Changes in myometrial and placental 15-hydroxyprostaglandin dehydrogenase with ovine parturition: production of prostaglandin metabolites *in vitro* and *in vivo*

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Summary. 15-Hydroxyprostaglandin dehydrogenase (PGDH) was measured *in vitro* in myometrium and maternal cotyledons from 6 sheep and in fetal cotyledons from 3 sheep before and after parturition. Maternal cotyledons contained more PGDH than fetal cotyledons or myometrium. At parturition, PGDH activity decreased in myometrium but increased in fetal and maternal placental tissue. The enzymatic activity observed *in vitro* did not result in a large, consistent release of prostaglandin F metabolites into the utero-ovarian vein *in vivo*, and such activity may have a strictly localized effect in ovine parturition.

Introduction

Much evidence has accumulated to implicate prostaglandins in the process of parturition in the sheep. Concentrations of prostaglandin (PG) F-2 α rise in uterine venous plasma and in several uterine tissues during lambing (Liggins & Grieves, 1971; Thorburn, Nicol, Bassett, Shutt & Cox, 1972). Uterine activity can be stimulated by chronic or acute infusion of PGF-2 α (Liggins, Fairclough, Grieves, Kendall & Knox, 1973; Mitchell, Flint & Turnbull, 1976a) and inhibited by meclofenamic acid, a PG synthetase inhibitor (unpublished). These elevated PG concentrations could result from increased synthesis or decreased catabolism. High rates of PGF catabolism have been demonstrated *in vitro* in some uterine tissues from pregnant sheep (Keirse, Hicks & Turnbull, 1976a), but it is not known whether the catabolic activity changes with the onset of parturition. To assess such changes we have measured rates of PG catabolism *in vitro* before and after parturition, and examined arterio-venous differences in the concentrations of major metabolites of PGF-2 α .

Materials and Methods

Cotyledons and myometrium were obtained at hysterotomy from 6 ewes between 120 and 140 days of gestation. Parturition occurred spontaneously or was induced by intrafetal administration of dexamethasone (Oradexon: Organon, Morden: 1 mg/24 h) or synthetic ACTH (Synacthen: Ciba, Horsham: 0.1 mg/24 h) from the 5th day after operation. All lambs were born alive. Within 15 min of lambing the ewes were killed by intravenous administration of thiopentone sodium (Pentothal: Abbott, Kent) and further samples of cotyledons and myometrium were again obtained. Cotyledons were separated manually into fetal and maternal components. Freshly obtained myometrium and maternal cotyledons from all 6 ewes and fetal cotyledons from 3 of them were used for determination of 15-hydroxyprostaglandin dehydrogenase (PGDH). Blood samples for PG measurements were obtained by means of utero-ovarian venous and carotid or aortic catheters (the latter inserted via the femoral artery) from 3 of the 6 ewes described above and a further 2 animals, all between 120 and 140 days of gestation. Venous and arterial samples obtained within 3 min of each other were immediately centrifuged to obtain plasma which was stored at -20°C.

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15-Hydroxyprostaglandin dehydrogenase was measured as described by Keirse *et al.* (1976b). Weighed portions of tissue were homogenized in 7 vols 0.1 M-potassium phosphate containing 20 mM-ethylenediaminetetraacetic acid, pH 7.4, at 0°C. After heating to 37°C for 5 min, 1.6 ml unfractionated homogenate was added to 0.4 ml medium containing 0.5 mM-[9-³H]PGF-2 α (sp.act. 25 Ci/mol, prepared from Prostin F-2 α (Upjohn, Crawley) and [9-³H]PGF-2 α , sp.act. 15 Ci/mmol: Radiochemical Centre, Amersham) and 10 mM-nicotinamide adenine dinucleotide (NAD⁺). Samples (0.2 ml) removed at 0, 3, 6, 9, 12, 18, 24, 36, 48 and 72 min were added to 0.5 ml ethanol containing formic acid (0.5%, v/v). Prostaglandins were extracted twice with 5 ml chloroform and the extracts subjected to thin-layer chromatography in chloroform:methanol:acetic acid:water (90:8:1:0.8, by vol.) to separate PGF-2 α from its metabolites. The percentage of PGF-2 α metabolized, calculated on the basis of ³H recovered in the PGF-2 α and combined metabolite fractions, was plotted against incubation time. PGDH activity was obtained by linear regression analysis of all points which corresponded to the initial reaction velocity; at least 5 time points were used and all *r* values were >0.97. Duplicate homogenization and incubation at tissue concentrations ranging from 50–200 mg/ml gave values which were within 10% of the mean (*n* = 10).

Plasma levels of PG metabolites were measured by radioimmunoassay. The assay for 13,14-dihydro-15-keto-PGF-2 α (DHKF) has been described previously (Mitchell *et al.*, 1976b). Similar procedures were used to assay 15-keto-PGF-2 α (KF) and 13,14-dihydro-PGF-2 α (DHF); the antibodies were raised in rabbits against antigens prepared by conjugating these compounds with bovine serum albumin (Caldwell, Burstein, Brock & Speroff, 1971). Tritiated metabolites were made by the method used by Mitchell *et al.* (1976b) to prepare [³H]DHKF.

Results

The results of PGDH determinations, expressed in ng PGF-2 α metabolized/g tissue (wet wt)/min are shown in Table 1. Although PGDH activity varied considerably between individual sheep, the maternal cotyledons consistently contained higher PGDH concentrations than fetal cotyledons or myometrium. These findings confirm those made previously in experiments in which the enzyme was not measured at *V*_{max} (Keirse *et al.*, 1976a). In all ewes, the PGDH activity changed markedly and in a consistent manner with parturition, increasing in the maternal and fetal cotyledons and decreasing in myometrium (Table 1).

To assess whether large amounts of PGF-2 α are metabolized by the pregnant uterus *in vivo* arterio-venous concentration gradients across the uterus for endogenous KF, DHF and DHKF were

Table 1. 15-Hydroxyprostaglandin dehydrogenase activity (expressed as ng PGF-2 α metabolized/g wet tissue/min) in myometrium, maternal and fetal cotyledons from sheep before (B) and after (A) parturition

Animal	Gestational age at operation (days)	Myometrium			Maternal cotyledon			Fetal cotyledon		
		B	A	A:B	B	A	A:B	B	A	A:B
1	140	3292	1487	0.45	6195	9558	1.54	N.D.	N.D.	—
2	135	531	389	0.73	1770	3292	1.86	N.D.	N.D.	—
3	125	1699	212	0.12	3257	7009	2.15	N.D.	N.D.	—
4	125	956	283	0.30	2513	5062	2.01	1558	5089	3.27
5	120	1451	637	0.44	3646	6619	1.82	2726	5735	2.10
6	122	2973	2124	0.71	4000	7823	1.96	885	1097	1.24
Mean		1817	855	0.48	3563	6561	1.89	1723	3977	2.20
± S.E.M.		±449	±317	±0.09	±620	±889	±0.08	±538	±1452	±0.59
Statistical difference by two-tailed <i>t</i> test		<i>P</i> < 0.005			<i>P</i> < 0.001			<i>P</i> > 0.05		

N.D. = not determined.

determined in 5 sheep before and during parturition. Levels of KF and DHF were low in arterial and venous samples during parturition: KF, <0.3 ng/ml, DHF, <0.05 ng/ml. There was no arterio-venous difference for either metabolite before or during lambing. The results of the DHKF measurements are given in Table 2. In 2 of the 5 animals there was no significant arterio-venous difference, despite utero-ovarian venous PGF levels being elevated at birth; in the other 3 animals a small but significant concentration gradient was observed.

Table 2. Plasma concentrations (ng/ml) of 13,14-dihydro-15-keto-PGF-2 α during pregnancy in the sheep

Animal	No. of paired arterial and venous samples	Conc. of 13,14-dihydro-15-keto-PGF-2 α (range of values)		Utero-ovarian venous:arterial (mean $\pm$ S.E.M.)	Statistical difference by one-tailed <i>t</i> test	
		Aortic or carotid	Utero-ovarian venous		<i>t</i>	<i>P</i>
3	6	5.19–35.41	4.84–36.10	1.04 $\pm$ 0.046	1.04	N.S.
4	5	3.17– 5.05	4.92– 6.32	1.44 $\pm$ 0.052	7.33	<0.001
5	17	1.18–10.76	1.42–13.84	1.43 $\pm$ 0.073	5.38	<0.001
7	12	0.88–16.40	0.76–12.70	1.32 $\pm$ 0.219	1.39	N.S.
8	6	2.67– 8.50	7.45–18.68	2.67 $\pm$ 0.424	3.63	<0.001

Discussion

The lack of any consistent, large arterio-venous difference across the uterus for the PG metabolites measured may be due to several factors. It is possible that inappropriate metabolites were examined in the present study. This seems unlikely, however, because KF and DHKF together account for over 95% of the PGF metabolites formed *in vitro* (Keirse *et al.*, 1976a). Secondly, it appears possible that a large proportion of the PGF-2 α produced during parturition is not accessible to the catabolic enzymes. Furthermore, if PGF-2 α were homogeneously distributed in an assumed intracellular water space of 300–400 μ l/g wet tissue and readily available to the enzymes, the endogenous concentration in the maternal cotyledon during parturition (150–500 ng PGF-2 α /g; Liggins & Grieves, 1971) would be low in comparison to the K_m of PGDH for PGF-2 α (1–2 μ M compared with a K_m of 20–60 μ M for human placental PGDH; see Hansen, 1976). Whatever the reason may be, the net output of the PGF metabolites from the pregnant uterus *in vivo* is rather small, particularly when compared to that of PGF.

We have previously drawn attention to differences between ovine and human uterine tissues in the localization of PGDH (Keirse *et al.*, 1975) and it now appears that there may also be quantitative differences in PGDH between these species. The highest PGDH activities observed in the present study were at least 10-fold lower than those present in the human placenta, in which PGDH activities, measured in a similar way, were 207 ± 18 (S.E.M., $n = 44$) nmol PGF-2 α /g wet tissue/min (Keirse *et al.*, 1976b). The increase in PGDH activity in fetal and maternal cotyledons after birth may result from a need to protect the fetus against the high concentrations of PGF known to be associated with ovine parturition. Liggins & Grieves (1971) have shown that, after induction of parturition, concentrations of PGF-2 α increase first in maternal cotyledons and later in myometrium and they have suggested that PGF-2 α originates mainly in the maternal cotyledons and then reaches the myometrium. The decrease in myometrial PGDH activity with parturition, causing lower catabolism, could contribute to the higher accumulation of PGF-2 α in myometrium during lambing. Together with the increase in placental PGDH activity this decrease in myometrial PGDH activity could represent a redistribution of PG metabolism, which even without causing arterio-venous differences in the concentration of the major metabolites of PGF-2 α , may be of importance in ovine parturition.

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