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Possible involvement of oxytocin and its receptor in the local regulation of prostaglandin secretion in the cat endometrium

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ABSTRACT

Ovarian originated oxytocin (OT) is involved in several reproductive process, amongst them its role in the regulation/modulation of the estrous cycle in several species has been demonstrated. Although the systemic role of endometrial originated prostaglandins (PGs), especially prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), is equivocal in cats, their possible involvement in the local regulation of uterine events during the estrous cycle is uncertain. We examined the spontaneous and LH-stimulated OT production in cultured luteal cells, the spatial and temporal arrangement of OT receptors (OTR) in a cat endometrium and, finally the effects of OT on PG secretion and prostaglandin-endoperoxide synthase (PTGS2) expression in the feline cultured endometrial cells. Uteri together with ovaries were collected from adult domestic cats ($n = 27$) at different stages of the estrous cycle, after routine ovariohisterectomy procedures. The endometrial and luteal cells were separated enzymatically. Luteinizing hormone (LH) augmented OT secretion in cultured luteal cells 2-fold compared with control ($P < 0.05$). Oxytocin receptor was abundantly expressed in different ovarian structure, as well as in uterine tissues collected at early/developing and mid-luteal phase. The secretion of $PGF_{2\alpha}$ by endometrial epithelial cells was increased by OT at a dose 10^{-7} M ($P < 0.001$). Atosiban (specific OTR blocker) alone did not affect PG secretion but atosiban in combination with OT abolished the stimulating effect of OT on $PGF_{2\alpha}$ secretion. Oxytocin augmented PGE_2 secretion at a dose 10^{-7} M and 10^{-6} M in the endometrial stromal cells ($P < 0.001$). The treatment with atosiban did not abrogated positive effect of OT on PGE_2 production in the stromal cells. Effect of OT on *PTGS2* mRNA expression, the rate-limiting enzyme in PG production, was examined by Real Time-PCR and *PTGS2* mRNA expression was significantly affected by OT in both epithelial and stromal cell cultures ($P < 0.01$). The present observations have shown that OT is locally produced by the early/developing corpora lutea and that corpora lutea delivered OT may regulate PG secretion in a cat endometrium especially at early- and mid-diestrus, by affecting *PTGS2* mRNA expression.

1. Introduction

Apart from involvement in labor induction and lactation, oxytocin (OT) also plays role in the social behavior and the estrous cycle (Gimp and Fahrenholz, 2001). Oxytocin is a nonapeptid that acts as a neurotransmitter and peripheral or as a local hormone. It is synthesized and secreted

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mainly by paraventricular nucleus in the hypothalamus, but significant amounts of this hormone are produced locally in the ovaries (Flint and Sheldrick, 1982; Berisha and Schams, 2005). Oxytocin receptor (OTR) belongs to the rhodopsin-like superfamily of G protein coupled receptors (GPCRs) (Gimpl et al., 2008). The OTR couples to $G\alpha_q$ and $G\alpha_i$ proteins (Strakova and Soloff, 1997) that activate together with $G\beta_\gamma$ the phospholipase C-β isoforms (Gimpl et al., 2008). Consequently the intracellular calcium concentration increases and activates several protein kinases finally inducing secretion of prostaglandins (PGs) (Gimp and Fahrenholz, 2001; Gimpl et al., 2008).

Oxytocin-mediated changes of PG secretion have been shown in the endometrium of cow (Asselin et al., 1996; Okuda et al., 2002), sheep (Sheldrick and Flint, 1984) and pig (Blitek and Ziecik, 2004; Franczak and Bogacki, 2009) and extra- and intragonadal OT has been shown to trigger prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release by the endometrial epithelial cells at the end of the luteal phase in ruminants (Silvia et al., 1991). More recently it was shown that OT plays only the secondary role in regression of CL in the cow (Hansel and Blair, 1996). In the pseudopregnant rat, OT has been demonstrated to mediate luteolytic activity, via uterine $PGF_{2\alpha}$ (Cao and Chan, 1993) and this effect was mediated through OTR, since it was blocked by a specific OTR antagonist (Motta et al., 1996). In contrast to ungulates, the life-span of the CL in the pseudopregnant cat seems not to be dependent on the uterine factors (Wheeler et al., 1988), and similarly has been observed in nonpregnant dog in which hysterectomy did not disturbed normal ovarian function (Hoffmann et al., 1992). Based on the data collected so far, in nonpregnant bitch, luteolysis seems to be rather a permissive process than an actively regulated one (Hoffmann et al., 2004; Kowalewski et al., 2008).

In cats exogenous OT is advocated as a medical management of dystocia in order to induce myometrial contractions (Pretzer, 2008). However, either the local synthesis and possible involvement of OT in the life-span of CL or affecting of the endometrial PG synthesis for the regulating of myometrium contractility and uterine vascularity remains uncertain in this species. To check whether OT is involved in the modulation/regulation of several reproductive events in sexually active, non-pregnant cat, was investigated: (1) spontaneous and LH-stimulated OT production in cultured luteal cells and spatial arrangement of OT receptor (OTR) in a cat ovary collected at diestrus (luteal phase), (2) spatial and temporal arrangement of OTR in a cat endometrium, (3) the effects of OT on $PGF_{2\alpha}$ and PGE_2 secretion by feline cultured endometrial cells, especially focusing on (4) the effects of OT on prostaglandin-endoperoxide synthase (PTGS2) expression which is the rate-limiting enzyme for PG production.

2. Materials and methods

All procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (No. 41/2007/N). All materials used were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA), unless otherwise stated.

2.1. Animals and collection of uterine tissue

Uteri together with ovaries and oviducts were collected from adult domestic cats ($n = 27$), at estrus, early/developing (Days 10–12), mid-luteal (Days 15–20) and late-luteal (Days 30–35) estrous phase after routine ovariohysterectomy procedures at the local veterinary clinic in Olsztyn, Poland, and Okayama, Japan, the uteri were transported to the laboratory within 1 hour (h) after surgery in 0.9% NaCl in ice bath. The stage of the estrous cycle was confirmed by the macroscopic observation of the ovaries and uterus as described previously (Karja et al., 2002) and by progesterone measurement in the circulating blood. Day 0 means estrus onset monitored clinically.

2.2. Isolation and culture of luteal cells

Corpora lutea ($n = 10$) were removed from ovaries of three queens at the early/developing luteal stage (10–12 days after the estrus onset), and diced with scalpel blade. Tissues from each animal was pooled and stirred for 60 min in Medium 199 containing collagenase IA (1.5 mg/ml) and 0.005% DNAse I, then filtered by nylon mesh (150 and 80 μm) to remove undissociated tissue fragments as described previously (Miyamoto et al., 2002). The crude suspension of luteal cells was washed three times by centrifugation for 10 min at $1000 \times g$ with Medium 199. The cells were counted in haemocytometer and assessed for viability by 0.05% trypan blue exclusion. The cell viability exceed 85% and purified luteal cells were suspended (2×10^5/ml) in 48-well culture plate in DMEM/Ham's F-12 (D/F) with 10% fetal calf serum (FCS) without phenol red and cultured for up to 48 h in a humidified atmosphere of 5% CO_2 in air at 37.5 °C (Hereaus BB6060, Hanau, Germany). Incubation medium was supplemented additionally with ascorbic acid (20 μg/ml), transferrin (5 μg/ml) and sodium selenite (5 ng/ml; ICN Pharmaceuticals, City, USA). All media contained gentamycin (20 μg/ml; ICN Pharmaceuticals, USA).

2.3. Isolation and culture of endometrial epithelial and stromal cells

Both epithelial and stromal cells from feline endometrium were enzymatically separated as described previously (Siemieniuch et al., 2010). In short, the enzymatic solution consisting of collagenase IA (0.05%), deoxyribonuclease (DNase I, 0.005%) and dispase (0.05%) was used for the epithelial cells isolation in a peristaltic pump system (Siemieniuch et al., 2010). After collection of the epithelial cells, the endometrial fragments were minced into small pieces (approximately 1 mm^3) and digested by stirring for 60 min in 50 ml of sterile Hanks' Balanced Salt Solution (HBSS) containing collagenase and DNase I for isolation of the stromal cells. The dissociated cells were then filtered through nylon mesh (100 and 80 μm) to remove undissociated tissue fragments. The cells were seeded at a density of 2×10^5 viable cells/ml in 75-cm^2 culture flasks (Greiner Bio-One, Monroe, NC) and were cultured at 37.5 °C in a humidified atmosphere of 5% CO_2 in air. All procedures in respect to cell culture