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Short communication

Effects of oxytocin on progesterone secretion by hen granuloza cells

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Oxytocin (OT) dose-dependently increased progesterone secretion in granulosa cells (GCs) of preovulatory follicles F_2 and F_3 in laying hens. The effects of OT 1, 5 and 10 mIU were weakest at 24 h and strongest at 72 h after cultivatin of granulosa cells. LH stimulated the OT-induced progesterone secretion while FSH inhibited the OT effect. It is concluded that OT is an intraovarian regulator of steroidogenesis in the preovulatory follicles of laying hens.

Key words: oxytocin, progesterone secretion, hens preovulatory follicles, granulosa cells, gonadotropins.

The nonapeptide oxytocin (OT) originally detected in the magnocellular neurons of the hypothalamus has recently been found in human ovaries and in the ovaries of many mammalian species [3, 4, 8, 9]. In the ewe and in the cow the plasma levels of OT are low during the follicular phase and increase during the luteal phase. These fluctuations are due to changes in the ovarian OT [8]. Chandrasekher and Fortune [1] and Voss and Fortune [6] have reported OT stimulation of progesterone secretion in bovine GCs isolated from preovulatory follicles. This stimulant effect of OT is blocked by the oxytocin antagonist. The authors suggest that OT is an intraovarian regulator of steroidogenesis in mammalian preovulatory follicles. We failed to find data about the synthesis of OT- or OT-like peptides in the ovaries of birds and about the OT effect on steroidogenesis in granulosa cells. This stimilated us to study the effect of OT on isolated GCs from hen preovulatory follicles, as well as the possible modulating action of gonadotropins on OT-induced progesterone secretion.

Material and methods

Hens were killed by cervical dislocation and the F_2 and F_3 large preovulatory follicles were removed and immediately placed in ice-cold 0,9% saline. GCs were collected and dispersed with 0,3% Collagenase (type 2) diluted in sterile DMEM (Flow Labs) as described by Tilly and Jonson [5]. Cell number and via-

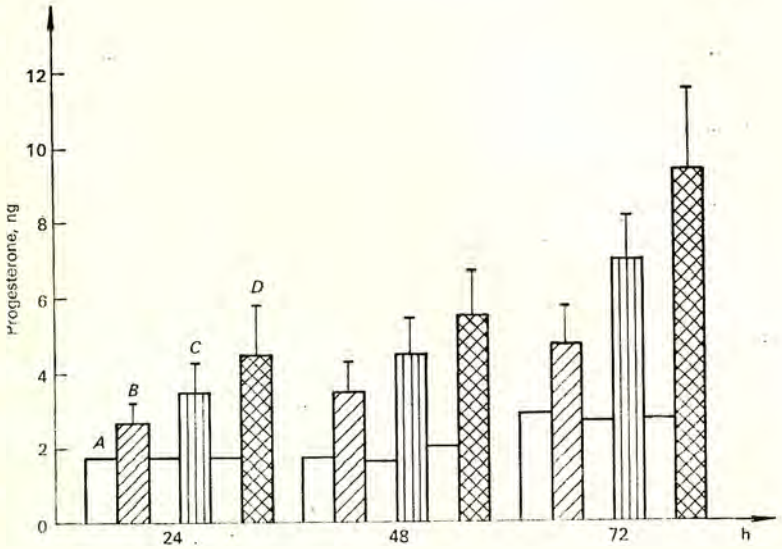

Fig. 1. Secretion of progesterone (nanograms per 400 000 cells ± SEM; n=9 cultures) by granulosa cells in the presence of oxytocin during 24, 48, and 72 h after cultivation
A — control; *B* — oxytocin 1 mIU; *C* — oxytocin 5 mIU; *D* — oxytocin 10mIU

bility were estimated by a hemacytometer and viability was evaluated using the trypan blue exclusion technique. In all cases viability was greater than 90%. Finally the cell suspension placed in sterile DMEM incubation medium supplemented with 5% fetal calf serum (FCS) (Difco Labs), 50 IU/ml penicillin, 50 µg/ml streptomycin, 2,5 µg fungisone to give a final concentration of 400 000 viable cells per 1 ml medium aerated with 5% CO_2 and 95% O_2 at 39°C.

OT (Richter) was added to the cultures in increasing doses to acheive final concentration of 10 m_1 U, GCs were incubated in the above described medium with the addition of OT in the presence or in the absence of gonadotropins — 300 ng/ml LH (Boehringer mannheim) or 250 ng/ml FSH (Boehringer mannheim).

Progesterone assay: At the end of cultivation (96 h) the medium was centrifuged and the supernatants were stored at — 20°C until progesterone assay. The concentration of progesterone in the medium from cultured GCs was determined by the method of K a n c h e v et al. [2] using rabbit antiserum (RD/4.10) at a dilution of 1:10 000. The antiserum was prepared against progesterone — 11-succinyl-BSA. The sensitivity of the method was 10 pg per tube.

Results and discussion

Oxytocin (OT) dose dependently increased the progesterone production in cultured GCs from F_2 and F_3 large preovulatory follicles of the ovum sequence. The 24h-treatment of GCs with 10 mIU OT led to a twofold increase of progesterone secretion as compared to the controls, while the 72h-treatment caused a fourfold increase of this secretion (Fig. 1). These results are in agreement with the data of other authors [1, 6] concerning the effect of OT on the progesterone secretion in GCs bovine preovulatory follicles.

Gonadotropins exerted a different effect on the OT-induced progesterone secretion in GCs from F_2 and F_3 large preovulatory follicles: LH increased the

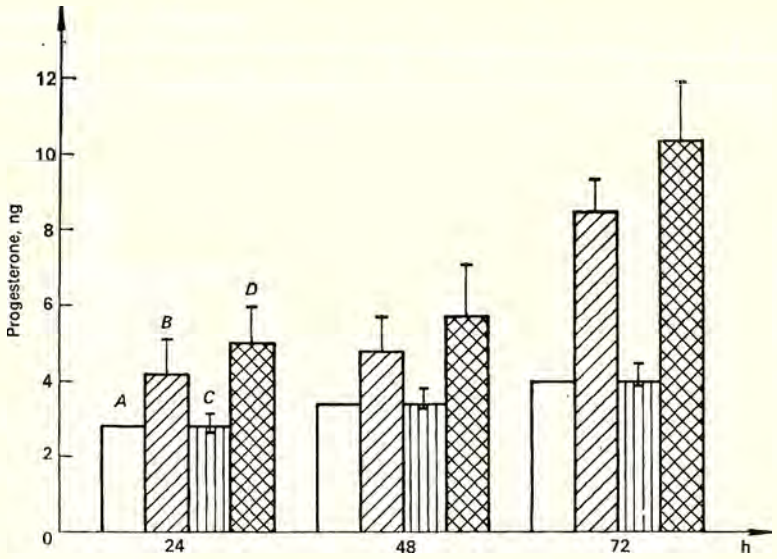

Fig. 2. Secretion of progesterone (nanograms per 400 000 cells ± SEM; n=9 cultures) by granulosa cells cultured with oxytocin in the presence of LH or FSH
A — control; *B* — oxytocin 10 mIU; C — oxytocin+FSH; *D* — oxytocin+LH

effect of 10 mIU OT on the progesterone secretion, while FSH decreased it. Chandrasekher and Fortune [1] and Voss and Fortune [6] also found an increase of progesterone secretion induced by OT in the presence of LH and a decrease of the OT effect in the presence of FSH.

The present results strongly suggest not only a paracrine but also an autocrine role of OT in birds. Like in the mammalian preovulatory follicles, in the preovulatory follicles of birds OT performs as an intraovarian regulator of steroidogenesis.

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