EFFECTS OF INSULIN AND INSULIN-LIKE GROWTH FACTOR-I ON OVARIAN GRANULOSA CELL FUNCTION IN CATTLE

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Story in Brief

The direct effect of insulin and insulin-like growth factor-I on ovarian granulosa cell function in cattle was evaluated by using a serum-free culture system. Granulosa cells were obtained from small (1 to 5 mm) follicles collected from cattle and cultured for 4 days. Culture medium was collected for quantification of progesterone, and cell numbers were determined. Both insulin and IGF-I significantly increased granulosa cell numbers and progesterone production after 2 days of treatment. We conclude that insulin and IGF-I may have direct effects on ovarian function in cattle.

(Key Words: Insulin, Insulin-like Growth Factor-I, Granulosa Cells, Progesterone, Cattle.)

Introduction

Recent attempts to improve production efficiency in domestic animals has concentrated on the use of bovine somatotropin (BST) (Enright et al., 1989). Long-term BST treatment is associated with increased secretion of IGF-I (Gluckman et al., 1987). This increase in IGF-I secretion is thought to affect reproductive function in cows. Therefore, we set out to compare the effects of insulin and IGF-I on cell growth and progesterone production of bovine granulosa cells.

Materials and Methods

Ovaries were obtained at a nearby commercial slaughterhouse from beef and dairy cattle. Granulosa cells from 1 to 5 mm follicles were collected by aspiration using a needle and syringe and washed three times in serum-free medium. At each wash, cells were separated from medium via centrifugation

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(200 x g for 10 min). Medium was a 1:1 mixture of Dulbecco's Modified Eagles Medium and Ham's F10 containing 0.12 mM gentamicin and 38.5 mM sodium bicarbonate. Between 2 and 4 x 10^5 viable cells were added to plastic multiwell plates containing 1 ml medium. Cultures were kept at 37°C in a 95% air- 5% CO_2 atmosphere, and medium was changed every 24 hours. To obtain optimal attachment, cells were maintained in the presence of 10% fetal calf serum for the first 48 hours of culture (Langhout et al., 1991). After this time, granulosa cells were washed twice with 0.5 ml serum-free medium and incubations continued in serum-free medium with or without added hormones. Hormonal treatments were maintained for 2 days.

At the termination of each experiment, numbers of cells were determined using a Coulter counter. Culture medium was also collected for determination of concentrations of progesterone (as a measure of steroidogenic capacity of cells) by radioimmunoassay.

Experimental data are presented as the least squares means ± SE of measurements from replicated experiments. Each experiment was replicated three times with four replicates per treatment within an experiment. Progesterone production was expressed as [ng·(10^5 cells)$^{-1}$·(24 h)$^{-1}$] using cell numbers at the termination of the experiment for the calculation.

Results

In serum-free medium with no additional hormones, recombinant human IGF-I had a dose-dependent effect on cell numbers and progesterone production, compared with control (Figure 1A and 2A). The doses of 20, 200 and 400 ng/ml of IGF-I caused significant increases in cell numbers (Figure 1A) and progesterone production (Figure 2A). Similarly, bovine insulin (.1, 1, and 10 μg/ml) increased cell numbers (Figure 1B) and progesterone production (Figure 2B).

Discussion

Results of the present study suggest that insulin and IGF-I have direct effects on bovine granulosa cells. Previous in vitro studies have shown that insulin and IGF-I can enhance (by 2- to 20-fold) progesterone production by rat (Davoren et al., 1985) and porcine (Maruo et al., 1988) granulosa cells. This is the first report comparing the effects of insulin and IGF-I on granulosa cell proliferation and steroidogenesis in cattle.

Although the present and previous studies establish granulosa cells as a site of insulin and IGF-I action, its physiologic relevance remains unclear. Average concentrations of insulin in beef and dairy cattle are usually less than 10 ng/ml, and thus fall below the effective doses of insulin used in the present

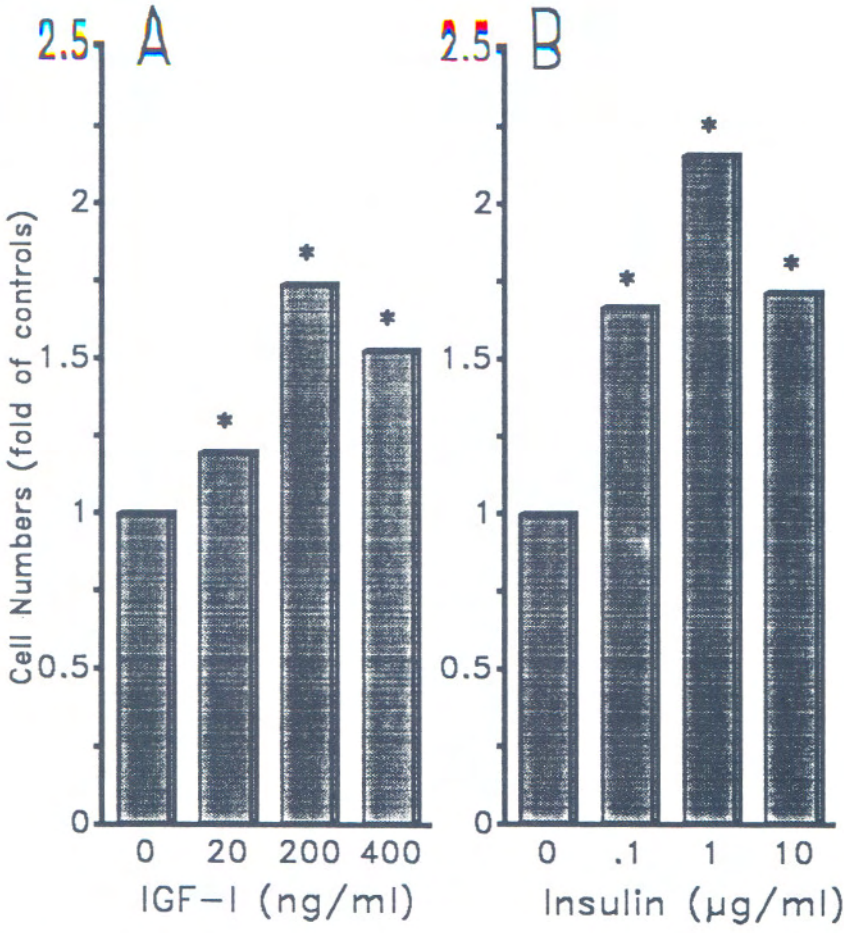

Figure 1. Effect of human recombinant IGF-I (Amgen Biologicals) (Panel A) and bovine insulin (Sigma Chemical Co.) (Panel B) on granulosa cell numbers at d 4 of culture. Granulosa cells were obtained from small follicles and treated within IGF-I or insulin. Values are least squares means and expressed as fold of controls without insulin or IGF-I. *, mean differs ($P < .05$) from treatment without addition of insulin or IGF-I. Pooled SE = 0.15-fold of controls for Panel A and pooled SE = 0.08-fold of controls for Panel B.

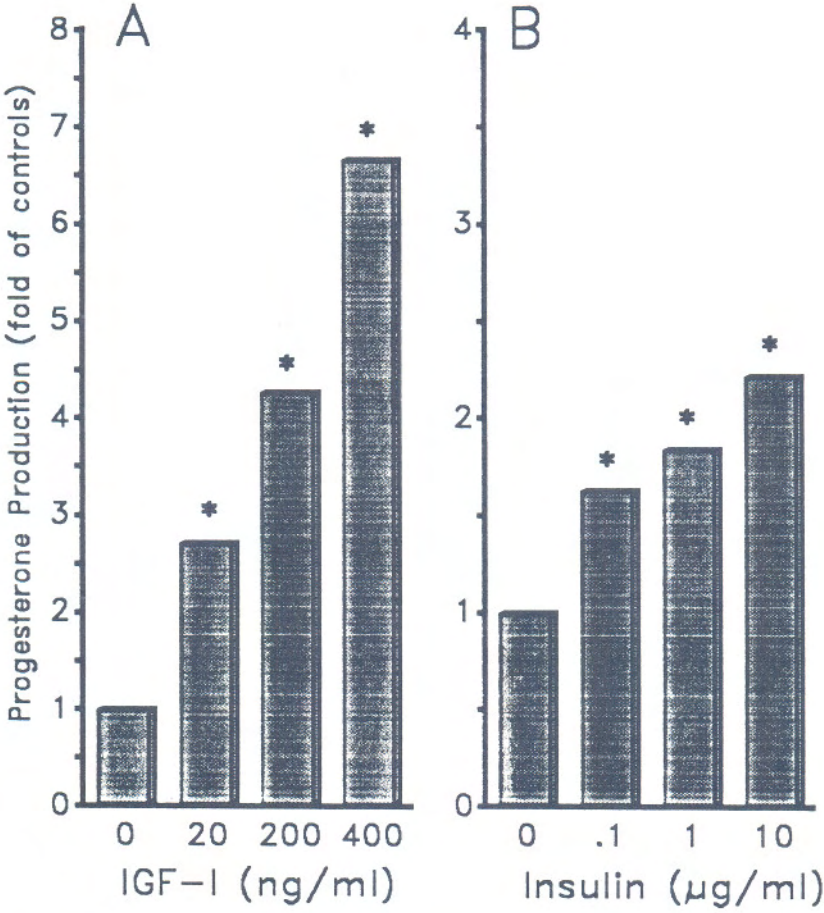

Figure 2. Effect of IGF-I (Panel A) and bovine insulin (Panel B) on granulosa cell progesterone production. Granulosa cells were obtained from small follicles and treated as in Figure 1. Values are least squares means and expressed as fold of controls without insulin or IGF-I. *, Mean differs ($P < .05$) from treatment without addition of insulin or IGF-I. Pooled SE=0.6-fold of controls for Panel A and pooled SE=0.2-fold of controls for Panel B.

study. However, in cattle IGF-I levels usually exceed 100 ng/ml (Gluckman et al., 1987). Thus, IGF-I may be a more physiologically relevant promoter of ovarian follicular function than insulin in cattle.

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