P45

EFFECTS OF ESTRADIOL-17β ON OXYTOCIN RECEPTORS AND PROSTAGLANDIN F2α PRODUCTION IN CULTURED BOVINE ENDOMETRIAL EPITHELIAL CELLS

Haruka ORIBE, Yoko MIYAMOTO and Kiyoshi OKUDA
Laboratory of Reproductive Endocrinology, Department of Animal Science, Faculty of Agriculture
Okayama University, Okayama 700-8530, Japan

Introduction

In bovine endometrium, oxytocin (OT) from corpus luteum stimulates the release of prostaglandin (PG) F2α via the epithelial OT receptor, resulting in regression of the corpus luteum. Concentrations of PGF2α increase immediately following injection of OT either early or late in the estrous cycle in heifers, but the response is much less during the middle of the estrous cycle[1]. Although OT receptors are absent during the mid-stage of the estrous cycle, their concentrations increase rapidly on Days 17-18, at the time of luteolysis, and reaches a maximum at estrus[2]. These reports showed that the rise in OT receptor concentrations increases the uterine responsiveness to OT. Therefore, the regulation of OT receptor expression plays a crucial role in determining the timing of the onset of luteolysis[3].

Although it has been well demonstrated in ruminants that estradiol-17β (estradiol) administration *in vivo* stimulates endometrial OT receptor expression and enhances responsiveness to OT[4-6], the action of estradiol at the cellular level in the endometrium is poorly documented.

To investigate the role of estradiol in regulating PGF2α synthesis in the endometrium, the present study examined the effects of estradiol on OT receptor expression and PGF2α production in response to OT treatment in cultured bovine endometrial epithelial cells.

Materials and Methods

Cell preparation and Cell culture

Epithelial cells obtained from bovine endometrium in the early stage of the estrous cycle

(Days 2-5) were cultured as described previously[7]. When the cells were confluent (6-7 days after the start of the culture), 0.02% trypsin solution was added to the cells to remove the stromal cells. After removal of the stromal cells, 0.25% trypsin solution was then added to the epithelial cells to collect the pure epithelial cells. The cells were removed, adjusted to a density of $1x10^5$/ml, and placed in 48-well cluster dishes and flasks in fresh DMEM/Ham's F-12 supplemented with 10% calf serum (CS), 20 μg/ml gentamicin and 2 mg/ml amphotericin B until confluency was reached. The culture media were then replaced with fresh DMEM/Ham's F-12 without phenol red and sodium bicarbonate containing 10% steroid-free CS, 20 μg/ml gentamicin, 2 mg/ml amphotericin B and 1 nM estradiol. The cells were cultured for 24 h and then provided for the following experiments.

Experiment 1: At the end of culture, membranes of epithelial cells were prepared. To evaluate the effects of estradiol on the concentration and affinity of OT receptors in bovine epithelial cells, radioreceptor assays were performed with the epithelial membranes.

Experiment 2: To evaluate the effect of estradiol on PGF2α production in response to OT in bovine endometrial epithelial cells, the culture media were replaced at 24 h with fresh DMEM/Ham's F-12 without phenol red and sodium bicarbonate containing 5 ng/ml sodium selenite, 0.5 mM ascorbic acid, 5 μg/ml transferrin, 20 μg/ml gentamicin, and 0.1% [w/v] BSA and the cells were cultured for 4 h with or without 100 nM OT. At the end of culture, the culture media were stored at –30 C until assays for PGF2α. The DNA contents were estimated by a spectrophotometric method as described previously[8]. DNA contents were used to standardize the results.

Membrane Preparation

The membranes of the cultured bovine epithelial cells were prepared as described previously[9].

Radioreceptor assay

$[d(CH_2)_5,Tyr(Me)^2,Thr^4,Tyr\text{-}NH_2^9]$-vasotocin was iodinated by a modified lactoperoxidase method[10]. Preliminary experiments to determine the conditions for the radioreceptor assay with bovine endometrial epithelial membranes were performed as described previously[11]. For the Scatchard analysis, the membranes were incubated with increasing concentrations of unlabeled OT and 20 000 cpm ^{125}I-OVT (0.8 nM). All incubations were performed in 155 μl assay buffer (mHBSS containing 5 mM $MgCl_2$ and 0.1% BSA, pH 7.5) for 30 min at 20 C. The incubation was terminated by transferring the tubes to ice-cold water and by adding the assay buffer to the tubes; bound- and free-tracers were separated by centrifugation at 3000 g for 40 min at 4 C. Supernatants were decanted immediately. The

pellets were counted for ^{125}I in a γ-counter.

PGF2α determination

Concentrations of PGF2α in the culture media were determined directly with an enzyme immunoassay as described previously[11].

Statistical Analysis

The data obtained on binding of ^{125}I-OVT to endometrial epithelium were analyzed with the LIGAND program[12] using non-linear iterative curve fitting procedures[13]. The data are shown as the mean±SEM. The statistical significance of differences between the meansof control and treated groups were assessed by Student's t-Test or an analysis of variance followed by Fisher's PLSD as a multiple comparison test. For the statistical analyses of differences in the specific binding of OT and PGF2α secretion, the percentages relative to the control were used.

Results and Discussion

The conditions for the radioreceptor assay on bovine epithelial membranes were initially validated. It was confirmed that maximal binding was reached after 30 min at 20 C [data not shown]. Therefore, further assays were performed for 30 min at 20 C. Specific binding increased with increasing protein concentrations. A linear relationship was established in the amount of binding from 1 to 100 μg protein/155 μl [data not shown]. Figure 1 shows the displacement curves of ^{125}I-OVT with three peptides related to OT and two peptides unrelated to OT. The binding was highly specific for OT.

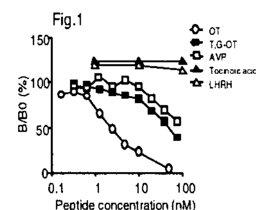

Fig. 1. Competitive binding to bovine epithelial membrane of ^{125}I-OVT and unlabeled OT (open circles), [Arg^8]-vasopresin (open squares), [Thr^4,Gly^7]-OT (solid squares), tocinoic acid (solid triangles), and LHRH (open triangles) on vovine endometrial epithelial membranes.

Scatchard plots of the binding data (^{125}I-OVT/OT) were linear [Fig. 2a]. Analysis with the LIGAND program revealed that the mean affinities (K_d) of the control and estradiol-treatment groups were 5.1±0.12 nM (n=3) and 4.8±0.12 (n=3) nM, respectively. The capacities of the binding sites (B_{max}) of the control and estradiol-treatment groups were 15.8±0.92 (n=3) and 34.7±3.85 (n=3) fmol/μg protein [Fig. 2b], respectively. The concentrations of OT receptor in the estradiol-treated epithelial cells were significantly higher than those in the control without any apparent changes in binding affinity [Fig. 2, $P<0.05$]. These results suggest that estradiol increases OT receptor concentration in the endometrial epithelium in cattle as suggested in ewes. Moreover, a 24 h

pretreatment with estradiol resulted in an increase of the PGF2α production in response to OT [Fig. 3, P<0.05].

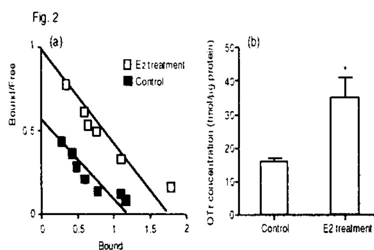

Fig. 2. (a) Representative Scatchard plots for competitive bindings of ^{125}I-OVT and unlabeled OT on bovine endometrial epithelial membranes obtained from the control or estradiol-treatment group. Each line represents the means of duplicate detaminations from one of three independent experiments. (b) Effect of estradiol on OT receptor concentration in bovine endometrial epithelial membranes (Mean ± SE, n=3). The value of the estradiol-treatment group is expressed as a percentage of the control. Asterisk indicates significant differences from the control (P<0.05), as determined by Student's t-Test.

The overall results suggest that estradiol plays a role as a regulator of PGF2α by means of modulating the OT receptor concentration in the bovine endometrial epithelium.

References

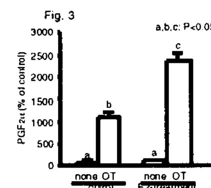

Fig. 3. Effect of estradiol on PGF2α secretion in response to OT by cultured bovine endometrial epithelial cells (Mean ± SE, n=3). The values of OT-treated control and estradiol-treatment group are expressed as a percentage of the non-treated control. Different superscript letters indicate significant differences (P<0.05), as determined by an analysis of variance followed by Fisher's PLSD as a multiple comparison test.

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