INTRODUCTION

It has long been recognized that androgens play an important role in the regulation of ovarian function. Outside of their use as substrate for estradiol production, it has been demonstrated that androgens themselves synergize with follicle stimulating hormone (FSH) to increase estradiol and progesterone production within granulosa cells (1, 2). Accordingly, granulosa cells express high levels of androgen receptor (AR). Recently, AR knockout female mice and granulosa cell specific AR knockouts were shown to be sterile, have impaired folliculogenesis, and develop premature ovarian failure (3-5). These findings establish that androgens are important players in the regulation of female fertility, particularly in granulosa cell function.

In this report we examined the effect of androgens on the expression of two enzymes essential for follicle growth and ovulation: Aromatase (CYP19) and P450scc (Figure 1). Aromatase catalyzes the synthesis of estrogen whereas P450scc is crucial for the production of progesterone. Aromatase and P450scc expression is augmented by FSH in granulosa cells as part of the differentiation program induced by this hormone. FSH action within granulosa cells is potentiated by androgens. However, it is not clear whether androgens have any effect on the expression of these enzymes in the absence of FSH. Moreover, the molecular link between FSH signaling and the cascade of events triggered by AR activation remains largely unknown. To investigate the mechanism of action that androgens have within the ovary, undifferentiated granulosa cells were cultured in serum free medium in the presence or absence of androgens. The effects of these treatments on aromatase and P450scc were investigated. Our results demonstrate for the first time that androgens have a direct effect on the expression of genes involved in the differentiation of granulosa cells.

RESULTS

Figure 1: Testosterone (T) stimulates aromatase and P450scc expression in granulosa cells. A & B: qPCR; C: Western Blot; D: Northern Blot

Figure 2: Testosterone specifically stimulates aromatase and P450scc but not SF-1. Similarly, testosterone synergizes with FSH only on the induction of aromatase and P450scc

Figure 3: Testosterone (T) and androstenedione (A2) are significantly more effective than estradiol (E2) and dihydrotestosterone (DHT) in the stimulation of aromatase and P450scc

Figure 4: Androgen receptor antagonists, Hydroxyflutamide (HOF) and Bicalutamide (CDX) as well as androgen receptor knockout (shAR) prevent testosterone stimulation of aromatase and P450scc. Fulvestrant (ICI) an estrogen receptor antagonist had no effect.

Figure 5: A) Testosterone increases aromatase promoter activity and synergizes with FSH on the activation of this promoter. B) Mutation of NRHS and CDS prevented testosterone effects on promoter activity.

Figure 6: Testosterone but not DHT increases LRH-1 mRNA expression and promoter activity. This effect is prevented by the knockdown of androgen receptors.

Figure 7: Knockdown of LRH-1, but not SF-1, prevents aromatase induction by testosterone. Granulosa cells were infected with shRNA against LRH-1 (shLRH-1), SF-1 (shSF-1) or a control shRNA (shLUC).

CONCLUSIONS

➢ Testosterone stimulates aromatase and P450scc independently of FSH.
➢ Testosterone stimulation does not seem to be mediated by its conversion to estradiol or DHT since these compounds are less effective.
➢ The androgen receptor is required for gene stimulation by testosterone.
➢ Testosterone stimulation of aromatase is mediated by the induction of LRH-1 expression.

MATERIALS AND METHODS

Granulosa cell culture: Undifferentiated granulosa cells were obtained from freshly pregnant immature rats treated with 1.5 mg of estradiol for three days. Cells were then cultured in Dulbecco’s modified Eagle’s medium-Ham’s F-12 (50:50) plus 10% FBS and 1% streptomycin and penicillin. After plating, granulosa cells were treated with steroids for 48 or 96 hours.

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