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Evaluation of Phospholipase C enzyme activity in goat endometrial cells

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ABSTRACT

The phospholipase C enzyme is now known to have has six isoforms i.e phospholipase C (γ, δ β, ε, ζ, η). Activation of phospholipase C results in the generation of inositol phosphates and increased intracellular calcium along with production of diacylglycerol. Diacylglycerol remains bound to the membrane, and IP_3 is released as a soluble structure into the cytosol. The posterior pituitary hormone vasopressin activates the various phospholipase enzymes including the phospholipase C. In this article the technique to estimate the phospholipase C activity in goat endometrial cells is described.

Keywords: phospholipase C, endometrial cells, IP_3, calcium

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INTRODUCTION

The two posterior hormones oxytocin and vasopressin are closely related and have emerged from a common ancestral gene. Their hormone receptors are expressed in the uterus [7]. All placental mammals produce mostly arginine vasopressin. The roles of these hormones in the goat uterus are not well defined. Vasopressin has some homology with oxytocin receptor [4]. Both oxytocin and vasopressin receptors are present in non-pregnant and pregnant myometrium [8]. The data of the present study are attributed to myometrium and contribution of AVP receptors to the binding of OT would be negligible [5, 6]. In this study the technique for estimating the phospholipase C activity in goat endometrial cells is described.

MATERIALS AND METHODS

Endometrium (40-50g) can be collected aseptically from one randomly selected uterine horn and placed in incomplete Hank's balanced salt solution. Goat Endometrial tissue can be extirpated immediately and obtained from a slaughter shop.

Cell culture:

Enriched populations of glandular epithelial, stromal and luminal epithelial cells were obtained using differential enzyme digestion and sieve separation as shown [1, 2]. Cellular viability at plating, determined by trypan blue exclusion, was approximately 95, 87 and 92 % for the enriched populations of stromal, glandular epithelial and luminal epithelial cells, respectively. Medium (RPMI-1640; Gibco BRL, Grand Island, NY) was supplemented with 20% fetal bovine serum (Gibco BRL) for luminal epithelial cells and with 8 % fetal bovine serum for glandular epithelial and stromal cells. Medium was replaced after 3 days with fresh medium containing 5% fetal bovine serum. On the next day, the medium was replaced with fresh serum-free medium and experiments were performed. The purity of the cell populations was determined by immunofluorescent staining for cytokeratin [1] and exceeded 93, 91 and 96 % for the glandular epithelial, stromal, and luminal epithelial cells, respectively.

Evaluation of Phospholipase C in goat endometrial cells:

Activity of phospholipase C in stromal, glandular epithelial and luminal epithelial cells was examined in response to treatment with control vehicle, (Sigma Chemical Co., St Louis, MO), 100 nmol lysine-vasopressin (Sigma), 200 nmol and 400 nmol arginine-vasopressin (Sigma) or for 30 min. Cells from 5 goats were used and each treatment was performed on duplicate wells for each cell type. Phospholipase C activity was measured by incorporation of [^{3}H]inositol into total inositol phosphates as described previously [1, 9]. In brief, the medium was replaced at the end of the 30 min treatment period with 2 ml ice-cold 15% (w/v) trichloroacetic acid and the cells were placed on ice for 25 min. The cell lysates were then collected and analysed for incorporation of [^{3}H]inositol into total inositol phosphates by anion exchange chromatography. Data for activity of phospholipase C were expressed as total [^{3}H]inositol phosphates (d.p.m. per

well). The radioactive gamma counter (Perkin Elmer) can be used to determine the radioactive counts.

Table 1: Effect of treatment with vehicle, arginine vasopressin and lysine vasopressin on glandular epithelial cells of goat endometrial cells

Pretreatment	Treatment	[^{3}H] IP_3 (d.p.m)
1) RPMI media	RPMI media	2455
2) RPMI media	AVP (200nM)	2790
3) RPMI media	AVP (400nM)	2910
4) RPMI media	LVP (100nM)	2523

DISCUSSION

The major contractant pathways in uterine smooth muscle target activation of phospholipase C (PLC), release of intracellular calcium and stimulation of calcium entry. Many uterine contractant receptors, including those for oxytocin, endothelin, prostaglandins (FP, EP1 and EP3 receptors), angiotensin and bradykinin utilize seven-transmembrane domain GTP binding protein (G-protein) coupled receptors that transmit signals into the cell via interaction with heterotrimeric G-proteins [16]. This article shows a novel technique to evaluate the phospholipase C activity in the goat endometrial cells after cell culture. There are not many studies on the goat endometrial cells although this tissue can be obtained easily from the slaughter house. The above mentioned article nicely describes the technique for culturing the cells from the endometrial cells. Previous studies conducted on pig endometrial cells showed that lysine vasopressin and arginine vasopressin did not appreciably increase the phospholipase C activity in the endometrial cells. A small study conducted by this author suggests that there is less effect of vasopressin analogues on phospholipase C activity in the endometrial cells of the goat (Table 1). This suggests that vasopressin receptors are not adequately coupled to the phospholipase C enzyme in these cells. Phospholipid metabolism is still under intensive scientific scrutiny and several studies are published regularly [14, 5, 13]. Vasopressin is coupled to the PI-3 kinase enzyme [10] and varied effects of vasopressin and its effects on physiology and gene expression have been studied extensively [12]. It seems that the glandular epithelial cells showed better response to the stimulation of phospholipase C as compared to the stromal and luminal epithelial cells. Thus in summary this article describes a novel method for estimating the phospholipase C activity in goat endometrial cells.

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