Insulin-Like Growth Factor-I and Transforming Growth Factor-β Regulate the Differentiation of Purified Ovarian Theca-Interstitial Cells in Serum-Free Medium

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Introduction

The ovarian theca-interstitial cells (TIC) are the source of androgens which are essential for follicle estrogen synthesis (1). Regulation of TIC androgen synthesis is critical for normal follicle development and ovulation. Although luteinizing hormone (LH) is the principal regulator of TIC androgen production (1), recent evidence suggests that intraovarian factors may modulate LH action by autocrine and paracrine mechanisms. Two such factors are insulin-like growth factor-I (IGF-I) secreted by granulosa cells (2) and transforming growth factor-β (TGF-β) produced by TIC (3). Although it has been shown that IGF-I increases LH-stimulated TIC androgen synthesis (4,5), the effects of TGF-β on TIC are unknown. The purpose of these studies was to test the effect of TGF-β on LH and IGF-I stimulated TIC androgen synthesis.

Materials and Methods

Four days after hypophysectomy, 25-day-old Sprague-Dawley rats were killed by cervical dislocation and the ovaries dispersed into a cell suspension with collagenase/DNase solution (6). The TIC were purified by centrifugation through 1.055 g/ml Percoll (7) and then cultured (1 x 10^5 viable cells/well) up to 6 days in 96-well microtest plates (4) containing ovine LH (G3-330BR provided by Dr. H. Papkoff), human TGF-β (Collaborative Research) and/or IGF-I (AMGen). The medium, collected every 2 days, was assayed for androsterone (the principal androgen produced by this cell type), androstenedione, testosterone, progesterone and estradiol by specific radioimmunoassays (6). The dose-response curves were analyzed using the Allfit program (8).
Results and Discussion

Recently we developed the first model system in which highly purified and hormone responsive TIC can be cultured in serum-free medium (7). This important advance now permits us to study ovarian TIC without the complications of serum-derived or granulosa cell-secreted growth factors. In these experiments we utilized this model to study the effects of TGF-β and IGF-I on TIC androgen synthesis.

Figure 1 shows that TGF-β caused a dose-related inhibition of LH-stimulated androsterone synthesis. When TIC were treated with a saturating concentration of LH (50 ng/ml), androsterone synthesis was stimulated approximately 200-fold above basal levels. TGF-β alone (0.01-10 ng/ml) caused no change in basal androsterone production (Fig. 1). However, TGF-β caused a dose-related inhibition of LH-stimulated androsterone production which reached 65% at 10 ng/ml of TGF-β. The ED₅₀ (0.1 ± 0.06 ng/ml) for TGF-β inhibition of LH-stimulated androsterone synthesis agreed with the ED₅₀ observed for physiological effects of TGF-β in other tissues (9). This result suggests that the TGF-β inhibition is mediated by a TGF-β receptor on the TIC.

We next examined the time course of TGF-β action. As shown in Figure 2, TGF-β alone (10 ng/ml) had no effect on basal androsterone secretion. LH stimulated androsterone production 200-fold at 2 days, and 130-fold at 4 and 6 days. Treatment of the TIC with TGF-β

![Fig. 1. Effect of TGF-β on TIC androgen synthesis. Purified TIC were cultured in the presence and absence of a saturating concentration of LH (50 ng/ml) with increasing concentrations of TGF-β (0-10 ng/ml) for 2 days. The data are the mean ± SEM of 3 experiments with 4 replicates each.](image-url)