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Messenger RNA expression of angiotensin-converting enzyme, endothelin, cyclooxygenase-2 and prostaglandin synthases in bovine placentomes during gestation and the postpartum period

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Abstract

The bovine placenta contains local vasoactive-related systems, including angiotensin-converting enzyme (ACE), endothelin-1 (ET-1), ET-A receptor (ETAR) and ET-B receptor (ETBR), as well as arachidonic acid (AA) cascade-related enzymes, such as cyclooxygenase-2 (Cox-2), prostaglandin E-synthase (PGES) and prostaglandin F-synthase (PGFS). The mRNA expression of these molecules was examined in bovine placentomes (caruncles and cotyledons) collected immediately (0 h) and 6 h after spontaneous parturition from 15 cows with early (fetal membranes released within 6 h of parturition) or late (fetal membranes released 6–12 h after parturition) detachment, as well as from 15 pregnant cows at a slaughterhouse. Significant differences were observed in expression of ET-1, ETAR and ETBR mRNAs between gestation and the postpartum period in both caruncles and cotyledons. Significant differences were also found between 0 and 6 h postpartum in the expression of ETBR mRNA in the early detachment group and PGES mRNA in the early and late detachment groups. Compared to PGFS, both Cox-2 and PGES exhibited opposite mRNA expression patterns during gestation and the postpartum period. The vasoactive-related peptide systems and AA cascade-related enzymes may mediate placental development and fetal membrane detachment after parturition in cattle.

Keywords: Cattle; Retained placenta; Vasoactive-related systems; Arachidonic acid cascade-related enzymes

Introduction

Retention of fetal membranes (RFM) affects herd health, milk production and reproductive efficiency (Laven and Peters, 1996; Grohn et al., 1998). Several local factors in placentomes might be involved in the aetiology of bovine RFM during the cascade of endocrine events that occurs after the onset of parturition (Thorburn et al., 1977; Lye, 1996). However, the physiology of the processes involved in placental release is not completely understood (Kankofer, 2002).

The bovine maternal and fetal placental units (caruncles and cotyledons, respectively) produce factors that are important local regulators of placental function (Hoffmann and Schuler, 2002). Angiotensin-converting enzyme (ACE), which transforms angiotensin (Ang) I into bioactive Ang II, is present in the bovine uterus and placental tissues, indicating the existence of a local renin–angiotensin system (RAS) in the bovine uteroplacental unit (Feng et al., 2000; Schauser et al., 2001). Endothelin (ET)-1 is an endothelial cell-derived vasoconstrictive peptide that may be involved in uterine stimulation during parturition in humans and

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rats (Horwitz et al., 1995; Dotsch et al., 2001). ACE and ET-1 are thought to be involved in the regulation of angiogenesis, uterine contractions and uterine growth throughout pregnancy in cattle (Schauser et al., 2001).

ET-1 has dual effects on blood vessels through the ET-A receptor (ETAR) and ET-B receptor (ETBR) (Sakurai et al., 1990; Sumner et al., 1992; Karaki et al, 1993). ETAR is expressed in vascular smooth muscle and activation by ET-1 and ET-2 mediates vasoconstriction (Sumner et al., 1992). ETBR is found in vascular endothelial cells and is activated by ET-1, ET-2 and ET-3 (Sakurai et al., 1990). No information is available on the expression of these receptors, the enzymes related to local vasoactive peptide systems and related receptors in bovine placentomes during the pre- and postpartum periods.

Prostaglandins (PGs) are actively synthesised in the bovine endometrium and placental tissues; they regulate uterine motility and play important roles as local factors necessary for placental expulsion (Gross et al., 1991). There is a complex chain of events in the arachidonic acid (AA) cascade during the pre- and postpartum periods in the bovine placentome (Parent et al., 2003; Arosh et al., 2004; Schuler et al., 2006). Disturbances in PG metabolism have been associated with RFM (Slama et al., 1994; Kankofer, 1999; Wischral et al., 2001; Kankofer, 2002). Little information is available on expression of AA cascade-related enzymes in the bovine placentome from early gestation to the postpartum period.

Previously, we reported the concentrations of several local factors in placental tissues after parturition from cows with or without RFM and suggested that the vasoactive peptides Ang II and ET-1 play tissue-specific roles in the placenta and probably amplify the local endocrinological cascade, involving oxytocin, the oxytocin receptor and $PGF_{2\alpha}$, which are necessary for normal placental separation in cattle (Takagi et al., 2002). As a first step towards understanding the activity of local vasoactive peptides in bovine placentomes during gestation and the postpartum period, we analysed the mRNA expression of vasoactive-related systems (ACE, ET-1, ETAR and ETBR) and AA cascade-related enzymes (cyclooxygenase-2, Cox-2; PGE-synthase, PGES and PGF-synthase, PGFS) in caruncles and cotyledons during gestation and the postpartum periods.

Materials and methods

Animals and sample collection

Placental tissues were collected from Holstein cows ($n = 15$, 24–63 months of age) at Obihiro University Farm. The experimental procedures complied with the Guide for Care and Use of Agriculture Animals of Obihiro University.

As a preliminary trial, we examined the effects of the manual separation of placentomes (0, 6 and 12 h after parturition) on postpartum recovery in three cows; we confirmed the absence of vaginal bleeding after separation and did not detect any uterine disorders during an examination conducted 1 month after parturition. Based on the above preliminary observations, postpartum placentomes were manually collected from the uterus via the vagina at 0 h (immediately after) and 6 h after spontaneous parturition. In the cows in this experiment, no clinical uterine disorders were revealed, either by postpartum endoscopic examination of the vagina and the external os of the cervix with a speculum at 24 h after parturition or by postpartum uterine examination with an ultrasound scanner at 1 month after parturition.

The fetal and maternal membranes of the placentomes were manually separated, rinsed with cold saline solution, immediately frozen in liquid nitrogen and then stored at –80 °C until processing. The cows were divided into two groups, depending on the time of fetal membrane expulsion: the early detachment group, in which the fetal membranes were released within 6 h of parturition ($n = 10$) and the late detachment group, in which the fetal membranes were released 6-12 h after parturition ($n = 5$).

Uteri of pregnant Holstein cows ($n = 15$) were collected from the local slaughterhouse and used to examine mRNA expression during the gestation period. The pregnant uteri were opened and the crown-rump lengths of the fetuses were recorded to estimate fetal age. Relatively large single placentomes (1 cm × 1 cm) were also collected at the slaughterhouse.

Placental tissues were divided into three groups according to the criteria for the estimation of fetal age (Boos et al., 2003): the first (up to 3 months of age, $n = 4$), second (4–6 months of age, $n = 6$) and third (7–9 months of age, $n = 5$) trimester groups. The fetal and maternal membranes of the placenta were manually separated, rinsed with cold saline solution, immediately frozen in liquid nitrogen and then stored at −80 °C until processing.

RNA extraction and reverse transcription

Total cellular RNA was extracted from each frozen tissue specimen with TRIzol reagent, treated with DNase (SV total RNA Isolation System, Promega) and frozen at −20 °C in RNA Storage Solution (Ambion). RNA was quantified by measuring absorbance at 260 nm ($A_{260\,nm}$) and its purity was determined by the $A_{260\,nm}/A_{280\,nm}$ ratio using a spectrophotometer.

Single-stranded cDNA was reverse-transcribed from total RNA (0.5–5 μg) using a first strand cDNA Synthesis Kit for real time polymerase chain reaction (PCR) (Roche Diagnostics) and a random primer. The reverse transcriptase (RT) cycle comprised annealing for 10 min at 25 °C, cDNA synthesis for 60 min at 42 °C and inactivation for 5 min at 99 °C. Solutions were then stored at −20 °C.

Quantitative PCR

The genes encoding ACE, ET-1, ETAR, ETBR, Cox-2, PGES and PGFS were quantified by real time PCR in a LightCycler (Roche Diagnostics) using a commercial kit (LightCycler FastStart DNA Master SYBR Green I, Roche Diagnostics). Primers were designed using Primer-3 based on bovine sequences (Table 1).

The amplification programme consisted of initial activation for 15 min at 95 °C, followed by 40 cycles of PCR (denaturation for 15 s at 94 °C, annealing for 30 s at 58 °C and extension for 20 s at 72 °C). To quantify the target genes, a series of standards were constructed by amplifying a fragment of DNA (~700 bp) containing the target sequence by real-time PCR (100–150 bp).

The PCR products were subjected to electrophoresis and the target band was purified using the SUPRECTM-01 DNA purification kit (TaKaRa Bio). In every PCR run, 3–5 stepwise-diluted DNA standards were included. The values were normalised using β-actin as an internal standard.

Statistical analysis

All data are expressed as the means ± standard error (SE). The relative mRNA expression of each gene from both the caruncles and cotyledons was analysed by ANOVA using the General Linear Model procedure of SAS.

The total results obtained for the gestation and postpartum samples were analysed. In the overall analysis, we considered the following sources