THE EFFECT OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 ON STEROIDOGENESIS OF BOVINE THECAL CELLS

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Story in Brief

To determine if insulin-like growth factor binding protein-3 (IGFBP-3) can directly modulate the hormone-dependent steroidogenesis of thecal cells in vitro, thecal cells from large ($\geq$ 8 mm) follicles were collected from cattle and cultured for 4 d. IGFBP-3 inhibited IGF-I-induced thecal cell progesterone and androstenedione production by 52% and 89%, respectively. In contrast, IGFBP-3 had no effect (P>.10) on LH-induced progesterone or androstenedione production by thecal cells. Higher doses of IGF-I overcame the inhibitory effects of IGFBP-3 on IGF-I-induced progesterone and androstenedione production by thecal cells. IGFBP-3 directly inhibited ^{125}I-IGF-I binding to thecal cells. We conclude that IGFBP-3 may play an important role in regulating thecal cell steroidogenesis during follicular development in cattle.

(Key Words: Insulin-like Growth Factor Binding Proteins, Thecal Cells, Bovine.)

Introduction

The insulin-like growth factor (IGF) system is comprised of IGF-I and -II, IGF receptors, and IGF-binding proteins (IGFBPs) (for review see Spicer and Echternkamp, 1995). Both IGF-I and IGF-II have been shown to be relevant promoters of ovarian follicular function of several species including cattle. In most species, IGF-I stimulates mitogenesis of granulosa and thecal cells in the absence of gonadotropins, whereas in the presence of gonadotropins, IGF-I stimulates steroidogenesis of granulosa and thecal cells. IGFBP-3 and IGFBP-2 are the first and second most prevalent IGFBP, respectively, in ovarian follicular fluid of pigs, cattle and sheep (Spicer and Echternkamp, 1995). Previous studies have not evaluated the effect of IGFBPs on steroidogenesis of thecal cells in domestic animals, although previous studies have shown that IGFBPs can directly inhibit FSH-induced steroid production by rat granulosa cells (Ui et al., 1989; Bicsak et al., 1990; Liu et al., 1993). Therefore, we set out to test the hypothesis that IGFBP-3 can modulate steroidogenesis of bovine thecal cells in vitro.

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Materials and Methods

Ovaries from beef and dairy cattle were obtained at a local commercial abattoir. After transport to the laboratory on ice (< 120 min), the ovaries were processed and thecal cells were obtained from large (≥ 8 mm) follicles as previously described (Stewart et al., 1995). Approximately 2×10^5 viable cells were added to plastic multiwell plates containing 1 ml of medium [a 1:1 (vol/vol) mixture of Dulbecco's Modified Eagle Medium and Ham's F-12 containing gentamicin and sodium bicarbonate]. Cultures were maintained at 38.5°C in a 5% CO_2 atmosphere. To obtain optimal attachment, cells were cultured in the presence of 10% fetal calf serum (FCS) for the first 2 days with a change of medium occurring on day 1. After this time, cells were washed twice with .5 ml of serum-free medium and cultured in serum-free medium with or without added hormones for an additional 48 h (i.e., from d 2 to 4 of culture) without changing media.

Experiment 1 was conducted to evaluate the dose-response effect of IGFBP-3 on the action of LH and IGF-I on thecal cell steroidogenesis. Thecal cells were cultured for 2 days in 10% FCS, and then cultured in serum-free medium for an additional 2 days with LH (100 ng/ml), IGF-I (0 or 30 ng/ml) and IGFBP-3 (0, 200 or 400 ng/ml).

Experiment 2 was conducted to evaluate the effect of IGFBP-3 on the dose-response to IGF-I on thecal cell steroidogenesis. Thecal cells were cultured for 2 days in 10% FCS, and then cultured in serum-free medium for an additional 2 days with LH (100 ng/ml), IGFBP-3 (0 or 200 ng/ml) and IGF-I (0, 30 or 100 ng/ml).

Experiment 3 was conducted to determine if IGFBP-3 inhibits the binding of ^{125}I-IGF-I to its receptor. Thecal cells were cultured for 3 days in 10% FCS, washed twice with serum-free medium and then treated with 0, 3, 10, 30 or 100 ng/ml of IGFBP-3 during the binding assay.

Numbers of thecal cells were determined at the termination of experiments using a Coulter counter. Concentrations of androstenedione and progesterone in culture medium collected on d 4 of culture were determined by radioimmunoassay.

Experimental data are presented as the least squares means ± SE of measurements for triplicate culture wells from three or more experiments. Each experiment was performed with different pools of thecal cells collected from 20 to 30 ovaries for each pool. Each well was a replicate, and each experiment contained three replicates per treatment. When steroid production was expressed as nanograms or picograms/10^5 cells per 48 h, cell numbers at the termination of the experiment were used for this calculation. Data for each response criteria were analyzed by least squares analysis of variance. Mean

comparisons were done using Fisher's protected least significant difference procedure.

Results

Experiment 1.   The dose-response effect of IGFBP-3 on IGF-I- and LH-induced thecal cell steroidogenesis is shown in Figure 1.   In the absence of IGFBP-3, 30 ng/ml of IGF-I increased (P<.01) thecal cell numbers by 1.3-fold, and LH-induced progesterone and androstenedione production by 1.7- and 3.2-fold, respectively (Figure 1).   In the absence of IGF-I, IGFBP-3 had no effect (P>.10) on thecal cell numbers, or LH-induced progesterone or androstenedione production.   In contrast, 200 and 400 ng/ml of IGFBP-3 inhibited (P<.05) the IGF-I-induced increase in thecal cell progesterone production and androstenedione production by 76 and 59%, 52 and 64%, and 89 and 49%, respectively (Figure 1).

Experiment 2.   To determine if higher doses of IGF-I could overcome the inhibitory effects of IGFBP-3, we conducted studies summarized in Figure 2. In the absence of IGFBP-3, 30 and 100 ng/ml of IGF-I increased (P<.001) LH-induced progesterone and androstenedione production in a dose-dependent manner (Figure 2).   In the absence of IGF-I, IGFBP-3 had no effect (P>.10) on LH-induced progesterone or androstenedione production.   However, 200 ng/ml of IGFBP-3 reduced (P<.05) androstenedione production stimulated by 30 ng/ml of IGF-I and 100 ng/ml of LH.   However, in the presence of 100 ng/ml of IGF-I and LH, IGFBP-3 had no effect (P>.10) on androstenedione production (Figure 2).   Basal and IGF-I-induced progesterone production was not affected by IGFBP-3, although IGFBP-3 significantly inhibited IGF-I-induced progesterone in one of the three experiments summarized in Figure 2A (data not shown).

Experiment 3.   To determine if IGFBP-3 inhibits binding of ^{125}I-IGF-I to its receptor in thecal cells, we conducted studies summarized in Figure 3.   As depicted in Figure 3, 3 to 100 ng/ml of IGFBP-3 inhibited (P<.05) specific ^{125}I-IGF-I binding to thecal cells.

Discussion

We found that IGFBP-3 had no effect on LH-induced androstenedione or progesterone production by bovine thecal cells.   However, we observed that IGFBP-3 reduced the stimulatory effect of IGF-I on progesterone and androstenedione production, and that these inhibitory effects of IGFBP-3 were overcome with higher doses of IGF-I.   Although previous studies have not evaluated the effect of IGFBPs on thecal cell function, previous studies have shown that purified porcine IGFBP-2 and IGFBP-3, and purified rat IGFBP-4 and IGFBP-5 can inhibit FSH-induced estradiol and progesterone production by cultured rat granulosa cells (Ui et al., 1989; Bicsak et al., 1990; Liu et al.,

1993). Also, purified porcine IGFBP-3 significantly decreased hCG-induced estrogen production and attenuated the inhibitory effect of hCG and IGF-I on follicular cell apoptosis in intact rat follicles (Chun et al., 1994). Collectively, it seems that IGFBPs consistently inhibit FSH-induced estradiol production by granulosa cells of rats; whether this is true for domestic animals remains to be determined.

Levels of IGFBP-2 and other lower molecular weight IGFBPs in follicular fluid increase as follicles become atretic and lose their ability to produce estradiol, whereas IGFBP-3 levels do not change in bovine and porcine follicles (Spicer and Echternkamp, 1995). Recently, recombinant human IGFBP-3 has been shown to inhibit the ability of FSH to suppress apoptosis in cultured rat follicles (Flaws et al., 1995). Thus, in vitro and in vivo data suggest that the presence of IGFBP-3 and increased IGFBP-2 in follicles undergoing atresia may hasten the atretic process by inhibiting androstenedione and estradiol production, and that IGF-I at physiological concentrations can attenuate this inhibitory effect. The mechanism by which IGFBP-3 antagonizes the stimulatory action of IGF-I on follicular steroidogenesis in vivo is unclear; however, based on the present studies, it is reasonable to propose that the mechanism involves the binding of systemic and endogenously produced IGF-I.

Whether IGFBP-3 is acting as an endocrine or paracrine regulator of ovarian follicular function in cattle is uncertain. As mentioned, IGFBP-3 is found in bovine, porcine and ovine follicular fluid. Also, IGFBP-3 levels are lower in follicular fluid than peripheral blood, and do not change as follicles enlarge and become estrogen active in sheep and cattle (Spicer and Echternkamp, 1995). Collectively, these results indicate that IGFBP-3 may be produced by ovarian follicular cells of some species and thus may act as autocrine or paracrine regulators, as well as endocrine regulators, of ovarian follicular steroidogenesis during follicular growth and atresia. Regardless of the source of IGFBPs, imbalances between IGFs and IGFBPs, such as during severe feed restriction, may be involved in the pathology of reproductive inefficiency in cattle and therefore warrant further study.

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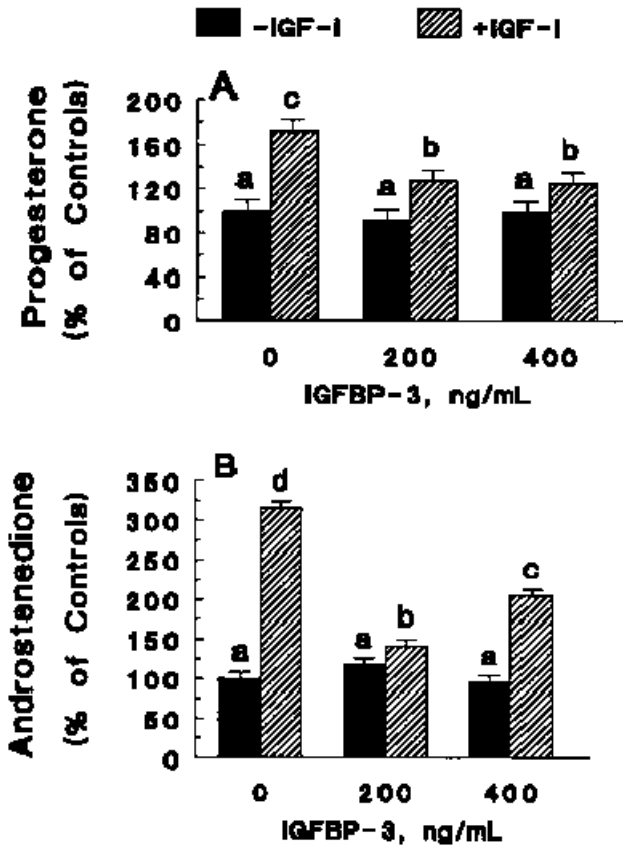
Figure 1. Effects of IGFBP-3 on LH- and IGF-I-stimulated progesterone (Panel A) and androstenedione (Panel B) production by thecal cells (Experiment 1). Thecal cells were cultured for 2 days in the presence of 10% fetal calf serum, and then treated in serum-free media with 100 ng/ml of LH, IGF-I (0 or 30 ng/ml) and IGFBP-3 (0, 200 or 400 ng/ml) for an additional 2 days. Values are means of three separate replicate experiments, and expressed as a percentage of control cultures (0 ng/ml of IGF-I and IGFBP-3). Within a panel, means without a common superscript differ (P < .05).
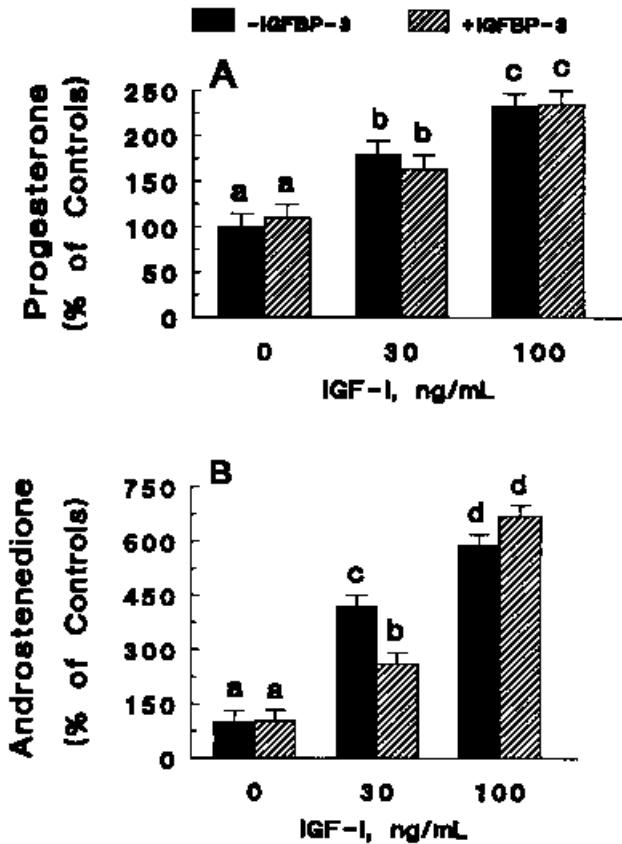
Figure 2. Effects of IGFBP-3 on IGF-I-stimulated progesterone (Panel A) and androstenedione (Panel B) production by thecal cells (Experiment 2). Thecal cells were cultured for 2 days in the presence of 10% fetal calf serum, and then treated in serum-free media with 100 ng/ml of LH, IGF-I (0, 30 or 100 ng/ml) and IGFBP-3 (0 or 200 ng/ml) for an additional 2 days. Values are means of four separate replicate experiments, and expressed as a percentage of control cultures (0 ng/ml of IGF-I and IGFBP-3). Within a panel, means without a common superscript differ (P < .05).
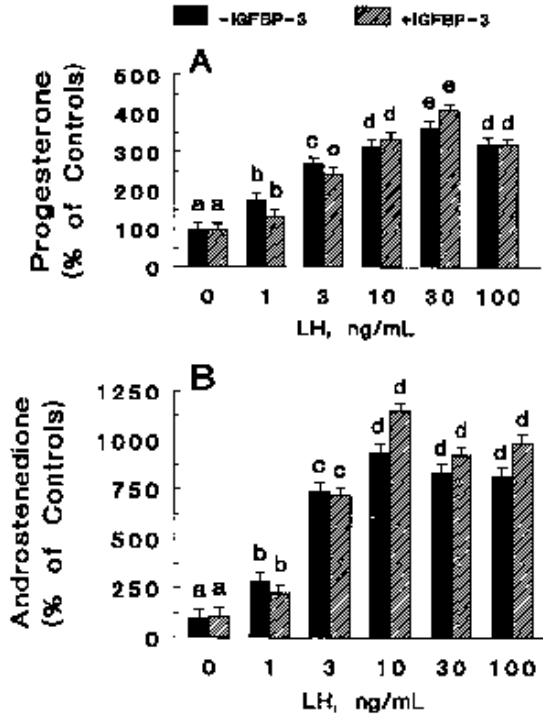
Figure 3. Effects of IGFBP-3 on ^{125}I-IGF-I binding to thecal cells (Experiment 3). Thecal cells were cultured for 3 days in the presence of 10% fetal calf serum, washed with serum-free media, and then treated with 0, 3, 10, 30 or 100 ng/ml of IGFBP-3 (•—•) during the binding assay. Values are means of three separate replicate experiments and expressed as a percentage of control (0 ng/ml of IGFBP-3) incubations. [*] Means differs from Control (P < .05).