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ORIGINAL ARTICLE

Effects of growth hormone (GH) and growth hormone releasing hormone (GHRH) on progesterone and estradiol release from cultured rat granulosa cells

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Abstract

OBJECTIVES: It has been reported that GHRH-GH-IGF-1 system plays an important role in the regulation of ovarian follicular development and maturation.

METHODS: In order to evaluate the direct effects of growth hormone releasing hormone (GHRH) and growth hormone (GH) on steroidogenesis, the effects of GHRH and GH on progesterone and estradiol release from cultured rat granulosa cells were examined. The progesterone and estradiol in supernatants were measured with RIA methods.

RESULTS: Our results demonstrated that the addition of GH to the culture medium produced a marked stimulation of progesterone and estradiol. The stimulating effects were observed after administration of GH in all concentrations: 1, 10, 100 nM during 60 and 120 mins of incubation. During 240 mins of incubation the minimal stimulation of progesterone and estradiol was found. However, GHRH administered in 1, 10 and 100 nM did not change progesterone and estradiol release from cultured granulosa cells.

CONCLUSION: Growth hormone (GH) but not GHRH has direct stimulating effects on progesterone release from cultured rat granulosa cells.

Introduction

Some evidence is accumulated that the actions of the gonadotrophins on the ovary were modulated by intra- and extraovarian factors including insulin and the insulin-like growth factors. In vitro insulin acts synergistically with the gonadotrophins to enhance both granulosa and theca-interstitial cell differentiation by its interaction with insulin and insulin-like growth factor –1 (IGF-1) – receptors [1].

It has been reported that growth hormone (GH) and IGF-1 in experiments *in vivo* and *in vitro* may play a role in the regulation of ovarian follicular development and maturation [2–9].

Studies *in vivo* showed that GH augments the response of the human ovary to stimulation by gonadotrophins [10, 11].

Yoshimura et al. [9] demonstrated that GH was able to stimulate follicular growth, oocyte maturation and estradiol production in perfused rabbit ovaries in the absence of gonadotrophins. The authors studied the mechanism of stimulating action of GH and presented data indicating that GH stimulates follicle growth and oocyte maturation in the *in vitro* perfused rabbit ovaries and may amplify gonadotrophin action by enhancement of the ovarian IGF-1 production [10].

It has been demonstrated that human GH modulates and regulates intraovarian reproductive processes via the GHRH/GH/IGF-1 axes [11].

The aim of this study was to evaluate the direct effects of GH and GHRH on progesterone and estradiol release from cultured rat granulosa cells.

Material and methods

Effects of GHRH and GH on progesterone and estradiol production by cultured granulosa cells were examined.

The ovaries from WKY rats were collected under aseptic conditions. Isolated ovaries were washed with PBS, supplemented with a mixture of antibiotics and then they were rubbed through a sieve (mesh 50). Granulosa cells were treated with 0.15 collagenase and 0.1% hyaluronidase in Hank's buffer at 37°C, for 30 min and digested in a buffer containing: 0.02% EDTA, 0.1% glucose, 0.1% NaCl, 0.19% NaHCO3 and 0.1% trypsin at 37°C for the next 30 min. Dispersed cells were washed with culture medium (RPMI, containing 0.5% BSA and 10% fetal calf serum) and seeded in culture medium in 96-well culture plates. The cells were then cultured for three days in a humidified atmosphere of 95% air and 5%CO_2 at 37°C. After that period the medium was removed and cells were cultured under serum-free conditions with GHRH and GH in varying concentrations: 1, 10, 100 nM. The control culture group was cultured in physiological solution. Cell cultures were maintained for 240 mins. Culture supernatants were then decanted and stored until a hormone analysis. That method was conducted according to details described previously [12–16]. The experiments were repeated 4 times, 2x105 /ml cells were present in each culture. For the statistical analysis the unpaired Student's T-test and the analysis of variance were used, as appropriate.

Results

Effects of GH on progesterone release from cultured granulosa cells were presented in Fig. 1.

GH markedly stimulated progesterone in all concentrations (1,10, 100 nM). The maximal effects were observed after administration of GH in 100 nM concentration during 60 and 120 mins of incubation, but increased release of progesterone was also observed during 240 mins of incubation.

Effects of GH on estradiol release were demonstrated in Fig. 2. GH administered in all concentrations stimulated estradiol release during 60 and 120 mins of incubation. The minimal stimulating effects were found during 240 mins of incubation.

Effects of GHRH on progesterone and estradiol release were shown in Figs. 3 and 4.

GHRH administered in 1, 10, 100 nM concentrations did not change the release of progesterone and estradiol concentrations during 60 and 120 mins of incubation.

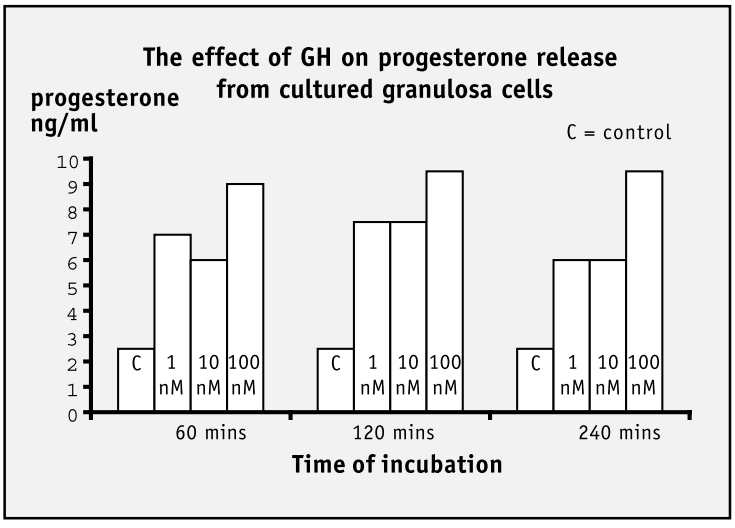
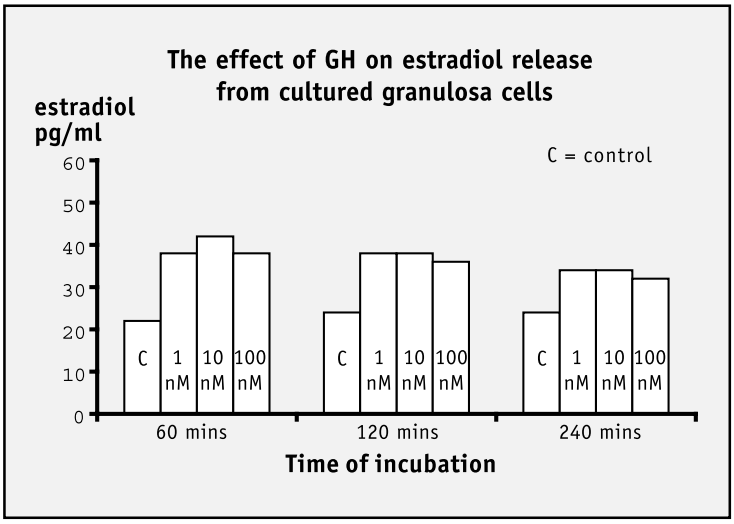


Fig. 1.



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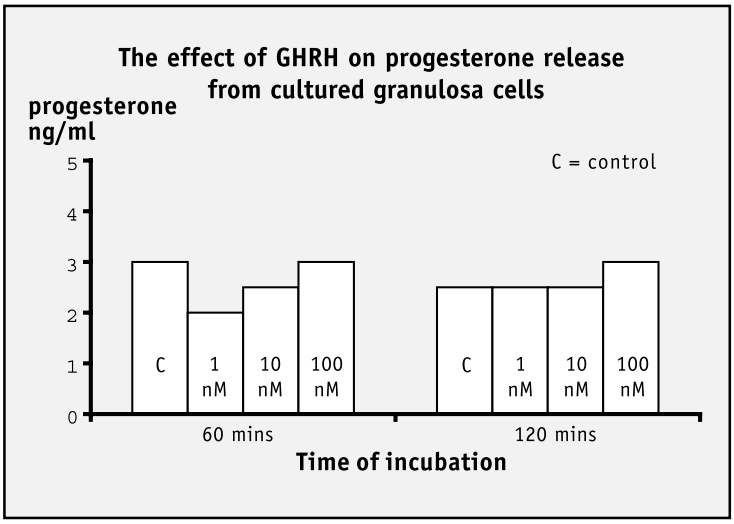

Fig. 3.

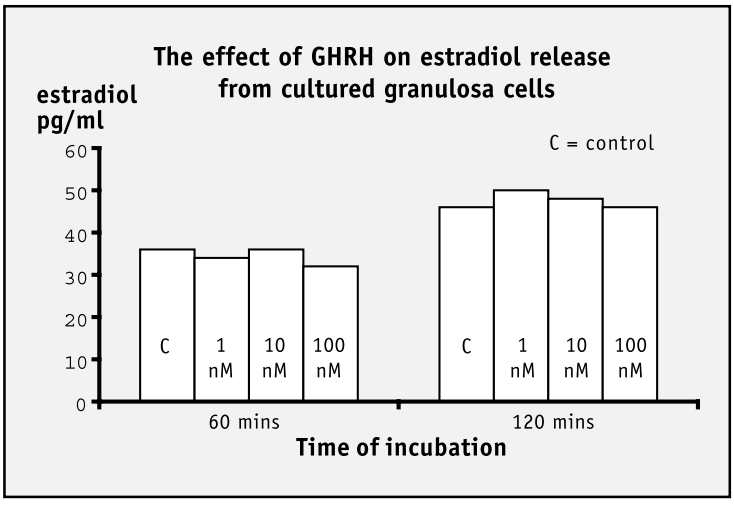

Fig. 4.

Discussion

Our results demonstrated that the addition of GHRH to culture medium did not change the release of progesterone and estradiol.

It has been commonly accepted that GHRH may be effective through the release of GH from the pituitary and subsequent increases of IGF-1.

The studies of Hugues et al. [17] showed that co-treatment with GHRH and FSH induced enhancement in plasma IGF-1 concentrations and steroid production by cultured granulosa cells, but the addition of GHRH to culture medium did not alter steroid production, which in contrast to IGF-1 significantly increased steroidogenesis.

The clinical applications of the above results indicated the beneficial effects of GHRH therapy in vitro fertilization, because GHRH enhances the hormonal ovarian response to hMG [18]. A significant increase of follicular fluid IGF-1 levels and plasma GH after GHRH administration was observed by Valpe et al. [19]. Their results suggest that GHRH supported the ovarian response to gonadotrophins through stimulation of the GH-IGF-1 axis.

However, despite stimulation of GH-IGF-1 axis, concomitant treatment with GHRH does not improve the ovarian response to FSH in poorly responsive women during in vitro fertilization (IVF) [20].

The addition of GH to culture medium in our studies markedly stimulated progesterone and estradiol from cultured granulosa cells.

Yoshimura et al. [10] accumulated the evidence that GH may amplify gonadotrophin action in the follicular development and ovulation through stimulation of ovarian IGF-1 production.

It has been shown that granulosa cells are able to synthesize IGF-1 [21, 22]. IGF-1 and insulin may play a role in the normal balance between folliculogenesis and atresis. They are mitogenic for granulosa cells and synergize with FSH to increase LH receptors, aromatase activity and progesterone production by granulosa cells in both rats and humans [6, 23].

Adashi et al. [7] documented the presence of IGF-1 and IGF-2 receptor mRNA in granulosa and theca-interstitial cells.

IGF-1 plays a role in amplifying the effect of FSH in rat granulosa cells [6, 21, 22]. The studies of Erickson [24, 25] indicate that IGF-1 is

a potent stimulator of FSH-induced aromatase activity in granulosa cells culture. Mason et al. [26] demonstrated that IGF-1 is able to regulate IGF-BP-1 and estradiol secretion by human granulosa cells.

Bergh et al. [27] found that IGF-1 stimulated progesterone and estradiol production in cultured human granulosa cells. Erickson [25] demonstrated that FSH and IGF-1 stimulated estradiol production by granulosa from patients with PCOS. These findings argue that IGF and their binding proteins are involved in the intraovarian regulation of ovarian function.

Conclusion

Growth hormone (GH) but not GHRH have direct stimulating effects on progesterone and estradiol release from cultured rat granulosa cells.

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