REGULATION OF HEAT SHOCK-INDUCED ALTERATIONS IN THE RELEASE OF PROSTAGLANDINS BY THE UTERINE ENDOMETRIUM OF COWS

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ABSTRACT

Maternal heat stress in cattle may disrupt pregnancy by elevating uterine prostaglandin F2α (PGF2α) secretion. The objectives of this study were to determine the effects of elevated temperature (42°C) in vitro upon 1) prostaglandin secretion by endometrial tissue; 2) the actions of extracellular regulators of uterine PGF [conceptus secretory proteins (bCSPs) and platelet-activating factor, (PAF)]; 3) the activity of the cyclooxygenase-endoperoxidase enzyme complex (PG synthetase); and 4) the activity of the endometrial PG synthesis inhibitor present in the endometrium from pregnant cattle. Endometrial explants at Day 17 of the estrous cycle produced more PGF than PGE2 while elevated temperature caused increased PGF secretion but did not affect PGE2 secretion. Elevated temperature did not reduce the ability of bCSPs or PAF to suppress release of PGF. The heat shock-induced increase in PGF at Day 17 was not due to the direct effects on PG synthetase, because PGF production from a cell-free cotyledonary microsomal enzyme preparation was reduced at elevated temperature. The activity of the cytosolic inhibitor of cyclooxygenase present in the endometrium of Day-17 pregnant cows could be reduced but not eliminated at 42°C. We conclude that in vitro heat stress induces PGF secretion from the bovine uterine endometrium at Day 17 after estrus. This increase is not accompanied by the loss of regulatory capacity of conceptus products or increased activity of PG synthetase.

Key words: endometrium, heat shock, prostaglandin, platelet-activating factor

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INTRODUCTION

Heat stress-induced hyperthermia can reduce embryonic survival (1-3). Accordingly, this is a major cause of the reduced pregnancy rate in cattle during the summer in regions associated with elevated ambient temperatures (4-6). One physiological event that may be sensitive to heat stress is rescue of the corpus luteum from luteolysis. Heat stress from 8 to 16 days after insemination altered the uterine environment (7), reduced the weight of corpora lutea and impaired conceptus growth (7,8). During this period, the conceptus secretes the anti-luteolysin, bovine trophoblast protein-1 (bTP-1) (9), a protein that causes suppression of uterine prostaglandin (PG) F secretion and luteal maintenance (10,11). Depressed PGF secretion by bTP-1 may be mediated by an inhibitor of PG synthesis that appears in uterine endometrial cells during pregnancy (12) and after exposure to bTP-1 (13). Secretion of bTP-1 was inhibited by in vitro heat stress (14). In another study, conceptus weight was significantly reduced when cows were exposed to elevated temperature from Day 8 to 16 of gestation, although no depression was observed for subsequent embryonic secretion of bTP-1 during in vitro culture at 37°C (7). Hyperthermia could also alter PGF secretion directly. Basal and oxytocin-induced PGF secretion by the cultured uterine endometrium from cyclic and pregnant cows at Day 17 after estrus was increased by exposure to elevated temperature (14-16). Further, nonpregnant cows or cows with retarded embryos at Day 17 after estrus displayed increased PGF secretion in response to oxytocin during heat stress in vivo (16).

Increased PGF release is not a general response of PG-producing tissues to heat shock, since explants of bovine uterine endometrium collected at estrus and explants of ovine uterine and placental tissues collected during the third trimester of pregnancy failed to respond in this fashion (17,18). It remains unclear how heat stress acts to increase PGF secretion by the endometrium at Day 17 after estrus in cyclic and pregnant cows. One possibility is that there is a thermally-induced increase in the reaction rate of enzymes converting arachidonic acid to PGs. A second possibility, that increased mobilization of arachidonic acid precursor accounts for this increase, is unlikely since the production of PGE2 and PGF were affected differentially by heat shock, and release of PGF from explants was sensitive to temperature even when excess arachidonic acid was present in the culture medium (14). A third possibility for tissues from pregnant cattle is that heat shock results in loss of endometrial responsiveness to regulators of uterine PGF synthesis. The first and third possibilities were tested in the present experiments. One regulator tested was the array of proteins secreted by Day-17 bovine conceptuses (bovine conceptus secretory proteins; bCSPs), which have been shown to suppress PGF secretion from the cultured endometrium (13,19). Another putative inhibitor tested was platelet-activating factor (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine; PAF). Platelet-activating factor is a potent mediator of inflammation which exerts effects through a receptor-mediated signal transduction that apparently utilizes cyclooxygenase and lipoxygenase products as second messengers (20), and which has been shown to reduce PGF secretion from cultured endometrial explants from cows at Day 17 of the estrous cycle (21). Finally, the possibility that the cytosolic inhibitor of cyclooxygenase found in the endometrium
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at Day 17 of pregnancy (12) loses activity in response to elevated temperature was investigated.

MATERIALS AND METHODS

Materials

A modified Eagle's minimum essential medium (MEM) was prepared as described previously (22). All medium components except insulin (Sigma) \(^a\) were from GIBCO \(^b\). \([5,6,8,11,12,14,15^-{H}]\)PGF\(_{2\alpha}\) (SA, ~180 Ci/mmol) and \([5,6,8,12,14,15^-{H}]\)PGE\(_2\) (SA, ~170 Ci/mmol) were purchased from Amersham Corp. \(^c\) Radioinert PGE\(_2\), PGF\(_{2\alpha}\), arachidonic acid and platelet-activating factor (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine) were purchased from Sigma. Rabbit antiserum to PGF\(_2\alpha\) was kindly provided by Dr. T.G. Kennedy (University of Western Ontario). Cross reactivity of this antibody with other F series prostaglandins has been reported (12) and data in the present experiments are expressed as F series prostaglandins (PGF). Sheep antiserum to PGE\(_2\) was provided by N.R. Mason (Eli Lilly Research Laboratories, Indianapolis, IN).

Animals

Cyclic, grade Brangus cows were slaughtered at Day 17 after estrus (estrus = Day 0) and the reproductive tracts collected. Uterine horns were opened anterior to the external bifurcation, intercaruncular endometrium was dissected free from myometrium, blotted on sterile gauze, cut into 2 to 3 mm\(^3\) cubes and cultured as described below. Endometrial tissue from cyclic cows rather than pregnant cows was utilized in experiments with PAF and bCSPs to avoid confounding the effects of previous in vivo exposure to secretory products of the conceptus.

Preparation of bCSPs

Bovine conceptus secretory proteins (bCSPs) were obtained as described by Helmer et al. (9). Cows were observed for estrus, bred by natural service and slaughtered at Day 17 after estrus. Following exsanguination, reproductive tracts were removed, transported to a laminar flow hood and trimmed free of excess tissue. The cervix was clamped and a sterile syringe fitted with a 16-ga needle used to flush 40 ml of sterile modified MEM into the uterine lumen through the tubal-uterine junction of the cornu contralateral to the corpus luteum. Conceptuses were flushed through an opening at the anterior tip of the ipsilateral cornu into a sterile 100-mm petri dish. Conceptuses were transferred to a sterile 100-mm petri dish containing 15 ml modified MEM and incubated for 24 h under an atmosphere of 50% N\(_2\), 47.5% O\(_2\) and 2.5% CO\(_2\) (v/v/v) in the dark on rocking platforms.

\(^a\)Sigma, St. Louis, MO.
\(^b\)GIBCO, Grand Island, NY.
\(^c\)Amersham Corp., Arlington Heights, IL.
Incubation was terminated by the removal of conceptuses from the modified MEM. The medium was centrifuged at 12,000 g for 10 min. Supernatant fractions from several cultures were pooled and dialyzed (Mr cutoff = 1000) against modified MEM. The protein content of conceptus-conditioned, dialysed culture medium was determined by the method of Lowry et al. (23) using BSA as a standard. Medium was frozen at -20°C.

Explant Culture

Explants of intercaruncular uterine endometrium (250 mg of 2 to 5 mm$^3$ pieces per dish) were placed in sterile plastic 100-mm Petri dishes and cultured in 8.5 ml of modified MEM under an atmosphere of 50% N$_2$, 45% O$_2$ and 5% CO$_2$ (v/v/v). Cultures were maintained in the dark on rocking platforms. Ten explant cultures were prepared from each of three cows for examination of the effects of PAF and bCSP. All cultures contained 100 μg arachidonic acid. Stock solution of PAF (10 μg/ml) was prepared in modified MEM that additionally contained 10 μg/ml BSA. The bCSP stock was prepared at a concentration of 800 μg protein/ml modified MEM. Duplicate cultures were prepared with one of five treatments: 1) no treatment, 2) 2.5 μg PAF, 3) 5 μg PAF, 4) 10 μg PAF, or 5) 800 μg bCSPs. All cultures were modified to contain the same BSA concentration (10 μg/culture). Cultures were incubated for 6 h at 39°C (homeothermic temperature) followed by a 6-h period at either 39°C or 42°C (heat shock). Cultures were stopped by the separation of tissue and medium during centrifugation (700 g; 30 min; 4°C). Culture supernatants were stored at -20°C until analyzed for PGF and PGE$_2$ by radioimmunoassay.

Cell-free System

To examine the effects of elevated incubation temperature upon the activity of the cyclooxygenase-endoperoxidase enzyme complex and the prostaglandin synthesis inhibitor found in the Day-17 pregnant bovine endometrium, a microsomal preparation of placental cotyledon collected at term from post-parturient cows was prepared as described by Gross et al. (12). This preparation, called the cotyledonary PG-generating system, is rich in cyclooxygenase-endoperoxidase enzyme complex and is capable of converting arachidonic acid to PGs (12). Periparturient bovine cotyledons were collected within 1 h of parturition and placed in sterile saline. Tissue (20 g/40 ml) was added to 0.05 M Tris-buffered 0.25 M sucrose, pH 7.4, and homogenized. Homogenates were centrifuged (800 g; 15 min; 4°C) and filtered through cheesecloth. Filtrates were centrifuged (9000 g; 15 min; 4°C). Then, supernates were centrifuged (100,000 g; 60 min; 4°C) to yield a microsomal fraction and a high-speed cytosolic supernatant. Microsomes were suspended in 0.1 M potassium phosphate, pH 7.5, and stored in aliquots at -70°C.

The cytosolic inhibitor of PG synthesis was prepared from bovine endometrial tissue collected at Day 17 of pregnancy. Tissue (10 g/20 ml) was homogenized and subjected to centrifugation as described for cotyledonary tissue above (12) to obtain a high-speed cytosolic supernatant. The cytosolic supernatant, previously demonstrated to contain an inhibitor of endometrial PG synthesis (12), was stored in aliquots at -70°C.
Cotyledonary microsomes were incubated for 60 min at 1) 39°C, 2) 39°C in the presence of the high-speed supernatant of Day-17 endometrial tissue from pregnant cows, 3) 42°C, 4) 42°C in the presence of the high-speed supernatant, 5) 39°C with the high-speed supernatant which had been preincubated at 42°C for 30 min, or 6) 42°C with high-speed supernatant preincubated at 42°C for 30 min. Each treatment was performed in six replicates using cotyledonary microsomes (500 mg tissue equivalent; 0.5 ml in 0.1 M potassium phosphate, pH 7.5) with and without endometrial high-speed supernatant (250 mg tissue equivalent; 0.5 ml) and 100 µg arachidonic acid (0.1 ml) at a final volume of 2 ml (0.1 M potassium phosphate) as described by Gross et al. (12). The incubations were terminated by addition of 0.25 ml absolute ethanol, and the samples were centrifuged to remove ethanol precipitable material. Supernates were assayed for PGF.

Measurement of Prostaglandins

Culture supernates were assayed for PGF and PGE₂ using radioimmunoassay procedures described previously (12). The assay for PGF₂α used an antibody described by Kennedy (24) that also recognized PGF₁α. Data are therefore reported as PGF.

Statistical Analysis

Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System (25). The model used for analysis of PAF and bCSP effects upon PG secretion by the Day-17 endometrium included cow, treatment (bCSP treatment or PAF dosage), incubation temperature, and interactions. Analysis of endometrial PG synthesis inhibitor data included effects of treatment, incubation temperature and interaction. For both models, the residual was used as the error term for all effects.

RESULTS

Temperature Response of Day-17 Endometrium to bCSP and PAF

Overall, explants produced greater quantities of PGF than PGE₂ (Figure 1). An elevated incubation temperature resulted in a significant increase in PGF secretion (P < 0.02) while not affecting PGE₂ secretion. Addition of bCSPs at 800 µg/culture resulted in a suppression of PGF release (P < 0.01) at both 39°C (631 ± 188 versus 134 ± 51 ng PGF/g tissue/24 h) and 42°C (813 ± 88 versus 322 ± 170 ng PGF/g tissue/24 h). There was no treatment by temperature interaction (P > 0.10), and the magnitude of the bCSP-induced decrease was similar at 39°C (-497 ng) and 42°C (-491 ng); PGE₂ secretion was not measured in bCSP cultures.

The PAF reduced secretion of PGF (P < 0.02) but had no effect on PGE₂ at 39°C and 42°C (Figure 1). There was a treatment by temperature interaction (P < 0.02) for PGF. Elevated temperature shifted the dose-response to PAF to the left (Figure 2). At both 39°C and 42°C, maximum inhibition of PGF was about 25%. At 42°C, inhibition of PGF release was maximal at 2.5 µg PAF and higher.
doses of PAF did not increase the inhibition. In contrast, at 39°C, maximal inhibition similar to that seen at 42°C did not occur until 5 to 10 μg PAF.

Cell-free System

Production of PGF by the microsomal prostaglandin-generating system prepared from term cotyledon was reduced at elevated temperature (Table 1). Addition of the high-speed cytosolic supernatant prepared from Day-17 pregnant endometrium depressed (P < 0.01) the PG generating system at both 39°C and 42°C. Endometrial cytosolic supernatant that had been preincubated at 42°C for 30 min also suppressed PGF secretion (P < 0.01). There was a treatment by temperature interaction (P < 0.01) because the magnitude of suppression of PGF release by the cytosolic inhibitor was not as great for the generating system incubated at 42°C as at 39°C.

![Prostaglandin (ng/g tissue)](image)

Figure 1. Effect of PAF and incubation temperature on secretion of PGF (panel A) and PGE2 (panel B) by endometrial explants collected from 3 cyclic cows at Day 17 after estrus. Data are expressed as means ± standard error. Elevated temperature increased PGF release (P < 0.02) but not PGE2 secretion. There was a treatment by temperature interaction (P < 0.02) for PGF.
Figure 2. Dose-response of PGF secretion at 39°C and 42°C to increasing concentrations of PAF. Data are the same as for Figure 1 but are expressed as inhibition of PGF release to demonstrate the treatment by temperature interaction (P<0.02) that occurred because elevated temperature shifted the response to the left.

Table 1. Prostaglandin F production from cotyledonary prostaglandin generating system incubated at 39 or 42°C in the presence or absence of endometrial prostaglandin synthesis inhibitor.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGF synthesis (ng/90 min)</th>
<th>Inhibitory activity (% reduction in PGF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39°C</td>
<td>42°C</td>
</tr>
<tr>
<td>no inhibitor</td>
<td>8.5</td>
<td>6.6</td>
</tr>
<tr>
<td>+ inhibitor</td>
<td>5.3</td>
<td>4.3</td>
</tr>
<tr>
<td>+ heat-treated inhibitor</td>
<td>6.0</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Data are expressed as means ± standard error of six incubations of one preparation of inhibitor and generating system. PGF synthesis was affected by temperature (P<0.01), treatment (P<0.01) and treatment by temperature (P<0.01).
Our hypothesis that elevated temperature would cause extracellular regulatory molecules to lose their capacity to suppress endometrial PGF release was not borne out. Bovine conceptus secretory proteins suppressed PGF in a similar fashion at both 39°C and 42°C (i.e., there was no treatment by temperature interaction). Additionally, elevated temperature increased the ability of PAF to suppress endometrial PG secretion since the dose-response relationship was shifted to the left at elevated incubation temperature. Results indicate that PAF can only partially inhibit PGF (at saturation by ~25%). Additionally, it could be inferred that elevated temperature did not cause denaturation of PAF but likely decreased the equilibrium dissociation constant of PAF with its receptor to reduce the maximum effective dosage.

Elevated temperature decreased the activity of the endometrial inhibitor of PG synthesis present at Day 17 of pregnancy. Putney et al. (16) found similar results when the endometrium was subjected to elevated temperature and the isolated inhibitor was tested for activity. Reduced function of this proteinaceous inhibitor of the cyclooxygenase-endoperoxidase enzyme complex (12) might account partially for increased PGF secretion in pregnant animals but cannot account for heat-induced PGF release from endometrium of cyclic animals. The temperature-induced increase of PGF secretion by the endometrium was also not due to a direct effect of temperature on reaction rate of cyclooxygenase activity, because the production of PGF from a cell-free preparation of cyclooxygenase-endoperoxidase enzyme complex from periparturient cotyledon was depressed by elevated temperature. In fact, results indicate that the temperature-induced increase in PGF secretion at Day 17 after estrus occurs in the face of potential partial denaturation of components of the enzyme complex.

As was found previously (14-16), elevated temperature did not increase PGE2 secretion, indicating that elevated temperature affects PG secretion in some manner specific for PGF. At Day 17, most PGF is released from the endometrial epithelium, while most of the PGE2 originates in the stroma (26). Perhaps high temperature affects these two cell types differently or preferentially enhances the activity of endoperoxide F reductase. The increase in PGF secretion due to elevated temperature did not occur because of increased 9-keto reductase activity converting PGE2 to PGF (27) since PGE2 secretion did not decrease upon exposure of explants to 42°C.

As alluded to earlier, temperature-induced secretion of PGF is not a common response of PG-producing tissues. It remains unclear how elevated temperature induces an increase in PGF release from the uterine endometrium at Day 17 of pregnancy or the estrous cycle, but not in the bovine endometrium at estrus (17), or in the uterine and placental tissues of sheep during mid- and late pregnancy (18). One possible mechanism for heat-induced PGF release at Day 17 could involve effects on phosphoinositide turnover. Heat shock alters membrane dynamics (28) and stimulates phosphoinositide turnover directly (29). Because oxytocin may act to increase endometrial PGF secretion at Day 17 via increased phosphatidylinositol turnover (30), a heat-induced increase in turnover might also
result in PGF secretion. Data from Putney et al. (16) indicate that heat shock enhances actions of oxytocin on the endometrium of pregnant and cyclic cows at Day 17.

Another underlying cause for the unique response of the Day-17 bovine endometrium to heat-shock may be associated with membrane composition. Curl (31) found an increase in the total phospholipid and phosphatidylcholine content in the endometrium between Days 17 and 19 after estrus. Some changes in the endometrium such as increased phosphatidylcholine content (31) indicate a progressive increase in fluidity and permeability of the cell membrane as phospholipids with bulky polar head-groups reduce close packing in the membrane bilayer (28). Furthermore, phospholipid composition of the membrane may itself regulate cyclooxygenase activity, since fatty acids influence activity of the enzyme complex (32).

In conclusion, elevated temperature induces secretion of PGF from uterine endometrium collected at Day 17 after estrus. Heat shock in vitro does not compromise the capacity of extracellular regulatory molecules involved in suppression of PGF release by pregnant bovine endometrial tissue at Day 17. Both proteinaceous (bTP-1) and lipid (PAF) regulators retained their suppressive activity at elevated temperature and PAF may have increased inhibitory activity at 42°C. The regulatory capacity of the inhibitor of PG synthesis from the Day-17 pregnant endometrium was depressed by heat treatment. The heat-induced increase in PGF secretion is likely not due to increased cyclooxygenase enzyme catalysis, since heat-shock reduced activity of a cell-free preparation of the cycloosygenase-endoperoxidase enzyme complex.

REFERENCES


