MODULATION OF STEROIDOGENESIS IN HUMAN OVARIAN GRANULOSA CELLS DURING AGING

R. Denkova, V. Bourneva, K. Baleva, E. Yaneva, B. Nikolov, I. Christov, I. Ivanov.

Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
E-mail: timanova@bas.bg

Objective. To investigate the *in vitro* effect of endothelin-1 (ET-1) on the steroid production (progesterone [P] and estradiol [E2]) by cultured human granulosa cells (GCs) during aging.

Material and methods. Human ovarian GCs and granulosa-luteal cells (GLCs) were isolated from ovaries of female patients (young and premenopausal) undergoing surgery for non-ovarian benign gynecological conditions. Cells were cultured with ET-1 in the presence or in the absence of FSH. The concentrations of P and E2 in conditioned media were determined by means of RIA.

Results. In human GCs and GLCs obtained from young and premenopausal women, ET-1 *in vitro* can significantly reduce the FSH stimulated biosynthesis of P, whereas the basal P biosynthesis is only insignificantly diminished. The *in vitro* application of ET-1 have only a sparse inhibitory effect on both the basal and FSH stimulated biosynthesis of E2 in GCs from the two patient groups.

Conclusions. Our findings support the opinion that ET-1 is a local regulator of ovarian steroidogenesis which might modulate the steroid production and the stimulatory effect of FSH in cultured GCs and GLCs obtained from women at various ages.

Key words: Human ovarian steroidogenesis – Granulosa cells – Granulosa-luteal cells – Endothelin-1 – FSH – Tissue culture

Even though pituitary gonadotropins are essential for follicular development and differentiation, it has become increasingly apparent that intraovarian regulatory mechanisms involving locally produced factors may also play an important role in ovarian functions. There are some data showing that endothelin-1 (ET-1), a 21 amino acid peptide isolated from vascular endothelial cells is synthesized and released also from ovarian granulosa cells (GCs) (Kamada et al. 1993). Endothelin-1 was found in high concentration in ovarian follicles and corpora lutea from various species and its both receptors subtypes (ETA and ETB) have been localized in the same ovarian structure (Mancina et al. 1997). *In vivo* and *in vitro* studies have reported ET-1 actions on ovarian steroid secretion and proposed this potent vasoconstrictor peptide as a new autocrine/paracrine modulator of ovarian functions (Furger et al. 1995; Kamada et al. 1995; Calogero et al. 1998; Mamluck et al. 1999; Karam et al. 1999). Our previous studies using an animal model (porcine ovarian GCs in culture) demonstrated an autocrine effect of endothelin-1 in the ovarian follicular cell (Denkova et al. 1999, 2000).

The aim of the present study was to investigate the in vitro effect of endothelin-1 on the steroid production (progesterone [P] and estradiol [E2]) by cultured human GCs obtained from women at various ages.

Material and Methods

Human ovarian granulosa cells (GCs) and granulosa-luteal cells (GLCs) were isolated from ovaries of female patients (young, aged 27-31 years and premenopausal, aged 43-52 years) undergoing ovarian surgery for non-ovarian, benign gynaecological conditions. All patients gave their informed

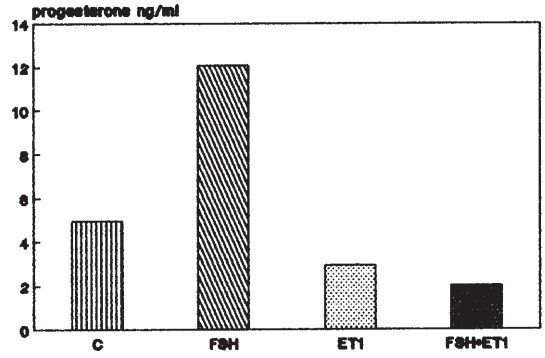

Fig. 1 In vitro progesterone production by GCs from young patients in the presence or absence of ET-1 and FSH. C (control) – culture without ET-1 and FSH

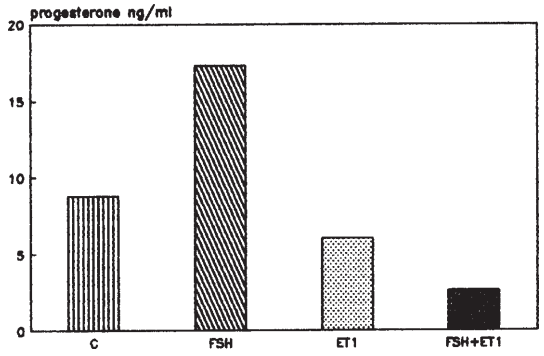

Fig. 3 In vitro progesterone production by granulosa-luteal cells from young patients in the presence or absence of ET-1 and FSH

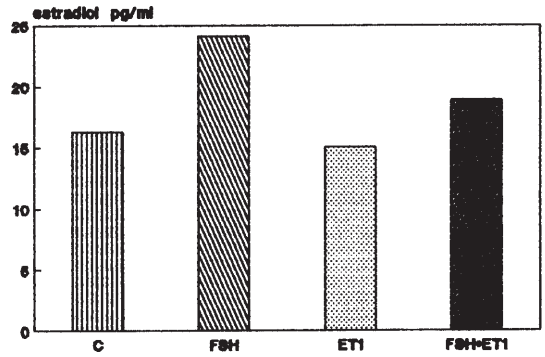

Fig. 2 In vitro estradiol production by GCs from young patients in the presence or absence of ET-1 and FSH

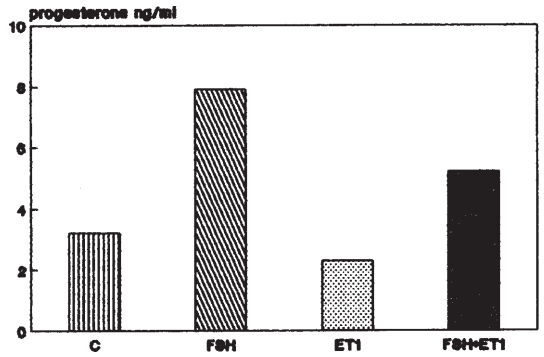

Fig. 4 In vitro progesterone production by GCs from premenopausal women in the presence or absence of ET-1 and FSH. C (control) – culture without ET-1 and FSH

consent concerning the use of their ovaries for research purposes.

Culture procedures.. Ovarian granulosa cells were isolated from antral follicles by the nonenzymatic needle puncture method (Channing and Ledwitz-Rigby 1975) and menstrual corpus luteum (Friden et al. 1999). Cells were cultured in sterile DMEM incubation medium supplemented with 5 % FCS (Difco Labs), 50 IU/ml penicillin, 5 µg/ml streptomycin and 2.5 µg/ml fungisone to give a final concentration of 6-10 viable cells. Granulosa cells and granulosa-luteal cells (GLCs) were incubated at a 37 ^{o}C under water saturated with 95 % air and 5 % CO_2 for 24 h. Cells were further cultured for additional 24 h in DMEM without serum, but supplemented with fibronectin (F) (8 µg/ml), insulin (I) (1 µg/ml), thrombin (T) (1 IU/ml), low density lipoprotein (LDL) (10 µg/ml) either with or without endothelin (10-7 M, Sigma-Aldrich Corp) and in the presence or in the absence of FSH (100 ng/ml, Sigma-Aldrich Corp).

Steroid assay. The concentration of progesterone (ng/ml) in the media from cultured GCs and GLCs was determined according to Kanchev et al. (1976) using rabbit antiserum (RD/4.'10) at a dilution of 1:10000. Estradiol (pg/ml) was estimated according to Dobson et al. 1974 (cit. by Kanchev et al. 1976)

Statistical evaluation: Summarized results of all experiments are shown. Statistical significance of differences was analyzed by Student's t-test.

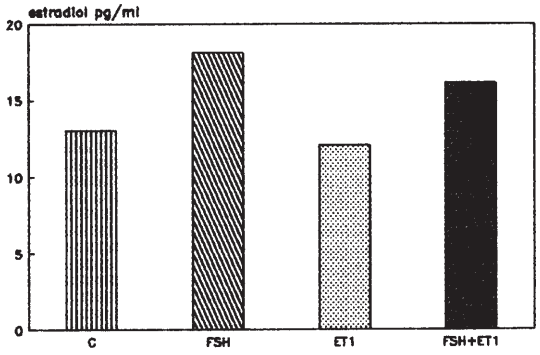

Fig. 5 In vitro estradiol production by GCs from premenopausal women in the presence or absence of ET-1 and FSH

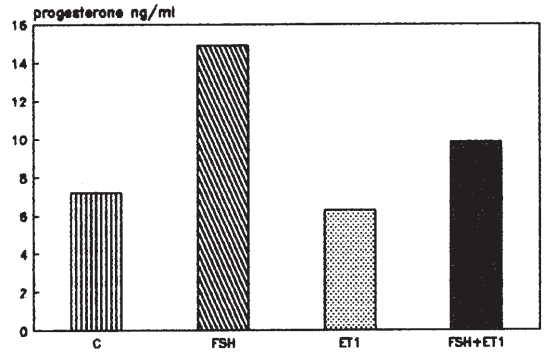

Fig. 6 In vitro progesterone production by GLCs from premenopausal women in the presence or absence of ET-1 and FSH

Results

In vitro treatment with ET-1 had a slight inhibitory effect on progesterone production by cultured GCs from young patients. Addition of FSH to cultured medium caused 5 fold decrease of P production in these cells ($P<0.001$) (Fig.1). No well expressed effect of GC treatment with ET-1 on estradiol release in the presence or absence of FSH was found ($P<0.01$) (Fig.2). The accumulation of P was not markedly suppressed in young woman granulosa-luteal cells treated with ET-1 ($P<0.05$) (Fig.3) which resulted in a significant reduction in the FSH supported accumulation of P in LGCs ($P<0.001$). *In vitro* administration of ET-1 provoked slight diminution of P production by GCs of premenopausal women ($P<0.01$) (Fig.4). Endothelin treatment decreased FSH stimulated accumulation of P secretion in the same cells ($P<0.001$). Both the basal and FSH stimulated E2 biosynthesis were only slightly reduced in the presence of ET-1 in GCS from premenopausal patients ($P<0.001$) (Fig. 5). The production of P in GLCs from premenopausal women was slightly decreased by ET-1 ($P<0.001$), whereas the cells cultured with this peptide in the presence of FSH showed considerable suppression of P release ($P<0.01$) (Fig.6).

Discussion

Our findings support the opinion that endothelin-1 is a local regulator of ovarian steroidogenesis which might modulate the steroid production and stimulatory effect of FSH with age. In human granulosa and granulosa-luteal cells obtained from young and from premenopausal women ET-1 *in vitro* significantly reduced the FSH-stimulated biosynthesis of progesterone, whereas the basal progesterone biosynthesis was only insignificantly diminished. Furthermore, ET-1 inhibitory effect on the FSH stimulated biosynthesis of progesterone was stronger in ovarian GCs and GLCs from young patients than in those from premenopausal women. On the other hand, ET-1 in vitro seemed to have a sparse inhibitory effect on both the basal and FSH stimulated biosynthesis of estradiol in GCs from two patients. Our studies underway should prove if the age related differences of ET-1 effects on progesterone biosynthesis in human ovarian granulosa and granulosa luteal cells are due to receptor alterations.

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Corresponding author: R. Denkova, M. D.
Bulgarian Academy of Sciences
Institute of Experimental Morphology
and Anthropology
G. Bonchev str. B-25
1113 Sofia, Bulgaria