperiod (see additional information in Frungieri et al. [20]). Hamsters were killed by asphyxia with carbon dioxide (CO_2) according to protocols for animal laboratory use, approved by the Institutional Animal Care and Use Committee (IBYME), following the National Institutes of Health (NIH) guidelines. At the time of sacrifice, testes were dissected and used for Sertoli cell purification. Alternatively, testes were fixed for at least 48 h in formaldehyde fluid followed by dehydration, and then embedded in paraffin wax for histological and immunohistochemical studies.

2.2. Hamster Sertoli cell purification and "in vitro" incubations

Testes from adult hamsters exposed to a SD photoperiod for 16 weeks were used to isolate Sertoli cells according to the method previously described by Schteingart et al. [80]. Briefly, approximately 2 g of decapsulated testes tissue were digested with 0.1% collagenase and 0.006% soybean trypsin inhibitor in Hanks' balanced salt solution for 10 min at room temperature. Seminiferous tubules were preserved, cut, and subjected 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 consisting of a 1:1 mixture of Ham's F12 and Dulbecco's modified Eagle medium (F12/DMEM), 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. An adequate aliquot of this cell suspension was preserved and DNA content was determined immediately. DNA levels were used to standardize the cell density in the cultures. Sertoli cells were cultured in 24-multiwell plates, 96-multiwell plates or in 30 mm tissue culture Petri dishes at a constant density of 15 μg DNA/cm^2, at 34 °C in a mixture of 5% CO_2–95% air. Sertoli cell-enriched cultures were routinely 87–90% pure as determined by light microscopy using antisera against myoid cell-specific α-smooth muscle actin (Sigma–Aldrich, St. Louis, MO, USA).

Sertoli cells were allowed to attach for 48 h in the presence of insulin and 2% fetal calf serum (FCS), and the medium was replaced at this time with fresh medium without phenol red, insulin or FCS. Stimulation was carried out at 34 °C in a mixture of 5% CO_2–95% air in the presence or absence of the following chemicals: testosterone (1 μM, Sigma–Aldrich), testosterone 3-(O-carboxymethyl) oxime/bovine serum albumin (testosterone-BSA, 1 μM, Sigma–Aldrich), ovine FSH (100 ng/ml oFSH-16, National Hormone and Pituitary Program, Baltimore, MD, USA), $PGF_{2\alpha}$ (1 μM, Sigma–Aldrich), 15d-$\Delta^{12,14}PGJ_2$ (0.1–1 μM, Cayman Chemical, Ann Arbor, MI, USA) or BSA (34 nM, Sigma–Aldrich).

In some experiments, Sertoli cells were pre-incubated for 1 h in the presence of the antiandrogen bicalutamide (10 μM, Casodex, ICI 176 334, AstraZeneca Pharmaceuticals, Macclesfield, England), the mitogen activated protein kinase kinase 1/2 (MEK 1/2) specific inhibitor U0126 (10 μM, Sigma–Aldrich), the selective COX2 inhibitor meloxicam (10 μM, Calbiochem, La Jolla, CA, USA), or the PPARγ antagonist bisphenol A diglycidyl ether (BADGE, 1 μM, Sigma–Aldrich).

In this study, U0126 and meloxicam stock solutions were prepared in dimethyl sulfoxide (DMSO) (ICN Biomedicals Inc., Aurora, OH, USA), whereas testosterone and BADGE stock solutions were prepared in absolute ethanol. These solutions were then further diluted in F12/DMEM medium. An appropriate volume of DMSO (1 μl DMSO/ml F12/DMEM medium) or absolute ethanol (0.03 μl absolute ethanol/ml F12/DMEM medium) was added to control experiments in order to account for possible effects of DMSO or ethanol.

Testosterone-BSA (28.9 mol testosterone per mol BSA) stock solution was prepared in 0.01 M phosphate-buffered saline (PBS, pH 7.4). To eliminate any potential contamination with free testosterone, the testosterone-BSA conjugate was incubated at 4 °C for 30 min in the presence of a 0.5% charcoal, 0.05% dextran suspension. After centrifugation at 1600*g* for 10 min, the supernatant

was collected and used in cell incubations. BSA alone (34 nM) was used as a control treatment.

FSH, $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ were dissolved in F12/DMEM medium, which was then used as vehicle for control incubations.

After incubations, cells were used either for RNA extraction followed by reverse transcription polymerase chain reaction (RT-PCR) or quantitative reverse transcription polymerase chain reaction (RT-qPCR), protein extraction followed by immunoblotting or [2,6-^{3}H]-2-deoxy-D-glucose ([^{3}H]-2-DOG) uptake studies. Media were frozen at −70 °C until PG levels were determined by immunoassay.

2.3. Immunohistochemical and immunocytochemical analyses

Testes from 90-day-old adult hamsters kept under a LD photoperiod, as well as from adult hamsters exposed to a SD photoperiod for 16 weeks (16SD), were examined by immunohistochemical assays. Groups of eight to ten animal testes were evaluated. After fixation, tissues were dehydrated and embedded in paraffin wax. Five micrometre sections obtained from three different levels were used for immunodetection of androgen receptors as previously described by Matzkin et al. [50]. Alternatively, formaldehyde-fixed Sertoli cells purified from adult hamsters exposed to SD photoperiod (as described in Section 2.2) were used for PPARγ immunodetection. In brief, endogenous peroxidase reactivity was quenched by a 20 min pre-treatment with 10% methanol, 0.3% H_2O_2 in PBS. Then, samples were permeabilized by a 5 min incubation with 0.5% saponin for detection of androgen receptors in paraffin sections and 0.05% saponin for immunodetection of PPARγ in formaldehyde-fixed Sertoli cells.

Non-specific proteins were blocked by subsequent incubation for 30 min with a protein block buffer (5% horse normal serum prepared in 1.5% milk in PBS for immunodetection of androgen receptors and 5% horse normal serum prepared in PBS for immunodetection of PPARγ). After several wash steps the incubation with the antiserum (polyclonal rabbit anti-androgen receptor serum, 1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA and monoclonal mouse anti-PPARγ serum, 1:100, Santa Cruz Biotechnology Inc.) diluted in incubation buffer (0.02 M $NaPO_4H_2.H_2O$, 0.15 M ClNa, sodium azide 1% BSA pH 7.6 for immundetection of androgen receptors and 2% horse normal serum in PBS for immunodetection of PPARγ) was carried out overnight in a humidified chamber at 4 °C. On the second day, cells and testicular sections were washed and incubated with biotinylated secondary antiserum (goat anti-rabbit IgG serum; 1:500 for immunodetection of androgen receptors and horse anti-mouse IgG serum; 1:500 for immunodetection of PPARγ, Vector Laboratories Inc., Burlingame, CA, USA) for 2 h at room temperature. Finally, immunoreactions were visualized with a 0.01% H_2O_2 and 0.05% 3,3-diaminobenzidine (DAB) solution (in 0.05 M Tris–HCl, pH 7.6) and an avidin–biotin-peroxidase system (Vector Laboratories Inc.).

Antigen retrieval, was performed by microwave irradiation of hamster testes sections in citrate buffer 0.01 M (pH 6.0), or by incubation of formaldehyde-fixed purified hamster Sertoli cells in 20 μg/ml proteinase K solution at 37 °C for 15 min.

For control purposes, either the first antiserum was omitted or incubation was carried out with normal non-immune sera.

2.4. Laser capture microdissection technique

Testicular sections from 90-day-old adult hamsters kept under a LD photoperiod, as well as from adult hamsters exposed to a SD photoperiod for 16 weeks were used. The sections were deparaffinized and immunostained with anti-androgen receptor serum (Santa Cruz Biotechnology Inc.) as described above. Subsequently, laser capture microdissection was performed according to Rossi et al. [68]. Briefly, the infrared (IR)-laser energy of Arcturus laser capture microdissection equipment (Applied Biosystems, Bedford, MA, USA) combined with the ultraviolet (UV) cutting laser were used for circumscription of androgen receptor-immunoreactive Sertoli cells. Then, a High Sensitive (HS) CapSure laser capture microdissection cap (Applied Biosystems) containing a thermoplastic film was placed over the target area. By pulsing the IR-laser through the cap, the thermoplastic film formed a thin protrusion that bridges the gap between the cap and tissue and adheres to the target cell. By lifting the cap, target cells now attached to the cap were removed. Approximately, 80–100 androgen receptor-positive cells were captured on each cap. As a negative control, androgen receptor-immunoreactive Sertoli cells were destroyed by a few laser shots and the remaining material was catapulted into the cap of a microfuge tube.

2.5. RT-PCR and RT-qPCR analyses

Total RNA was prepared from hamster Sertoli cells using the QIAGEN RNeasy mini kit (QIAGEN Inc., Valencia, MO, USA) following the manufacturer's instructions. RNA extraction from Sertoli cells obtained by laser capture microdissection was performed using the Paradise Plus Reagent system (Applied Biosystems) following the manufacturer's instructions. In order to improve RNA yield, samples were digested for 16 h at 37 °C in the presence of 20 μg Proteinase K (Applied Biosystems). Degradation of contaminating DNA was performed by incubation of the cell extract mixtures with RNase-free DNase (1 unit per μg RNA, Promega Corporation, Madison, WI, USA) at room temperature for 20 min.

RT-reaction was performed using dN6 random primers as described elsewhere [19]. An amount of 400 ng total RNA was used for each RT-PCR and RT-qPCR reaction.

RT-PCR analyses were performed using oligonucleotide primers for COX2 (5′-CTGGCGCTCAGCCATACAG and 5′-ACACTCATACATACACCTCGGT), FSH receptor (1° set: 5′-CCTTGCTCCTGGTCTCCTTG and 5′-CTCGGTCACCTTGCTATCTTG; 2° set: 5′-TGGTCTCCTTACTGGCAT and 5′-GGAAGACCCTGTCAGAGC), 18S (5′-ACACGGACAGGATTGACAGATT and 5′-CGTTCGTTATCGGAATTAACCA), prostanoid DP receptor (5′-TGCAACCTGGGTGCCATG and 5′-GGCTTGGAGGTCTTCCGA), prostanoid FP receptor (5′-CCAAACATGTGAAAATGATG and 5′-GCCTTGTCTGTGCTGCTG) and PPARγ (5′-GATACAAGTATGACCTG and 5'-GCTTTATCTCCACAGAC). When information about exon structure was available at GenBank, oligonucleotide primers were designed as homologous to regions of different exons. In this context, we designed oligonucleotide primers for COX2 to be homologous to areas of different exons.

PCR conditions were 95 °C for 5 min, followed by cycles of 94 °C for 1 min, 55–60 °C (annealing temperature) for 1 min and 72 °C for 1 min, and a final incubation at 72 °C for 5 min. PCR products were separated on 3% agarose gels, and visualized with ethidium bromide. The identity of the cDNA products was confirmed by sequencing using a fluorescence-based dideoxy-sequencing reaction and an automated sequence analysis on an ABI 373A DNA sequencer (Applied Biosystems).

qPCR assays were performed as described elsewhere [68] using oligonucleotide primers for COX2 (5′-CTGGCGCTCAGCCATACAG and 5′-ACACTCATACATACACCTCGGT) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNA (5′-TGCACCACCAACTGCTTAGC and 5′-GGCATGGACTGTGGTCATGAG). GAPDH was chosen as the housekeeping gene. Reactions were conducted using SYBR Green PCR Master Mix and the ABI PRISM 7500 sequence detector System (Applied Biosystems, Foster City, CA, USA). The reaction conditions were as follows: 10 min at 95 °C (one cycle), followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 60 °C and 1 min at 72 °C for COX2 and 10 min at 95 °C (one cycle), followed

by 40 cycles of 15 s at 95 °C, 30 s at 55 °C and 1 min at 60 °C for GAPDH. Following the mathematical model of Pfaffl [59], the fold change of mRNA COX2 expression was determined for each sample by calculating $(E\ target)^{\Delta Ct(target)}/(E\ housekeeping)^{\Delta Ct(housekeeping)}$, where E is the efficiency of the primer set, CT the cycle threshold, and ΔCt = Ct(normalization cDNA)-Ct(experimental cDNA). The amplification efficiency of each primer set was calculated from the slope of the standard amplification curve of log microlitres of cDNA per reaction vs Ct value over at least four orders of magnitude ($E = 10^{-(1/slope)}$; E GAPDH = 2.03 and E COX2 = 2.05 vs hamster Sertoli cells cDNA).

2.6. Immunoblotting

Sertoli cells were homogenized in 20 mM Tris–HCl (pH 8), 137 mM NaCl buffer containing 10% glycerol, 1% lysis buffer (NP40, Sigma–Aldrich), and 1% of a pre-formed mixture of protease inhibitors (P8340, Sigma–Aldrich). Protein concentrations were measured by the method of Lowry et al. [47]. Approximately 150 μg of protein aliquots were heated at 95 °C for 5 min under reducing conditions (10% mercaptoethanol), loaded onto tricine–SDS–polyacrylamide gels (10%), electrophoretically separated, and blotted onto nitrocellulose membranes, as described by Frungieri et al. [19]. Blots were incubated with rabbit polyclonal anti-COX2 serum (1:250, Cayman Chemical), mouse monoclonal anti-phospho mitogen activated protein kinases 1/2 (MAPK1/2) antibody (1:250, Cell Signaling Technology Inc., Beverly, MA, USA) or mouse monoclonal anti-actin antibody (1:5000, Calbiochem) and subsequently with peroxidase-labeled secondary antibodies (goat anti-rabbit IgG serum, 1:2500, Sigma–Aldrich, for COX2; 1:1000 sheep anti-mouse IgG serum, GE Healthcare, Piscataway, NJ, USA, for phospho-MAPK 1/2; and 1:2000 goat anti-mouse IgM serum, Calbiochem, for actin). Signals were detected with an enhanced chemiluminescence kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA).

2.7. PG immunoassays

Commercially available kits were used to determine PGD_2 (Cayman Chemical), $PGF_{2\alpha}$ (Cayman Chemical) and 15d-$\Delta^{12,14}PGJ_2$ (Assay Designs, Ann Arbor, MI, USA) levels in Sertoli cell incubation media, as described elsewhere [22,50,51].

For PGD_2 quantification, media were pre-treated with methoxylamine hydrochloride (MOX HCl) in order to prevent further PG chemical degradation and subsequently acidified using 1 M citrate buffer (final pH 4.7).

For $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ assays, media were acidified using 2 N HCl (final pH 3.5) before being injected into a 200-mg C18 column and then eluted with ethyl acetate.

Eluted fractions of incubation media from hamster Sertoli cells were evaporated to dryness under a nitrogen stream and reconstituted in assay buffer.

The minimum detectable immunoassay concentrations were 0.28, 0.64 and 0.01 femtomole (fmol)/tube for PGD_2, $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$, respectively. Intra-assay and inter-assay coefficients of variation were less than 10% and less than 15%, respectively. PG levels were expressed as fmol/μg DNA.

2.8. Measurement of 2-deoxyglucose uptake

Glucose transport was studied by analyzing the uptake of [^{3}H]-2-DOG, a non-metabolizable glucose analogue according to the method previously described by Riera et al. [64]. Cells were washed three times with glucose-free PBS at room temperature. Then, Sertoli cells were incubated for 30 min at 34 °C in 0.5 ml glucose-free PBS containing [^{3}H]-2-DOG (0.5 Ci/ml). Non-specific uptake was determined in incubations performed in the presence of a 10,000-fold higher concentration of unlabeled 2-DOG. At the end of the incubation period, dishes were placed on ice and extensively washed with ice-cold PBS. Cells were then dissolved in 0.5 M sodium hydroxide containing 0.4% sodium deoxycholate, and counted in a liquid scintillation spectrometer. [^{3}H]-2-DOG uptake was calculated as disintegration per minute (dpm) and normalized to DNA content (μg) in a parallel cell culture. DNA content was measured according to the method described by Labarca and Paigen [45]. Results are expressed as [^{3}H]-2-DOG uptake percentage (%) relative to the control (basal conditions).

2.9. Cell viability assays

A colorimetric cell viability assay (CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega Corporation) was performed in hamster Sertoli cells cultured on 96-multiwell plates and treated for 1 h with 15d-$\Delta^{12,14}PGJ_2$ as described elsewhere [22].

Sertoli cell viability was also evaluated by trypan blue exclusion. Cells were incubated for 1 h in the presence or absence of 15d-$\Delta^{12,14}PGJ_2$, subsequently treated for 5 min with 0.4% Trypan-blue and observed in a light microscope.

2.10. Statistical analyses

Statistical analyses were performed using ANOVA followed by Student's *t* test for two comparisons or Student-Newman–Keuls test for multiple comparisons. Data are expressed as mean ± S.E.M.

For immunoblotting studies, bands were quantified by densitometry and normalized to actin using SCION IMAGE (SCION Corporation, Frederick, MD, USA).

3. Results

3.1. Evidence for gene expression of COX2 in hamster Sertoli cells

A total of 80–100 androgen receptor-immunoreactive Sertoli cells were isolated from adult hamsters exposed to a LD photoperiod (Fig. 1A) and adult hamsters exposed to a SD photoperiod for 16 weeks (Fig. 1B) using laser capture microdissection. The RT-PCR analyses of these cells, followed by sequencing, indicated that androgen receptor-immunoreactive Sertoli cells express COX2. 18S and FSH receptor expression were used as positive controls.

The relative levels of mRNA COX2 expression, as determined by qRT-PCR experiments, was significantly higher in Leydig cells from hamsters kept under a LD photoperiod (mean ± SEM, 28.52 ± 5.69) than those detected in Leydig cells from hamsters exposed to a SD photoperiod for 16 weeks (4.25 ± 0.17, $p < 0.05$) or Sertoli cells from regressed animals (0.83 ± 0.09, $p < 0.05$).

3.2. Testosterone induction of COX2 expression and PG production in hamster Sertoli cells

Sertoli cells were isolated from testes of adult hamsters exposed to a SD photoperiod for 16 weeks. After being cultured in basal conditions for 48 h, Sertoli cells were incubated for 5, 15, 30 and 60 min in the presence or absence of testosterone (1 μM). After 5, 15 and 30 min incubations, testosterone did not alter COX2 expression but significantly increased MAPK 1/2 phosphorylation (Fig. 2A). After a 60 min treatment with 1 μM testosterone, both COX2 expression and MAPK 1/2 phosphorylation were markedly induced (Fig. 2A) but Akt phosphorylation was unaffected (data not shown). PGD_2 and $PGF_{2\alpha}$ release to the media was unaffected

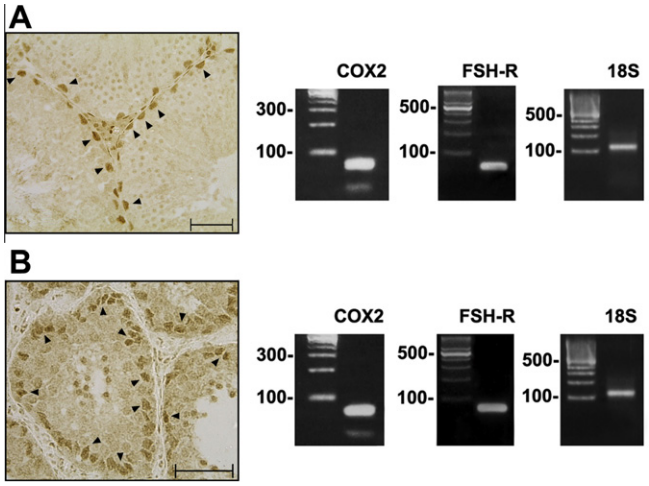

Fig. 1. COX2 expression in hamster Sertoli cells. Using the laser capture microdissection technique, androgen receptor-immunoreactive Sertoli cells were isolated from testes of (A) adult hamsters exposed to a LD photoperiod and (B) adult hamsters exposed to a SD photoperiod for 16 weeks (Bar, 50 μm). In each panel androgen receptor-immunoreactive Sertoli cells are shown (head arrows). These immunoreactive cells were initially circumscribed using the ultraviolet (UV) laser of the equipment and subsequently isolated using the infrared (IR) laser. The material was subjected to RT-PCR analysis. Expression of COX2, FSH receptor (FSH-R, Sertoli cell marker) and 18S (housekeeping gene) was detected in Sertoli cells of both hamsters kept under a LD photoperiod and animals exposed to a SD photoperiod.

by a 60 min testosterone treatment, while 15d-$\Delta^{12,14}PGJ_2$ production was significantly increased (Fig 2B).

3.3. Participation of membrane associated-androgen receptors in testosterone-mediated up-regulation of COX2 expression and PG production in hamster Sertoli cells

The incubation of Sertoli cells for 60 min in the presence of 1 μM testosterone-BSA conjugate, a plasma membrane-impermeant complex, significantly induced COX2 expression (Fig. 3A), MAPK 1/2 phosphorylation (Fig 3A) and 15d-$\Delta^{12,14}PGJ_2$ production (Fig. 3B). In contrast, no significant changes were observed in Akt phosphorylation when hamster Sertoli cells were incubated for 60 min in the presence of 1 μM testosterone-BSA (data not shown).

The stimulatory effects of testosterone on COX2 expression and MAPK 1/2 phosphorylation were reversed when 10 μM U0126 (a selective MEK 1/2 inhibitor) or 10 μM bicalutamide (a pure antiandrogen) were added to the incubation media (Fig. 3A). No stimulatory effect was observed when Sertoli cells were treated with 10 μM bicalutamide (data not shown) or 34 nM BSA alone. 10 μM U0126 produced a quite significant decrease in the phosphorylation levels of MAPK 1/2, but did not affect COX2 expression (data not shown).

3.4. FSH induction of COX2 expression and PG production in hamster Sertoli cells

After 60 min treatment with 100 ng/ml FSH, COX2 expression, as well as MAPK 1/2 phosphorylation, were significantly induced (Fig. 4A). Moreover, the stimulatory effect of FSH on COX2 expression and MAPK 1/2 was abolished in the presence of U0126 (Fig. 4A). At a shorter incubation time of 30 min, FSH had no effect on COX2 expression while MAPK 1/2 phosphorylation was increased (data not shown). $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ synthesis was significantly increased when Sertoli cells were incubated in the presence of FSH (Fig 4B). However, PGD_2 production remained unchanged (Fig. 4B).

3.5. Identification of PG receptors in hamster Sertoli cells

Sertoli cells purified from hamsters exposed to a SD photoperiod for 16 weeks were used to amplify, by RT-PCR, cDNA fragments that, after sequencing, were shown to correspond to PPARγ (the nuclear receptor for PGJ_2 derivatives), FP ($PGF_{2\alpha}$ receptor) and DP (PGD_2 receptor) (Fig. 5A). The presence of PPARγ receptors in isolated Sertoli cells from SD hamsters was confirmed by immunocytochemistry (Fig. 5B). Specific immunostaining was not detected in isolated SD hamster Sertoli cells when PPARγ antiserum was omitted (Fig. 5B).

3.6. Testosterone and FSH modulate glucose uptake in hamster Sertoli cells

Glucose transport was studied by analyzing the uptake of [^{3}H]-2-DOG, a non-metabolizable glucose analogue, in regressed hamster Sertoli cells. After 60 min incubation, testosterone (1 μM), testosterone-BSA conjugate (1 μM) and FSH (100 ng/ml) significantly stimulated glucose uptake in Sertoli cells (Fig. 6A). A further increase in glucose uptake was seen when Sertoli cells were pre-treated for 60 min with meloxicam (10 μM), a selective COX2 inhibitor (Fig. 6A). No significant changes in glucose uptake were detected after treatment with BSA (34 nM) or meloxicam (10 μM) alone (Fig. 6A).

A significant inhibition of [^{3}H]-2-DOG uptake by Sertoli cells was observed after 60 min incubation in the presence of $PGF_{2\alpha}$ (1 μM) or 15d-$\Delta^{12,14}PGJ_2$ (1 μM) (Fig. 6B).

The inhibitory effect exerted by 15d-$\Delta^{12,14}PGJ_2$ on glucose uptake was reverted in the presence of the PPARγ antagonist BADGE (Fig. 6B). In order to determine whether the modulatory effect exerted by FSH is mediated through 15d-$\Delta^{12,14}PGJ_2$/PPARγ, Sertoli cells pre-incubated for 60 min in the presence or absence of BADGE (1 μM), were then incubated for an additional 60 min with FSH (100 ng/ml). The stimulatory effect of FSH on glucose uptake was further stimulated when Sertoli cells were pre-treated with the PPARγ antagonist (Fig. 6B). BADGE alone did not exert an effect on glucose uptake (Fig. 6B).

3.7. 15d-$\Delta^{12,14}PGJ_2$ has no effect on hamster Sertoli cell viability

A colorimetric assay was used to evaluate the effect of 15d-$\Delta^{12,14}PGJ_2$ on hamster Sertoli cell viability. Values of the different experimental groups were expressed as arbitrary units and averaged (mean ± SEM, $n = 12$) as follows: basal 549.50 ± 17.31; 10 μM 15d-$\Delta^{12,14}PGJ_2$ 605.13 ± 23.05; 1 μM 15d-$\Delta^{12,14}PGJ_2$ 555.00 ± 19.53; 0.1 μM 15d-$\Delta^{12,14}PGJ_2$ 525.13 ± 11.84. No significant differences were seen between groups.

In addition, a trypan blue exclusion test also showed no effect of 15d-$\Delta^{12,14}PGJ_2$ on hamster Sertoli cell viability.

4. Discussion

This study provides new evidence for the existence of a COX2/15d-$\Delta^{12,14}PGJ_2$ system in hamster Sertoli cells and its up-regulation by FSH and testosterone. Our results indicate that testosterone exerts its stimulatory effect on COX2 expression through a non-classical mechanism that involves the presence of membrane associated androgen receptors and MAPK 1/2 activation. FSH action on COX2 is also mediated by phosphorylation of MAPK 1/2. Glucose uptake in hamster Sertoli cells was found to be positively regulated by FSH and testosterone. However, these hormones also modulate glucose uptake through an indirect negative mechanism exerted via COX2/15d-$\Delta^{12,14}PGJ_2$/PPARγ.

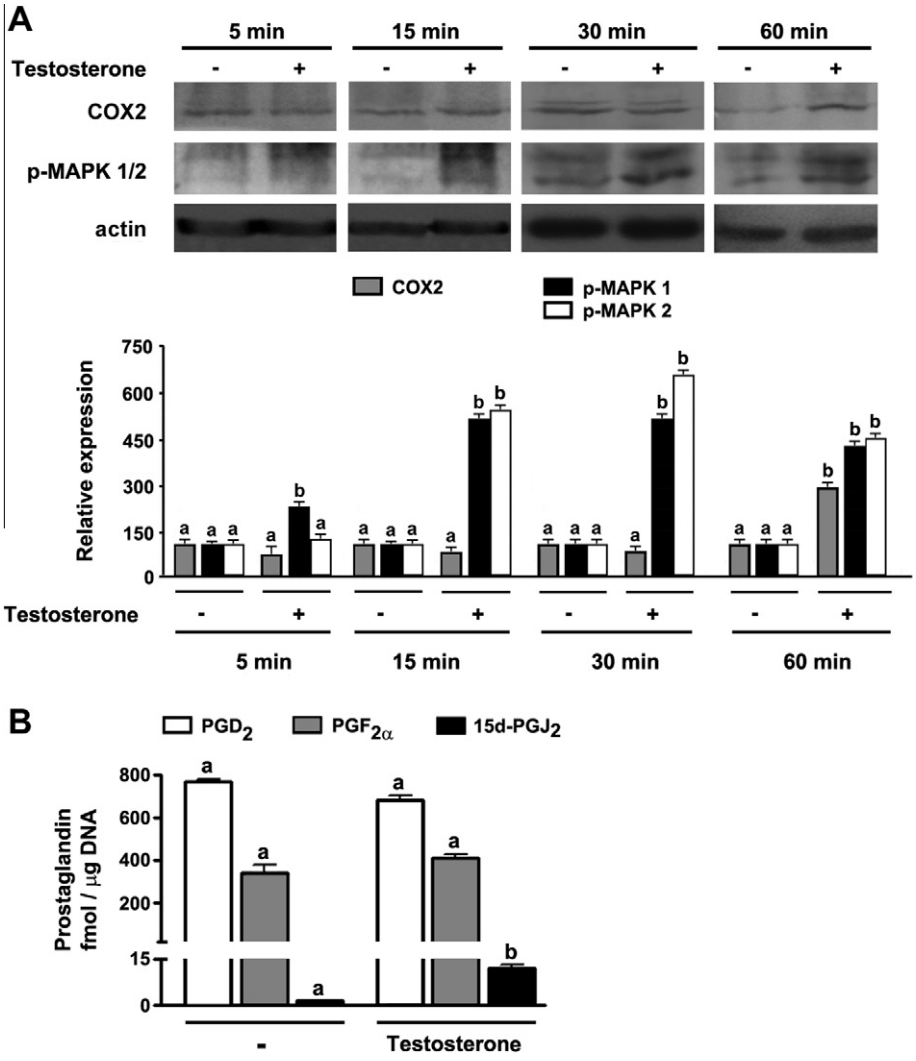

Fig. 2. Testosterone induction of COX2 expression and PG production in hamster Sertoli cells. Sertoli cells were isolated from testes of adult hamsters exposed to a SD photoperiod for 16 weeks and cultured in basal conditions for 48 h. (A) Sertoli cells were incubated for 5, 15, 30 and 60 min in the presence or absence of testosterone (1 μM). COX2 (72 kDa), phospho-MAPK 1/2 (p-MAPK 1/2, 44/42 kDa) and actin (42 kDa) protein levels were determined by immunoblotting. These representative immunoblots show results obtained from one of three experiments performed in different cell preparations that showed comparable results. Bar plot graph represents the mean ± S.E.M. and depicts the quantification by densitometry of the bands. Results are expressed as fold change relative to the control (basal conditions), which was assigned a value of 100, and normalized to actin. (B) Sertoli cells were incubated for 60 min in the presence or absence of testosterone (1 μM). PGD_2, $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ levels in the incubation media were determined by immunoassay. Bar plot graph represents the mean ± S.E.M. from one of three experiments performed in different cell preparations that showed comparable results. Different letters denote a statistically significant difference between groups ($p < 0.05$; Student-Newman–Keuls test).

Recent reports propose that COX2/PGs might be of relevance in male fertility pathology and/or physiology. With regard to Sertoli cells, it has been described the production of PGE_2, $PGF_{2\alpha}$ and PGI_2 from these somatic cells, as well as the expression of the prostanoid receptors EP1, EP2, EP3, EP4, IP and FP [10,33]. Furthermore, the expression of COX in Sertoli cells has been reported [44,92,93].

The Sertoli cells contact all stages of developing cells, extending processes from the basal membrane to within the central lumen of the epithelium [31]. Therefore, Sertoli cell preparations from testes of adult rodents are very often contaminated by the presence of germ cells. To reduce germ cell contamination in Sertoli cell cultures, animals that have not yet completed the first wave of spermatogenesis are commonly used as Sertoli cell donors. The Syrian hamster is a readily available experimental model that allows for isolation of Sertoli cells from adult testes limiting the contamination with germ cells to an absolute minimum, given the spontaneous germ cell line involution and the natural testicular Sertoli cell-enrichment that hamsters experience as a consequence of their exposure to a SD photoperiod for 12–16 weeks [3,20,83].

We have previously found positive COX2 immunostaining only in Leydig cells of the reproductively active seasonal breeder Syrian hamster. COX2 was not detected in Sertoli cells or Leydig cells of photoperiodically regressed animals [21]. However, in this study we have seen COX2 expression in hamster Sertoli cells of regressed animals using different experimental techniques such as PCR and immunoblotting. One plausible explanation for these discrepancies is that the levels of COX2 expression in Sertoli cells may be too low to be detected by immunohistochemistry. Actually, mRNA COX2 expression in Leydig cells from hamsters kept under a long day photoperiod is 5- to 10-fold higher and 30- to 40-fold higher than that detected in Leydig cells and Sertoli cells from regressed animals, respectively.

Sertoli cells are, within the seminiferous tubules, the major transducers of testosterone signals that are required to support germ cell survival and development [49]. Previously, immunohistochemical studies demonstrate the expression of androgen

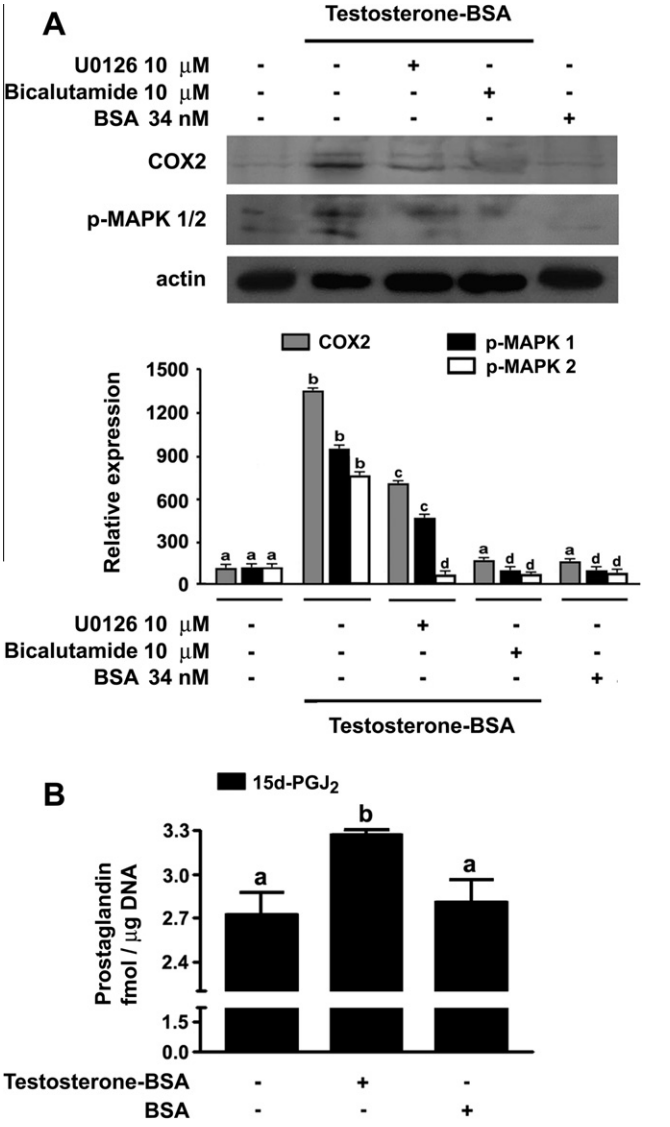

Fig. 3. Participation of membrane-associated androgen receptors in testosterone mediated-up-regulation of COX2 expression and PG production in hamster Sertoli cells. Sertoli cells were isolated from testes of adult hamsters exposed to a SD photoperiod for 16 weeks and cultured in basal conditions for 48 h. (A) Sertoli cells were incubated for 60 min in the presence or absence of testosterone-BSA (1 μM) and BSA (34 nM) either with or without the MEK 1/2 inhibitor U0126 (10 μM) or the antiandrogen bicalutamide (10 μM). COX2 (72 kDa), phospho-MAPK 1/2 (p-MAPK 1/2, 44/42 kDa) and actin (42 kDa) protein levels were determined by immunoblotting. These representative immunoblots show results obtained from one of three experiments performed in different cell preparations that showed comparable results. Bar plot graph represents the mean ± S.E.M. and depicts the quantification by densitometry of the bands. Results are expressed as fold change relative to the control (basal conditions), which was assigned a value of 100, and normalized to actin. (B) Sertoli cells were incubated for 60 min in the presence or absence of testosterone-BSA (1 μM) or BSA (34 nM). 15d-$\Delta^{12,14}PGJ_2$ levels in the incubation media were determined by immunoassay. Bar plot graph represents the mean ± S.E.M. from one of three experiments performed in different cell preparations that showed comparable results. Different letters denote a statistically significant difference between groups ($p < 0.05$; Student-Newman–Keuls test).

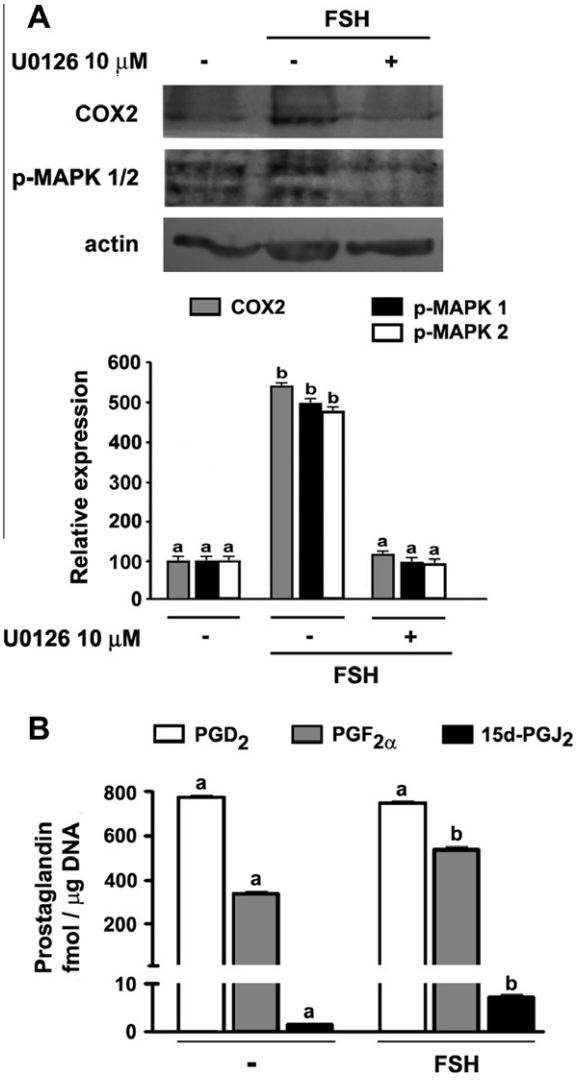

Fig. 4. FSH induction of COX2 expression in hamster Sertoli cells involves MAPK 1/2 activation. Sertoli cells were isolated from testes of adult hamsters exposed to a SD photoperiod for 16 weeks and cultured in basal conditions for 48 h. (A) Sertoli cells were incubated for 60 min in the presence or absence of FSH (100 ng/ml) either with or without the MEK 1/2 inhibitor U0126 (10 μM). COX2 (72 kDa), phospho-MAPK 1/2 (p-MAPK 1/2, 44/42 kDa) and actin (42 kDa) protein levels were determined by immunoblotting. These representative immunoblots show results obtained from one of three experiments performed in different cell preparations that showed comparable results. Bar plot graph represents the mean ± S.E.M. and depicts the quantification by densitometry of the bands. Results are expressed as fold change relative to the control (basal conditions), which was assigned a value of 100, and normalized to actin. Different letters denote a statistically significant difference between groups ($p < 0.05$; Student-Newman–Keuls test). (B) Sertoli cells were incubated for 60 min in the presence or absence of FSH (100 ng/ml). PGD_2, $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ levels in the incubation media were determined by immunoassay. Bar plot graph represents the mean ± S.E.M. from one of three experiments performed in different cell preparations that showed comparable results. Different letters denote a statistically significant difference between groups ($p < 0.05$; Student's t test).

receptors in Sertoli cells from both reproductively active and regressed Syrian hamsters [50].

In this work, testosterone significantly induced protein COX2 expression in Sertoli cells purified from regressed adult hamsters after 1 h incubation. It is well known that induction of both COX2 mRNA and protein are rapid events that occur within minutes [22,50]. We also analyzed the effect of testosterone on the production of certain PGs (PGD_2, $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$) relevant to testicular function regulation. In this context, testosterone stimulated the production of 15d-$\Delta^{12,14}PGJ_2$, which is known to induce fibrosis of the tubular wall [22], but did not affect the release of PGD_2 and $PGF_{2\alpha}$, eicosanoids previously associated to the modulation of testicular steroidogenesis in hamsters [21,79]. The differential effect of testosterone on PGD_2, $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ production could be related to additional modulations of other PG synthetic enzymes that need to be further investigated.

The classical mechanism of testosterone action in which this hormone activates gene transcription by causing the androgen

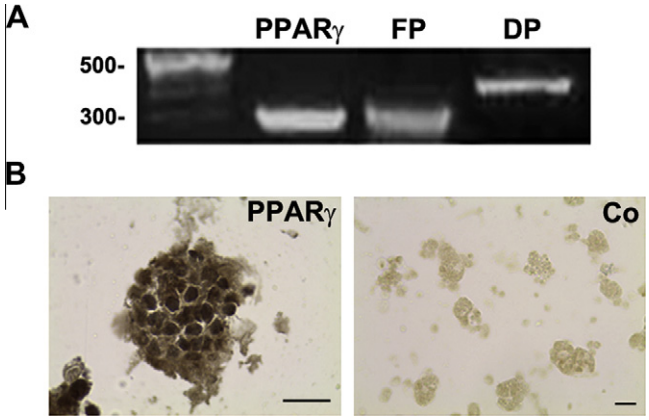

Fig. 5. Identification of PG receptors in hamster Sertoli cells. Sertoli cells were isolated from testes of adult hamsters exposed to a SD photoperiod for 16 weeks. (A) mRNA expression of PPARγ (the nuclear receptor for PGJ_2 derivatives), FP ($PGF_{2\alpha}$ receptor) and DP (PGD_2 receptor) was determined by RT-PCR. (B) Positive immunostaining for PPARγ was found in Sertoli cells purified from regressed hamsters. No reaction was observed when Sertoli cells were incubated only with normal non-immune serum and the conjugated antibody (Co, control). Bar, 20 μm.

receptor to translocate to the nucleus and bind specific DNA regulatory elements does not appear to fully explain testosterone regulation of testicular function. In addition to this classical pathway, there is growing evidence indicating that androgens are also able to trigger cellular processes through rapid, non-genomic mechanisms in different isolated cells and tissues, including testis and prostate (for a review see Foradori et al. [17]). Non-classical androgen actions are often associated to signaling pathways that lead to the activation of certain kinases such as the phosphoinositide-3 (PI3) kinase/serine threonine kinase (PI3K/Akt) and MAPK 1/2 [1,7–9,16,30,36,60,61,82]. In this context, we have recently described a stimulatory effect of testosterone on COX2 expression in hamster Leydig cells taking place within minutes through a non classical mechanism which involves MAPK 1/2 phophorylation [50]. As a consequence, we have investigated the participation of the Akt and MAPK 1/2 signaling pathways in the regulation of COX2 expression by testosterone in hamster Sertoli cells. Testosterone failed to activate Akt. However, our results indicate that testosterone stimulation of COX2 expression in hamster Sertoli cells is associated to MAPK 1/2 activation. The use of the antiandrogen bicalutamide indicated that testosterone action on COX2 expression and PG production in hamster Sertoli cells is mediated through androgen receptors. Rapid androgen actions seem to be mediated by membrane receptors. There is evidence for the existence of testosterone binding sites located in the plasma membrane of oocytes, prostate, osteoblast, skeletal muscle, T cells and more importantly, in Sertoli cells [4,15,16,25,35]. Members of the

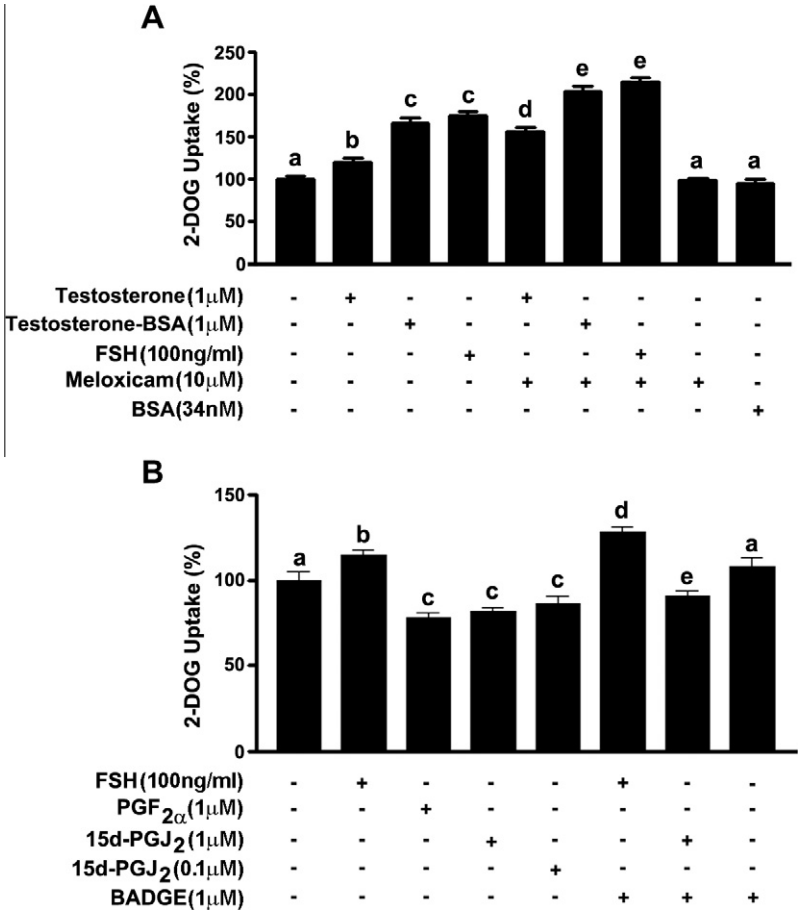

Fig. 6. Testosterone and FSH effect on glucose uptake in Sertoli cells: participation of COX2/PG system. Sertoli cells were isolated from testes of adult hamsters exposed to a SD photoperiod for 16 weeks and cultured in basal conditions for 48 h. (A) Sertoli cells were pre-incubated for 60 min in the presence or absence of meloxicam, a selective COX2 inhibitor (10 μM) followed by treatment for an additional 60 min with testosterone (1 μM), testosterone-BSA (1 μM), FSH (100 ng/ml) or BSA (34 nM). (B) Sertoli cells were pre-incubated for 60 min in the presence or absence of BADGE, a PPARγ nuclear receptor antagonist (1 μM) followed by an additional 60 min treatment with $PGF_{2\alpha}$ (1 μM), 15d-$\Delta^{12,14}PGJ_2$ (0.1 and 1 μM) or FSH (100 ng/ml). Cells were subsequently incubated for 30 min with 0.5 μCi/ml [2,6-^{3}H]-2-DOG, a non-metabolizable glucose analogue. [^{3}H]-2-DOG uptake was calculated as disintegration per minute (dpm) and normalized to DNA content (μg) in the cell culture. Results are expressed as [^{3}H]-2-DOG uptake percentage (%) relative to the control (basal conditions) which was assigned a value of 100%. Bar plots represent the mean ± SEM ($n = 3$). All groups were compared; different letters denote a statistically significant difference between groups ($p < 0.05$; Student-Newman–Keuls test).

MAPK pathway have also been shown to form complexes with androgen receptors on molecular scaffolds anchored to the plasma membrane [48,58]. Therefore, we analyzed the effect of a membrane-impermeable androgen analogue, testosterone-BSA, on COX2/PGs system in hamster Sertoli cells. Testosterone-BSA mimicked the effect previously observed with testosterone alone on COX2 expression and PG production suggesting that this androgen activates a membrane-associated androgen receptor. In support to our results, immunofluorescence studies, have found that a population of androgen receptors is localized, in a transient manner, to the plasma membrane after testosterone stimulation of rat Sertoli cells [8].

Thus, our results describe a non-classical modulation of COX2 expression and PG synthesis by testosterone in hamster Sertoli cells taking place through androgen receptors associated to the plasma membrane and MAPK 1/2 activation. Non-classical testosterone actions could complement and diversify the already known classical actions of this androgen and contribute to the regulation of spermatogenesis and fertility. Therefore, a concomitant action of testosterone on COX2/PGs system occurring via a classical mechanism cannot be discarded and should be further investigated.

FSH acts synergistically with testosterone to increase spermatogenesis efficiency and fertility [52]. *In situ* hybridization studies have confirmed that Sertoli cells are the only cell type expressing the FSH receptor in the testis; consequently the Sertoli cell is believed to be the only cell population target for a direct FSH regulation [41,56,62,69]. In this study, treatment of Sertoli cells with FSH induced protein COX2 expression as well as $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ production. These results are in agreement with those previously reported by Jannini et al. [34], showing a FSH-stimulated eicosanoid generation through the COX pathway but not the lipoxygenase pathway in immature rat Sertoli cells.

The interaction between FSH and its receptor activates numerous signaling pathways including the MAPK 1/2 pathway (for a review see Walker [89]). Both stimulatory and inhibitory roles for FSH on MAPK 1/2 phosphorylation have been described [11,12,53]. Such discrepancies seem to arise from the different ages of the animals used for Sertoli cell purification. In this study, we observed that FSH induced MAPK 1/2 phosphorylation in regressed adult hamster Sertoli cells. Furthermore, the stimulatory effect of FSH on COX2 expression was abolished in the presence of U0126, a potent and highly selective MEK 1/2 inhibitor, indicating that FSH action on COX2/PGs in hamster Sertoli cells is exerted via MAPK 1/2 activation.

Taking into account the results from this work, we could speculate that Sertoli cells purified from testes of reproductively inactive animals maintain the *in vitro* capability to induce COX2 expression and PG production in response to testosterone and FSH, despite the significant fall in the serum levels of these hormones, and testicular content of FSH receptors observed in regressed hamsters [3,20]. Whether receptors are more sensitive to FSH and/or testosterone or only a few of them are required to obtain a maximal response during the quiescence phase remains to be further investigated.

One of the main functions of Sertoli cells is to provide factors required to fuel germ cell metabolism. As mentioned before, Sertoli cell lactate production is exquisitely regulated by many hormones, cytokines, growth factors and nutrient milieu components [63–65]. Several biochemical mechanisms may contribute to an increase in lactate production and secretion; one of them is cellular uptake of glucose, the main carbon source for lactate synthesis. Facilitated Sertoli cell glucose transport across plasma membrane is mediated by carrier proteins termed glucose transporters (GLUT). So far, the expression of transporters GLUT1, GLUT3 and GLUT8 has been demonstrated in Sertoli cells [6,24,43,87].

In this study, we observed that both FSH and testosterone induce the uptake of [^{3}H]-2-DOG, a non-metabolizable glucose analogue, in hamster Sertoli cells. A FSH-mediated glucose uptake increase has been previously described in 20-day-old rat Sertoli cells [64]. Regarding androgen-mediated effects on glucose metabolism, Sato et al. [74] have described an up-regulation of GLUT4 expression in skeletal muscle cells; this transporter is not present in Sertoli cells.

Glucose uptake in Sertoli cells is also stimulated by certain cytokines and factors [14,23,53–55,64,87]. Taking into account the participation of AA, precursor in PG biosynthesis, in the regulation of Sertoli cell function [53], we evaluated a possible role of certain PGs on glucose uptake in hamster Sertoli cells. The presence of FP ($PGF_{2\alpha}$ receptor), DP (PGD_2 receptor) and PPARγ (the nuclear receptor for PGJ_2 derivatives) was initially detected in adult hamster Sertoli cells by RT-PCR and immunocytochemistry, in agreement with results obtained in immature rat Sertoli cells [10,33,72]. An inhibitory role exerted by 1 μM $PGF_{2\alpha}$ and 0.1–1 μM 15d-$\Delta^{12,14}PGJ_2$ on glucose uptake in regressed adult hamster Sertoli cells has been observed.

Previously, quantification of testicular PGF2α levels in Syrian hamsters allowed us to determine that physiological concentrations of PGF2α range between 100 pM and 1 μM [21]. Furthermore, recent unpublished data from our group suggests that doses from 1 to 10 μM 15d-$\Delta^{12,14}PGJ_2$ can be considered as physiological concentrations in the hamster testis. In addition, the inhibitory effect of 15d-$\Delta^{12,14}PGJ_2$ on glucose uptake was not due to the loss of hamster Sertoli cell viability.

15d-$\Delta^{12,14}PGJ_2$ and other PPAR ligands act classically through the nuclear receptor PPARγ triggering the transcription of target genes [18,42]. In addition to this established mechanism of action, growing evidence points to the existence of a rapid and non-genomic effect of PPAR ligands. It has previously been reported that 15d-$\Delta^{12,14}PGJ_2$ and thiazolidinediones (TZDs) can act within minutes to regulate the activity of certain kinases (jnk, erk, PI3/Akt and the AMP-activated protein kinase) in mammalian tissues [37,38,46,77,78,85]. Nevertheless, it remains unclear whether the non-genomic effects are actually PPARγ-dependent or PPARγ-independent [57,67]. We conclude from our experiments in which BADGE, a specific antagonist for PPARγ was used, that 15d-$\Delta^{12,14}PGJ_2$ action on glucose uptake after 1 h incubation is exerted via this nuclear receptor. In this regard, Saltiel and Olefsky [71] have described the participation of PPARγ in the maintenance of glucose homeostasis in adipose tissue. However, the actual role played by 15d-$\Delta^{12,14}PGJ_2$/PPARγ on Sertoli cell function had not been addressed so far.

Bearing in mind the fact that FSH and testosterone exerted a stimulatory role on glucose uptake by hamster Sertoli cells but $PGF_{2\alpha}$ and 15d-$\Delta^{12,14}PGJ_2$ showed an opposite effect, we decided to investigate this monosaccharide uptake in hamster Sertoli cells pre-incubated with meloxicam and/or BADGE, and subsequently stimulated in the presence of FSH or testosterone. Meloxicam, a selective COX2 inhibitor, further increased glucose uptake in Sertoli cells incubated with FSH or testosterone. A similar result was obtained when Sertoli cells were incubated with FSH and BADGE. Since an anti-androgenic *per se* effect of BADGE has been previously described by Satoh et al. [75], in this study we had to discard the possibility of using BADGE to elucidate a possible role of 15d-$\Delta^{12,14}PGJ_2$ in the regulatory mechanism exerted by testosterone on glucose uptake by hamster Sertoli cells.

Taken together, our results suggest that both hormones FSH and testosterone, modulate glucose entry into hamster Sertoli cells through a positive effect, but also via a negative indirect mechanism exerted by COX2/PGs. In the latter case, FSH and testosterone stimulate COX2 expression and subsequently, 15d-$\Delta^{12,14}PGJ_2$ and

$PGF_{2\alpha}$ production. Then, these PGs lead to a decrease in the monosaccharide uptake.

In summary, our work demonstrates the presence of a COX2/PGs system in hamster Sertoli cells and provides new insights into how FSH and testosterone modulate COX2 expression, PG production and glucose transport in these somatic tubular cells.

This previously unknown FSH-testosterone/COX2/PG system may serve as a local modulator of Sertoli cell activity and, consequently of spermatogenesis efficiency.

Acknowledgments

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