Role of Arachidonic Acid and Its Metabolites in the Regulation of Progesterone and Oxytocin Release from the Bovine Corpus Luteum (43487)

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Abstract. **We have examined the effects of arachidonic acid (AA) and some of its metabolites on progesterone (P_4) and oxytocin (OT) release by corpora lutea obtained from Holstein heifers at day 8 of the estrous cycle (Day 0 = estrus). The luteal cells were dispersed with collagenase and small and large cells were separated by unit gravity sedimentation and flow cytometry. After an 18-hr preincubation period, the cells were incubated in the presence of various treatments for 1 hr, followed by a 23-hr incubation period with no treatment. OT was secreted by the large, but not by the small, luteal cells into the incubation medium. AA elicited a significant ($P < 0.05$) release of OT from the large cells and P_4 from both the large and small cells within 1 hr of incubation, having a specific effect at a concentration of 10 μM. Larger doses (25 and 100 μM) of AA adversely affected the cell viability. Phospholipases A_2 (0.5 unit/ml) and C (0.05 unit/ml) and calcium ionophore A23187 (0.1 μM) stimulated OT release from the large cells to the same extent as AA (10 μM). Inhibition of the AA cyclooxygenase metabolic pathway by indomethacin did not affect AA-induced release of OT and P_4, although exogenous prostaglandins $F_{2\alpha}$ and I_2 (5–25 ng/ml) stimulated the release of OT. Lipoxygenase products of AA (hydroxyeicosatetraenoic acid and leukotrienes; 25 ng/ml) also stimulated OT release. Inhibition of the lipoxygenase metabolic pathway by nordihydroguaiaretic acid abolished AA-induced release of both OT and P_4. These results suggest that intracellular accumulation of free AA may modulate secretory functions in the bovine corpora lutea, including OT and P_4 release.**

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The corpus luteum (CL) of many species, including the cow, contains two morphologically and functionally distinct types of steroidogenic cells, designated small and large (1, 2). The large luteal cells, which produce progesterone (P_4), also synthesize and secrete a number of regulatory peptides, including oxytocin (OT) (3–5). The bovine CL exhibits temporal variation in OT mRNA levels during the estrous cycle, being maximal during the first week and then returning to low preovulatory levels by the late luteal phase (6, 7). It has been suggested that ovulation is the trigger for OT gene transcription, which could be regulated by the luteinizing hormone. A delay of 4–6 days between the peak in luteal levels of OT mRNA and the presence of OT in the bovine CL (8) suggests a maturational processing of the peptide after transcription and translation. Simultaneous release episodes of OT and P_4 are measured in the circulation during the early and midluteal phases of the cycle (9). However, OT levels start to decline before P_4 at luteolysis (10–12).

The CL is now considered to be the main source of OT during the estrous cycle in the domestic ruminants. However, little is known about the stimulus for OT posttranslational processing and it is uncertain how the luteal peptide secretion is controlled. In previous studies in both sheep and cattle, it was reported that

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cloprostenol, an analog of prostaglandin (PG) $F_{2\alpha}$, stimulated the secretion of luteal OT *in vivo* (13, 14). On the other hand, OT has been shown to stimulate the release of $PGF_{2\alpha}$ from uterine tissue (15–18), which suggests the existence of a feedback loop OT-$PGF_{2\alpha}$ involved in the control of luteolysis in ruminants (19). $PGF_{2\alpha}$ has also been shown to stimulate OT release from bovine (20), but not ovine (21, 22), luteal tissue slices incubated *in vitro*. However, the essential fatty acid arachidonic acid (AA), which is the precursor of $PGF_{2\alpha}$, has recently been shown to stimulate OT (21, 22) and P_4 (21) secretion by ovine luteal tissue slices. Interestingly, the bovine luteal tissue binds labeled AA with high affinity (23), and AA is present in large quantities in the CL, predominantly esterified to phospholipids (24). A recent study in our laboratory (25) showed that AA increased the intracellular concentration of calcium ions ($[Ca^{2+}]i$) in both small and large bovine luteal cells.

The aim of the present study was to further investigate the involvement of AA and its metabolites as potential secretory agonists in dispersed bovine luteal cell populations separated into large and small cells by flow cytometry.

Materials and Methods

Materials. Culture medium 199 and penicillin-streptomycin were purchased from Gibco Laboratories (Grand Island, NY). Collagenase was obtained from Worthington Biochemicals (Freehold, NJ). Ham's F12-HEPES, L-glutamine, insulin-transferrin-sodium selenite medium supplement, phospholipase A_2 (from porcine pancreas; 510 units/mg of protein), phospholipase C Type X1V (from *Clostridium perfringens*; 290 units/mg of protein), indomethacin, nordihydroguaiaretic acid (NDGA), arachidic acid (20:0), oleic acid (18:1), docosatetraenoic acid (22:4), and calcium ionophore A23187 were purchased from Sigma Chemical Co. (St. Louis, MO). Arachidonic acid (20:4), eicosapentaenoic acid (20:5), linoleic acid (18:2), linolenic acid (18:3), prostaglandin $F_{2\alpha}$, carbaprostacyclin, high-performance liquid chromatography mixtures of five hydroxyeicosatetraenoic acids ([HETE] 5-, 8-, 11-, 12-, and 15-HETE) and five leukotrienes ([LT] LTC_4, LTD_4, LTE_4, LTF_4, N-Ac-LTE_4) were all from Cayman Chemical (Ann Arbor, MI). P_4 and synthetic OT were from Calbiochem (San Diego, CA). ^{125}I-OT, ^{3}H-P_4, and ^{3}H-$PGF_{2\alpha}$ were from Dupont NEN (Boston, MA).

The fatty acids, prostaglandins, HETE, leukotrienes, calcium ionophore, indomethacin, and NDGA were dissolved in ethanol. The phospholipases were stored as a suspension in 3.2 *M* ammonium sulfate ($(NH_4)_2SO_4$). All the stock solutions in ethanol and $(NH_4)_2SO_4$ were diluted in culture medium just before the experiments. The final concentration of ethanol or $(NH_4)_2SO_4$ in the incubation medium did not exceed 0.1%. The other compounds were dissolved in the culture medium just prior to the experiments.

Tissue Dissociation and Cell Separation. Corpora lutea were obtained by enucleation from 16 normally cycling Holstein heifers under epidural anesthesia on Day 8 of the estrous cycle (Day 0 = estrus). Luteal tissues were dissociated with collagenase (2000 units/g of tissue) by a previously described procedure (26) with few minor modifications. After dispersion, the cells were separated first by unit gravity sedimentation (1), which yielded preparations of small luteal cells that were free of large cells but slightly contaminated with endothelial cells. The fractions containing the large cells were further purified, based on their fluorescent properties, by flow cytometry (26). The purity of the large cell preparations obtained by this method was 79 ± 7% (n = 16 CL). The viability of the small and large cell populations, as estimated by trypan blue dye exclusion (27), was 93 ± 3% for small and 73 ± 4% for large luteal cells.

Incubation Procedure. Incubations were conducted with 2×10^4 viable large cells or 1×10^5 viable small luteal cells in 0.5 ml of Ham's F12-HEPES medium containing penicillin-streptomycin (100 units/ml of penicillin and 100 μg/ml of streptomycin), L-glutamine (0.29 mg/ml), insulin (5 μg/ml), transferrin (5 μg/ml), and selenium (5 ng/ml) at 37.5°C under an atmosphere of 5% CO_2 and 95% air in a humidified incubator. The numbers of small and large cells used approximated the ratio in which they exist in midcycle CL. The cells were plated in 16-mm tissue culture wells (Corning, supplied by Krackeler Scientific, Albany, NY) pretreated for 1 hr with 10% fetal calf serum in Ham's F12 medium in order to facilitate cell adhesion to the plates (28).

After an 18-hr period of preincubation, the medium was withdrawn and the cells were incubated in fresh medium (0.5 ml) in the presence of the various test solutions for 1 hr and then in the absence of treatment for an additional 23-hr incubation period. Each treatment was performed on three replicates per CL for large and small cell fractions, respectively. Controls for the effect of vehicle solution (ethanol and $(NH_4)_2SO_4$) were also performed when necessary. The media removed after 1, 3, 6, and 24 hr of incubation were replaced with treatment-free media. The samples collected were immediately stored at −20°C for hormone analysis by radioimmunoassay (RIA), and at −80°C for lactate dehydrogenase enzyme assay. At the end of the incubation period (24 hr after treatment), the cell viability in each culture well was estimated by trypan blue dye exclusion.

Experimental Protocol. The study consisted of five experiments designed to examine the effects of: (i) exogenous AA and several other fatty acids; (ii) endogenous nonesterified AA generated by phospholipases;

(iii) inhibition of AA metabolism; (iv) products of AA metabolism; and (v) variation of intracellular calcium concentration on the secretion of OT, P_4, $PGF_{2\alpha}$, and 5-hydroxyeicosatetraenoic acid (5-HETE).

Hormone Determinations. OT release into the incubation medium was quantified by RIA as described previously (20). The antibody used was kindly supplied by Dr. Dieter Schams (Institute of Physiology, Technical University of Munich, FRG). The sensitivity of the assay was 0.125 pg/tube. The intra- and interassay coefficients of variation were 5.9% and 10.7%, respectively.

Concentrations of P_4 were measured by RIA as described previously (29). The sensitivity of the assay was 6.25 pg/tube. The intra- and interassay coefficients of variation were 8.2% and 9.3%, respectively.

Levels of $PGF_{2\alpha}$ were measured by RIA directly in 100-μl samples of incubation medium. The antibody used was purchased from Advanced Magnetics Inc. (Cambridge, MA). The cross-reactivity of this antibody at 50% displacement is less than 1% for arachidonic acid. The sensitivity of the assay was 7.8 pg/tube. The intra- and interassay coefficients of variation were 6.8% and 11.2%, respectively.

Levels of 5-HETE were measured directly in 50-μl samples of incubation medium by using a commercial radioimmunoassay kit (supplied by Advanced Magnetics). The cross-reactivity of the antibody at 50% displacement is 0.14% for arachidonic acid and less than 0.06% for various prostaglandins. The sensitivity of the assay was 4.9 pg/tube. The intra- and interassay coefficients of variation were 12.8% and 17.4%, respectively.

Lactate Dehydrogenase Assay. The release of the cytosolic enzyme lactate dehydrogenase (LDH), used to monitor cytolytic effects of treatments, was estimated spectrophotometrically (using a LKB Biochrom Ultrospec K, supplied by Pharmacia, Piscataway, NJ) in 100-μl samples of incubation medium by a previously described procedure (21, 30). All assays of LDH were carried out within 3 days after the experiments.

Statistical Analysis. The values (OT, $PGF_{2\alpha}$, 5-HETE, and P_4 concentrations, expressed in pg/ml or ng/ml) for each treatment were used for analysis using statistical analysis system, general linear model procedures (31). Data were analyzed by analysis of variance and the differences between individual means were assessed by Student-Newman-Keuls multiple range test. Differences with a $P < 0.05$ were regarded as statistically significant.

Results

OT was secreted by the large, but not by the small, luteal cells into the culture medium during the 18-hr period of pre-incubation. The mean (±SE; $n = 14$ CL) OT concentration was 902 ± 187 pg/ml. The mean P_4 concentrations measured after the preincubation period from the large ($n = 14$ CL) and small ($n = 5$ CL) luteal cells were 257 ± 30 and 68 ± 9 ng/ml, respectively.

Effects of Exogenous AA and Other Fatty Acids. Exogenous AA stimulated OT secretion from the large ($n = 10$ CL), but not from the small ($n = 5$ CL), luteal cells during the first hour of incubation (Fig. 1). Doses of 10, 25, and 100 μM significantly ($P < 0.05$) increased OT release from 42.3 ± 2.7 pg/ml (control) to 62.8 ± 5.3, 78.6 ± 7.6, and 96.2 ± 13.0 pg/ml, respectively. There was no effect of 5 μM AA.

In addition to enhancing OT secretion by the large cells, AA (10–100 μM) significantly ($P < 0.05$) increased P_4 release from both the large and small cells within 1-hr of incubation (Table I). The degree of stimulation of P_4 secretion from the large cells (1.56 ± 0.05-fold; 24.0 ± 3.2 vs 15.4 ± 2.0 ng/ml for control) achieved with 10 μM AA was comparable to that for the small cells (1.44 ± 0.03-fold; 10.7 ± 1.2 vs 7.5 ±

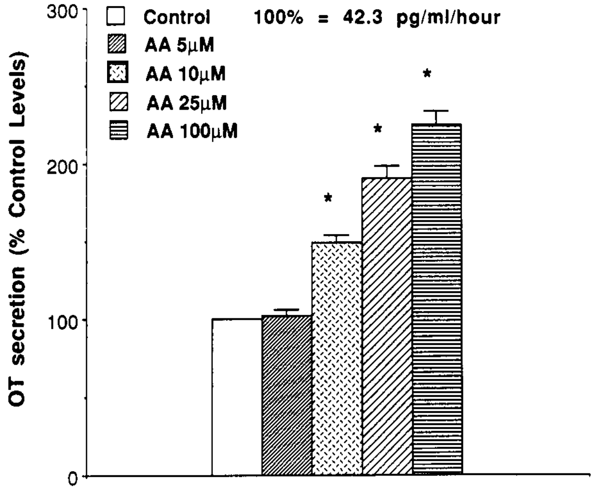

Figure 1. Increases in OT release in the culture medium after 1 hr of incubation in the presence of AA (5, 10, 25, and 100 μM). The values, expressed as percentage of control (basal levels), are the mean ± SE for large luteal cells from 10 corpora lutea at Day 8 of the estrous cycle. *$P < 0.05$, compared with control.

Table I. Effects of Exogenous AA on P_4 Release by Bovine Large and Small Luteal Cells

Treatment	P_4 secretion (% control)[a]	
	Large cells	Small cells
Control	100	100
AA		
5 μM	118 ± 10	ND
10 μM	156 ± 5[b]	144 ± 3[b]
25 μM	168 ± 5[b]	ND
100 μM	166 ± 7[b]	208 ± 19[b]

[a] The values, expressed as percentage of control (basal levels), are the mean ± SE. ND, not determined.

[b] $P < 0.05$, compared with control.

0.9 ng/ml for control). No prolonged AA stimulatory effect on either OT or P_4 secretion was seen in the medium collected after 3, 6, and 24 hr of incubation after treatment (data not shown).

Basal secretion of $PGF_{2\alpha}$ and 5-HETE by the large cells into the incubation medium was below the detection limit of our assays. The release of 5-HETE was stimulated to 1757 ± 291 pg/ml by 10 μM AA within 1 hr of incubation. However, only the highest doses of AA used (25 and 100 μM) stimulated the secretion of $PGF_{2\alpha}$ to 95 ± 32 and 292 ± 15 pg/ml, respectively.

At a dose of 10 μM, AA had no apparent deleterious effect on cell viability, as indicated by (i) the low and nonsignificant LDH activity in the medium within 1 hr of incubation, (ii) the secretion of both OT and P_4 at rates similar to controls at 3, 6, and 24 hr of incubation, and (iii) no increase in intracellular trypan blue staining at 24 hr after treatment. However, hormone secretion and cell viability were adversely affected by 25 and 100 μM AA, as observed after 24 hr of incubation. Consequently, only 10 μM AA were used in subsequent experiments. The saturated fatty acid arachidic (20:0) and several unsaturated fatty acids with shorter (oleic (18:1), linoleic (18:2), linolenic (18:3)), similar (eicosapentaenoic (20:5)), and longer (docosatetraenoic (22:4)) chain lengths all failed to stimulate OT release when added at a concentration of 10 μM (n = 5 CL) (Table II). They were also without effect on P_4 secretion. None of the fatty acids had an effect on the secretion of $PGF_{2\alpha}$, except for docosatetraenoic acid, which increased $PGF_{2\alpha}$ to 276 ± 45 pg/ml within 1 hr of incubation (Table II). The release of 5-HETE in response to fatty acids other than AA was not determined.

Effects of Phospholipases A_2 and C. The involvement of endogenous nonesterified AA on the stimulation of OT and P_4 secretion from the large cells was investigated by using the enzymes phospholipase (PL) A_2 and phospholipase C, which are known to release AA from cellular phospholipids (32–34). Both phospholipases caused a dose-dependent stimulation of OT secretion (n = 5 CL) (Fig. 2), without affecting the cell viability. The degree of stimulation of OT secretion achieved with the lowest effective dose of each PL was comparable to that attained with AA (10 μM). PLC was more potent than PLA_2, stimulating ($P < 0.05$) OT release at a dose (0.05 unit/ml) 10 times less than that of PLA_2 (0.5 unit/ml). Neither PLC nor PLA_2 stimulated the secretion of P_4. The phospholipases were also without effect on $PGF_{2\alpha}$ and 5-HETE release.

Inhibition of AA Metabolism. The effects of inhibition of AA metabolism on AA-induced release of OT and P_4 from the large cells were investigated by using inhibitors of the cyclooxygenase pathway (indomethacin) and the lipoxygenase pathway (NDGA). When AA (10 μM) was added to the large luteal cells (n = 5 CL) treated previously for 60 min with indomethacin at a dose (30 μM) known to inhibit prostaglandin production, there was no effect of the inhibitor on the stimulation of OT (Fig. 3A) or P_4 (Fig. 3B) release induced by AA within 1 hr of incubation. Indomethacin had no significant effect on basal OT and P_4 release. In contrast, NDGA (5 μM), which inhibited 5-HETE release in response to 10 μM AA, also blocked the effect of AA on both OT (Fig. 3A) and P_4 (Fig. 3B) release, with OT and P_4 values not different from control levels. NDGA had no significant effect on the basal secretion of either hormone. Indomethacin and NDGA, alone or in combination with AA, did not cause any apparent deleterious effect on cell viability.

Effects of AA Metabolites. The involvement of AA metabolites of the cyclooxygenase and lipoxygenase pathways in the stimulation of OT and P_4 secretion from the large cells was determined by using the prostaglandins $F_{2\alpha}$ and I_2 (prostacyclin) and high-performance liquid chromatography mixtures of five HETE (5-, 8-, 11-, 12-, 15-) and five leukotrienes (LTC_4, LTD_4, LTE_4, LTF_4, N-Ac-LTE_4). At the 5-ng/ml level, only $PGF_{2\alpha}$ significantly ($P < 0.05$) stimulated the release of OT (1.67 ± 0.07-fold; 51.5 ± 6.8 vs 30.9 ± 3.9 pg/ml for control) (n = 4 CL) (Table III). PGI_2 and the HETE and leukotriene mixtures significantly ($P < 0.05$) in-

Table II. Effects of Several Fatty Acids (10 μM) on OT, P_4, and $PGF_{2\alpha}$ Release by Large Luteal Cells[a]

Treatment	OT secretion (% control)	P_4 secretion (% control)	$PGF_{2\alpha}$ secretion (pg/ml)
Control	100	100	ND
Arachidonic acid	152 ± 6[b]	149 ± 5[b]	ND
Oleic acid	104 ± 3	107 ± 10	ND
Linoleic acid	107 ± 4	112 ± 7	ND
Linolenic acid	116 ± 10	101 ± 9	ND
Arachidic acid	103 ± 7	94 ± 3	ND
Eicosapentaenoic acid	115 ± 3	119 ± 5	ND
Docosatetraenoic acid	110 ± 3	117 ± 5	276 ± 45

[a] The values, expressed as percentage of control (basal levels) for OT and P_4 release and as pg/ml for $PGF_{2\alpha}$ release, are the mean ± SE (n = 5 CL). ND, not detectable.

[b] $P < 0.05$, compared with control.

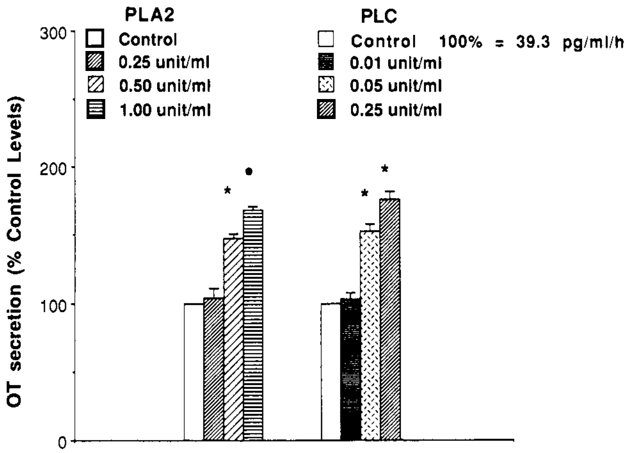

Figure 2. Increases in OT release in the culture medium after 1 hr of incubation in the presence of PLA_2 (0.25., 0.50, and 1.00 unit/ml) and PLC (0.01, 0.05, and 0.25 unit/ml). The values, expressed as percentage of control (basal levels), are the mean ± SE for large luteal cells from five corpora lutea at Day 8 of the estrous cycle. $^*P < 0.05$, compared with control.

creased the release of OT, but only at a higher dose level (25 ng/ml). Neither $PGF_{2\alpha}$, PGI_2, the five HETE, nor the leukotrienes stimulated the release of P_4 by the large luteal cells at the doses used. None of these metabolites had any measurable effect on cell viability.

Effect of Variation of Intracellular Calcium Concentration. The involvement of calcium in the stimulation of OT and P_4 secretion from the large cells was investigated by using the calcium ionophore A23187. OT secretion was significantly ($P < 0.05$) stimulated 1.59 ± 0.06-fold by A23187 (0.1 μM) with no deleterious effect on cell viability (n = 3 CL) (Fig. 4). There was no additive or synergistic effect of A23187 and AA when added in combination in stimulating OT release. P_4, $PGF_{2\alpha}$, and 5-HETE release were not affected by A23187 at the dose used.

Discussion

The present study, the first in which OT release has been studied in purified large and small luteal cell preparations, is also the first to demonstrate the effects of AA on bovine luteal secretion.

Exogenous AA (10–100 μM) significantly stimulated the release of OT from the large luteal cells of CL obtained from heifers during the midluteal phase of the cycle. However, no detectable basal or stimulated OT release from the small cells was measured. These results might have been expected, since OT is synthesized only by the large luteal cells (3-5). On the other hand, AA significantly stimulated P_4 release from both the small and large luteal cells, which suggests that the fatty acid may have a role in luteal functions other than OT release.

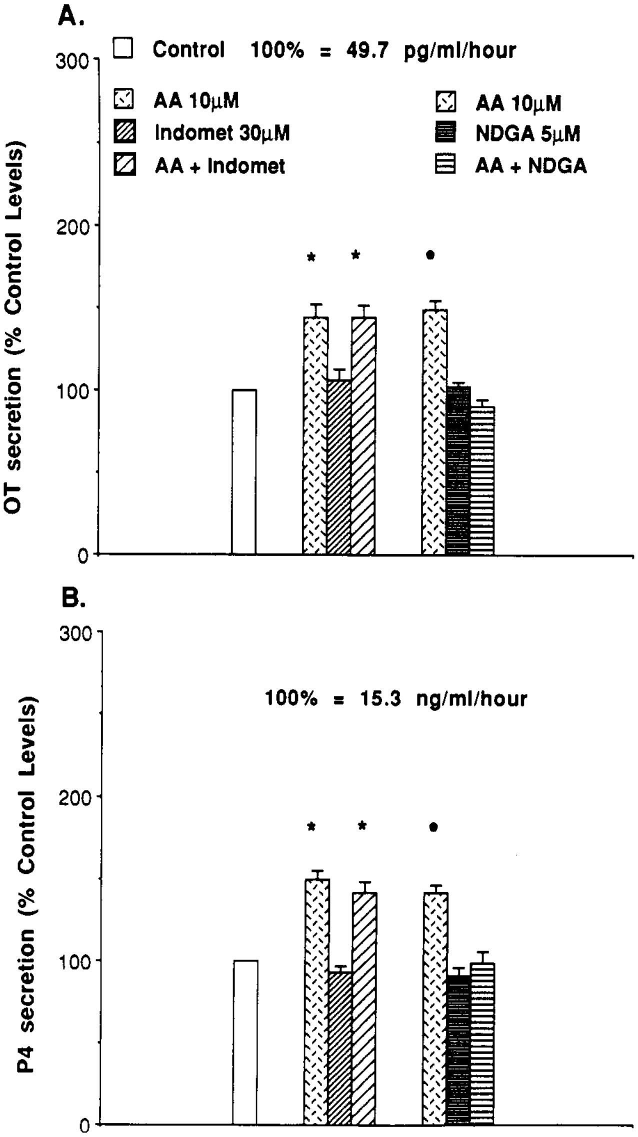

Figure 3. Increases in (A) OT and (B) P_4 release in the culture medium after 1 hr of incubation in the presence of AA (10 μM), indomethacin (30 μM), indomethacin + AA, NDGA (5 μM), and NDGA + AA. The values, expressed as percentage of control (basal levels), are the mean ± SE for large luteal cells from five corpora lutea at Day 8 of the estrous cycle. $^*P < 0.05$, compared with control.

Higher levels of AA (25 and 100 μM) had deleterious effects (data not shown), as reported by McCann and Flint (21) for ovine luteal tissue. However, our study shows that AA at a lower dose (10 μM), which had previously been shown to increase $[Ca^{2+}]i$ (25), stimulated OT and P_4 release without any apparent deleterious effect on the bovine luteal cells. The cells treated with 10 μM AA for 1 hr continued to secrete OT and P_4 at the same rates as control cells for the 24-hr posttreatment period, and there was no evidence of LDH leakage into the incubation medium or increased intracellular trypan blue staining. AA appeared to act in a specific manner in inducing OT and P_4 release; several unsaturated fatty acids (10 μM) with either shorter, similar, or longer chain lengths to AA failed to stimulate OT and P_4 release from the large cells.

Table III. Effects of AA Metabolites on OT Release by Large Luteal Cells

Treatment	OT secretion^a (% control)
Control	100
$PGF_{2\alpha}$	
5 ng/ml	167 ± 7^b
25 ng/ml	173 ± 9^b
PGI_2	
5 ng/ml	114 ± 9
25 ng/ml	190 ± 5^b
HETEs	
5 ng/ml	95 ± 8
25 ng/ml	171 ± 13^b
Leukotrienes	
5 ng/ml	83 ± 5
25 ng/ml	209 ± 21^b

[a] The values, expressed as percentage of control (basal levels), are the mean ± SE ($n = 4$ CL).
[b] $P < 0.05$, compared with control.

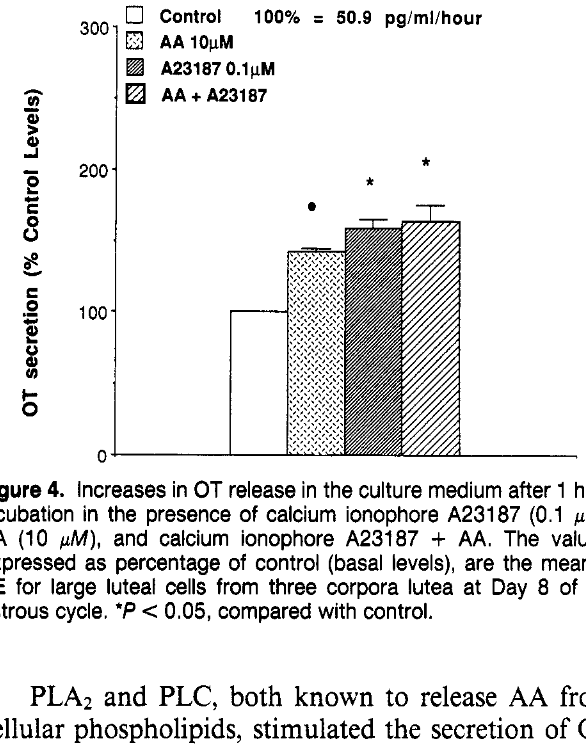

Figure 4. Increases in OT release in the culture medium after 1 hr of incubation in the presence of calcium ionophore A23187 (0.1 *μM*), AA (10 *μM*), and calcium ionophore A23187 + AA. The values, expressed as percentage of control (basal levels), are the mean ± SE for large luteal cells from three corpora lutea at Day 8 of the estrous cycle. $^*P < 0.05$, compared with control.

PLA_2 and PLC, both known to release AA from cellular phospholipids, stimulated the secretion of OT from the large cells to the same extent as 10 *μM* AA. PLC was more potent than PLA_2, stimulating OT release at a dose 10 times less than that of PLA_2. Neither PLA_2 nor PLC had a deleterious effect on cell viability at the doses used. The PLA_2-induced OT release may be due to an increase in intracellular nonesterified AA. The stimulatory effect of PLC, resulting from phosphoinositide hydrolysis may be attributed to the activation of a protein kinase C by diacylglycerol and/or the enhanced $[Ca^{2+}]i$ mobilization caused by inositol trisphosphate (32, 35). The hydrolysis of phosphoinositide by PLC could also lead to intracellular accumulation of nonesterified AA through further metabolism of diacylglycerol. In contrast to exogenous AA, the phospholipases did not induce the secretion of 5-HETE or $PGF_{2\alpha}$ and had no effect on P_4 release. The lack of effect of the enzymes on P_4 secretion remains unexplained.

It was shown previously that AA increased $[Ca^{2+}]i$ in both small and large bovine luteal cells (25). In the large cells, AA increased $[Ca^{2+}]i$ in one phase, representing extracellular mobilization only, whereas in the small cells, the fatty acid mobilized $[Ca^{2+}]i$ first in an intracellular phase followed by a second extracellular phase. In the present study, the calcium ionophore A23187 (0.1 *μM*), used to raise $[Ca^{2+}]i$, stimulated the release of OT from the large cells to the same extent as 10 *μM* AA. The increase in $[Ca^{2+}]i$ may have enhanced OT exocytosis and/or could have been involved in the posttranslational conversion of OT prohormone.

The mechanism(s) by which P_4 is released from the CL is still not completely understood. However, both basal production and hormone-stimulated production of P_4 in the bovine large luteal cells appear to be calcium-dependent, whereas only hormonally stimulated release of P_4 is calcium dependent in the small cells (36, 37).

The ability of bovine luteal tissue to produce prostaglandins both *in vivo* and *in vitro* has been well established (23, 38, 39). It has also been shown that a high concentration of 5-HETE is present in the luteal tissue (40). In the present study, the inhibitor of cyclooxygenase, indomethacin, did not affect the stimulatory effect of AA (10 *μM*) on the secretion of OT and P_4 by the large cells. Indomethacin was also without effect on the basal release of the two hormones and AA (10 *μM*) did not induce a detectable release of $PGF_{2\alpha}$ (used as an indicator of the cyclooxygenase metabolism of AA). However, the fatty acid docosatetraenoic acid (10 *μM*), which has been shown to have no effect on either OT or P_4 secretion, stimulated the release of $PGF_{2\alpha}$. Thus, it appears unlikely that the AA-induced secretion of OT and P_4 from the large cells was mediated via the formation of endogenous $PGF_{2\alpha}$ or some other metabolic products of the cyclooxygenase pathway.

In contrast, AA (10 *μM*) significantly stimulated the release of 5-HETE from the large cells, which indicates that the fatty acid was preferentially metabolized by the lipoxygenase rather than by the cyclooxygenase pathway. The inhibitor of lipoxygenase, NDGA, abolished AA-stimulated OT and P_4 release, which suggests that lipoxygenation of AA could have a role in the secretion of both OT and P_4 as suggested previously (22).

Although AA-induced stimulation of OT appeared not to result from prostaglandin formation, the addition of low doses of $PGF_{2\alpha}$ and PGI_2 significantly stimulated the release of OT without affecting cell viability. Similar effects of $PGF_{2\alpha}$ on OT release from bovine luteal tissue slices have been reported (20). $PGF_{2\alpha}$ has been shown recently to increase intracellular calcium concentration (41), polyphosphoinositide metabolism (42–44), and AA release from cultured rat luteal cells via the activation of phospholipase A_2 (45, 46). These effects of $PGF_{2\alpha}$ could explain its stimulatory action on OT secretion. On the other hand, our data showed no effect of $PGF_{2\alpha}$ or PGI_2 on P_4 secretion by large cells, suggesting that OT and P_4 secretion from the large luteal cells are controlled differently.

The products of the lipoxygenase metabolic pathway, HETE and leukotrienes, significantly stimulated the release of OT at a dose as low as 25 ng/ml, which was approximately 70 times less than the AA-induced release of 5-HETE in the incubation medium of the large cells. These findings support a role of the lipoxygenase pathway for OT release. However, neither the HETE nor the leukotrienes stimulated the release of P_4. These latter findings are not consistent with our observations of the inhibitory effect of NDGA on the AA-induced release of P_4. The lack of P_4 response to HETEs and leukotrienes (as well as of prostaglandins) can be tentatively attributed to the fact that P_4 secretion is less sensitive than OT to the action of these AA metabolites at the doses used in the present study.

In summary, these data are best interpreted as indicating that AA, which causes an increase (within the physiological range) of intracellular Ca^{2+} ions, may play a second messenger role in bovine corpus luteum secretory functions. Although $PGF_{2\alpha}$ and PGI_2 stimulate the release of luteal OT, the stimulatory effects of AA on both OT and P_4 do not result from prostaglandin formation. However, the failure of AA to cause OT release after blockage of the lipoxygenase pathway with NDGA and the sensitivity of the large cells to lipoxygenase products may suggest a more specific role of these compounds in OT secretion.

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