Effects of arginine- and lysine-vasopressin on phospholipase C activity, intracellular calcium concentration and prostaglandin F_{2α} secretion in pig endometrial cells

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Oxytocin and vasopressin are related peptides that have receptors in the uterus. Species from families other than Suidae produce only arginine-vasopressin; in contrast, pigs apparently express both arginine- and lysine-vasopressin. The aim of this study was to determine whether arginine- or lysine-vasopressin would activate phospholipase C, increase intracellular calcium concentration [Ca²⁺]_i and stimulate PGF_{2α} production in enriched cultures of stromal, glandular epithelial and luminal epithelial cells from pig endometrium. Cells were obtained from gilts on day 16 after oestrus by differential enzymatic digestion and sieve separation. After 96 h in culture, the cells were treated with 0 or 100 nmol arginine- or lysine-vasopressin l⁻¹. The responses to 100 nmol oxytocin l⁻¹ and 100 nmol GnRH l⁻¹ were used as positive and negative controls, respectively. Consistent with previous results, oxytocin stimulated phospholipase C activity ($P < 0.05$), increased [Ca²⁺]_i ($P < 0.05$) and promoted PGF_{2α} secretion ($P < 0.05$) from stromal and glandular epithelial cells. Activity of phospholipase C, [Ca²⁺]_i and PGF_{2α} release were also increased ($P < 0.05$) by arginine-vasopressin in stromal

cells, but the responses were less ($P < 0.01$) than those induced by oxytocin. An oxytocin antagonist attenuated the [Ca²⁺]_i response of stromal cells to both oxytocin and arginine-vasopressin. Sequential treatment of cells with oxytocin and arginine-vasopressin indicated that oxytocin desensitized the response to oxytocin, but arginine-vasopressin did not similarly desensitize the response to oxytocin. In glandular and luminal epithelial cells, arginine-vasopressin did not stimulate phospholipase C activity, [Ca²⁺]_i or PGF_{2α} secretion. Neither GnRH nor lysine-vasopressin induced phospholipase C activity, increased [Ca²⁺]_i or stimulated PGF_{2α} production in any endometrial cell type. These results indicate that oxytocin receptors can bind arginine-vasopressin more readily than they bind lysine-vasopressin. Type 1 vasopressin receptors may also exist in endometrium predominantly on cells other than stromal, glandular epithelial and luminal epithelial cells, as in previous studies both arginine-vasopressin and lysine-vasopressin stimulated phospholipase C activity in endometrial explants to a similar extent as oxytocin.

Introduction

Oxytocin and vasopressin are two closely related neurohypophyseal nonapeptides that have emerged from a common ancestral gene (Acher *et al.*, 1995; Conklin *et al.*, 1999) and both have receptors in the uterus (Maggi *et al.*, 1997). The roles of these hormones in the pig uterus are still unclear. The common ancestral peptide is arginine-

vasotocin (Acher *et al.*, 1995) and the mechanisms regulating the physiological responses to arginine-vasotocin and oxytocin appear to be conserved across classes of vertebrate. For example, arginine-vasotocin activates phospholipase C in the uterus of two species of reptile and the response develops similarly near the time of oviposition or parturition (Mirando and Guillelte, 1991). Oviposition in birds is also associated with increased concentrations of arginine-vasotocin and PGF_{2α} in the peripheral circulation and the induction of oviposition by arginine-vasotocin in birds may be mediated by secretion of prostaglandins (Olson *et al.*, 1986; Saito *et al.*, 1987). These mechanisms appear to be similar to those commonly shared among mammalian species during parturition (Fuchs *et al.*, 1995; López Bernal *et al.*, 1995).

In addition to its role in parturition (Jenkins and Nussey, 1991; Fuchs *et al.*, 1995; López Bernal *et al.*, 1995),

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oxytocin is also involved in regulation of the oestrous cycle of domestic ungulates by stimulating release of the uterine luteolysin, $\text{PGF}_{2\alpha}$ (Silvia *et al.*, 1991; Mirando *et al.*, 1996; McCracken *et al.*, 1999). Oxytocin induces pulsatile secretion of $\text{PGF}_{2\alpha}$ during late dioestrus in sheep (Hooper *et al.*, 1986), an event that is required to promote luteolysis (Schramm *et al.*, 1983; Zarco *et al.*, 1988). In pigs, endometrial responsiveness to oxytocin develops before luteolysis is initiated during late dioestrus (Carnahan *et al.*, 1996; Edgerton *et al.*, 1996). Responsiveness is maximal in stromal cells, intermediate in glandular epithelial cells and not evident in luminal epithelial cells (Uzumcu *et al.*, 1998; Braileanu *et al.*, 1999), although this cellular pattern of response to oxytocin is opposite to that which occurs in endometrium from mares and cows (Watson *et al.*, 1992; Asselin *et al.*, 1996).

All placental mammals from families other than Suidae apparently produce only arginine-vasopressin. In contrast, pigs are reported to express arginine-vasopressin in the corpus luteum (Pitzel *et al.*, 1984; Choy and Watkins, 1988) and lysine-vasopressin in the central nervous system (Popenoe *et al.*, 1952; Heller, 1960). Structurally, lysine-vasopressin and arginine-vasopressin differ only in possessing lysine or arginine, respectively, at position 8; oxytocin has leucine at position 8 and isoleucine in position 3 (Heller, 1960). Type one vasopressin (V_1) receptors are apparently present in pig endometrium, as arginine-vasopressin and lysine-vasopressin induce phospholipase C activity in endometrial explants, although neither peptide promotes $\text{PGF}_{2\alpha}$ secretion (Whiteaker *et al.*, 1994; Ludwig *et al.*, 1998). However, it is not known which cell types are responsive to arginine- and lysine-vasopressin. Therefore, the aim of the present study was to determine the effects of lysine- and arginine-vasopressin on phospholipase C activity, $[\text{Ca}^{2+}]_i$ and $\text{PGF}_{2\alpha}$ secretion in enriched populations of stromal, glandular epithelial and luminal epithelial cells collected from pig endometrium on day 16 of the oestrous cycle.

Materials and Methods

Animals

Peripubertal crossbred gilts (Yorkshire, Landrace, Large White, Duroc and Hampshire) were observed daily for standing oestrous behaviour in the presence of an intact boar and onset of second oestrus was designated as day 0. Hysterectomy was performed on day 16 after second or third oestrus as described by Whiteaker *et al.* (1994) and Ludwig *et al.* (1998). Endometrium (20–25 g) was collected aseptically from one randomly selected uterine horn and placed in incomplete Hank's balanced salt solution.

Cells

Enriched populations of stromal, glandular epithelial and luminal epithelial cells were obtained using differential enzyme digestion and sieve separation as described by

Zhang *et al.* (1991) and modified subsequently by Uzumcu *et al.* (1998) and Braileanu *et al.* (1999). Cellular viability at plating, determined by trypan blue exclusion, was approximately 97, 86 and 93% 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 10% 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 (Uzumcu *et al.*, 1998) and exceeded 95, 90 and 97% for the stromal, glandular epithelial and luminal epithelial cells, respectively.

Experiment 1

Activity of phospholipase C in stromal, glandular epithelial and luminal epithelial cells was examined in response to treatment with control vehicle, 100 nmol oxytocin l^{-1} (Sigma Chemical Co., St Louis, MO), 100 nmol arginine-vasopressin l^{-1} (Sigma), 100 nmol lysine-vasopressin l^{-1} (Sigma) or 100 nmol GnRH l^{-1} (Bachem California, Torrance, CA) for 30 min. Cells from seven gilts were used and each treatment was performed on duplicate wells for each cell type. Oxytocin and GnRH served as positive and negative controls, respectively. Phospholipase C activity was measured by incorporation of $[\text{^3H}]$ inositol into total inositol phosphates as described previously (Whiteaker *et al.*, 1994; Ludwig *et al.*, 1998; Uzumcu *et al.*, 1998). In brief, the medium was replaced at the end of the 30 min treatment period with 1 ml ice-cold 15% (w/v) trichloroacetic acid and the cells were placed on ice for 20 min. The cell lysates were then collected and analysed for incorporation of $[\text{^3H}]$ inositol into total inositol phosphates by anion exchange chromatography. Data for activity of phospholipase C were expressed as total $[\text{^3H}]$ inositol phosphates (d.p.m. per well).

Experiment 2

In Expt 2, $[\text{Ca}^{2+}]_i$ was measured in individual stromal, glandular epithelial and luminal epithelial cells in response to 100 nmol oxytocin l^{-1} , 100 nmol arginine-vasopressin l^{-1} or 100 nmol lysine-vasopressin l^{-1} . The cells were washed with serum-free RPMI-1640 and loaded with fluorescent Ca^{2+} indicator, Fura-2 am ($1 \mu\text{mol l}^{-1}$) (Molecular Probes, Eugene, OR) in buffer (140 mmol NaCl l^{-1} , 5 mmol KCl l^{-1} , 2 mmol $\text{CaCl}_2 \text{l}^{-1}$, 1 mmol $\text{MgCl}_2 \text{l}^{-1}$, 10 mmol Hepes l^{-1} and 6 mmol glucose l^{-1} , pH 7.4) containing 0.04% (v/v) pluronic acid F-127 (Molecular Probes) for 30 min at room temperature. The cells were washed with buffer for an additional 30 min before quantification of $[\text{Ca}^{2+}]_i$ as described by Braileanu *et al.* (1999). Buffer or oxytocin, arginine-vasopressin or lysine-vasopressin dissolved in buffer was administered at a constant flow rate of 5 ml min^{-1} . Treatments were performed

at room temperature and consisted of 90 s pulses that were applied at different intervals. Cells from five different pigs were used and 1–3 coverslips (≥ 15 cells per coverslip) per pig were analysed for each cell type. Pairs of images with excitation light at 340 nm and 380 nm and emission light at 510 nm were taken every 6 s with a CCD camera and analysed using the MetaFluor Imaging System Software (Universal Imaging Corp., West Chester, PA). Images were corrected for background fluorescence and the ratio values from individual cells within the image were converted to $[Ca^{2+}]_i$ using a standard curve generated in 150 mmol KCl/Hepes buffer I^{-1} (pH 7.4) using EGTA and $CaCl_2$. Fluorescent image intensities during oxytocin exposure were monitored periodically to ensure that $[Ca^{2+}]_i$ changes were due to reciprocal changes in the fluorescent intensities at 340 and 380 nm.

Experiment 3

$PGF_{2\alpha}$ secretion from stromal, glandular epithelial and luminal epithelial cells was measured in response to treatment for 3 h with control vehicle, 100 nmol oxytocin I^{-1} , 100 nmol arginine-vasopressin I^{-1} , 100 nmol lysine-vasopressin I^{-1} or 100 nmol GnRH I^{-1} . Cells from ten gilts were used and each treatment was performed in quadruplicate for each cell type. Concentrations of $PGF_{2\alpha}$ in 25 μ l medium were quantified by radioimmunoassay as described by Whiteaker *et al.* (1994) and Uzumcu *et al.* (1998). Intra- and interassay coefficients of variation were 6.4 and 16.5%, respectively.

Experiment 4

Stromal cells from five gilts were treated with 0, 33, 100 and 333 nmol arginine-vasopressin I^{-1} for 3 h to confirm the effects of 100 nmol arginine-vasopressin I^{-1} on phospholipase C activity and $PGF_{2\alpha}$ secretion by stromal cells that were observed in Expts 1 and 3, respectively. Cellular phospholipase C activity and $PGF_{2\alpha}$ secretion were determined as described for Expts 1 and 3, respectively.

Experiment 5

Stromal cells from four gilts were treated with 100 nmol oxytocin I^{-1} or 100 nmol arginine-vasopressin I^{-1} in the presence or absence of 100 nmol oxytocin antagonist I^{-1} (L-366,948; Merck, Sharp and Dohme Research Laboratories, West Point, PA) to investigate whether the response of stromal cells to arginine-vasopressin was mediated through oxytocin receptors. $[Ca^{2+}]_i$ was measured as described for Expt 2. Exposure to oxytocin antagonist was initiated 90 s before oxytocin or arginine-vasopressin treatment and continued for the duration of agonist treatment.

Stromal cells from these same gilts were exposed sequentially to oxytocin and arginine-vasopressin to determine whether prior exposure to one peptide desensitized the response to the other peptide. Cells were treated with 100 nmol oxytocin I^{-1} for 60 s and were treated

subsequently with 100 nmol arginine-vasopressin I^{-1} for 90 s starting 120 s after removal of oxytocin. Additional wells of cells were treated first with 100 nmol arginine-vasopressin I^{-1} for 60 s and then with 100 nmol oxytocin I^{-1} for 60 s starting 90 s after removal of arginine-vasopressin.

Statistical analyses

Data from all experiments were subjected to least squares ANOVA using the General Linear Models procedure of the Statistical Analysis System (SAS System for Windows 6.12, SAS Institute Inc., Cary, NC). Preplanned comparisons were performed using the PDIF option within the LSMEANS statement of the General Linear Models procedure. Least squares means and appropriate standard errors were generated from the ANOVA using the LSMEANS statement.

Results

Experiment 1

Oxytocin increased phospholipase C activity in stromal cells ($P < 0.01$, Fig. 1a) and glandular epithelial cells ($P < 0.05$, Fig. 1b), but not in luminal epithelial cells (Fig. 1c). In stromal cells, arginine-vasopressin increased ($P < 0.05$) phospholipase C activity (Fig. 1a), but the increase was less ($P < 0.01$) than that for oxytocin. Activity of phospholipase C was not increased by arginine-vasopressin in either glandular or luminal epithelial cells (Fig. 1b,c, respectively). Neither lysine-vasopressin nor GnRH altered phospholipase C activity in any cell type.

Experiment 2

In stromal cells, oxytocin induced a synchronous and transient increase in $[Ca^{2+}]_i$ ($P < 0.01$) in 95% of the cells analysed ($n = 150$ total cells, Fig. 2a). Administration of arginine-vasopressin to stromal cells increased $[Ca^{2+}]_i$ ($P < 0.01$); however, the mean amplitude of the peak and the proportion of cells responding were less ($P < 0.01$) than that for oxytocin. Treatment with lysine-vasopressin did not alter $[Ca^{2+}]_i$ in stromal cells (Fig. 2a). The effects of arginine-vasopressin and lysine-vasopressin were similar regardless of the order in which treatments were administered (Fig. 2b). In glandular epithelial cells, oxytocin induced a smaller increase in $[Ca^{2+}]_i$ (Fig. 2c) and did not influence $[Ca^{2+}]_i$ in luminal epithelial cells (data not shown). Neither arginine-vasopressin nor lysine-vasopressin increased $[Ca^{2+}]_i$ in glandular (Fig. 2c) or luminal epithelial cells (data not shown).

Experiment 3

Oxytocin increased $PGF_{2\alpha}$ secretion from stromal cells ($P < 0.05$) and glandular epithelial cells ($P = 0.06$) but not from luminal epithelial cells (Fig. 3a,b,c, respectively). For stromal cells, arginine-vasopressin also stimulated ($P < 0.05$) $PGF_{2\alpha}$ secretion (Fig. 3a). However, arginine-vasopressin did

Fig. 1. The effect of treatment with 100 nmol oxytocin I^{-1} , 100 nmol arginine-vasopressin I^{-1} (AVP), 100 nmol lysine-vasopressin I^{-1} (LVP) or 100 nmol GnRH I^{-1} on phospholipase C activity, as determined by accumulation of [³H]inositol phosphates, in (a) stromal cells, (b) glandular epithelial cells and (c) luminal epithelial cells from endometrium of seven cyclic gilts on day 16 after oestrus in Expt 1. Asterisks indicate that treatment stimulated phospholipase C activity compared with the control (* $P < 0.05$ and ** $P < 0.01$).

not alter $PGF_{2\alpha}$ release from either glandular or luminal epithelial cells (Fig. 3b,c, respectively). Neither lysine-vasopressin nor GnRH altered $PGF_{2\alpha}$ secretion from any cell type.

Experiment 4

Oxytocin increased phospholipase C activity ($P < 0.01$) and $PGF_{2\alpha}$ secretion ($P < 0.05$) in stromal cells (Fig. 4a,b, respectively). Activity of phospholipase C and $PGF_{2\alpha}$ secretion were increased by arginine-vasopressin in a concentration-dependent manner; phospholipase C activity was stimulated by 33 nmol arginine-vasopressin I^{-1} ($P < 0.05$), 100 nmol arginine-vasopressin I^{-1} ($P < 0.01$) and 333 nmol arginine-vasopressin I^{-1} ($P < 0.01$), but the

Fig. 2. The effect of treatment with 100 nmol oxytocin I^{-1} , 100 nmol arginine-vasopressin I^{-1} (AVP) and 100 nmol lysine-vasopressin I^{-1} (LVP) on intracellular free calcium concentration ($[Ca^{2+}]_i$) in (a,b) individual stromal cells and (c) glandular epithelial cells from endometrium of seven cyclic gilts on day 16 after oestrus in Expt 2. Each line represents the results for an individual cell and the results are from one representative experiment. In stromal cells, arginine-vasopressin increased $[Ca^{2+}]_i$ ($P < 0.01$), but the response was less ($P < 0.01$) than that for oxytocin. Only oxytocin increased ($P < 0.05$) $[Ca^{2+}]_i$ in glandular epithelial cells.

increases at all the concentrations of arginine-vasopressin examined were less than those promoted by oxytocin ($P < 0.05$) (Fig. 4a). Secretion of $PGF_{2\alpha}$ was stimulated by 100 nmol arginine-vasopressin I^{-1} ($P < 0.06$) and 333 nmol arginine-vasopressin I^{-1} ($P < 0.05$), but the increase resulting from 100 nmol arginine-vasopressin I^{-1} was less ($P < 0.05$) than that promoted by 100 nmol oxytocin I^{-1} (Fig. 4b).

Experiment 5

Exposure of stromal cells to oxytocin increased $[Ca^{2+}]_i$ ($P < 0.01$; 902 ± 52 nmol I^{-1} , $n = 70$ total cells; Fig. 5a),

Fig. 3. The effect of treatment with 100 nmol oxytocin l⁻¹, 100 nmol arginine-vasopressin l⁻¹ (AVP), 100 nmol lysine-vasopressin l⁻¹ (LVP) or 100 nmol GnRH l⁻¹ on PGF_{2α} secretion by (a) stromal cells, (b) glandular epithelial cells and (c) luminal epithelial cells from endometrium of ten cyclic gilts on day 16 after oestrus in Expt 3. Asterisks indicate that oxytocin or arginine-vasopressin stimulated PGF_{2α} secretion (**P* < 0.05). (^a*P* = 0.06.)

whereas the increase in [Ca²⁺]_i caused by arginine-vasopressin (*P* < 0.05; 407 ± 39 nmol l⁻¹, *n* = 83 total cells; Fig. 5c) was less (*P* < 0.05) than that caused by oxytocin. In the presence of the oxytocin antagonist L-366,968, the [Ca²⁺]_i response to oxytocin was significantly reduced (*P* < 0.05; 338 ± 49 nmol l⁻¹; Fig. 5a). The effect of the oxytocin antagonist was reversible as the oxytocin response recovered after the antagonist was washed from the cells (Fig. 5a). The oxytocin antagonist also reduced the [Ca²⁺]_i response to arginine-vasopressin (*P* < 0.05; 34 ± 9 nmol l⁻¹, *n* = 34 total cells; Fig. 5b). When cells were challenged with arginine-vasopressin within 2 min after a challenge to oxytocin, the [Ca²⁺]_i response to arginine-vasopressin was virtually eliminated (*P* < 0.05; 19 ± 5 nmol l⁻¹, *n* = 29 total cells; Fig. 6a). However, exposure of stromal cells to

Fig. 4. Effect of treatment with 100 nmol oxytocin l⁻¹ or 10–333 nmol arginine-vasopressin (AVP) l⁻¹ on (a) phospholipase C activity, as determined by accumulation of [3H]inositol phosphates, and (b) PGF_{2α} secretion by stromal cells collected from cyclic gilts on day 16 after oestrus in Expt 4. Asterisks indicate that oxytocin or arginine-vasopressin stimulated phospholipase C activity or PGF_{2α} secretion (**P* < 0.05 and ***P* < 0.01). (^a*P* < 0.06.)

arginine-vasopressin before oxytocin did not significantly affect the calcium response to oxytocin (856 ± 64 nmol l⁻¹, *n* = 41 total cells; Fig. 6b).

Discussion

Consistent with previous results, oxytocin stimulated phospholipase C activity, increased [Ca²⁺]_i and enhanced PGF_{2α} secretion in stromal cells and, to a lesser degree, in glandular epithelial cells (Uzumcu *et al.*, 1998; Braileanu *et al.*, 1999). Of the three populations of endometrial cells examined, only stromal cells were responsive to arginine-vasopressin. In these cells, arginine-vasopressin increased phospholipase C activity, [Ca²⁺]_i and PGF_{2α} secretion. In contrast, stromal cells were completely unresponsive to lysine-vasopressin. Neither arginine-vasopressin nor lysine-vasopressin stimulated phospholipase C activity, intracellular [Ca²⁺]_i or PGF_{2α} secretion in either glandular or luminal epithelial cells. These results indicate that oxytocin receptors on stromal cells of pig endometrium can recognize arginine-vasopressin but not lysine-vasopressin.

Receptors for oxytocin and vasopressin have been cloned and shown to be highly homologous members of a subfamily within the superfamily of G protein-linked receptors (Zingg, 1996). Oxytocin receptors and phospholipase C-linked V₁ receptors are present together in several tissues, including the

Fig. 5. The effect of oxytocin antagonist L-366,968 (100 nmol l⁻¹) on response of intracellular free calcium concentration ($[Ca^{2+}]_i$) to (a) 100 nmol oxytocin l⁻¹ and (b,c) 100 nmol arginine-vasopressin l⁻¹ (AVP) in stromal cells from endometrium of four cyclic gilts on day 16 after oestrus in Expt 5. Each line represents the results for an individual cell and the results are from one representative experiment. Treatment with L-366,968 reduced ($P < 0.05$) the responses to oxytocin and arginine-vasopressin.

uterus (Chen *et al.*, 1999), although information regarding the spatial and temporal expression of V_1 receptors in the endometrium of ungulates is currently unavailable. However, three lines of evidence from the present results support the contention that the actions of oxytocin and arginine-vasopressin on pig endometrial cells occur through specific oxytocin receptors, rather than V_1 receptors. Firstly, the $[Ca^{2+}]_i$ response to arginine-vasopressin was attenuated markedly by the selective oxytocin antagonist L-366,968. The response to oxytocin was also reduced by L-366,968, an effect that was reversible. Secondly, oxytocin completely desensitized the response to arginine-vasopressin when arginine-vasopressin was applied within 2 min of oxytocin treatment. Similarly, oxytocin completely desensitized the response to subsequent oxytocin treatments that occurred within 5 min of the first exposure (Braileanu *et al.*, 1999). In contrast, oxytocin and angiotensin II were unable to desensitize the response to each other but did desensitize their own response in a homologous manner (Braileanu *et al.*, 1998). These results indicate that oxytocin and arginine-vasopressin acted through the same receptors, whereas oxytocin and angiotensin II acted through two distinct receptor populations. Finally, stromal cells were responsive to arginine-vasopressin but were completely unresponsive to

Fig. 6. The effect of sequential treatment with (a) 100 nmol oxytocin l⁻¹ and 100 nmol arginine-vasopressin l⁻¹ (AVP) or (b) 100 nmol oxytocin l⁻¹ and 100 nmol arginine-vasopressin l⁻¹ on intracellular free calcium concentration ($[Ca^{2+}]_i$) in stromal cells from endometrium of four cyclic gilts on day 16 after oestrus in Expt 5. Each line represents the results for an individual cell and the results are from one representative experiment. Administration of oxytocin completely abolished ($P < 0.05$) the response to subsequent treatment with arginine-vasopressin, but arginine-vasopressin did not reduce the response to subsequent treatment with oxytocin.

lysine-vasopressin. It seems extremely unlikely that stromal cells possess V_1 receptors, which can discriminate between the subtle structural difference between arginine-vasopressin and lysine-vasopressin, as lysine-vasopressin is the principal vasopressin molecule secreted in pigs (Heller, 1960). Such an ability of V_1 receptors to discriminate between lysine-vasopressin and arginine-vasopressin would prevent lysine-vasopressin from activating these receptors in other tissues as well. Collectively, these results indicate that arginine-vasopressin acted through oxytocin receptors in the present study.

Previous studies with endometrial explants in pigs showed that lysine-vasopressin and arginine-vasopressin increased phospholipase C activity to a similar extent as did oxytocin, but did not increase $PGF_{2\alpha}$ secretion (Whiteaker *et al.*, 1994; Ludwig *et al.*, 1998). In the present study, arginine-vasopressin stimulated phospholipase C activity, intracellular $[Ca^{2+}]_i$ and $PGF_{2\alpha}$ secretion in stromal cells only, whereas all cell types were completely unresponsive to lysine-vasopressin. These apparently conflicting results can be reconciled readily by the facts that stromal cells comprise only a portion of the cells in the endometrium and that V_1 receptors may be present on cells other than stromal cells, such as blood vessel endothelial cells (Conklin *et al.*, 1999; Thibonnier *et al.*, 1999). Alternatively, the inability to detect an arginine-vasopressin-induced increase in $PGF_{2\alpha}$ secretion in previous studies (Ludwig *et al.*, 1998) is probably because arginine-vasopressin-responsive stromal cells represent only a portion of the endometrium and glandular and luminal epithelial cells secrete at least twice as much $PGF_{2\alpha}$ per cell as do stromal cells (Uzumcu *et al.*, 1998). Therefore, the high basal secretion of $PGF_{2\alpha}$ from

arginine-vasopressin-unresponsive cells in endometrial explants may have obscured the stimulatory effect of arginine-vasopressin on enriched stromal cells that was detected in the present study.

All effects of arginine-vasopressin on stromal and glandular epithelial cells in the present study were considerably less than the effects of oxytocin. This finding is probably attributable to the documented crossreactivity of arginine-vasopressin with oxytocin receptors (Whiteaker *et al.*, 1994; Rahmani *et al.*, 1997; Chen *et al.*, 1999) rather than through an action on V_1 receptors, as discussed previously. However, stromal and glandular epithelial cells were completely unresponsive to the concentration of lysine-vasopressin (100 nmol l^{-1}) that was used in the present study. Similarly, oxytocin was reported to be 50–75 times more potent than lysine-vasopressin in activating oxytocin receptors on pig myometrium (Yu *et al.*, 1995). Thus, the evolution of lysine-vasopressin in pigs appears to provide increased selectivity of oxytocin receptors for oxytocin over lysine-vasopressin compared with their relative inability to discriminate between oxytocin and arginine-vasopressin in other mammalian species.

Although the roles of lysine-vasopressin and arginine-vasopressin at the central or circulatory level are established (for example, vasoconstriction, liver glycogenolysis, platelet adhesion, adrenal secretion of angiotensin II and certain brain functions), it is not clear what roles they play within the uterus. In myometrium, vasopressin may mediate contractility (Yu *et al.*, 1995; Åkerlund *et al.*, 1998), whereas in endometrium it may mediate the release of $\text{PGF}_{2\alpha}$ or other uterotonic substances, such as endothelin (Maggi *et al.*, 1997). For example, arginine-vasopressin increased $\text{PGF}_{2\alpha}$ secretion from cultured vascular smooth muscle cells (Tahara *et al.*, 1997). The results of the present study indicate that arginine-vasopressin may also promote $\text{PGF}_{2\alpha}$ production in stromal cells of pig endometrium although the physiological role of arginine-vasopressin in this tissue is not clear. Regardless, the possibility that vasopressin acts as an antagonist of oxytocin action in pig endometrium was excluded because 100 nmol lysine-vasopressin l^{-1} did not reduce oxytocin-stimulated phospholipase C activity in endometrial explants; on the contrary, the stimulatory actions of lysine-vasopressin and oxytocin when applied together were additive (Ludwig *et al.*, 1998). Similarly, ongoing studies have indicated that $1 \mu\text{mol}$ lysine-vasopressin l^{-1} did not antagonize the action of 100 nmol oxytocin l^{-1} in endometrial explants from pigs (J. Hu, T. E. Ludwig and M. A. Mirando, unpublished).

In ruminants, endometrial expression of oxytocin receptors increases markedly during late dioestrus (Roberts *et al.*, 1976; Ayad *et al.*, 1991; Mirando *et al.*, 1993; Stevenson *et al.*, 1994), particularly in the luminal epithelium on which oxytocin receptors first appear as the luteolytic mechanism develops (Ayad *et al.*, 1991; Stevenson *et al.*, 1994). Development of endometrial responsiveness to oxytocin coincides with the temporal increase in oxytocin receptor expression (Roberts *et al.*,

1976; Mirando *et al.*, 1993) and epithelial cells are the predominant cell type responsive to oxytocin (Asselin *et al.*, 1996). However, the results of the present study confirmed those of Uzumcu *et al.* (1998) and Braileanu *et al.* (1999), who reported that stromal cells from pig endometrium were most responsive to oxytocin, luminal epithelial cells were completely unresponsive and glandular epithelial cells had an intermediate response. This occurred despite the fact that epithelial cells of pig endometrium expressed a greater number of receptors for oxytocin than did stromal cells during dioestrus (Boulton *et al.*, 1995). Thus, the pattern of response to oxytocin among cell types in pigs is opposite to that of endometrium from other ungulate species, in which epithelial cells secrete more $\text{PGF}_{2\alpha}$ in response to oxytocin than do stromal cells (horses: Watson *et al.*, 1992; cows: Asselin *et al.*, 1996). It appears that this pattern of response to oxytocin among the cell types described in the present and previous (Uzumcu *et al.*, 1998; Braileanu *et al.*, 1999) studies may reflect their physiological state *in vivo*, as stromal cells were similarly responsive to oxytocin during 12 h–12 days in culture and responsiveness of both epithelial cell types did not increase after several additional days of culture (G. T. Braileanu, S. M. Simasko, and M. A. Mirando, unpublished). However, the functional significance of this difference among species is not clear.

In conclusion, these results indicate that stromal cells isolated from pig endometrium on day 16 after oestrus are most responsive to oxytocin, less sensitive to arginine-vasopressin and completely unresponsive to lysine-vasopressin. However, both glandular and luminal epithelial cells are completely unresponsive to arginine-vasopressin and lysine-vasopressin. Collectively, these results indicate that oxytocin receptors can bind arginine-vasopressin more readily than lysine-vasopressin. The physiological role of arginine-vasopressin in pig endometrial stromal cells remains to be determined.

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