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FSH and bFGF regulate the expression of genes involved in Sertoli cell energetic metabolism

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ABSTRACT

The purpose of this study was to investigate if FSH and bFGF regulate fatty acid (FA) metabolism and mitochondrial biogenesis in Sertoli cells (SC). SC cultures obtained from 20-day-old rats were incubated with 100 ng/ml FSH or 30 ng/ml bFGF for 6, 12, 24 and 48 h. The expression of genes involved in transport and metabolism of FA such as: fatty acid transporter CD36 (*FAT/CD36*), carnitine-palmitoyltransferase 1 (*CPT1*), long- and medium-chain 3-hydroxyacyl-CoA dehydrogenases (*LCAD*, *MCAD*), and of genes involved in mitochondrial biogenesis such as: nuclear respiratory factors 1 and 2 (NRF1, NRF2) and transcription factor A (*Tfam*), was analyzed. FSH stimulated *FAT/CD36*, *CPT1*, *MCAD*, *NRF1*, *NRF2* and *Tfam* mRNA levels while bFGF only stimulated *CPT1* expression. A possible participation of PPARβ/δ activation in the regulation of gene expression and lactate production was then evaluated. SC cultures were incubated with FSH or bFGF in the presence of the PPARβ/δ antagonist GSK3787 (GSK; 20 μM). bFGF stimulation of *CPT1* expression and lactate production were inhibited by GSK. On the other hand, FSH effects were not inhibited by GSK indicating that FSH regulates the expression of genes involved in FA transport and metabolism and in mitochondrial biogenesis, independently of PPARβ/δ activation. FA oxidation and mitochondrial biogenesis as well as lactate production are essential for the energetic metabolism of the seminiferous tubule. The fact that these processes are regulated by hormones in a different way reflects the multifarious regulation of molecular mechanisms involved in Sertoli cell function.

1. Introduction

The process of spermatogenesis and consequently male fertility is dependent upon the somatic cells that are present in the testis. Leydig cells are essential because of androgen production, and Sertoli cells are absolutely necessary in order to provide an adequate and protected environment within the seminiferous tubules. Sertoli cell carbohydrate metabolism presents some interesting characteristics. Glucose is metabolized to lactate since germ cells situated beyond the blood testis barrier rely on Sertoli cell production of this hydroxyacid to obtain energy (Boussouar and Benahmed, 2004). Hence, glucose is not an essential source of energy for Sertoli cells; in fact it has been shown that this cell type can survive in culture for at least 48 h in the absence of glucose (Riera et al., 2009). In this metabolic context, it has been shown that the oxidation of fatty acids (FA) can yield much of the energy required by Sertoli cells (Jutte et al., 1985).

Needless to say, FA must enter into the cell in order to be metabolized. One of the proteins involved in FA uptake is *FAT/CD36*, an integral membrane glycoprotein which has been found in a wide variety of cells (Abumrad et al., 1993; Coburn et al., 2000; Bonen et al., 2004). Once FA are incorporated into the cells, they are activated by covalently linking to coenzyme A forming an acyl-CoA derivative. Thereafter, the carnitine-palmitoyltransferase 1 (*CPT1*) is responsible for the entrance of acyl-CoA into the mitochondria where β-oxidation takes place (Rasmussen and Wolfe, 1999). β-oxidation involves the stepwise removal of acetyl-CoA molecules from the shrinking FA chain. The first step is the α-β-dehydrogenation of the acyl-CoA by a family of specific chain length acyl-CoA dehydrogenases (Ghisla and Thorpe, 2004). This family includes, among others, long chain (*LCAD*) and medium chain (*MCAD*) dehydrogenases. The molecular events necessary for FA oxidation are strictly controlled and their regulation varies within different tissues (McGarry and Foster, 1980; Lopaschuk et al., 1994; Rasmussen and Wolfe, 1999). Additionally, an association between FA oxidation and regulation of mitochondrial biogenesis in various tissues has been observed

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(Deepa et al., 2013; O'Neill et al., 2013; Santillo et al., 2013). To this respect, it has been shown that regulation of mitochondrial biogenesis is a crucial mechanism for cellular adaptation in response to hormonal environment and energy deprivation. For instance, induction of mitochondrial biogenesis can be observed in skeletal muscle in response to exercise (Joseph et al., 2006), in brown adipose tissue in adaptative thermogenesis (Butow and Bahassi, 1999) and in white adipose tissue during differentiation (Wilson-Fritch et al., 2003). In Sertoli cells, a cell type that utilizes FA as the main source of energy, it is reasonable to assume that the genes involved in mitochondrial biogenesis and those involved in FA oxidation may be somehow associated and regulated by hormones.

It is well known that Sertoli cells are under the control of follicle-stimulating hormone (FSH) and a plethora of locally produced factors (Gnessi et al., 1997). Basic fibroblast growth factor (bFGF), which belongs to the family of locally produced peptides, regulates several biological processes in a wide range of tissues and organs including the testis (Han et al., 1993). We have previously observed that FSH and bFGF regulate several mechanisms involved in lactate production in Sertoli cells (Meroni et al., 2002; Riera et al., 2002, 2003). However, the possible participation of these hormones in the regulation of FA metabolism and in mitochondrial biogenesis in Sertoli cells has not been analyzed yet.

Recently, we have observed that pharmacological PPARα and PPARβ/δ activation regulates the expression of genes involved in FA metabolism such as *FAT/CD36*, *CPT1*, *LCAD* and *MCAD* in Sertoli cells. We have also observed that PPARβ/δ activation can simultaneously regulate the expression of the above-mentioned genes and lactate production. These results were interpreted as a reflection of a coordinated mechanism which will ensure the concomitant provision of energy to Sertoli and germ cells (Regueira et al., 2014). The mechanisms involved in a possible activation of this nuclear receptor under physiological conditions, which may include hormonal regulation, have not been analyzed yet in Sertoli cells.

In the present study, we investigated whether FSH and bFGF are able to regulate molecular mechanisms involved in FA metabolism and in mitochondrial biogenesis. In addition, in the case that FA metabolism and mitochondrial biogenesis are regulated by FSH and bFGF, if PPARβ/δ activation has any role in this hormonal regulation of Sertoli cell energetic metabolism.

2. Materials and methods

2.1. Materials

Human recombinant bFGF were purchased from Invitrogen (Life Technologies, Rockville, MD). Ovine FSH (NIH-oFSH-S-16) was obtained from the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD. GSK and all other drugs and reagents were purchased from Sigma–Aldrich (St Louis, MO, USA).

2.2. Sertoli cell isolation and culture

Sertoli cells from 20-day-old Sprague–Dawley rats were isolated as previously described (Meroni et al., 2002). Briefly, decapsulated testes were digested with 0.1% collagenase and 0.006% soybean 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 consisted of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's 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 25 cm^2 flasks, 6- or 24-multiwell plates (5 μg DNA/cm^2) at 34 °C in a mixture of 5% CO_2:95% air.

No myoid cell contamination was revealed in the cultures when an immunoperoxidase technique was applied to Sertoli cell cultures using a specific antiserum to smooth muscle α actin. Remaining cell contaminants were of germ cell origin and this contamination was below 5% after 48 h in culture as examined by phase contrast microscopy.

2.3. Culture conditions

Sertoli cells were allowed to attach for 48 h in the presence of insulin and medium was replaced at this time with fresh medium without insulin. Stimulation with FSH (100 ng/ml) or bFGF (30 ng/ml) was performed on day 3. Cells incubated for 6, 12, 24 or 48 h with FSH or bFGF in the absence or presence of 20 μM GSK3787 (GSK) harvested on day 5 were used to evaluate *Tfam*, *NRF1*, *NRF2*, *FAT/CD36*, *CPT1*, *LCAD*, *MCAD* mRNA levels and CPT1 protein levels. Cells treated for 24 or 48 h with FSH or bFGF in the absence or presence of GSK were used to perform fatty acid oxidation assay. The 48-h conditioned media obtained in the above-mentioned incubations were used to determine lactate production.

2.4. Real-time PCR (RT-qPCR)

Total RNA was isolated from Sertoli cells cultured in 6-multiwell plates with TRI Reagent (Sigma–Aldrich). The amount of RNA was estimated by spectrophotometry at 260 nm. Reverse transcription was performed on 2 μg RNA at 42 °C for 50 min with a mixture containing 200 U SuperScript II reverse transcriptase enzyme, 125 ng random primers and 0.5 mM dNTP Mix (Invitrogen).

RT-qPCR was performed by a Step One Real Time PCR System (Applied Biosystems, Warrington, UK). The specific primers for real-time PCR were: 5′-ACCAGGCCACATAGAAAGCA-3′ and 5′-CACCAATAACGGCTCCAGTAA-3′ for *FAT/CD36*; 5′-AAAGGTCTGGGAGTGATTGG-3′ and 5′-CCATTCTCCACCAAAAAGAGG-3′ for *LCAD*; 5′-CGAGCACAACACACAAAACC-3′ and 5′-TTCCTCTCTGGCAAACTTCC-3′ for *MCAD*; 5′-GGCAGAAACGCCTAAAGAAG-3′ and 5′-CCGAGGTCTTTTTGGTTTTC-3′ for *Tfam*; 5′-GCTCATCCAGGTTGGTACTG-3′ and 5′-TTTGTTCCACCTCTCCATCAG-3′ for *NRF1*; 5′-GGCAGAGACATTCCCATTTG-3′ and 5′-GATCAGGGGTGGTGAAGACT-3′ for *NRF2*; 5′-AGTTCTTTGCTGACCTGCTG-3′ and 5′-TTTATGTCCCCCGTTGACTG-3′ for *HPRT1*. Amplification was carried out as recommended by the manufacturer: 25 μl reaction mixture containing 12.5 μl of SYBR Green PCR Master mix (Applied Biosystems), the appropriate primer concentration and 1 μl of cDNA. The relative cDNA concentrations were established by a standard curve using sequential dilutions of a cDNA sample. The data were normalized to *HPRT1*. The amplification program included the initial denaturation step at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Fluorescence was measured at the end of each extension step. After amplification, melting curves were acquired and used to determine the specificity of PCR products. The comparative $\Delta\Delta$Ct method was used to calculate relative gene expression.

2.5. Northern blot analysis

Total RNA was isolated from Sertoli cells cultured in 25 cm^2 tissue culture flasks using the TRI Reagent (Sigma–Aldrich). The amount of RNA was estimated by spectrophotometry at 260 nm.

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