Prostaglandin H_2 synthase-1 and -2 expression in guinea pig gestational tissues during late pregnancy and parturition

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Increased intrauterine prostaglandin (PG) production is crucial for the initiation of parturition. To investigate the mechanisms controlling intrauterine PG synthesis, we examined the expression of the key PG biosynthetic isoenzymes, PG-H_2 synthase (PTGS)-1 and -2, in the amnion, visceral yolk sac (VYS), placenta and myo-endometrium of pregnant guinea pigs. This animal model was chosen because the hormonal milieu of pregnancy and the role of PGs in the hormonal control of parturition are similar to those in the human. PTGS1 mRNA abundance, measured by real-time RT-PCR, increased in the amnion and the placenta during the last third of gestation. During labour, PTGS1 mRNA levels decreased precipitously in all four tissues. PTGS1 protein abundance, assessed by immunoblotting, increased to high levels in the amnion and the placenta by the end of pregnancy and remained high during labour. PTGS2 mRNA expression was higher in the placenta than in the other tissues, but did not change before and during labour. PTGS2 protein expression decreased in the placenta and remained low in the other tissues during labour. Immunohistochemistry showed pervasive PTGS1 protein expression in the amnion and strong expression in the parietal yolk sac membrane (PYS) covering the placenta. PTGS2 was expressed in the PYS and the endometrium. The PTGS inhibitor piroxicam, administered in doses that inhibited PTGS1 but not PTGS2, significantly prolonged gestation. These data suggest that PGs generated by intrauterine PTGS1 are involved in the timing of birth in guinea pigs. The induction of PTGS1 in the amnion and the PYS is a critical event leading to labour in guinea pigs and models analogous changes in the human gestational tissues before labour.

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Prostaglandins (PGs) produced by the intrauterine tissues (placenta, fetal membranes, decidua/endometrium and myometrium) play pivotal roles in the onset of labour in mammalian species (Challis *et al.* 2000). The involvement of PGs in parturition is best characterized in mice, where increased production of $PGF_{2\alpha}$ by the endometrium causes luteolysis, which decreases circulating progesterone levels, triggering birth. Studies in knockout mice have shown that the induction of PG-H_2 synthase-1 (PTGS1, other synonyms are COX-1 and PGHS-1), an enzyme that catalyses the committing and rate-limiting step in PG biosynthesis, is the critical event that leads to the increased endometrial $PGF_{2\alpha}$ production at term (Reese *et al.* 2000; Tsuboi *et al.* 2000; Gupta *et al.* 2001). In women, the expression of the other isoenzyme, PTGS2 (COX-2, PGHS-2), increases in the fetal membranes (amnion and chorion laeve) before and during labour (Hirst *et al.* 1995; Mijovic *et al.* 1999; Slater *et al.* 1999). Furthermore, women give birth without a need for luteolysis and without a decrease in circulating progesterone levels. The differences between human and murine parturition suggest that the control of birth by PGs and progesterone is fundamentally different in the two species. For this reason, the utility of the mouse is limited in studies where new information on the hormonal mechanisms of birth is extrapolated from an animal model to humans.

Molecular phylogenetics has shown robustly that mouse and man belong to the same superorder of placental mammals (Euarchontoglires) (Carter, 2001; Springer *et al.* 2003). The cohort Euarchonta, which includes the Primates, and the cohort Glires, which includes the Rodenta and Lagomorpha (rabbit), separated approximately 87 million years ago, followed by the separation of the Rodent and Lagomorph orders. The

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Table 1. Gestational stages at which guinea pig tissues were collected

Group	Description
A	44–47 days gestation; 'early'
B	52–56 days; before attachment of visceral yolk sac (VYS) to the endometrium
C	54–64 days; following attachment of VYS to the endometrium
D	57–65 days; first day of palpable pubic symphysis (PS) separation
E	62–66 days; fifth day following PS separation
L	During labour, following the delivery of at least one pup

Each group comprised 6 pregnant animals.

caviomorph (guinea pig) and murid (rat, mouse) lineages of Rodenta diverged later (approx. 67 million years ago) according to the molecular data. In view of this timeline, it is remarkable that guinea pigs (*Cavia porcellus*) give birth in the presence of high circulating progesterone levels and without a requirement for prepartum luteolysis, like higher primates and unlike mice and rabbits (Challis *et al.* 1975; Thorburn & Challis, 1979). Moreover, circulating sex steroid levels in guinea pigs and women follow analogous patterns during pregnancy, and responses to progesterone antagonist treatments are similar (Challis *et al.* 1971; Tulchinsky *et al.* 1972; Elger *et al.* 1986). Guinea pigs are therefore considered the best non-primate species to model the steroid regulation of human pregnancy. The similarities extend to the role of PGs. PG administration induces labour and delivery in guinea pigs, like in women and other mammals (Elger & Hasan, 1985). The guinea pig gestational tissues (amnion, visceral yolk sac, placenta and myo-endometrium) produce labour-promoting PGs (PGE_2 and $PGF_{2\alpha}$), and the PG output of the placenta and the amnion increases with advancing pregnancy (Moussard *et al.* 1986; Schellenberg & Kirkby, 1997). Schellenberg and colleagues have demonstrated that the predominant intrauterine source of PGs is the amnion membrane and that PTGS activity in the amnion rises before and during labour as a result of increased enzyme synthesis (Schellenberg & Kirkby, 1997; Schellenberg *et al.* 2003). They have detected both PTGS1 and -2 mRNAs in the guinea pig amnion, but found no change in PTGS2 mRNA abundance during the last third of gestation. In the present investigation we have characterized the expression of the other PTGS isoenzyme, PTGS1, in the guinea pig amnion, visceral yolk sac (VYS; the anatomical equivalent of the chorion membrane), placenta and myo-endometrium in late pregnancy. We have measured PTGS1 mRNA and protein levels and determined the cellular localization of PTGS1 protein by immuno-histochemistry. We have also measured PTGS2 mRNA levels, protein abundance and protein localization in the same tissues to assess the relative importance of the two isoenzymes in intrauterine PG-production. Furthermore, we have tested the involvement of PTGS1 in the timing of labour by measuring the effect of selective PTGS1 inhibition on gestational length. The results show that PTGS1 is selectively induced in the guinea pig amnion and placenta at term and that PTGS1 activity is involved in the normal timing of birth. We suggest that PGs control parturition in guinea pigs in a manner that is analogous to humans, with amniotic PTGS1 performing a role corresponding to the role of amniotic PTGS2 in humans.

Methods

Materials

The sources and suppliers of the materials used are described in the Supplemental material.

Animals and tissue collection

Outbred tri-colour guinea pigs were time-mated at the Animal Services Unit of the University of Newcastle, Australia. The amnion and VYS membranes, placentae and the myo-endometria were collected at six consecutive stages of late gestation, listed in Table 1. These stages were based on the criteria described by Glasier & Hobkirk (1993). Tissues were collected from animals anaesthetized with xylazine hydrochloride (5–10 mg kg^{-1} s.c.) and ketamine hydrochloride (50–100 mg kg^{-1} s.c.). Anaesthesia was maintained with 2–4% halothane gas administered via a face-mask. Animals were killed immediately after tissue collection by an intravenous pentobarbitone overdose. The attachment of the VYS to the endometrium was determined during tissue collection to differentiate between Stages B and C. The separation of the pubic symphysis, which occurs 6–9 days before delivery in our colony, has been determined by gentle palpation. To determine the onset of labour, the pregnant dams were observed without disturbance using an infrared camera. Labouring guinea pigs were euthanized by CO_2 inhalation for 5 min following the delivery of at least one pup and tissues were collected from the mother and the undelivered pups. The animals have been monitored during all procedures to ensure that no pain and suffering occurred at any time. Six guinea pigs have been assigned to each gestational stage group, and each pregnancy produced one to six pups. For this reason, the number of maternal and fetal tissues obtained at each

gestational stage varied between 6 and 24. The details of animal husbandry, time-matings and the number of tissues processed for analysis at each stage are described in the online Supplemental material. All procedures were approved by the University of Newcastle Animal Care and Ethics Committee and were carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

RNA extraction and real-time RT-PCR

Total RNA was extracted with TRIZOL® reagent, column-purified and treated with DNase I. Complementary DNA (cDNA) was synthesized by reverse transcription using random hexamer primers.

The abundance of PTGS1 and PTGS2 mRNAs relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were determined by real-time PCR using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Primer sequences are listed in Supplemetary Table 1. The real-time PCR systems have been optimized to achieve equal efficiency of amplification of the target and reference mRNAs, and relative abundance was calculated after determining the threshold amplification cycle numbers (Livak & Schmittgen, 2001). The amplification products were characterized by dissociation curve analysis,

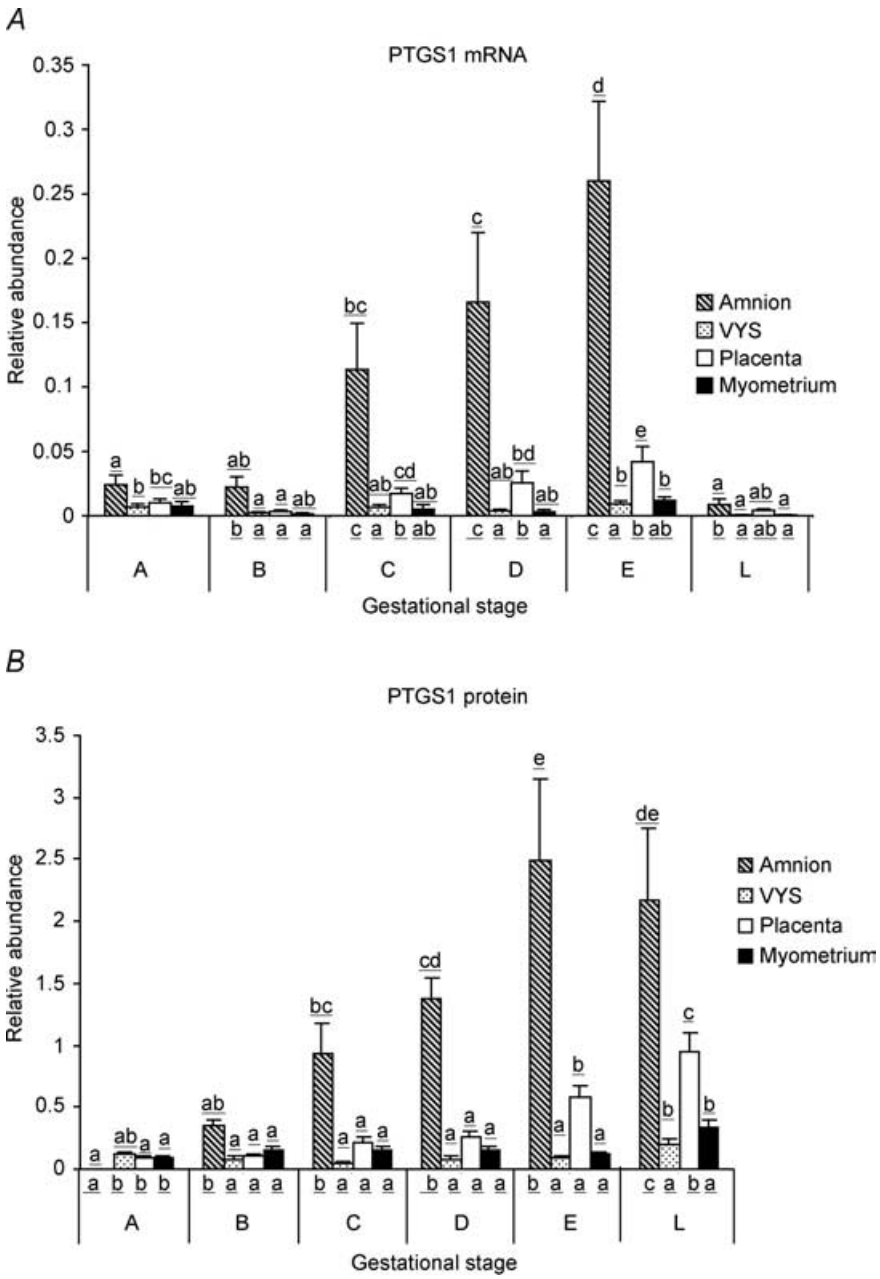

Figure 1. PTGS1 mRNA (*A*) and protein (*B*) relative abundance in guinea pig amnion, visceral yolk sac (VYS), placenta and myo-endometrium throughout late gestation and in labour
Means ± S.E.M. are presented, $n = 6$–24 per group as detailed in the online Supplemental material. Letters above the bars indicate significance levels between gestational stages for each tissue type, while letters under the bars indicate significance levels between tissue types at each gestational stage ($P < 0.05$, nested ANOVA using general linear models, with Tukey's multiple comparison test). Gestational stages are described in Table 1.

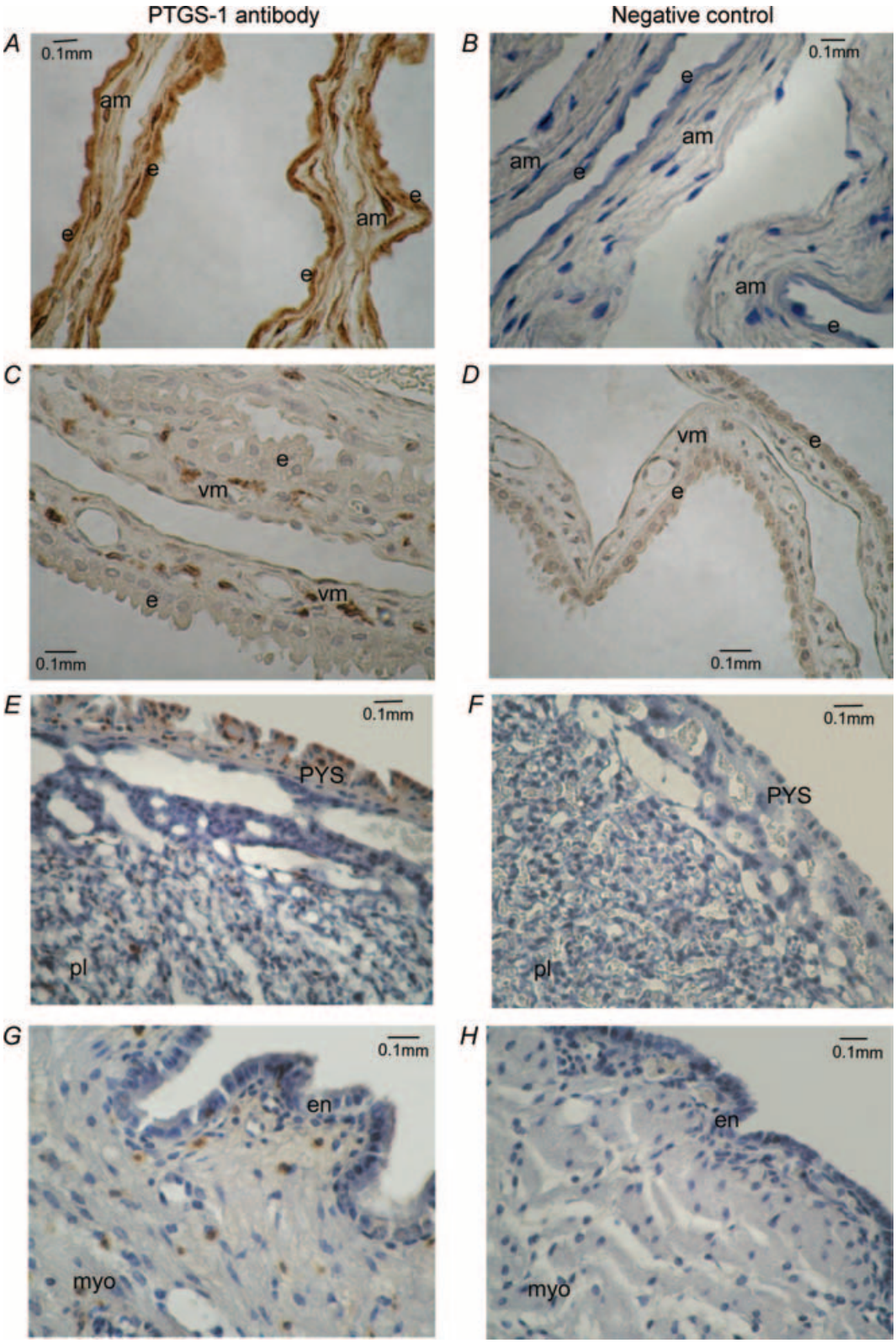

Figure 2. PTGS1 protein localization in guinea pig gestational tissues by immunohistochemistry
Tissue sections incubated with PTGS1 antibody are shown in panels *A*, *C*, *E* and *G*; negative controls (PTGS1 antibody preabsorbed with immunizing peptide) are shown in panels *B*, *D*, *F* and *G*. PTGS1 protein is indicated by brown staining; counterstaining is with Scott's blue. The tissues were obtained from animals at term

agarose gel electrophoresis and nucleotide sequencing. Procedural details and the results of PCR optimization and PCR product analysis are described in the online Supplemental material.

Immunoblotting and immunohistochemistry

PTGS1 and PTGS2 protein abundance in the amnion, VYS, placenta and myo-endometrium of guinea pigs were determined by immunoblotting. The intra- and inter-assay coefficients of variation for the quantitative immunoblotting systems were between 7% and 10%. PTGS1 and PTGS2 proteins were localized in formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry. The details of these procedures are described in the Supplemental material.

Inhibition of PTGS1 *in vivo*

Pregnant guinea pigs were injected with the preferential PTGS1 inhibitor piroxicam (Meade *et al.* 1993) or vehicle every day from the first day of pubic symphysis separation until delivery. Animals were randomly assigned to the piroxicam and vehicle treatment groups on the first day of pubic symphysis separation. The guinea pigs were killed following delivery, and blood was collected by cardiac puncture. PTGS1 and PTGS2 activity was measured in the collected blood by established procedures to verify the selective inhibition of PTGS1 by the piroxicam treatment protocol employed (Smith *et al.* 1998; Chan *et al.* 1999). Technical details are described in the Supplemental material.

Statistical analysis

The Skewness and Kurtosis Test for normality was used to assess the distribution of data (Intercooled Stata v 8.0 software; Stata Corporation, College Station, TX, USA). Where appropriate, data were transformed to normal equivalent deviates (NED) in order to achieve normal distribution (Rowe, 2002). Analysis of variance (ANOVA) using general linear models (GLM), with individual pregnancies nested within their gestational stage, was performed to assess gestational stage-dependent differences in mRNA or protein levels (Minitab release 12.1 software; Minitab Inc., State College, PA, USA). Differences between the gestational stage groups were resolved by Tukey's multiple comparison test if significant F-values were found with ANOVA. The number of tissues analysed by real time RT-PCR and immunoblotting at the various gestational stages were used as the n-values in the statistical analyses. The n-values for each group are listed in the Supplemental material. Student's t test was used to compare gestational length, PTGS1 activity and PTGS2 activity between vehicle- and piroxicam-treated animals. For all analyses, $P < 0.05$ was considered statistically significant.

Results

PTGS1 expression

PTGS1 messenger RNA. PTGS1 mRNA was detected by real time RT-PCR in all tissue types (Fig. 1*A*). At the earliest gestational stage studied (44–47 days, Stage A), PTGS1 mRNA relative abundance was not different among the tissues. As pregnancy advanced, PTGS1 mRNA abundance increased sharply in the amnion and reached significantly higher levels than in the VYS, placenta and the myo-endometrium. In labour, however, amniotic PTGS1 mRNA levels decreased precipitously (Stage L). Placental expression of PTGS1 mRNA also increased with advancing pregnancy, while the increase of PTGS1 mRNA levels in the VYS and the myo-endometrium was marginal. PTGS1 mRNA levels were significantly lower in these tissues during labour than at late gestation (Stage E), like in the amnion.

PTGS1 protein. PTGS1 protein abundance was measured in the particulate (microsomal) fractions by immunoblotting. The results are shown in Fig. 1*B*. At Stage A, PTGS1 protein levels were low in the VYS, placenta and myo-endometrium and were undetectable in the amnion. As gestation advanced, PTGS1 protein expression increased steadily in the amnion, reaching significantly higher levels than in the other tissues. Furthermore, PTGS1 protein levels remained high in the amnion during labour, in contrast with the cognate mRNA. Placental PTGS1 protein abundance also increased by late pregnancy (Stage E) and rose further in labour, despite the fall of PTGS1 mRNA levels in labouring animals. A small, but significant, increase of PTGS1 protein expression occurred during labour in the VYS and in the myo-endometrium.

PTGS1 protein localization. Representative immunohistochemical pictures showing PTGS1 protein localization in amnion, VYS, placenta and myo-endometrium are presented in Fig. 2. PTGS1 protein was pervasively expressed in the epithelium and mesoderm

prior to labour onset (Gestational Stage E). *A* and *B*, amnion; *C* and *D*, visceral yolk sac; *E* and *F*, placenta; and *G* and *H*, myo-endometrium. e, epithelium; am, amnion mesoderm; vm, visceral yolk sac mesoderm; PYS, parietal yolk sac; pl, placental labyrinthe; en, endometrium; myo, myometrium.

of the amnion membrane (Fig. 2*A*). In the VYS, PTGS1 protein was present in sporadic cells within the mesoderm, but the epithelium was devoid of positive staining (Fig. 2*C*). PTGS1 protein was abundantly expressed in the parietal yolk sac membrane (PYS) overlaying the placenta and in sporadic cells within the placental labyrinth (Fig. 2*E*), but not in other placental cells (trophoblast and endothelium). The endometrium and myometrium expressed PTGS1 only in isolated sporadic cells (Fig. 2*G*).

PTGS2 expression

PTGS2 mRNA. PTGS2 mRNA was present in all tissues examined, with the highest relative abundance in the placenta (Fig. 3*A*). There was a significant decrease in placental PTGS2 mRNA level in the late group (Stage D) compared to the earliest group (Stage A), but there was no difference in PTGS2 mRNA abundance between placentas collected at Stages B, C, D, E and L. PTGS2 mRNA levels were relatively low in the amnion and the myo-endometrium at Stage A and decreased further with advancing pregnancy. A slight, but significant, increase was observed, however, in the two tissues with labour.

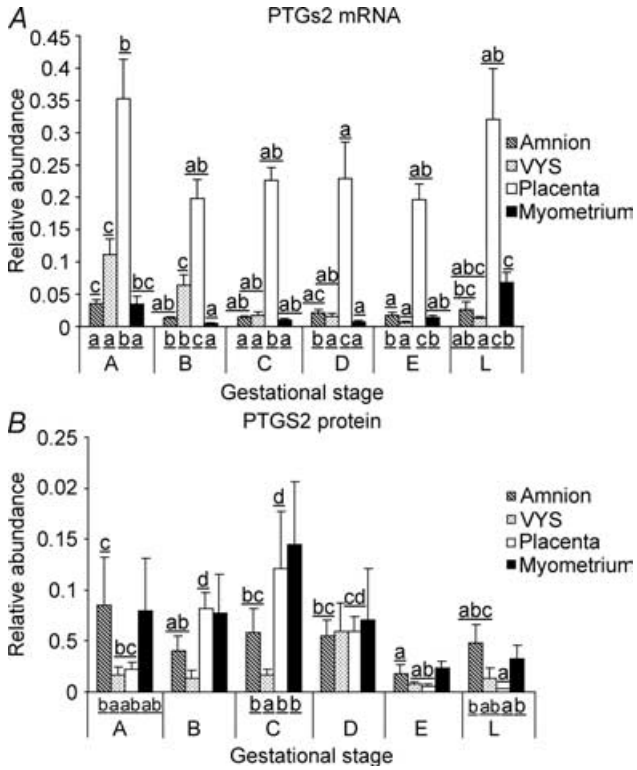

Figure 3. PTGS2 mRNA (*A*) and protein (*B*) relative abundance in amnion, VYS, placenta and myo-endometrium of guinea pigs throughout late gestation and in labour
Details are described in the legend to Fig. 1. Multiple comparisons are presented where a significant effect was found by ANOVA.

PTGS2 mRNA expression decreased in the VYS as gestation progressed and remained low during labour.

PTGS2 protein. PTGS2 protein levels, determined by immunoblotting, were variable between individual tissues, especially in the amnion and the myo-endometrium (Fig. 3*B*). In the placenta, statistical analysis showed a significant increase of PTGS2 protein abundance from Stage A to Stage C, followed by a decrease to low levels before labour (Stage E) and during labour (Stage L). PTGS2 protein was expressed in low and unchanging levels in the VYS. PTGS2 protein abundance in the amnion followed the trend of mRNA expression, exhibiting a significant decrease between Stages A and E. Variable expression in the myo-endometrium thwarted the statistical demonstration of differences during pregnancy, but a tendency for decreasing PTGS2 levels by term and in labour was observed. Between-tissue variance was significant in groups A, C and L, showing relatively low PTGS2 protein expression in the VYS at Stages A and C and diminished placental expression of enzyme protein during labour (L).

PTGS2 protein localization. Representative pictures showing the immunohistochemical localization of PTGS2 protein are presented in Fig. 4. In the placenta, PTGS2 expression was limited to the PYS membrane covering the placental surface (Fig. 4*A*). PTGS2 protein was also expressed in the endometrial layer of the uterus, showing stronger staining on the basal side (Fig. 4*C*). No staining of PTGS2 protein was seen in the amnion and the VYS (not shown).

PTGS inhibition *in vivo*

Selective inhibition of PTGS isoenzymes in pregnant animals may provide information about the involvement of PTGS1 and -2 in the control of gestational length. We have tested several PTGS inhibitors (nimesulide, valeryl salicylate, niflumic acid) for isoenzyme selectivity *in vitro*, but none of them was sufficiently selective towards guinea pig PTGS1 or -2. The preferential PTGS1 inhibitor piroxicam (Meade *et al.* 1993), however, showed good selectivity towards guinea pig PTGS1 in the preliminary *in vitro* experiments. We have injected pregnant guinea pigs with 5 mg kg^{-1} day^{-1} piroxicam starting on the first day of pubic symphysis separation, which is a gestational stage (Stage D) when PTGS1 is significantly induced in the amnion (Fig. 1). Piroxicam treatment resulted in a significant increase of the interval between pubic symphysis separation and delivery (Table 2). To verify the selective inhibition of PTGS1 *in vivo* by our piroxicam dose regimen, we have measured the activity of PTGS1 and PTGS2 in whole blood collected from the piroxicam-treated and vehicle-treated animals.

Table 2. The effects of piroxicam treatment on the interval between symphysis separation and birth and on the activity of PGHS1 and PGHS2

Parameter	Vehicle	Piroxicam	*P*
Days between symphysis separation and birth	8.5 ± 0.34 (6)	10.8 ± 0.95 (6)	0.043*
PGHS1 activity (ng TXB2 ml^{-1} (10 min)$^{-1}$)	211.33 ± 50.49 (3)	36.22 ± 18.77 (4)	0.014*
PGHS2 activity (ng PGE2 ml^{-1} (24 h)$^{-1}$)	4.489 ± 1.02 (4)	3.67 ± 0.65 (4)	0.522

Values are means ± S.E.M., with number of animals shown in parentheses. Guinea pigs were treated with vehicle (canola oil : DMSO 3 : 1) or piroxicam (5 mg kg^{-1} day^{-1}) s.c. from the first day of pubic symphysis separation until delivery. *Significant difference calculated by the two-tailed *t* test.

The results in Table 2 show that PTGS1 activity was significantly reduced in the piroxicam-treated guinea pigs while PTGS2 activity was not significantly different between the two groups. These data suggest that PTGS1 activity is required for timely onset of labour in guinea pigs. The length of labour and the well-being of the mother and the newborns were not affected by the piroxicam or vehicle (canola oil : DMSO 3 : 1) treatment.

Discussion

Increased PG synthesis in the uterus is critical for labour onset in guinea pigs, as in other mammalian species (Elger & Hasan, 1985). It has been shown that PGE_2 and $PGF_{2\alpha}$ production increases in the amnion and the placenta of guinea pigs by late gestation, and the activity of PTGS, the committing and rate limiting enzyme of the PG biosynthetic pathway, rises dramatically in guinea pig amnion before and during labour (Moussard *et al.* 1986; Moussard *et al.* 1987; Schellenberg & Kirkby, 1997). PTGS2 mRNA has been detected in the amnion, but its level did not change at term (Schellenberg *et al.* 2003). Our results are in agreement with these findings and extend them to PTGS2 protein abundance, which was either unchanged or decreased to low levels in the four gestational

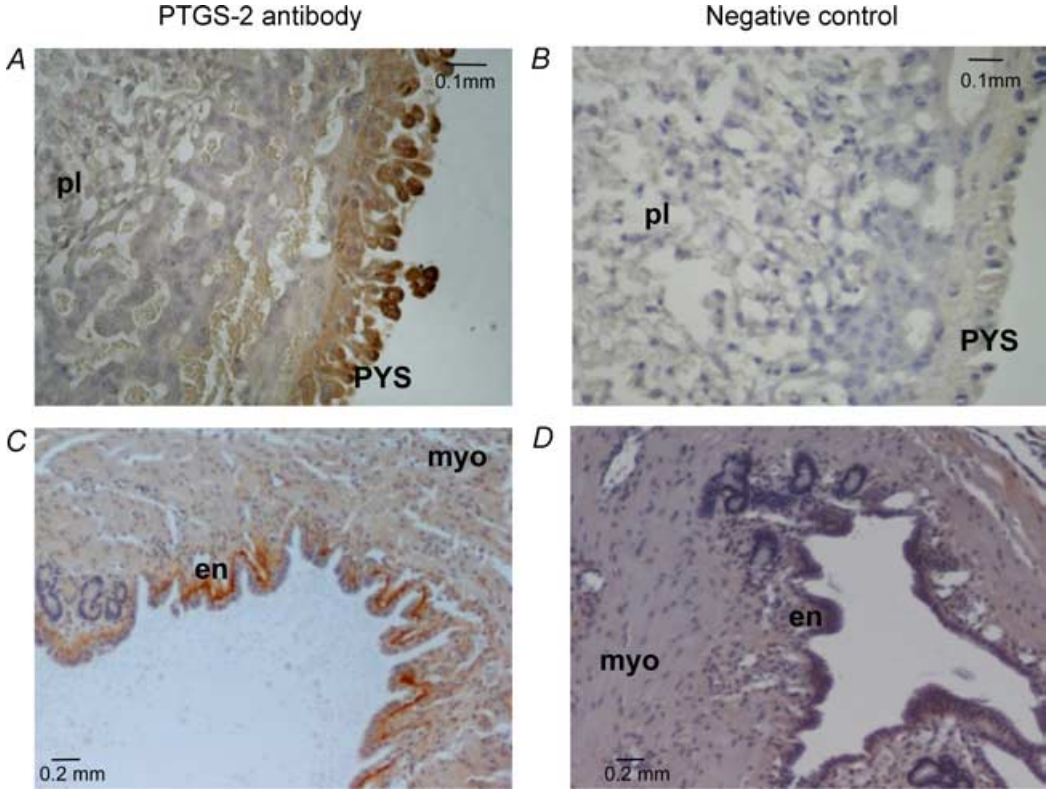

Figure 4. PTGS2 protein localization in guinea pig placenta and myo-endometrium by immunohistochemistry
Details are described in the legend to Fig. 2. *A* and *B*, placenta; *C* and *D*, myo-endometrium. Tissues were obtained from animals at Gestational Stage A. PYS, parietal yolk sac; pl, placental labyrinthe; en, endometrium; myo, myometrium.

tissues by late pregnancy and labour. We have shown, however, that the expression of the other PTGS isoenzyme, PTGS1, increases in guinea pig amnion, VYS, placenta and myo-endometrium by term and, at the protein level, during labour. Moreover, selective inhibition of PTGS1 activity *in vivo* significantly delayed delivery. Collectively, our data demonstrate that PTGS1 expression rises in the guinea pig uterus during late pregnancy and this enzyme generates PGs that control the timing of birth in this species.

PTGS1 mRNA and protein are expressed at higher levels in the amnion and the placenta than in the VYS and the myo-endometrium. This suggests that the former two tissues are the principal sources of labour-promoting prostaglandins. In the placenta, strong PTGS1 protein expression has been localized to the PYS membrane, which constitutes the surface layer of the placenta and faces the uterine wall. Increased PTGS1 expression in the amnion and the PYS ensures that the whole surface of the feto-placental unit increases its PG-producing capacity by the end of gestation. PTGS1 protein has also been localized to sporadic single cells in the VYS, placenta and myo-endometrium. Although we have not characterized these cells in the present investigation, the possibility exists that they correspond to resident or migrating inflammatory cells. Further work is needed to determine whether inflammatory mechanisms are involved in guinea pig parturition similarly to other mammalian species including humans (Dudley, 1999).

Figure 1 shows that PTGS1 mRNA abundance exhibited a similar overall trend of change in all four tissues towards term: major up-regulation in the amnion and a significant increase in the placenta, VYS and myo-endometrium. Increasing mRNA expression was accompanied by increased protein abundance in the amnion and the placenta, suggesting that PTGS1 protein expression was influenced by mRNA levels in late gestation. During labour, however, mRNA abundance decreased precipitously in all four tissues, while protein abundance either increased (VYS, placenta, myo-endometrium) or remained unchanged (amnion). Thus, protein turnover plays a critical role maintaining high PTGS1 protein levels during labour. Protein-level regulation of PTGS2 expression occurs in the placenta and the VYS too, as indicated by the disparate changes of PTGS2 mRNA and protein abundance in these tissues (Fig. 3). PTGS regulation at the protein synthesis level has been suggested in a former study by Schellenberg *et al.* (2003), who assessed *de novo* PTGS synthesis rates in amnion explants pretreated with aspirin and concluded that the PTGS enzyme accumulates in the amnion *in vivo* during late gestation. Thus, regulation of mRNA and protein synthesis collectively leads to increased PTGS1 and decreased PTGS2 abundance in the gestational tissues by term, resulting in the dominance of PTGS1 over PTGS2 around the time of labour.

Pregnancy maintenance in guinea pigs does not require a functioning corpus luteum during the second half of gestation, because a luteo-placental shift of progesterone production occurs around the 4th week of pregnancy (Csapo *et al.* 1981). This is analogous to the luteo-placental shift of progesterone production in early human pregnancy and suggests that the endocrine mechanisms that maintain and terminate pregnancy are similar in the two species. Intrauterine PGs, for example, should promote parturition by a mechanism that does not involve luteolysis and systemic progesterone withdrawal, which are essential actions of labour-promoting PGs in mice. Interestingly, the amnion membrane is the major intrauterine source of PGs in guinea pigs as well as in humans. The exact role of these PGs is unknown, but recent experiments suggest that PGs may decrease progesterone responsiveness in cultured human myometrial cells (Madsen *et al.* 2004) and decidua explants (Goldman *et al.* 2005) by modulating progesterone receptor isoform expression. Decreasing progesterone responsiveness of target tissues may cause 'functional' progesterone withdrawal even when circulating progesterone levels are high. The guinea pig can be an ideal animal model to test the possibility that PGs contribute to functional progesterone withdrawal by down-regulating progesterone responsiveness *in vivo*.

The mechanisms that stimulate PTGS expression in the human or guinea pig amnion are unknown. It has to be noted, though, that in the human amnion, the inducible PTGS isoenzyme, PTGS2, is up-regulated at labour (Hirst *et al.* 1995; Mijovic *et al.* 1999; Slater *et al.* 1999), while in the guinea pig gestational tissues, PTGS1 is induced. This may be a case of evolutionary convergence where two different isoforms of an enzyme have been recruited to perform homologous functions in two species. PTGS1 is generally considered a constitutive enzyme; however, up-regulated PTGS1 mRNA expression is not unique to guinea pig gestational tissues; it occurs in murine endometrium between 15 and 17 days of pregnancy (Tsuboi *et al.* 2000).

An important further example of evolutionary convergence relates to the function of the fetal membrane that is positioned between the amnion and the uterine wall. In humans, this membrane is the chorion leave, which is of trophoblastic origin, while in guinea pigs, the corresponding membrane is the VYS, which is derived from the endoderm of the embryo. PG-metabolic activity and enzyme expression are high and PTGS expression is relatively low in both tissues (Keirse *et al.* 1978). Apparently, the chorionic and VYS membranes perform a homologous function in the two species, which is to control the transfer of uterotonic PGs from the amnion to

the myometrium. The expression of the PG-inactivating enzyme prostaglandin dehydrogenase (PGDH) has been reported to decrease in the human chorion and the guinea pig VYS before labour (Van Meir *et al.* 1997; Welsh *et al.* 2002).

In conclusion, our study reinforces the view that gestational length is controlled in an analogous fashion by PGs in guinea pigs and humans. The similarities, together with the similarities of progesterone action, indicate that guinea pigs can serve as a relevant non-primate model for studies where conclusions and inferences are extrapolated to the physiology and pathophysiology human birth.

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Supplemental material

The online version of this paper can be accessed at:
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and consists of a detailed description of the materials and methods used, and the procedures and the data validating the real-time RT-PCR and immunoblotting procedures are presented. There are also four figures:

Supplementary Figure 1. Representative dissociation curves for PCR amplification products for PTGS1 (*A*), PTGS2 (*B*) and GAPDH (*C*) mRNAs.

Supplementary Figure 2. PCR amplification efficiency plot of PTGS1 (*A*) and PTGS2 (*B*) cDNA, relative to GAPDH cDNA

Supplementary Figure 3. Immunoblot detection of PTGS1 protein in guinea pig myo-endometrium

Supplementary Figure 4. Immunoblot detection of PTGS2 protein in guinea pig placenta

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