Production of prostaglandin by late-gestation porcine placental cells *in vitro*

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Summary. Fifteen sows were assigned to three groups of five each, according to gestational age (109 days, 114 days or labour). Two fetuses per sow were chosen at random, and amnion, allantochorion, amniochorion, amniotic fluid and fetal urine were collected. Tissues were enzymatically dispersed and incubated for 1, 2, 3 or 4 h and the prostaglandin (PG) content of the supernatant medium was measured by radioimmunoassay. In general, all placental cell types produced at least three times more prostaglandin E (PGE) and 6-keto-PGF_{1 α} than PGF. Production did not vary across gestational age, except that production of 6-keto-PGF_{1 α} was lower in cells collected during labour, resulting in a relative increase in PGF and PGE. Amniochorion cells had a lower *de novo* capacity to synthesize PG than did allantochorion or amniochorion, whereas treatment of allantochorion with preterm amniotic fluid, preterm or term fetal urine resulted in increased PG output. These results demonstrate that porcine placental cells can synthesize and metabolize prostaglandin in late gestation but suggest that their capacity to produce PGI₂ (as measured by 6-keto-PGF_{1 α}) is lower than for other prostaglandins during labour.

Keywords: prostaglandin; placenta; pig; fetal fluids

Introduction

In many mammalian species, prostaglandins (PGs) are considered to be mediators of events that lead to increased uterine activity during labour (Novy & Liggins, 1980; Bazer & First, 1983). In sheep (Thorburn & Challis, 1979) and sows (Silver *et al.*, 1979; Randall *et al.*, 1986), higher PG concentrations in uterine venous blood than in uterine arterial blood at the end of pregnancy suggest that the gravid uterus is an important source of PGs. Ovine myometrial and placental tissues collected during parturition have a greater capacity for PG production *in vitro* than those obtained before labour (Mitchell & Flint, 1978). Bovine placental tissues in late gestation produce PGs *in vitro* (Gross *et al.*, 1985; Gross & Williams, 1988), the synthetic capacity differing among tissues (Gross & Williams, 1988). In pigs, PG production by placental tissues *in vitro* has not been studied.

This study determined the capacity of late-gestation porcine placental cells to produce PGs *in vitro* and examined the effects of gestational age and fetal fluids on PG production.

Materials and Methods

Animals

Fifteen Yorkshire gilts (160.9 $\pm$ 17.3 kg) were allocated to three groups according to gestational age (day 109, day 114 (not in labour) or in labour). Sows were designated in labour when the first piglet was delivered vaginally; none of

*Reprint requests.

the sows used on day 114 showed any of the preparturient behavioural changes that normally precede the myometrial activity associated with labour (Taverne, 1979). Gestational age for the group in labour ranged from 113 to 116 days, with an average of 114.6 days.

Collection of tissue and fluid

For sample collection, gilts underwent Caesarean sections under general halothane anaesthesia. Two fetuses per sow were chosen at random for sampling. Amniotic fluid uncontaminated with blood and fetal urine was collected, frozen and stored at -20°C ; approximately 30 ml² of amnion, allantochorion and amniochorion were sampled and transferred to Hank's balanced salt solution (HBSS) at pH 7.3, cooled to 4°C , with 0.035% sodium bicarbonate.

Cell dispersion

Tissues from each fetus were minced, transferred to culture tubes, centrifuged for 7 min at 180 *g* and resuspended in HBSS (4 ml ml⁻¹ packed tissue) containing 0.5% collagenase type II, 0.1% protease type XIV and 0.1% hyaluronidase type I-S, and incubated in a shaking water bath at 37°C for 2 h (Marcus *et al.*, 1984). After incubation for 1 h approximately 1 mg DNAase type I was added to the suspension. Fetal calf serum (20% v/v) was added at the end of the incubation. Cell suspensions were filtered twice through four layers of cheese cloth and centrifuged for 10 min at 180 *g*.

Red blood cells were removed by centrifugation of cell pellets in iso-osmotic HBSS containing 52% v/v percoll (Sigma Chemical Co., St Louis, MO, USA) PVP-coated silica (Percoll-Sigma) for 15 min at 1750 *g*. Placental cells were washed three times in HBSS and the resulting cell pellets were resuspended in Eagles' minimum essential medium (MEM) supplemented with 1% v/v 100 $\times$ nonessential amino acid solution, 50 000 units penicillin G sodium, 50 mg streptomycin sulfate and 625 μg amphotericin B l⁻¹ of MEM (Marcus *et al.*, 1984). Cell yield and viability were estimated by diluting an aliquot of the final cell suspension in MEM containing 0.001% fluorescein diacetate. An overall viability of 87% was obtained.

Cell incubation

Cell suspensions were diluted with MEM, with or without additions, to provide 4×10^5 viable cells ml⁻¹. Cells were cultured in 24-well plastic plates (Falcon), with four replicates per time point, for 0, 1, 2, 3 or 4 h at 37°C under 95% O₂ and 5% CO₂. In addition, allantochorion cells were cultured in MEM containing preterm or term fetal urine or preterm amniotic fluid at a final concentration of 25% v/v for 0 or 4 h only. Incubation was stopped by storing the cell suspensions at -20°C . Before analysis, suspensions were thawed in water cooled to 4°C and centrifuged at 2000 *g* for 15 min to remove cell debris.

Radioimmunoassay

Concentrations of PGs in culture media were estimated without extraction using specific radioimmunoassays for 13,14-dihydro-15 keto-PGF_{2 α} (PGFM), PGE, PGF and 6-keto-PGF_{1 α} (Olson *et al.*, 1983). As the antibodies to PGE₂ (17%) and PGF_{2 α} (53%) reacted with the 1 and 2 series, and PGFM reacted with 15-keto-PGF_{1 α} (20%), results are expressed as PGE and PGF. All other cross reactivities were <0.5%. Standard solutions ($n = 4$ dose⁻¹) ranging from 12.5 to 500 pg were prepared in 100 μl MEM and added to 12 $\times$ 75 mm culture tubes containing 100 μl phosphate-buffered saline (0.1 mol l⁻¹) with 0.1% gelatin. Subsequently, 100 μl PG antiserum and 100 μl [³H]PG (5000 c.p.m.) in phosphate-buffered saline were added and the tubes were incubated overnight at 5°C . Bound PG was separated from the free fraction by adding 0.5 ml 0.5% dextran-charcoal to the incubates, which were then centrifuged for 10 min at 1500 *g*. Radioactivity in supernatants (0.65 ml) was measured for 5 min in a scintillation counter. Intra- and inter-assay coefficients of variation varied from 1.9 to 3.6% and from 20.2 to 28.5%, respectively, for the four PGs measured. All results are expressed as net PG concentrations, i.e. concentration estimated after 1, 2, 3 or 4 h of incubation minus that obtained at 0 h of incubation. An additional subtraction of PG concentrations found in fetal fluids was carried out when cells were treated with any of these fluids.

Statistical analyses

Data were analysed using the general linear models procedure (SAS, 1982). The effect of sow was nested within gestational age, and fetus was nested within sow and within gestational age. Tissue, incubation period and treatment were cross-classified with fetus within sow within gestational age. Sow and fetus were considered as random effects. Comparisons within effects were carried out using least-squares mean *t* test. Significant differences were declared at probability levels <5% (Steel & Torrie, 1980).

Results

Untreated cells

Within tissue and gestational age effects, amnion had a lower capacity ($P < 0.05$) to produce PGs than either amniochorion or allantochoion, which did not differ (Tables 1 and 2). Consistent differences due to gestational age within tissues were not detected, with the exception of 6-keto-PGF_{1α} at labour, for which the concentration was approximately 2.5, 3 and 10 times lower ($P < 0.05$) in media of allantochoion, amniochorion and amnion cells, respectively, compared with day 109 (Table 2). A strong tendency towards a lower production of 6-keto-PGF_{1α} at labour was apparent even at 0 h (Table 1). Production of PGE, PGFM and 6-keto-PGF_{1α} tended to increase proportionally with duration of incubation, while PGF appeared to have reached a peak at 2 h of incubation and then decreased (Table 3). The most abundant PG in the incubates of all tissues was PGE, followed by 6-keto-PGF_{1α}; PGF and PGFM were present in much lower amounts.

Table 1. Least-squares means ($\pm$ SEM) of prostaglandin (PG) concentrations (pg in 4×10^5 cells) at 0 h in cultures of porcine placental tissues of different gestational ages

	Day 109	Day 114	Labour
Amnion			
PGFM	96.6 $\pm$ 54	102.1 $\pm$ 53	60.0 $\pm$ 53
PGE	1880.5 $\pm$ 843	850.2 $\pm$ 855	658.5 $\pm$ 854
PGF	556.3 $\pm$ 373	204.3 $\pm$ 373	409.7 $\pm$ 363
6-keto-PGF _{1α}	1336.3 $\pm$ 767	626.7 $\pm$ 715	122.9 $\pm$ 694
Allantochoion			
PGFM	112.0 $\pm$ 51	206.0 $\pm$ 50	142.6 $\pm$ 52
PGE	4699.9 $\pm$ 831	3310.1 $\pm$ 833	2438.8 $\pm$ 843
PGF	1122.5 $\pm$ 378	1031.9 $\pm$ 364	843.0 $\pm$ 384
6-keto-PGF _{1α}	2730.0 $\pm$ 767	2676.4 $\pm$ 705	927.5 $\pm$ 714
Amniochorion			
PGFM	95.3 $\pm$ 53	177.8 $\pm$ 61	122.4 $\pm$ 53
PGE	4704.8 $\pm$ 831	3100.5 $\pm$ 1008	2446.0 $\pm$ 854
PGF	1258.0 $\pm$ 367	643.1 $\pm$ 430	791.7 $\pm$ 385
6-keto-PGF _{1α}	2921.6 $\pm$ 767	2910.1 $\pm$ 834	938.6 $\pm$ 695 ^a

^a $P < 0.1$ compared with day 109 or 114 within row.

Treated cells

Treatment of allantochoion cells from the 15 gilts (30 fetuses) with fetal fluids resulted in a significant change in PG output: treatment with preterm, term fetal urine or preterm amniotic fluid resulted in increased production ($P < 0.05$) of PGE, 6-keto-PGF_{1α} (Fig. 1(a)) and PGF (Fig. 1(b)) compared with controls. PGFM did not change with the addition of either preterm fetal urine or amniotic fluid, while term fetal urine reduced ($P < 0.05$) PGFM production (Fig. 1(b)).

Discussion

These data demonstrate that porcine placental tissues have the capacity to produce PGs *in vitro*, as do sheep (Mitchell & Flint, 1978; Evans *et al.*, 1982; Risbridger *et al.*, 1985; Olson *et al.*, 1985)

Table 2. Least-squares means ($\pm$ SEM) of net prostaglandin (PG) production (pg in 4×10^5 cells) by porcine placental tissues of different gestational ages after incubation for 4 h

	Day 109	Day 114	Labour
Amnion			
PGFM	56.2 $\pm$ 51	68.3 $\pm$ 52	85.9 $\pm$ 53
PGE	868.1 $\pm$ 425	514.1 $\pm$ 445	206.5 $\pm$ 438
PGF	240.4 $\pm$ 167	151.8 $\pm$ 180	-32.2 $\pm$ 173
6-keto-PGF _{1α}	1090.6 $\pm$ 191 ^b	323.6 $\pm$ 177	137.5 $\pm$ 182 ^c
Allantochoion			
PGFM	462.7 $\pm$ 134 ^a	286.9 $\pm$ 136 ^a	351.4 $\pm$ 134 ^a
PGE	1772.5 $\pm$ 501 ^a	1664.9 $\pm$ 501 ^a	2500.0 $\pm$ 517 ^a
PGF	349.1 $\pm$ 171	135.0 $\pm$ 182	-41.3 $\pm$ 191
6-keto-PGF _{1α}	2520.9 $\pm$ 382 ^a	1697.5 $\pm$ 358 ^a	1013.5 $\pm$ 358 ^{a,c}
Amniochoion			
PGFM	412.2 $\pm$ 119 ^a	259.5 $\pm$ 140 ^a	329.2 $\pm$ 121 ^a
PGE	3774.1 $\pm$ 783 ^a	2223.8 $\pm$ 912 ^a	2482.1 $\pm$ 808 ^a
PGF	625.5 $\pm$ 178	305.1 $\pm$ 208	44.5 $\pm$ 187
6-keto-PGF _{1α}	2513.4 $\pm$ 502 ^a	1978.7 $\pm$ 524 ^a	907.0 $\pm$ 464 ^{a,c}

^a*P* < 0.05 compared with amnion within gestational age.^b*P* < 0.05 compared with day 114 or labour within row.^c*P* < 0.05 compared with day 109 within row.**Table 3.** Least-squares means ($\pm$ SEM) of net prostaglandin (PG) production (pg in 4×10^5 cells) by porcine allantochoion of different gestational ages after incubation for 1, 2, 3 or 4 h

		Incubation time (h)			
		1	2	3	4
PGFM	d 109	117.8 $\pm$ 44	221.3 $\pm$ 54	293.1 $\pm$ 75	462.7 $\pm$ 133
	d 114	50.7 $\pm$ 58	126.3 $\pm$ 62	207.5 $\pm$ 72	286.9 $\pm$ 136
	Labour	67.6 $\pm$ 69	129.6 $\pm$ 60	214.2 $\pm$ 77	351.4 $\pm$ 134
PGE	d 109	1486.3 $\pm$ 239	2363.8 $\pm$ 436	1835.5 $\pm$ 409	1772.0 $\pm$ 501
	d 114	993.6 $\pm$ 238	1562.6 $\pm$ 443	1615.0 $\pm$ 416	1664.4 $\pm$ 501
	Labour	936.0 $\pm$ 376	1328.0 $\pm$ 497	1740.9 $\pm$ 454	2500.0 $\pm$ 517
PGF	d 109	284.6 $\pm$ 198	658.8 $\pm$ 189	566.3 $\pm$ 177	349.1 $\pm$ 171
	d 114	229.1 $\pm$ 226	513.8 $\pm$ 200	433.2 $\pm$ 175	135.1 $\pm$ 182
	Labour	245.5 $\pm$ 303	274.2 $\pm$ 206	263.4 $\pm$ 180	-41.3 $\pm$ 191
6-keto-PGF _{1α}	d 109	907.3 $\pm$ 382	1004.1 $\pm$ 461	1240.0 $\pm$ 623	2520.9 $\pm$ 382
	d 114	996.9 $\pm$ 441	385.5 $\pm$ 652	1391.3 $\pm$ 593	1697.5 $\pm$ 358
	Labour	412.3 $\pm$ 536	695.0 $\pm$ 443	1137.4 $\pm$ 602	1013.5 $\pm$ 358 ^a

^a*P* < 0.05 compared with day 109 within PG.

and cows (Gross *et al.*, 1985; Gross & Williams, 1988). Tissues in contact with the endometrium produced significantly more PGs than the amnion, which is not in contact with it. The predominant PGs produced were PGE and 6-keto-PGF_{1 α} , PGs of the F series being produced in small amounts (Tables 1 and 2). This is consistent with the large arteriovenous difference in umbilical plasma concentrations of PGE in pigs (Silver *et al.*, 1979). Negative PGF values after 4 h of incubation could be attributed to the activity of 15-dehydrogenase-13-14 reductase (Lands, 1979) and/or PGE₂-9-keto-reductase (Watson *et al.*, 1979; Niesert *et al.*, 1987) which may enable the cells to convert PGF_{2 α} into PGFM or PGE, respectively (Table 3). Except for a reduction in output of

Fig. 1. Least-squares means of net prostaglandin (PG) output by porcine allantochorion treated with various fluids * $P < 0.05$ compared with control: (a) (□) PGE and (■) 6-keto-PGF_{1α} and (b) (□) PGFM and (■) PGF.

6-keto-PGF_{1α} by all cells collected during labour, the capacity of amnion, allantochorion and amniochorion to produce PGs *in vitro* did not change with gestational age. This is different from reports on sheep (Risbridger *et al.*, 1985; Olson *et al.*, 1984, 1985) and human (Olson *et al.*, 1983), where, in general, the placenta had greater PG synthetic capacity *in vitro* when collected during labour, although Mitchell (1981) found little variation in placental PG synthetic capacity related to gestational age. The gestational ages selected in the present study covered periods when maternal plasma progesterone concentrations are high (day 109), progesterone and PG concentrations are low (day 114) and PG concentrations are greatest (in labour) (Randall *et al.*, 1986).

The decrease in PGI₂ production, as measured by 6-keto-PGF_{1α}, may reflect selective suppression, which results in a relative increase in PGF and PGE output. PGI₂ may inhibit spontaneous uterine activity in humans, sheep and rats (Omini *et al.*, 1978, 1979; Lye & Challis, 1982; Kawano & Mori, 1988), although its action in pigs is unknown. This relative decrease of an inhibitory PG during labour could be part of a mechanism to enhance uterine activity, and it may also suggest an independent regulatory mechanism for PGI₂, PGE and PGF synthetase.

As PGs are evanescent compounds (Lands, 1979), it is reasonable to expect them to act as local hormones. The allantochorion and amniochorion, which had the highest PG production *in vitro*, form most of the placenta in this species and are in direct juxtaposition to the endometrium (Noden & de Lahunta, 1985). This could facilitate the passage of PGs to the endometrium and myometrium. However, little is known about PG movement across the pig placenta and it is not known whether placental PGs act as substrates for the increased uterine PG production seen during labour (Randall *et al.*, 1986).

Treatment of allantochorion with preterm amniotic fluid, preterm or term fetal urine resulted in an increase in net production of all PGs except PGFM. The increase was greater for PGF than for PGE or 6-keto-PGF_{1α} (Fig. 1). The nature of the stimulant and whether it resulted from reduced catabolism, increased synthesis or a combination of both cannot be determined from these data. Porcine fetal fluids stimulated PG production in placental cells regardless of gestational age, unlike human fetuses, in which urine obtained during labour had a greater capacity to stimulate PG synthesis (Casey *et al.*, 1983; Strickland *et al.*, 1983) and which may have resulted from the presence of a protein-associated (Casey *et al.*, 1983) or a growth-factor-like substance (Casey *et al.*, 1984) in the fetal urine. Porcine fetal urine and amniotic fluid contain oestrogens during late gestation (Choong & Raeside, 1974; Robertson *et al.*, 1985) and these have also been shown to stimulate PGF_{2α} production by human endometrium *in vitro* and *in vivo* (Kelly & Abel, 1980; Schatz & Gurpide, 1983; Schatz *et al.*, 1986, 1987). As the fetal urinary bladder connects with the allantoic cavity and the amniotic sac (Noden & de Lahunta, 1985), fetal urine may have contact with amniochorion and allantochorion cells. If these cells behave *in vivo* as they do *in vitro*, these as yet uncharacterized substances in fetal urine may play a role in modulating PG metabolism in the fetal membranes.

We have shown that the porcine placenta produces PGs in abundance, PGE being predominant, and that placental tissues in direct apposition to the endometrium (allantochorion and amniochorion) produce more PGs than the amnion. The lower PGI₂ production in cells collected during labour leads to a proportional increase in PGE and PGF. Fetal urine and amniotic fluid have the capacity to stimulate the production of PGs by placental cells, but the stimulatory factor remains to be characterized.

We are grateful to R. Lucis, Y. Barbeau, G. Raby and E. Meredith for technical assistance. Thanks also to T. A. Louis (School of Medicine, East Carolina University), N. R. Mason (Lilly Research Lab., Indianapolis), K. T. Kirton (Upjohn, Kalamazoo) and C. W. Leffler (University of Tennessee, Memphis) for their kind donations of PG antisera.

Supported in part by a grant from le Conseil des recherches en pêche et agroalimentaire du Québec (MCA-86-A-2019).

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Received 18 February 1991