Heat Stress-Induced Alterations in the Synthesis and Secretion of Proteins and Prostaglandins by Cultured Bovine Conceptuses and Uterine Endometrium

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ABSTRACT

Effect of in vitro heat stress on protein and prostaglandin synthesis and secretion by bovine conceptuses and endometrium was examined. Conceptuses (n=11) and endometrium (n=10) obtained on Day 17 of pregnancy were cultured at thermoneutral (39°C, 24 h) or heat stress (39°C, 6 h; 43°C, 18 h) temperatures in medium supplemented with L-[4, 5-3H] leucine (100 μCi) and arachidonic acid (10 μg/ml). Radiolabeled protein secreted into culture medium increased with time in both groups. Heat stress reduced (p<0.001) incorporation of [3H]leucine into intracellular and secreted proteins by conceptuses but did not alter incorporation of [3H]leucine by endometrium. In particular, heat stress reduced by 72% the secretion of bovine trophoblast protein-1, the conceptus polypeptide believed to cause extension of luteal lifespan. Two-dimensional, sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that heat stress altered the array of proteins in endometrial and conceptus tissues, as evidenced by the induction of "heat-shock proteins." Endometrial secretion of prostaglandin F (p<0.001) and conceptus secretion of prostaglandin E2 (p<0.05) increased in response to heat stress. Sensitivity of bovine conceptuses and endometrium to heat stress in vitro suggests that infertility associated with maternal heat stress may be caused, partially, by alterations in signals required for maintenance of the corpus luteum during early pregnancy.

INTRODUCTION

High ambient temperatures and humidity often result in periods of transient infertility in cattle (Gwazdauskas et al., 1973; Ingraham et al., 1974; Thatcher, 1974; Badinga et al., 1985). The early bovine embryo is extremely sensitive to maternal heat stress. Intermittent thermal stress from 30 h after the onset of estrus until Day 7 of pregnancy increased the incidence of abnormal or retarded embryos recovered from dairy heifers (Putney et al., 1988). Furthermore, maternal heat stress between Days 8 and 16 after insemination reduced conceptus weight and caused a trend towards increased pregnancy failure in beef cattle (Biggers et al., 1987).

The cause of heat stress-induced embryonic mortality is not known. Chromosomal abnormalities (Waldbieser and Chrisman, 1986) and congenital defects (Trujano and Wrathall, 1985; Mirkes, 1987) are associated with heat stress. Also, embryonic mortality in hyperthermic cows may be due to thermal-induced alterations in synthesis of conceptus proteins involved in embryonic development and maternal recognition of pregnancy. In addition, alterations in the secretory activity of the uterine endometrium in response to severe hyperthermia may alter embryonic development and contribute to hyperthermia-induced pregnancy failure. Since heat stress retards embryonic development (Putney et al., 1988), it is possible that embryonic mortality may result, in part, from failure of embryos to produce biochemical signals at the proper time to prevent corpus luteum (CL) regression. For example, the preattachment conceptus secretes an array of proteins that are involved in maintenance of the CL and continuation of pregnancy (Northey and French,
1980; Betteridge et al., 1984; Bartol et al., 1985; Knickerbocker et al., 1986a). These proteins may act locally on the gravid uterine horn to attenuate the synthesis and release of luteolytic prostaglandin (PG) F2α from the endometrium by stimulating the production of an endometrial inhibitor of prostaglandin synthesis (Gross et al., 1988a).

Little information is available on the effects of heat stress on the biochemical processes within the developing bovine conceptus and maternal endometrium. This study examined whether heat stress in vitro alters protein and prostaglandin synthesis and secretion by conceptuses and endometrium obtained on Day 17 of pregnancy to determine whether heat stress-induced alterations in these functions of conceptus and endometrial tissues play a role in embryonic mortality.

**MATERIALS AND METHODS**

**Materials**

Radioisotopes L-[4,5-3H]leucine (specific activity [SA] = \( \sim 150 \) Ci/mmol), \( [^{125}I] \)-Na (SA = \( \sim 16.9 \) Ci/µg of 1), \( [5,6,8,12,14,15-3H] \)PGF2α (SA = \( \sim 160-180 \) Ci/mmol) and \( [5,6,8,12,14,15-3H] \)PGE2 (SA = \( \sim 140-170 \) Ci/mmol) were purchased from Alfersham Corporation (Arlington Heights, IL). Radioinert PGF2α and PGE2 were purchased from Sigma Chemical Company (St. Louis, MO). Protein A was obtained from Genzyme (Boston, MA), and coupled to \( 1^{25}I \) with IODO-GEN (Pierce Chemical Company, Rockford, IL). A PD-10 column was purchased from Pharmacia Inc. (Piscataway, NJ). Arachidonic acid was purchased from Sigma. Rabbit antiserum to PGF2α was provided by Dr. T. G. Kennedy, University of Western Ontario, and sheep antiserum to PGE2 was provided by N. R. Mason from the E. L. Lilly Research Laboratories (Indianapolis, IN). Rabbit antiserum to ovine trophoblast protein-1 (anti-oTP-1), which exhibits binding to bovine trophoblast protein-1 (bTP-1) (Helmer et al., 1987), was obtained from F. W. Bazer, University of Florida. Preparation of a modified Eagle's minimum essential medium (MEM) and supplies for tissue culture were as described by Godkin et al. (1982), except that medium was additionally supplemented with 1% (v/v) MEM vitamin mix, purchased from Gibco (Grand Island, NY). Spectrapor membrane dialysis tubing was purchased from Spectrum Medical (Los Angeles, CA). Whatman 3MM paper (Whatman, Clifton, NJ) was utilized for trichloroacetic acid (TCA) precipitation using TCA from Fisher Scientific (Orlando, FL). Nitrocellulose membrane (BA85, 0.45 µm) was purchased from Schleicher and Shuell (Keene, NH). Supplies for polyacrylamide gel electrophoresis (PAGE) and Western blotting were as follows: tris (hydroxymethyl)aminomethane (Tris) base, Nonidet P-40, and N,N,N',N'-tetramethyl ethylenediamine were purchased from Sigma; sodium salicylate, 2-mercaptopethanol, glycine, and ammonium peroxysulfate were purchased from Fisher; acylamide, urea, dithiothreitol, sodium dodecyl sulfate (SDS), and amido black 10B were purchased from Research Organics (Cleveland, OH); and bis-acylamide, gelatin, and Tween-20 were purchased from Bio-Rad (Richmond, CA). Carrier ampholytes used in isoelectric focusing were purchased from Serva (Heidelberg, FRG).

**Collection of Conceptuses and Uterine Endometrium**

Beef cattle (Angus or Brangus) were used for collection of bovine conceptuses and endometrium. Cattle were observed for estrous behavior and bred by natural service to Angus bulls. Animals were slaughtered on Day 17 after estrus and reproductive tracts were recovered. Conceptuses were flushed from uteri with 50 ml MEM, according to procedures described by Helmer et al. (1987). Conceptuses were weighed, placed in fresh MEM, and cultured as described below.

After flushing of the conceptus, the uterine horn ipsilateral to the corpus luteum was opened longitudinally along the antimesometrial border and endometrial slices were excised. Intercaruncular endometrium was dissected free from myometrial tissue, blotted on sterile gauze, weighed, and cultured in MEM.

**In Vitro Culture**

Conceptuses and endometrium were transferred to sterile plastic petri dishes and cultured as described by Basha et al. (1979). Endometrial explants (500 mg; \( \sim 2-3 \) mm\(^3\)) or whole Day 17 conceptuses were cultured in 20 ml of MEM supplemented with 100 µCi L-[4,5-3H]leucine and 200 ng arachidonic acid under an atmosphere of 47.5% O\(_2\), 50% N\(_2\), and 2.5% CO\(_2\) (v/v/v). Cultures were maintained in the dark on rocking platforms. Control cultures were maintained at 39°C for 24 h. Control cultures were incubated
under conditions representing normal body temperature of the cow, such as when an animal is in a thermoneutral environment. Accordingly, this treatment was referred to as "thermoneutral culture." Heat-stressed cultures were acclimated at 39°C for 6 h and then placed at 43°C for 18 h. Culture medium from both treatment groups was sampled (1 ml) at 0, 3, 6, 9, 12, 18 and 24 h after initiation of culture. Samples of medium were stored at -70°C until assayed for incorporation of [3H]leucine into protein and concentrations of prostaglandins.

Preparation of Culture Medium and Tissue for Analysis

At termination of culture, endometrial and conceptus tissues were isolated from culture medium by centrifugation (3500 x g, 4°C, 30 min) and decanting of supernatant. Tissues were homogenized in 50 mM Tris-acetate buffer (8 ml, pH 7.5) that contained 1 mM phenylmethylsulfonyl fluoride, 1 mM ethylenediamine tetraacetic acid and 2% (v/v) Nonidet P-40. Homogenates were stored at -70°C until used for subsequent analyses. To remove low molecular weight compounds and unincorporated radiolabeled precursors, culture medium recovered at the end of incubation was dialyzed extensively (three changes of 4 liters) against deionized-distilled water by using dialysis tubing with a 6000-8000 molecular weight exclusion limit.

Protein Determination

Incorporation of [3H]leucine into secreted and intracellular proteins was determined by trichloroacetic acid (TCA) precipitation. Samples (50 µl) of solubilized tissue and conditioned culture medium were placed onto Whatman 3MM paper (previously saturated with 20% TCA [w/v]) and allowed to dry. Precipitation of proteins onto filter paper and removal of nonproteinaceous compounds was accomplished by serial washings of the filter paper with 20% TCA, 5% TCA and 95% ethanol as described by Mans and Novelli (1961). Radioactivity of precipitated protein was determined by scintillation spectrometry.

Electrophoresis

One-dimensional polyacrylamide gel electrophoresis in the presence of SDS and 2-mercaptoethanol (1D-SDS-PAGE) was performed according to the buffer system of Laemmli (1970). Separation of proteins was by electrophoresis in 12.5% (w/v) polyacrylamide gels. Two-dimensional (2D) SDS-PAGE was performed according to a modification of the method described by Roberts et al. (1984). Samples were dissolved in 0.01 ml of 5 mM K2CO3 containing 9.3 M urea, 2% (v/v) Nonidet P-40 and 0.5% (w/v) dithiothreitol, and resolved in the first dimension by isoelectric focusing in 4% (w/v) acrylamide tube gels containing N,N'-diallyltartardimide, 8.0 M urea, 2% (v/v) Nonidet P-40, and 5.1% (v/v) ampholines (pI: 3–10, 5–7, and 9–11; 50:36:16 by volume, respectively). First dimension gels were equilibrated in 0.07 M Tris-HCl buffer (pH 6.8), containing 1% (w/v) SDS and 1% (v/v) 2-mercaptopropanol, and subjected to electrophoresis in the second dimension in 12.5% (w/v) polyacrylamide slab gels according to the procedure of Laemmli (1970). Proteins were localized by Coomassie Brilliant Blue R-250 staining and fluorography as described by Roberts et al. (1984). Fluorographs were prepared with sodium salicylate as a fluor and Kodak XAR film.

Western Blotting

Conceptus proteins present in culture supernatants were resolved (1D SDS-PAGE) on 12.5% polyacrylamide gels. Slab gels were equilibrated for 15 min in 25 mM Tris-HCl buffer (pH 6.8) containing 200 mM glycine and 20% (v/v) methanol, overlaid with a nitrocellulose membrane (BA85, 0.45 µm), and subjected to electrophoresis (200 mA, for 24 h at 4°C) toward the cathode. After electrophoretic transfer, nitrocellulose membranes were stained with amido black (Harper et al., 1986) and immunoblotted. Nonspecific binding of proteins to nitrocellulose was blocked with 10 mM Tris (pH 7.6) containing 3% (w/v) gelatin, 0.8% (w/v) NaCl and 0.05% (v/v) Tween-20. Blocked membranes were incubated (2 h at room temperature) with rabbit antiserum to oTP-i or with normal rabbit serum at dilutions of 1:100 in incubation buffer (10 mM Tris-HCl, pH 7.6, containing 1% [w/v] gelatin, 0.8% [w/v] NaCl and 0.05% [v/v] Tween-20). After incubation, membranes were washed (30 min) in incubation buffer to remove unbound antiserum and further incubated (2 h at room temperature) with 125I-labeled Protein A (10⁶ cpm/ml). Membranes were rinsed with distilled H2O, washed (24 h at 4°C) with Tris-HCl (pH 7.6), dried, and visualized by autoradiography to detect...
Protein-A antibody-antigen complexes bound on the nitrocellulose membrane.

**Iodination of Protein A**

Protein A was iodinated by a modification of the procedure of Markwell and Fox (1978). Briefly, 20 µg IODO-GEN was added to 975 µl of 0.02 M KPO₄ (pH 7.0) containing 0.4 M NaCl. Next, 20 µg Protein A and 5 µl of carrier-free [¹²⁵I]-Na (500 µCi) were added to the reaction tube and incubated for 20 min. Unreacted ¹²⁵I was separated from radioiodinated Protein A by chromatography on a Pharmacia PD-10 column with 1% gelatin in phosphate-buffered saline used as the eluent.

**Quantification of Proteins Separated by PAGE**

Conceptus secretory proteins present in culture medium were resolved by 1D SDS-PAGE on 12.5% gels. Individual lanes, representing separate conceptus cultures, were isolated and sequentially sectioned into 2-mm slices to generate a profile of radioactive proteins. Slices were individually solubilized by incubation in 0.4 ml H₂O₂ for 2 h at 70°C and mixed with scintillation fluid; radioactivity was determined by scintillation spectrometry.

**Measurement of Prostaglandins**

Conceptus- and endometrium-conditioned culture medium was analyzed for PGF₂α with a radioimmuno-assay (RIA) procedure (Knickerbocker et al., 1986b) modified to use an antibody characterized by Kennedy (1985). Standard curves were prepared in MEM with known amounts of radioinert PGF₂α (10–5000 pg). An antiserum dilution of 1:5000, with a minimum sensitivity of 25 pg per tube, was used. Cross-reactivities of the PGF₂α antiserum with other prostaglandins were 24% for PGE₁, 1.7% for PGF₂α, and <0.1% for PGFM, PGF₁α, and arachidonic acid. Correction for nonspecific binding due to the presence of [³H]leucine in samples was done as described for the PGF RIA. An inhibition curve containing PGF₂ (5 ng/ml) was assayed serially in 25-, 50-, 100-, 200-, and 300-µl volumes (final volume of 300 µl with blank MEM) with [³H]leucine (approx. 25,000 cpm). This inhibition curve was parallel to the standard curve, with the test for homogeneity of regression indicating that the curves did not differ. Inter- and intraassay coefficients of variation were 17.7 and 12.9%, respectively.

**Statistical Analyses**

Effect of treatment on conceptus and endometrial secretory activity was analyzed by least-squares analysis of variance utilizing the General Linear Models procedure of the Statistical Analysis System (SAS, 1985). Analysis of conceptus data was conducted with a nested design, with conceptuses nested within treatment. Endometrial data were modeled with cow cross-classified across treatment groups. Protein and prostaglandin secretion rates were characterized by polynomial regressions for time trends. Tests for homogeneity of regressions were used to detect differences in secretion rates due to treatment.

**RESULTS**

**Quantitative Protein Synthesis and Secretion**

To examine the effect of heat stress on protein synthetic rate of conceptus and endometrial tissues,
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Cultures were maintained at 39°C for 6 h followed by an additional 18-h incubation at either 39°C (thermotropic) or 43°C (heat stress) in the presence of [3H]leucine. Secretion of proteins by tissues was determined by measuring incorporation of [3H]leucine into TCA-precipitable proteins released into culture medium. Conceptus and endometrial tissues remained viable throughout the duration of culture, as suggested by continued accumulation of newly synthesized, [3H]leucine-labeled proteins secreted into culture medium (Fig. 1). Regression analysis indicated that protein secretion rates varied over the duration of culture according to tissue type (endometrium < conceptus) and temperature treatment. Prior to initiation of heat stress, secretion of proteins by tissues within heat-stress treatment groups was similar to that of thermoneutral control tissues (conceptus: 4888 ± 3363 vs. 7654 ± 3684; endometrium: 1161 ± 154 vs. 1135 ± 154 dpm/mg tissue/6 h). Elevation of incubation temperature from 39°C to 43°C reduced radiolabeled protein synthetic capacity of conceptus tissues, resulting in a 54.2% decrease (p<0.01) in incorporation of [3H]leucine into secretory proteins and a 66.8% decrease (p<0.01) in incorporation into conceptus tissue proteins at the end of culture (Table 1). In contrast, heat stress of endometrium did not effect the rate of incorporation of [3H]leucine into either secretory or tissue proteins.

Qualitative Analysis of Proteins

Qualitative differences in [3H]leucine incorporation into proteins synthesized by conceptus and endometrial tissues were evaluated. Electrophoretic examination (2D SDS-PAGE) of radiolabeled tissue proteins revealed a complex spectrum of newly

![Figure 1](image_url)

**FIG. 1.** Incorporation of [3H]leucine into polypeptides released into culture medium during 24 h of culture by endometrial and conceptus tissues. Proteins present in culture medium (50 μl) were trichloroacetic acid (TCA)-precipitated onto filter paper, and [3H]leucine-labeled proteins were measured by scintillation spectrometry. Shown are least squares means and best fit (conceptus: r = .89, p<0.001; endometrium: r = .94, p<0.001) regression line calculated by multiple regression (solid line, thermonutral; dashed line, heat shock).

<table>
<thead>
<tr>
<th>Tissue Type X Treatment</th>
<th>Tissue Wet wt (mg)</th>
<th>Radiolabeled Protein (dpm/mg tissue/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Secreted</td>
<td>Tissue</td>
</tr>
<tr>
<td>Endometrium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermoneutral</td>
<td>10</td>
<td>500</td>
</tr>
<tr>
<td>Heat Stress</td>
<td>10</td>
<td>500</td>
</tr>
<tr>
<td>Conceptus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermoneutral</td>
<td>5</td>
<td>81.2 ± 12.3</td>
</tr>
<tr>
<td>Heat Stress</td>
<td>6</td>
<td>64.8 ± 8.2</td>
</tr>
</tbody>
</table>

* (p<0.01) for thermoneutral vs. heat stress for conceptuses.

**TABLE 1.** Incorporation of [3H]leucine into trichloroacetic acid-precipitable protein in tissue and medium collected after 24 h of culture by conceptuses and endometrium cultured at thermoneutral (39°C) or heat-stress (43°C) temperatures (least squares mean ± SEM).
synthesized, radiolabeled proteins. Representative fluorographs of protein patterns presented in Figure 2 are typical of all tissue samples analyzed. Heat stress altered the array of proteins present in conceptus and endometrial tissues. In particular, heat stress caused conceptuses to synthesize proteins with apparent molecular weights of 70,000 (pI 6.2) and 91,000 (pI 5.6). These "heat-shock proteins" were also apparent in low levels in tissues from endometrial cultures maintained at 39°C and were present in more abundant amounts in endometrial tissues from cultures incubated at 43°C.

Conditioned medium from the conceptus and endometrial cultures were analyzed by 1D SDS-PAGE and radiolabeled proteins localized by fluorography (Fig. 3 and 4). The predominant radiolabeled polypeptides detected in conceptus culture supernatants appeared as three bands of 22,000, 24,500 and 26,000 Mr; these proteins appeared to be bTP-1. The predominant proteins secreted by endometrium were of 29,000, 38,000 and 50,000 Mr. Heat stress did not appear to alter the spectrum of radiolabeled proteins secreted into culture medium by conceptuses or endometrium.

Quantitative Analysis of Proteins

To quantitatively evaluate the relative radiolabeling of secretory protein species present in culture medium, equal volumes of conceptus-conditioned medium from each conceptus were electrophoretically separated (1D SDS-PAGE), and the resolved proteins were solubilized from the gel matrix in sequential 2-mm slices and counted for radioactivity. Profiles of radioactivity were similar between conceptuses within the same treatment group and hence were averaged to examine treatment effects (Fig. 5). The predominant radiolabeled polypeptides (representing 27.5% of total radioactivity) appeared as a single peak with an apparent molecular weight range of 22,000–26,000. This protein peak appears similar in molecular weight to those in the predominant protein bands detected by fluorography (Fig. 3) as well as those in the major radioactive peak solubilized from 1D SDS-PAGE gels (Fig. 5). Collectively, these different experimental approaches confirm that proteins within this molecular weight range may in part represent the bTP-1 component of conceptus secretory proteins. Heat stress of the conceptus resulted in a marked reduction in secretory proteins transferred to nitrocellulose that bound oTP-1 antiserum, as indicated by a decrease in [125I]-Protein A labeling of antibody-bound proteins detected by autoradiography. Minor cross-reactivity of antiserum with a polypeptide species of 66,000 Mr was detected. This protein most likely represents bovine serum albumin present in conceptus cultures. No detectable antibody-protein complexes were observed on autoradiograms of nitrocellulose blots incubated with nonimmune rabbit serum.

Prostaglandin Secretion

To examine the effect of heat stress on prostaglandin synthetic rate of conceptus and endometrial tissues, samples of medium were analyzed for PGF and PGE2 by RIA. It has been shown previously that with bTP-1 were reduced 71.7% (p<0.01) relative to proteins obtained from thermoneutral conceptuses (7391 ± 698 vs. 26,099 ± 2758 dpm/mg tissue/24 h).

Immunoblotting

As a final test of whether bTP-1 secretion was reduced by heat stress, antiserum to oTP-1, an immunologically related species (Helmer et al., 1987), was used to detect bTP-1 in culture medium by immunoblotting. Autoradiograms of immunoblots are depicted in Figure 6. Visual appraisal of autoradiograms indicated that oTP-1 antiserum bound specifically to protein species with an apparent molecular weight range of 22,000–26,000. These antibody-reactive proteins were of similar molecular weight to those in the predominant protein bands detected by fluorography (Fig. 3) as well as those in the major radioactive peak solubilized from 1D SDS-PAGE gels (Fig. 5). Collectively, these different experimental approaches confirm that proteins within this molecular weight range may in part represent the bTP-1 component of conceptus secretory proteins. Heat stress of the conceptus resulted in a marked reduction in secretory proteins transferred to nitrocellulose that bound oTP-1 antiserum, as indicated by a decrease in [125I]-Protein A labeling of antibody-bound proteins detected by autoradiography. Minor cross-reactivity of antiserum with a polypeptide species of 66,000 Mr was detected. This protein most likely represents bovine serum albumin present in conceptus cultures. No detectable antibody-protein complexes were observed on autoradiograms of nitrocellulose blots incubated with nonimmune rabbit serum.
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ENDOMETRIUM

CONCEPTUS
most PGF and PGE₂ present in culture medium of endometrial explants is derived from de novo synthesis of PG during culture (Thatcher et al., 1984). Endometrium maintained at 39°C synthesized and released prostaglandins primarily during the first 3 h of incubation (Fig. 7). Concentrations of PGE₂ detected in culture medium of thermoneutral endometrium increased throughout culture, resulting in higher \((p<0.01)\) levels of PGE₂ than PGF (Table 2) at the end of culture. Prior to heat stress, secretion of prostaglandins by endometrial tissues was similar to that by thermoneutral control tissues (heat stress vs. thermoneutral, PGF: \(3.4 \pm 0.5\) vs. \(2.8 \pm 0.5\); PGE₂: \(2.5 \pm 0.5\) vs. \(4.1 \pm 1.5\) pg/ml/mg tissue/6 h). Elevation of incubation temperature from 39°C to 43°C stimulated endometrial PGF release, resulting in a 125.5% increase \((p<0.01)\) in concentrations detected in culture medium. Concentrations of PGE₂ were not affected significantly by heat stress. As a result,

TABLE 2. Concentrations of prostaglandin (PG) F and PGE₂ in medium supernatants collected after 24 h of culture of conceptuses and endometrium at thermoneutral or heat-stress temperatures (least squares mean ± SEM).

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>X treatment</th>
<th>n</th>
<th>PGF (pg/ml/mg tissue/24 h)</th>
<th>PGE₂ (pg/ml/mg tissue/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrium</td>
<td>Thermoneutral</td>
<td>10</td>
<td>(5.6 ± 0.7)</td>
<td>(13.1 ± 2.9)</td>
</tr>
<tr>
<td></td>
<td>Heat stress</td>
<td>10</td>
<td>(74.2 ± 17.9^{**})</td>
<td>(18.6 ± 5.4)</td>
</tr>
<tr>
<td>Conceptus</td>
<td>Thermoneutral</td>
<td>5</td>
<td>(21.5 ± 8.8)</td>
<td>(12.2 ± 4.1)</td>
</tr>
<tr>
<td></td>
<td>Heat stress</td>
<td>6</td>
<td>(9.6 ± 3.3)</td>
<td>(43.9 ± 33.8^{*})</td>
</tr>
</tbody>
</table>

\(^{*}(p<0.05)\) For thermoneutral vs. heat stress for conceptuses. Due to a large coefficient of variation, data were transformed to Log₁₀ prior to analysis.

\(^{**}(p<0.001)\) For thermoneutral vs. heat stress for endometrium.

Fig. 3. One-dimensional polyacrylamide gel electrophoresis of secretory proteins present in conceptus culture medium. Samples were medium supernatants of individual conceptuses collected after 24 h of culture. After dialysis \((M_r \text{ cutoff } = 6000 \text{ to } 8000)\), proteins were separated by SDS-PAGE using 12.5% (w/v) polyacrylamide gels. Radiolabeled proteins were localized by fluorography. Equal volumes (100 µl) of medium were loaded per lane. Fluorographic exposure time differed for conceptuses (43°C > 39°C) in order to examine qualitative differences in radiolabeled polypeptides in medium between treatments. Note that the major \(^{1}H\)leucine-labeled proteins identified in conceptus secretions were reduced by heat stress (arrows) and that overall incorporation was much reduced.

Fig. 4. One-dimensional polyacrylamide gel electrophoresis of secretory proteins present in endometrial culture medium. Samples were medium supernatants of endometrium collected after 24 h of culture. After dialysis \((M_r \text{ cutoff } = 6000 \text{ to } 8000)\), proteins were separated by SDS-PAGE using 12.5% (w/v) polyacrylamide gels. Radiolabeled proteins were localized by fluorography. Equal volumes (100 µl) of medium were loaded per lane.
heat-stressed endometrium secreted more (p<0.01) PGF than PGE₂.

Although conceptuses released prostaglandins during culture, there was a trend towards decreased PGF and PGE₂ concentrations in medium as the duration of culture increased at 39°C (Fig. 7). Imposition of heat stress, however, stimulated conceptus production of PGE₂, resulting in a 360% increase (p<0.05) in concentrations detected in culture medium. Concentrations of PGF were not affected by heat stress.

DISCUSSION

Physiological studies have demonstrated that early stage embryos are extremely sensitive to high environmental temperature and humidity (Alliston and Ulberg, 1961; Dutt, 1963; Alliston et al., 1965; Elliott et al., 1968; Elliott and Ulberg, 1971; Ulberg and Sheenan, 1973; Putney et al., 1988) resulting in increased mortality among stressed embryos. The present study has shown that in vitro elevation of incubation temperature of later Day 17 bovine conceptuses from 39° to 43°C reduces total protein synthetic capacity while enhancing the synthesis of heat-shock or heat-stress proteins. Similar effects of in vivo thermal stress on subsequent in vitro protein production by conceptuses have been noted in swine (Wetteman et al., 1984). These in vitro data suggest that high environmental temperature may severely alter conceptus metabolic activity in vivo and lead to reduced growth rates and failure of conceptuses to produce biochemical signals in adequate amounts required for preventing CL regression. Smaller conceptuses may not develop the biosynthetic capacity required to properly signal the maternal system to maintain CL function, as evidenced by findings that heat stress caused reduced CL weight and a trend towards increased pregnancy failure (Biggers et al., 1987).

FIG. 5. Electrophoretic profile of [³H]leucine-labeled polypeptides accumulated into conceptus culture medium after 24 h of culture. After dialysis (Mr cutoff = 6000 to 8000), proteins were separated by 1D SDS-PAGE using 12.5% (w/v) polyacrylamide gels and were solubilized from the slab gel matrix with H₂O₂. Equal volumes (100 µl) of conceptus-conditioned medium from each conceptus were loaded per lane. Shown are mean profiles of radiolabeled proteins (dpm/mg tissue) for thermoneutral (solid line) and heat-stressed (broken line) conceptuses. Note that peaks in detected radioactivity, corresponding to predominant proteins identified by 1D SDS-PAGE and fluorography, were reduced (p<0.05) by heat stress (arrows).

FIG. 6. Immunoblotting of bovine trophoblast protein-1 (bTP-1) released into conceptus culture medium during 24 h of culture. After dialysis (Mr cutoff = 6000 to 8000), proteins were separated by 1D SDS-PAGE using 12.5% (w/v) polyacrylamide gels, electrophoretically transferred to nitrocellulose membrane, and immunoblotted with either nonimmune (not shown) or anti-ovine trophoblast protein-1 (oTP-1) rabbit serum. Equal volumes (100 µl) of conceptus-conditioned medium from each conceptus were loaded per lane. Antibody-protein complexes bound to nitrocellulose were detected by ¹²⁵I-Protein A labeling of antibody and autoradiography. Note specific binding of oTP-1 antibody to bTP-1 was reduced by heat stress (arrow).
FIG. 7. Secretion of prostaglandins (PGF and PGE₂) into culture medium by endometrium and conceptuses. Samples of conditioned medium (300 µl) were analyzed for prostaglandins by radioimmunoassay. Shown are profiles of PGF and PGE₂ for thermoneutral (solid line) and heat-stressed (broken line) endometrium and conceptuses. Endometrial secretion of PGF (p<0.001) and conceptus secretion of PGE₂ (p<0.05) increased in response to heat stress.

Conceptus-conditioned culture medium was enriched in a group of low molecular weight proteins (20,000–26,000 Mr). Antiserum to oTP-1 cross-reacts immunologically with several components of the array of low molecular weight proteins present in culture medium. This complex of proteins is referred to as bTP-1 complex and is believed to be involved in preventing luteal regression during early pregnancy (Helmer et al., 1987). Heat stress, by altering total protein synthetic capacity of conceptuses, induced a marked reduction in secretory proteins, particularly proteins within the bTP-1 complex. This may compromise successful rescue of the CL from regression, since this event may depend directly upon the appropriate timing and quantity of secretory proteins produced by the conceptus.

Concentrations of prostaglandins released by tissues maintained at 39°C were similar to those synthesized in vitro by bovine endometrium from Day 17 of pregnancy (Thatcher et al., 1984). Heat stress of endometrial tissues resulted in a marked increase in release of PGF into culture medium, possibly due to alterations in membranes resulting in increased mobilization of substrates for prostaglandin biosynthesis. The primary cellular site for the action of heat damage on tissues is located in membranes (Bowler et al., 1973; Hahn, 1982), causing alterations in membrane lipid composition (Anderson and Parker, 1982) as well as increases in membrane fluidity, phospholipase activity, and phosphoinositide turnover (Calderwood et al., 1987). Heat-induced increases in the turnover of membrane phospholipids and the release of fatty acids, such as arachidonic acid, may provide substrates for prostaglandin synthesis (Flint et al., 1986). Similar increases in endometrial PGF₂₀ production in response to heat stress have been reported in vivo with gilts (Wetteman et al., 1984; Hoagland and Wetteman, 1984). Since maintenance of luteal function in cattle is associated with alterations in endometrial prostaglandin production (Thatcher et al., 1984; Gross et al., 1988b), increased endometrial prostaglandin secretion in response to thermal stress may compromise CL function and initiate earlier luteal regression.

Conceptuses maintained at a high incubation temperature synthesized proteins not synthesized by control tissues. The predominant heat-shock protein was of 70,000 Mr, similar to that found in other heat-shocked mammalian tissues (Nover, 1984). Considerable research has focused on the identification and characterization of heat-shock proteins. The most prominent of these proteins, the mammalian 70,000 Mr heat-shock protein, is synthesized by mouse (Wittig et al., 1983), rat (Mirkes, 1987), and rabbit (Heikkila and Schultz, 1984) embryos maintained at high incubation temperatures. While of uncertain function, it has been assumed that heat-shock proteins may play an essential role in cellular homeostasis and thermotolerance during periods of environmental stress (Loomis and Wheeler, 1980; Li and Werb, 1982).

Elevation of incubation temperature of endometrial explants did not appear to alter protein synthetic rate of tissues. However, endometrial tissues in both treatment groups synthesized proteins similar in molecular weight (70,000 and 91,000 Mr) to heat-shock proteins identified in heat-stressed conceptuses. The intensity of these endometrial proteins was enhanced in tissues exposed to heat-stress culture conditions. The presence of a 70,000 Mr heat-stress protein in control tissues was not unexpected. Two members of the 70,000 Mr heat-shock “family” of proteins have been identified (Welch et al., 1982): a constitutively produced 73,000 Mr heat-shock protein that is produced at homeothermic temperatures but whose production is amplified during heat stress, and an inducible 72,000 Mr protein that is
produced as a result of tissue shock. Alternatively, trauma of tissue slicing or incubation conditions of the present experiment may have resulted in some expression of heat-shock proteins by endometrium in both treatment groups. Similarly, Hightower and White (1981) reported that several high molecular weight, stress-induced proteins were synthesized by sliced mammalian tissues in vitro but not synthesized by tissues in vivo.

In summary, elevation in tissue incubation temperature from 39° to 43°C induced a large reduction in conceptus protein synthesis and secretion and stimulated release of PGF by pregnant endometrium. These in vitro results suggest that exposure of pregnant cows to high environmental temperature and humidity, as often occurs during summer months of the year, may disrupt the balance between conceptus and endometrial biochemical factors responsible for maintenance of pregnancy.

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