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Relaxin and Sertoli cell proliferation

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Summary

Immature Sertoli cells proliferate and several factors affect their number, including the follicle stimulating hormone (FSH), testosterone, estradiol and several paracrine growth factors. Using a primary culture of Sertoli cells isolated from 15-day old Wistar rats we have shown that relaxin stimulates Sertoli cell proliferation through the activation of MEK/ERK1/2 and PI3K/AKT pathways. In contrast, FSH inhibited both ERK1/2 and AKT phosphorylation. Furthermore, FSH strongly increased cAMP production, whereas relaxin inhibited basal cAMP production. Our results indicate that in rat Sertoli cells from 15-day old rats relaxin and FSH affect the same signaling pathways in opposite directions. Interplay between both hormones may be important to control the proliferation and differentiation of Sertoli cells.

Key words

Relaxin, FSH, Sertoli cells, cell proliferation.

Sertoli cells are an important source of hormones, nutrients and growth factors essential for normal spermatogenesis. Immature Sertoli cells proliferate, and their number is important to determine the number of germ cells in the adult life (Sharpe et al., 2003). Our laboratory works with the hypothesis that relaxin may play an autocrine/paracrine role in the testis, based on two main evidences: 1) Relaxin knockout mice show an arrest of testicular growth and infertility (Samuel et al., 2003); 2) Relaxin and RXFP1 are expressed in the testis (Filonzi et al., 2007; Cardoso et al., 2010).

Relaxin mRNA levels are about 7 times higher in the testis of prepubertal 15-day old compared to adult 120-day old rats (Cardoso et al., 2010), suggesting that relaxin is especially important in immature animals. In the testis of 15-day old rats relaxin precursor is mainly found in Sertoli cells (Cardoso et al., 2010). We therefore designed studies to investigate the role of relaxin using a primary culture of Sertoli cells (Lucas et al., 2008) isolated from the testis of prepubertal 15-day old rats. The presence of the relaxin precursor and the relaxin receptor RXFP1 in the cultured Sertoli cells was confirmed by confocal microscopy. Relaxin increased the incorporation of ^{3}H-thymidine, which indicates that it stimulates cell proliferation (Cardoso et al., 2010; Nascimento et al., 2012). The relaxin-mediated effect on cell proliferation was inhibited by inhibitors of the MEK/ERK1/2 and PI3K/AKT pathways (Nascimento et al., 2012).

Considering that FSH is a major regulator of Sertoli cell proliferation, we investigated the effect of relaxin and FSH, isolated or combined, on the incorporation of

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^{3}H-thymidine , activation of cAMP production, and ERK1/2 and AKT phosphorylation. We did not detect an additive or synergistic effect between relaxin and FSH on ^{3}H-thymidine incorporation (Cardoso et al., 2010). Whereas FSH strongly increased cAMP production, short periods (5 to 20 minutes) of exposure to relaxin did not affect cAMP production, and longer periods of exposure (30 min) inhibited cAMP production. Whereas FSH progressively inhibited basal and relaxin-induced activation of ERK1/2 phosphorylation, with a peak after 30 min, relaxin induced a rapid and transient increase of ERK12 phosphorylation, with a peak after 5 min (Nascimento et al., 2012). Finally, relaxin stimulated AKT phosphorylation whereas FSH inhibited the basal and the relaxin-induced AKT phosphorylation.

FSH does not affect cAMP production (Bhattacharya et al., 2012) and increases ERK1/2 phosphorylation (Crépieux et al., 2001) in Sertoli cells from 5 day old rats. Therefore, the activation of these signaling pathways by FSH is dependent on the developmental stage of Sertoli cells. Sertoli cells from 15-day old rats are about to cease proliferation and start differentiation. At this particular stage relaxin stimulates while FSH inhibits signaling pathways that favor cell proliferation. We propose that the interplay between relaxin and FSH signalling in Sertoli cells may be important to determine the end of cell proliferation and the beginning of cell differentiation (Figure 1).

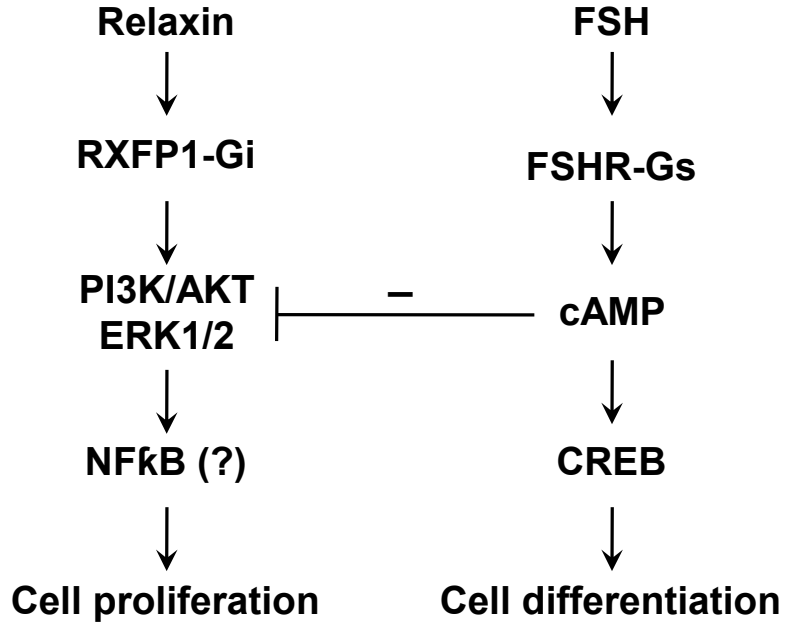

Figure 1. A model of the possible interplay between FSH and relaxin to regulate Sertoli cell proliferation and differentiation. RXFP1, relaxin family peptide receptor; FSHR, FSH receptor. CREB, cAMP response element-binding protein; NFκB, nuclear factor-kappaB. The involvement of NFκB on relaxin effect in Sertoli cells still needs to be confirmed.

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