Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

HIF involvement in the regulation of rat Sertoli cell proliferation by FSH

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ARTICLE INFO

Article history:
Received 21 May 2018
Accepted 30 May 2018
Available online xxx

Keywords:
Sertoli cell
Proliferation
HIF
FSH

ABSTRACT

The final number of Sertoli cells reached during the proliferative periods determines sperm production capacity in adulthood. It is well known that FSH increases the rate of proliferation of Sertoli cells; however, little is known about the transcription factors that are activated by the hormone in order to regulate Sertoli cell proliferation. On the other hand, Hypoxia Inducible Factors (HIFs) are master regulators of cell growth. HIFs are dimers of HIF-β and HIF-α subunits. Considering that HIF-β is constitutively expressed, HIF transcriptional activity is regulated through the abundance of HIF-α subunits. To date, three HIF-α isoforms have been described. The association of the different HIF-α subunits with HIF-β subunit constitutes three active transcription factors —HIF-1, HIF-2 and HIF-3— which interact with consensus hypoxia-response elements in the promoter region of target genes. Hypoxia has been classically considered the main stimulus that increases HIF transcriptional activity, however, regulation by hormones under normoxic conditions was also demonstrated. The aim of this work has been to investigate whether HIFs participate in the regulation of rat Sertoli cell proliferation by FSH. Sertoli cells obtained from 8-day old rats were cultured in the absence or presence of FSH. It has been observed that FSH increases HIF transcriptional activity and HIF-2α mRNA levels without modifying either HIF-1α or HIF-3α expression. Incubations with FSH have been also performed in the absence or presence of a pharmacological agent that promotes HIF-α subunit degradation, LW6. It has been observed that LW6 inhibits the FSH effect on proliferation, CCND1 expression and c-Myc transcriptional activity. Altogether, these results suggest that HIFs might be involved in the regulation of Sertoli cell proliferation by FSH.

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1. Introduction

As the unique somatic cells within the seminiferous tubules, Sertoli cells play essential roles in regulating normal spermatogenesis. The number of Sertoli cells in the adult testis determines both testis size and daily sperm production. This relationship occurs because each Sertoli cell has a fixed capacity for the number of germ cells that it can support [1]. In rodents, Sertoli cells proliferate during fetal and neonatal life up to 15 days of age. Around 15–18 days of age the blood-testis barrier is formed and Sertoli cells keep on maturating to attain the ability to sustain full spermatogenesis. In addition to the master role of FSH, Sertoli cell proliferation and maturation are regulated by a number of hormonal cues, which include among others, thyroid hormone, retinoic acid, activin, and testosterone [2]. FSH actions can be demonstrated during fetal and the whole postnatal life. FSH regulates Sertoli cell proliferation during fetal and early postnatal life and its maturation after cessation of mitosis at puberty [3]. The molecular mechanisms underlying FSH action on Sertoli cell proliferation have not been fully elucidated yet. In this respect, there are scarce studies which analyze FSH-activated transcription factors involved in Sertoli cell proliferation. Within these studies, the participation of c-Myc in the regulation of Sertoli cell proliferation by FSH has been

Abbreviations: BrdU, bromo-deoxyuridine; CCN, cyclin; FSH, follicle stimulating hormone; HIF, hypoxia inducible factor; HRE, hypoxia-response element; LW6, 3-[2-(4-adamantan-1-yl-phenoxy)-acetylamino]-4-hydroxy-benzoic acid methyl ester; VHL, von Hippel-Lindau.

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https://doi.org/10.1016/j.bbrc.2018.05.206
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demonstrated [4].

The heterodimeric hypoxia-inducible factors (HIFs) are critical transcriptional regulators of cell physiology. HIFs consist of an α subunit (HIF-1α, HIF-2α or HIF-3α) and a β subunit (HIF-β) [5]. While HIF-β is expressed constitutively, HIF-α levels are regulated by hypoxia and by different hormones under normoxic conditions. Consequently, HIF activity is regulated through the amount of α subunit in the cell. HIF-1α and HIF-2α are closely related and their interaction with HIF-β subunit result in HIF-1 and HIF-2, respectively. Both active transcription factors bind to hypoxia-response elements (HRE) and stimulate target genes such as those related to angiogenesis, energy metabolism, cell growth and cell cycle progression [6]. HIF-3α is the most distantly related subunit in its primary structure and the active transcription factor HIF-3 functional role still has to be elucidated.

Classically HIF-α levels and consequently HIF transcriptional activity have been associated with hypoxic conditions. However, some years ago, regulation by hormones of HIF transcriptional activity under normoxic conditions was demonstrated. Different stimuli such as heregulin, IGF1 and insulin, among others, were shown to elevate HIF-1α protein levels and consequently HIF transcriptional activity in several tissues and cell types [7–9].

In the last decade, the expression of HIF-1α and HIF-2α in Sertoli cells was demonstrated [10–12]. It is worth mentioning that postnatal mouse HIF-2α ablation leads to male infertility, reduced testis size and seminiferous tubule number [10]. Despite the decrease in seminiferous tubules number, only the number of Sertoli cells per tubule was analyzed, while the total number of Sertoli cells per testis was neglected. Considering that the final number of Sertoli cells is the result of the proliferative periods and that FSH regulates the proliferation of Sertoli cells, it would of interest to evaluate whether HIFs are involved in the mechanism of action of the hormone. To our knowledge, no reports are available linking FSH actions, Sertoli cell proliferation and HIF transcriptional activity. Hence, the aim of this study was to analyze the participation of HIFs in the regulation of Sertoli cell proliferation by FSH.

2. Materials and methods

2.1. Materials

3-[2-(4-adamantan-1-yl-phenoxy)-acetylamino]-4-hydroxy-benzoic acid methyl ester (LW6) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Ovine FSH (NIH-oFSH-S-16) was obtained from the National Hormone and Pituitary Program, NIDDK (Bethesda, MD, USA). Tissue culture media and all other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sertolicell isolation and culture

Eight-day-old Sprague-Dawley rats (*Rattus norvegicus*) were obtained from an animal care unit (Animal Care Laboratory, Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina). Animals were killed by CO_2 asphyxiation according to protocols for animal laboratory use following the principles and procedures outlined in the National Institute of Health Guide for Care and Use of Laboratory Animals.

Sertoli cells were isolated as we previously described [13]. The Sertoli cell suspension, collected by sedimentation, was resuspended in culture medium consisted of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium, supplemented with: 10 μg/ml transferrin, 5 μg/ml insulin, 5 μg/ml vitamin E and 4 ng/ml hydrocortisone. Sertoli cells were cultured in either 8-well Lab-Tek Chamber slide, 6- or 24-multiwell plates (15 μg DNA/cm^2) at 34 °C in a mixture of 5% CO_2:95% air.

2.3. Culture conditions

Cells were allowed to attach for 48 h in the presence of insulin, then medium was replaced with fresh medium without insulin. Sertoli cells treated for 24 h with FSH (100 ng/ml) in the absence or presence of LW6 10 μM were used to evaluate bromo-deoxyuridine (BrdU) incorporation. In order to determine HIF-1α, HIF-2α and HIF-3α mRNA levels, cells were cultured for 4 days under basal conditions or pretreated for 2, 4 or 6 h with FSH (100 ng/ml). For cyclin (CCN) D1, CCND2, CCND3 and c-Myc mRNA levels determination, cells were cultured for 4 days under basal conditions or pretreated for 4 h with FSH (100 ng/ml) in the absence or presence of LW6 (10 μM). As for von Hippel-Lindau (VHL) protein levels determination, cells were cultured for 4 days under basal conditions or pretreated for 4 or 24 h with LW6 (10 μM).

2.4. Trypan blue exclusion assay

The monolayers were washed twice with PBS and then cells recovered by trypsinization. Cell suspension was then centrifuged for 5 min at 400 × g and the cell pellet recovered. Cells were resuspended in fresh medium and 0.4% Trypan Blue was added. Cells that excluded the colorant —viable— and those that resulted stained —not viable— were counted in a Neubauer chamber.

2.5. Reverse transcription real-time PCR (RT-qPCR)

RNA was isolated and RT was performed as indicated in Ref. [14] using 200 U M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The cDNAs enconding HIF-1α, HIF-2α, HIF-3α, CCND1-3 and c-Myc were amplified using specific primers (Table 1). qPCR was performed by a Step One Real Time PCR System (Applied Biosystems, Warrington, UK). Amplification was performed as indicated in Ref. [14]. The data were normalized to β-actin (BACT). The comparative ΔΔCt method was used to calculate relative gene expression.

2.6. Western blot

Cells were washed once with PBS at room temperature followed by lysing on ice in 200 μl lysis buffer (Tris 10 mM pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% w/v Triton X100, 100 mM NaF, 10 mM $Na_4P_2O_7$, 10 mM Na_3VO_4 and complete Mini EDTA-free protease inhibitor cocktail tablets -Roche Diagnostics, Mannheim, Germany-) for 30 min. Cell extracts were obtained by centrifugation at 16000 × g for 30 min at 4 °C. Samples were treated and run on a 10% SDS-PAGE (10% acrylamide/bisacrylamide for the resolving gel and 4.3% acrylamide/bisacrylamide for the stacking gel) as we previously described [15]. Membranes were incubated with Purified Mouse anti-VHL (556347BD Pharmingen, San Diego, CA, USA) and anti-β-Tubulin (T8328 Sigma-Aldrich). Band intensities were estimated by densitometric scanning using NIH Image software (Scion Corporation, Frederick, MD, USA).

2.7. Transfection and luciferase reporter assay

Cells were plated in 24-multiwell plate and 48 h later transfected with the plasmids pGL3PGK6TKpLUC [16], pGL3-E-box [17] or PGL3-Basic (Promega Corporation, Madison, USA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The plasmid pMIR-REPORT™ Beta-galactosidase Reporter Control Vector(Applied Biosystems, Warrington, UK)expressing the LacZ gene was used for normalization. 24 h after transfection, the medium was replaced and cells were stimulated with FSH (100 ng/ml) for 24 h in the absence or

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Table 1
Rat-specific primers for RT-qPCR.

Gene	Primer Sequence	Product Size (bp)	Accession Number
HIF-1α	Fwd:5′-TCAAGTCAGCAACGTGGAAG-3′ Rev:5′-ACGTCATAGGCGGTTTCTTG-3′	105	NM_024359.1
HIF-2α	Fwd:5′-GGCCAAACATGGAGGATATG-3′ Rev:5′-AGACCACCACGTCATTCTTCTC-3′	134	NM_023090.1
HIF-3α	Fwd:5′-TATCATCTGCGTCCACTTCC-3′ Rev:5′-CGAGTATGTTGCTCCGTTTG-3′	84	NM_022528.2
CCND1	Fwd:5′-CTACCGCACAACGCACTTTC-3′ Rev:5′-AAGGGCTTCAATCTGTTCCTG-3′	85	NM_171992.4
CCND2	Fwd:5′-CTGACCAAGATCACCCACAC-3′ Rev:5′-CTCTTGACGGAACTGCTGAAG-3′	99	NM_022267.1
CCND3	Fwd:5′-GAAACCACACCCCTGACTATTG-3′ Rev:5′-AGGTCCCACTTGAGCTTCC-3′	113	NM_012766.1
c-Myc	Fwd:5′-CACCAGCAGCGACTCTGAA-3′ Rev:5′-GACCCTGACTCGGACCTCTT-3′	102	NM_012603.2
BACT	Fwd:5′-TGGCACCACACTTTCTACAAT-3′ Rev:5′-GGTACGACCAGAGGCATACA-3′	189	NM_031144.3

HIF-α: hypoxia inducible factor alpha subunit; *CCN*: cyclin; *BACT*: β-actin; Fwd: forward; Rev: reverse; bp: base pairs.

presence of LW6 (10 μM). Finalized the incubation, cells were washed with PBS and total lysates prepared using passive lysis buffer. Samples were processed with the Dual-Luciferase Reporter system (Promega Corporation) and luciferase activity measured using Junior LB9509 luminometer (Berthold Technologies, Bad Wildbad, Germany). β-galactosidase activity was determined spectrophotometrically by analyzing the hydrolysis of o-nitrophenyl b-D-galactopyranoside (ONPG). Firefly luciferase activity was normalized to β-galactosidase activity and expressed as arbitrary units.

2.8. Bromo-deoxyuridine (BrdU) incorporation

Sertoli cells were incubated with BrdU (10 μM) for the last 24 h of culture. Subsequently, cells were washed with PBS and fixed with methanol:acetic acid (3:1) for 30 min for immunocitochemistry. DNA denaturation was assessed by incubating the fixed cells with 70% ethanol/0.2 MNaOH for 3 min followed by cold 70% and absolute ethanol (1 min each), thus allowing the plate to dry. In order to hydrate the cells, three rinses with PBS were performed for 3 min. Endogenous peroxidase and nonspecific sites were blocked by incubations with H_2O_2 in 3% methanol and horse serum in PBS for 30 min each. Finally, cells were incubated with monoclonalBrdU antibody (1/200; Dakko, Glostrup, Denmark) in wet chamber at 4 °C overnight. Immunoperoxidase staining was performed according to the manufacturer's recommended protocol for universal labeled streptavidin biotin/system horseradish peroxidase (VECTASTAIN® Elite ABC HRP Kit, Vector Laboratories, Burlingame, CA, USA). Nuclei were counterstained with hematoxylin, and over 1000 cells were counted per group. The percentage of proliferating (BrdU-positive) Sertoli cells was calculated as (BrdU-positive cells/total Sertoli cells) x 100.

2.9. Other assays

DNA was determined by the method of Labarca and Paigen [18].

2.10. Statistical analysis

All experiments were run in triplicates and repeated three times. One-way ANOVA followed by Tukey-Kramer test using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA, USA) was performed. Probabilities<0.05 were considered statistically significant.

3. Results and discussion

3.1. FSH upregulates HIF-2α expression in Sertoli cells able to proliferate

As Hypoxia Inducible Factors (HIFs) markedly contribute to providing signals for proliferation and differentiation and HIF-1α and HIF-2α are expressed in Sertoli cells [10–12], we decided to evaluate whether both expression and transcriptional activity of HIFs were modulated by FSH. In order to analyze a possible regulation of HIFs expression by FSH, cultured Sertoli cells isolated from 8-day-old rats were incubated for 2, 4 or 6 h in the absence or presence of 100 ng/ml FSH and HIF-α subunit mRNA levels were determined. Fig. 1 shows that FSH increased HIF-2α (B) while it did not modify either HIF-1α (A) or HIF-3α (C) mRNA levels. In order to evaluate if FSH upregulation of HIF-2α mRNA levels was accompanied by a higher HIF transcriptional activity, Sertoli cell cultures previously transfected with a reporter plasmid which contains six copies of HRE upstream of the luciferase gene (pGL3PGK6TKpLUC) were incubated for 24 h in the absence or presence of FSH and thereafter luciferase activity was evaluated. Fig. 1D shows that FSH augmented HIF transcriptional activity in Sertoli cells. Based on this set of results, it is tempting to speculate that the increase in HIF transcriptional activity produced by FSH might be mediated by the increase of HIF-2α expression.

Generally, the rise in HIF transcriptional activity by hypoxia is always accounted for by an increase in the levels of the two major isoforms of HIF-α, HIF-1α and HIF-2α. However, it has been shown that HIF-1α and HIF-2α expressions are differentially regulated by hormones in normoxia. For example, insulin only increases HIF-1α in adipocytes [19] while IGF1 augments HIF-2α but not HIF-1α subunit in osteoblasts [20]. Noticeably, we have recently demonstrated that FSH upregulates the expression of HIF-1α and HIF-2α in Sertoli cells obtained from 20-day-old rats [21], a life-period in which rat Sertoli cells are under terminal maturation. Moreover, these cells have lost the proliferative ability and have established blood-testis barrier. On the other hand, the present investigation shows that in Sertoli cells obtained from 8-day-old rats, a life-period in which Sertoli cells are actively proliferating, FSH upregulates only HIF-2α levels. Curiously, it has been shown that while HIF-2α promotes tumor growth in renal clear cell carcinoma xenografts [22]; overexpression of stable HIF-1α in this model inhibits it [23]. The latter findings led the authors of these studies to speculate that the different HIF-α subunits may have in fact opposite effects on the regulation of cell proliferation. Our results

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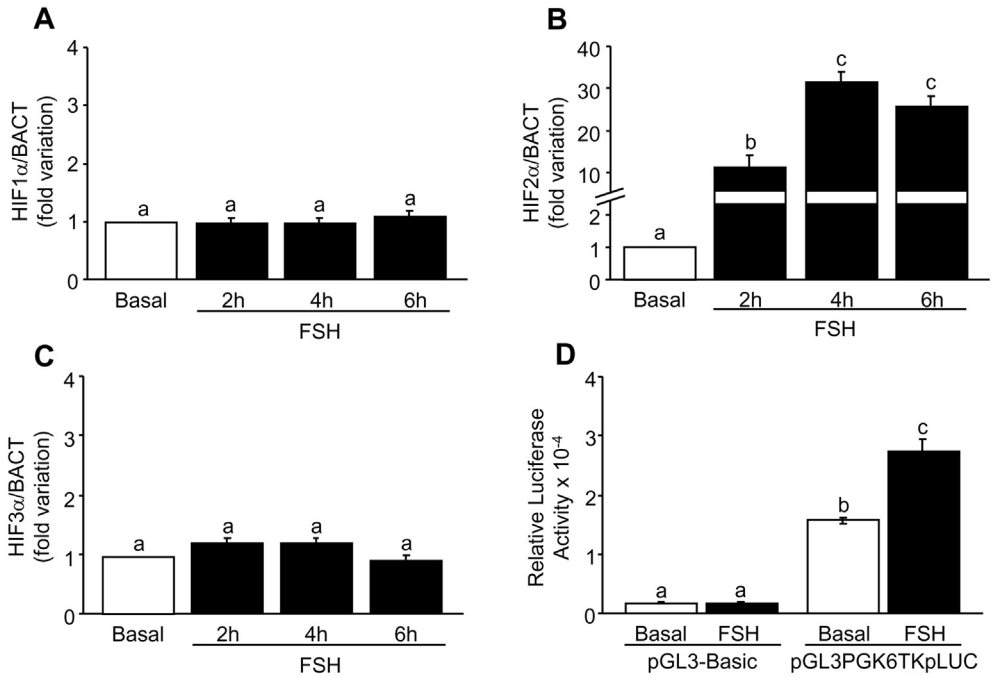

Fig. 1. Effect of FSH on HIF-1α, HIF-2α and HIF-3α mRNA levels and HIF transcriptional activity.Sertoli cells were treated with FSH (100 ng/ml) for 2, 4 or 6 h. RT-qPCR was performed using HIF-1α **(A)**, HIF-2α **(B)**, HIF-3α **(C)** and BACT primers. Relative gene expression is shown as fold-variation relative to Basal (means ± S.D., n = 3, different letters indicate statistical significance, $P < 0.05$). **(D)** Sertoli cells transiently transfected with pGL3PGK6TKpLUC/pMIR-REPORT™ Beta-galactosidase Reporter Control Vector or pGL3-Basic/pMIR-REPORT™ Beta-galactosidase Reporter Control Vector were treated with FSH (100 ng/ml) for 24 h. Results are expressed as Relative Light Units for Firefly luciferase activity normalized to β-galactosidase activity. Data represent the means ± S.D. of triplicate incubations in one representative experiment out of three (different letters indicate statistical significance, $P < 0.05$).

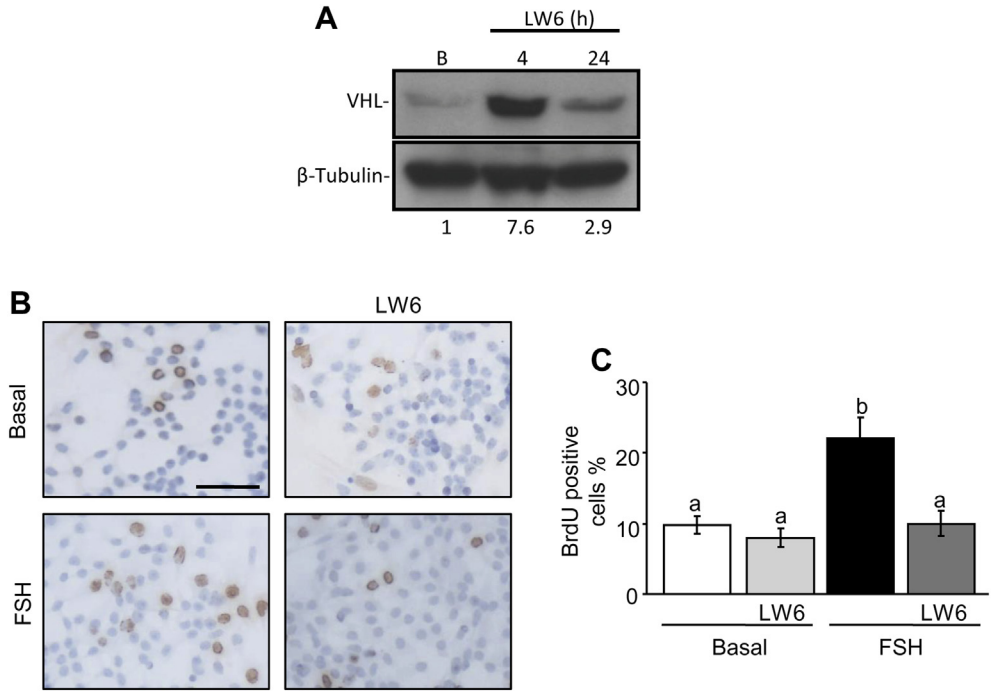

Fig. 2. Effect of LW6 on FSH-stimulated bromo-deoxyuridine (BrdU) incorporation.
(A) Sertoli cells were treated with 10 μM LW6 for 4 or 24 h. Western blot analysis was performed using anti-VHL and anti-β-Tubulin antibodies. The figure shows an autoradiography representative out of three independent experiments and the numbers at the bottom of each lane indicate the fold variation (VHL/β-Tubulin) relative to Basal (B).
(B) Sertoli cell monolayers were incubated under basal conditions or stimulated for 24 h with FSH (100 ng/ml) in the absence or presence of LW6 (10 μM). Incorporated BrdU was detected immunochemically using an anti-BrdU antibody by microscopy. Representative images of Sertoli cells showing immunoreactivity for BrdU (brown) counterstained with hematoxylin (blue). Scale bar: 50 μm.
(C) Each column represents the percentage of BrdU-positive cells (1000 cells/group). Results are presented as mean ± SD of triplicate incubations in one representative experiment out of three (different letters indicate statistical significance, $P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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are consistent with the latter concept as FSH, distinctly to the regulation exerted in terminal maturing rat Sertoli cells, upregulates only HIF-2α levels in proliferating Sertoli cells.

3.2. HIFs are involved in the regulation of Sertoli cell proliferation by FSH

The next set of experiments were designed to analyze whether HIF activity was indeed necessary for the regulation of Sertoli cell proliferation by FSH. A pharmacological agent that promotes the degradation of HIF-α subunits —LW6— was used for this purpose. First of all, the effect of LW6 on cell viability and on the expression levels of von Hippel-Lindau protein, which is involved in the ubiquitination and degradation of HIF-α subunits [24], was tested. LW6 (10 μM) did not modify Sertoli cell viability in 24-h incubations as evaluated by a Trypan blue exclusion test (Basal: 26 ± 4; LW6: 28 ± 5, number of Trypan Blue stained cells x 10^{-3}/well). On the other hand and as expected, LW6 increased VHL protein (Fig. 2A). Next, the effect of LW6 on the proliferative action of FSH was evaluated. Fig. 2B and C shows that LW6 suppressed FSH-stimulated BrdU incorporation. LW6 promotes the degradation of all HIF-α isoforms through the increase in VHL expression [25]. However, considering that FSH only increases HIF-2α levels, it is reasonable to think that our results reflect the participation of HIF-2 in FSH-regulated Sertoli cell proliferation. It is worth mentioning that postnatal HIF-2α ablation leads to male infertility, which is accompanied by reduced testis size and weight [10]. The authors pointed out that the observed effects were caused by the incapacity of Sertoli cells to establish the blood-testis barrier. However, histological analysis of HIF-$2\alpha^{\Delta/\Delta}$ mice testes cross-sections revealed a reduction in seminiferous tubule number per testis, which might indicate a decrease in Sertoli cell and germ cell numbers. Altogether, these latter and the results presented herein highlight the relevance of HIF-2 in the regulation of Sertoli cell physiology.

3.3. FSH increases cyclin (CCN) D1 expression and c-Myc transcriptional activity in a HIF dependent manner

In the last set of experiments the participation of HIFs in FSH regulation of cyclins (CCN) D and c-Myc expression was evaluated. CCND and the transcription factor c-Myc are associated with G1/S cell cycle transition and it is well known that FSH mitogenic effect triggers upregulation of CCND1 and c-Myc enabling the progression of cell cycle in immature Sertoli cells [4,26]. Fig. 3 shows that FSH increased CCND1 (A) and CCND2 (B) without modifying CCND3 (C) mRNA levels and that LW6 impaired FSH effect on CCND1 expression. The latter results suggest that FSH induces CCND1 in a HIF-dependent manner.

It has been well documented that there are two functional HRE sequences in the CCND1 promoter [27]. Despite close similarities between the HIF-α isoforms, it has been shown that activation of HIF-1 or HIF-2 has opposing effects on CCND1 gene expression. In this respect, while the association of HIF-2 with CCND1 gene promoter leads to gene induction [23], the association of HIF-1 leads to repression of this gene [27]. The latter observations and our results lead us to postulate that HIF-2, but not HIF-1, may be the transcription factor utilized by FSH to augment CCND1 expression in proliferating Sertoli cells.

Finally, and as for c-Myc expression, Fig. 4A shows that LW6 did not modify the c-Myc mRNA levels stimulated by FSH. This result is consistent with the idea that FSH induces c-Myc in a HIF-independent manner.

The regulation of c-Myc by HIFs has been intensively investigated. In addition to the well-known actions of HIF as transcription

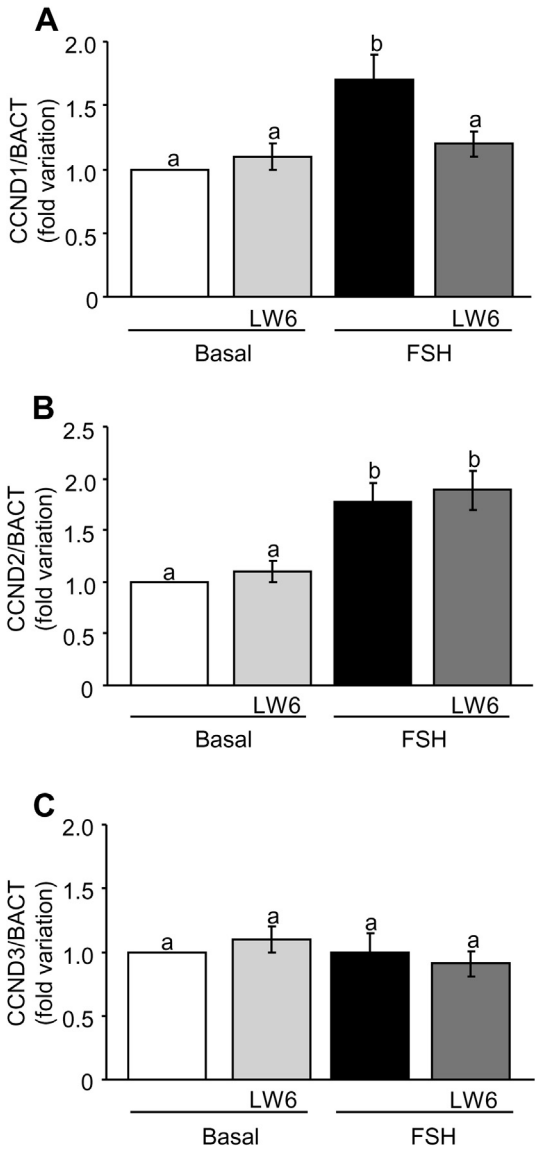

Fig. 3. Effect of LW6 on FSH-stimulated CCND1 and CCND2 mRNA levels. Sertoli cells were maintained under basal conditions or stimulated with FSH (100 ng/ml) for 4 h in the absence or presence of LW6 (10 μM). RT-qPCR was performed using CCND1 **(A)**, CCND2 **(B)**, CCND3 **(C)** and BACT primers. Relative gene expression is shown as fold-variation relative to Basal (different letters indicate statistical significance, $P < 0.05$).

factor, Gordan et al. [28] reported that HIF-α subunits exert transcriptional-independent roles. While HIF-2α enhances c-Myc transcriptional activity by stabilizing the complex c-Myc/Max in renal carcinoma cells, NTH3T3 cells, HEK293 cells, and embryonic epithelial cells, HIF-1α competes with c-Myc for available cellular Max. In this way, HIF-1α inhibits the ability of c-Myc to interact with E-box sequences in the DNA. On the other hand, HIF-1, but not HIF-2, induces Mxi1—c-Myc antagonist— leading to cell cycle arrest at G1/S phase [29,30]. Considering that c-Myc transcriptional activity does not only depend on c-Myc expression levels, we decided to evaluate c-Myc transcriptional activity under conditions of increased HIF-α subunit degradation like those produced by LW6. Sertoli cell cultures previously transfected with a reporter plasmid which contains four E-box elements —c-Myc recognition sites in the target gene promoters [31]— upstream of the luciferase gene (pGL3-E-box) were incubated for 24 h under basal conditions or stimulated with FSH (100 ng/ml) in the absence or presence of

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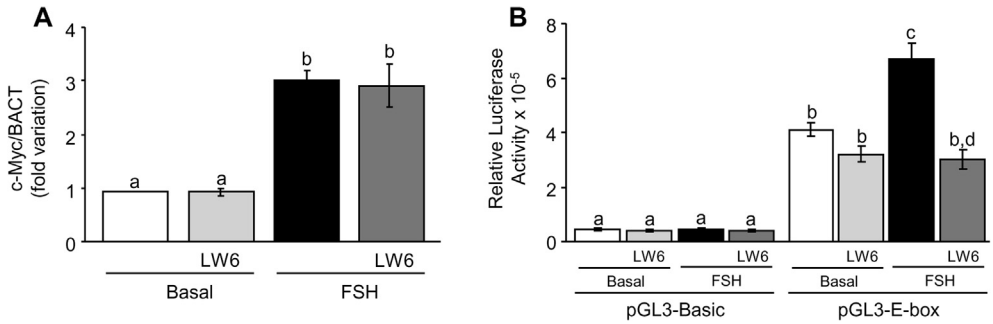

Fig. 4. Effect of LW6 on FSH-stimulated c-Myc mRNA levels and c-Myc transcriptional activity.
(A) Sertoli cells were maintained under basal conditions or stimulated with FSH (100 ng/ml) for 4 h in the absence or presence of LW6 (10 μM). RT-qPCR was performed using c-Myc and BACT primers. Relative gene expression is shown as fold-variation relative to Basal (means ± S.D., n = 3, different letters indicate statistical significance, $P < 0.05$).
(B) Sertoli cells transiently transfected with pGL3-E-box/pMIR-REPORT™ Beta-galactosidase Reporter Control Vector or pGL3-Basic/pMIR-REPORT™ Beta-galactosidase Reporter Control Vector were maintained under basal conditions or stimulated with FSH (100 ng/ml) for 24 h in the absence or presence of LW6 (10 μM). Results are expressed as Relative Light Units for Firefly luciferase activity normalized to β-galactosidase activity. Data represent the means ± S.D. of triplicate incubations in one representative experiment out of three (different letters indicate statistical significance, $P < 0.05$).

LW6 (10 μM). Fig. 4B shows that the effect of FSH on luciferase activity was blocked by LW6. This latter observation indicates that while HIF does not participate in c-Myc expression it does so in transcriptional activity stimulated by FSH.

Altogether, the results of the present investigation suggest an important role of HIFs in the mechanism of action utilized by FSH to regulate Sertoli cell proliferation. The participation of other transcriptional factors regulated by FSH on Sertoli cell proliferation cannot be ruled out. This research has intended to shed light on the mechanisms triggered by FSH that converge in the regulation of cell cycle progression in immature Sertoli cells.

Conflicts of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Acknowledgements

To Dr. Ratcliffe (Oxford, UK) and Dr. Berg (Leipzig, Germany) for providing pGL3PGK6TKpLUC and pGL3-E-box plasmids, respectively.

This work was supported by National Agency of Scientific and Technological Promotion (PICT2014/0945) and National Research Council (PIP112-201501-00127).

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Please cite this article in press as: A. Gorga, et al., HIF involvement in the regulation of rat Sertoli cell proliferation by FSH, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.206