Effect of PGI-2 on uterine activity *in vivo* in non-pregnant ovariectomized goats (*Capra hircus*)

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Summary. Jugular administration of 200 µg PGI-2 salt significantly reduced spontaneous uterine activity in ovariectomized, oestrogen-primed goats; the effect was acute and persisted for about 3 h. Peripheral plasma concentrations of 6-keto-PGF-1α, the stable metabolite of PGI-2, decreased to 50% of initial values after 30 min; but at the start of uterine recovery were in excess of 2 ng.ml⁻¹. Uterine reactivity to both oxytocin and PGF-2α after PGI-2 administration was unaffected.

Keywords: prostacyclin; uterus; motility; goat; PGF-2α; oxytocin

Introduction

Prostaglandins (PG) of the E and F series are generally considered to be the key prostanoids involved in the modulation of uterine activity. However, a role for prostacyclin (PGI-2), a potent vasodilator and inhibitor of platelet aggregation (Moncada *et al.*, 1976), has to be defined. PGI-2 is present in the myometrium and endometrium of non-pregnant and pregnant animals including rats, guinea-pigs, sheep (Jones *et al.*, 1977; Williams *et al.*, 1978), goats (Cooke & Homeida, 1987) and women (Omini *et al.*, 1979). In-vitro experiments have produced conflicting results: PGI-2 may either inhibit (Omini *et al.*, 1979) or stimulate and then inhibit the pregnant human myometrium (Wikland *et al.*, 1983); myometrial activity in the rat, however, is stimulated (Williams *et al.*, 1979). Lye & Challis (1982) showed that PGI-2 inhibited both the electrical and mechanical activity of the non-pregnant sheep uterus *in vivo*, but responsiveness to PGF-2α and oxytocin were unaffected.

The aims of this study were to examine in ovariectomized goats the effect of PGI-2 on spontaneous uterine activity, and also the effects of PGI-2 on uterine reactivity to both PGF-2α and oxytocin.

Materials and Methods

Animals and treatments. Four mature female goats of mixed breeding but similar age (3–4 years) and weight (40–45 kg) were used. The animals, anaesthetized with sodium thiopentone intravenously and maintained with fluothane and oxygen, were ovariectomized through a left-flank incision. A soft polyethylene, air-filled balloon (27 × 5 mm; Portex Ltd, Hyde, Kent, UK) attached to a more rigid 40-cm length of cannula was inserted into the uterus *via* a small incision in the uterine wall; intrauterine pressure was monitored by a Bell and Howell pressure transducer and recorded on a Devices MX-2 machine. Details of the techniques have been described previously (Jones & Knifton, 1975). Following a post-surgical recovery period of 10 days all 4 animals were treated daily with 60 µg oestradiol benzoate (Intervet, Cambridge, UK) intramuscularly (i.m.) for 7 days.

On the 6th day of oestradiol administration 200 µg PGI-2 sodium salt (Upjohn Co., Kalamazoo, MI, USA) in 1 ml sterile saline (0.9% w/v NaCl) were injected into the jugular vein of each goat using 23-gauge needles. Uterine activity was recorded for 30 min before and for 2.5 h after PGI-2 administration. Jugular venous blood was collected by direct venepuncture into chilled heparinized tubes immediately before treatment and then at 10-min intervals for 120 min. Plasma was separated, snap-frozen in CO₂-acetone and stored at –20°C; samples were later analysed for the

Fig. 1. Intrauterine pressure in an ovariectomized goat before and after administration of 200 µg PGI-2 salt.

Fig. 2. Mean ($\pm$ s.e.m.) uterine activity (●) and jugular venous plasma concentrations of 6-keto-PGF-1 α (○) in 4 ovariectomized goats before and after administration of 200 µg PGI-2 salt at time 0.

6-oxo-PGF-1 α , the stable metabolite of PGI-2. Before administration of PGI-2, 1 ml saline had been injected to assess whether the drug vehicle produced any change in uterine motility.

After a period of recovery (4 h) when uterine activity returned to that observed in the pre-injection phase, dose-response relationships were determined to doses of oxytocin (Universal Biologicals, Cambridge, UK) and PGF-2 α (Lutalyse: Upjohn Co.); both were suitably diluted in saline so that each was contained in a maximum of 1 ml, and were injected into the jugular vein. To minimize possible time-related effects doses of oxytocin and PGF-2 α were administered in random order; each was separated by a 15-min interval. The same sequence of injections was then repeated 20 min after administration of 200 µg PGI-2 as described above.

Table 1. Mean ($\pm$ s.e.m.) uterine responses (Montevideo units) in 4 ovariectomized, oestradiol-treated goats to i.v. oxytocin and PGF-2 α before and after treatment with 200 μ g PGI-2 i.v.

Oxytocin (mU)	Response		PGF-2 α (μ g)	Response	
	Before	After		Before	After
0	290 $\pm$ 26*	28 $\pm$ 8	0	276 $\pm$ 25*	25.6 $\pm$ 5
50	510 $\pm$ 41	552 $\pm$ 36	1	453 $\pm$ 41	418 $\pm$ 40
100	742 $\pm$ 61	726 $\pm$ 51	2	663 $\pm$ 45	675 $\pm$ 45
200	879 $\pm$ 71	1051 $\pm$ 80	4	1036 $\pm$ 77	1210 $\pm$ 84

* $P < 0.001$ compared to corresponding value after treatment.

Uterine responses were expressed in Montevideo units (Caldeyro-Barcia *et al.*, 1957), i.e. the product of mean amplitude and the total number of contractions in a 10-min period.

Assay of 6-keto-PGI-1 α . A specific radioimmunoassay for 6-keto-PGF-1 α was used as described by Liggins *et al.* (1980), utilizing their antiserum (4V-208) at a dilution of 1:10 000; 6-keto [5,8,9,11,12,14,15(n)- 3 H]PGF-1 α (5000 c.p.m./tube) was obtained from Amersham International (Bucks, UK). Standard curves were constructed using pure 6-keto-PGF-1 α (Upjohn). The recovery of added tracer was $77 \pm 15\%$ (mean $\pm$ s.e.m., $n = 9$) and results were corrected for extraction losses; the recovery of standards (20, 50, 500, 1000 pg) added to goat plasma was 19.1 ± 1.9 , 52.6 ± 7.8 , 489 ± 15.7 and 893 ± 22.8 pg (mean $\pm$ s.e.m., $n = 6$). The non-specific binding was 8%, the sensitivity of the assay 10 pg/tube, and the intra- and inter-coefficients of variation were 6.2 and 12.6%, respectively ($n = 12$). Antiserum specificity has been reported previously (Liggins *et al.*, 1980).

Data analysis. Data were analysed using two-way ANOVA and Duncan's multiple range test, and Student's *t* test for paired data.

Results

Injection of 1 ml saline vehicle did not have any effect on uterine motility in the 4 goats. Figure 1 shows a representative trace of the effects of PGI-2 (200 μ g) on uterine motility in one ovariectomized goat during treatment with oestradiol benzoate. There was a rapid decline in activity within 10 min, with a peak effect after about 30 min. Activity returned slowly and was still significantly ($P < 0.01$) below pre-injection values after 2.0 h. The results for all 4 animals are presented in Fig. 2. Concentrations of 6-keto-PGF-1 α decreased progressively to 50% of initial values after about 30 min; at the start of the recovery period concentrations were still greater than 2 ng.ml $^{-1}$. Table 1 shows the mean responses to various doses of intravenous oxytocin and PGF-2 α before and after treatment with 200 μ g PGI-2. Reactivity to both agents, at all dose levels, was not significantly ($P < 0.05$) affected, i.e. the dose-response curves for both oxytocin and PGF-2 α were unaltered by PGI-2, even though spontaneous activity was significantly ($P < 0.001$) reduced.

Discussion

The results of this study show that PGI-2 inhibits spontaneous uterine motility in the goat; similar results have been reported for the sheep (Lye & Challis, 1982). Such an acute effect of PGI-2 may be of importance in rendering the uterus quiescent before implantation when progesterone concentrations are very low (Thorburn & Schneider, 1972; Irving *et al.*, 1972). This is supported by several observations. While PGI-2 is present in the goat corpus luteum, highest concentrations of its metabolites, 6-keto-PGF-2 α , have been found in the myometrium and endometrium, and concentrations increase significantly during early pregnancy (Cooke & Homeida, 1987); in this respect it may be beneficial to compare uterine activity, and responses to PGI-2, in non-pregnant and early pregnant goats. PGI-2 has also been shown to regulate uterine blood flow in the rat (Kennedy &

Zamecnik, 1978), to assist blastocyst implantation in women (Kelly, 1981), and be luteotrophic in the cow both *in vitro* and *in vivo* (Milvae & Hansel, 1980).

The prolonged effect of PGI-2 on the goat uterus was surprising considering its short half-life of 3 min in blood or biological buffers (Dusting *et al.*, 1978). It is possible that 6-keto PGF-2 α is responsible for the inhibitory effect, but the uterus recovered when plasma concentrations of the metabolite were in excess of 2 ng.ml⁻¹, and also it has been reported that 6-keto-PGF-2 α has no effect on human myometrial activity *in vitro* (Omini *et al.*, 1979). However, bearing in mind the possible species differences in uterine responses to PGI-2 referred to above, it would be useful to examine the effects of the metabolite on uterine motility in ruminants.

As observed in ovariectomized ewes (Lye & Challis, 1982) both oxytocin and PGF-2 α were able to overcome the inhibitory effects of PGI-2, suggesting that prostacyclin has little effect on receptors to these hormones. This contrasts to the action of progesterone which blocks uterine responsiveness to both oxytocin and PGF-2 α (R. G. Cooke & A. M. Homeida, unpublished observations), probably by inhibiting receptor formation (McCracken *et al.*, 1984).

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References

- Caldeyro-Barcia, R., Sica-Blanco, Y., Poseiro, J.J., Gonzales-Panizza, V., Mendez-Bauer, C., Fielitz, C., Alvarez, H., Pose, S.V. & Hendricks, C.H. (1957) A quantitative study of the action of synthetic oxytocin on the non-pregnant human uterus. *J. Pharm. exp. Ther.* **121**, 18–31.
- Cooke, R.G. & Homeida, A.M. (1987) Alterations in reproductive tissue concentrations of oxytocin and prostaglandins during early pregnancy in the goat. *J. vet. Med.* **A34**, 415–419.
- Dusting, G.L., Moncada, S. & Vane, J.R. (1978) Disappearance of prostacyclin (PGI₂) in the circulation of the dog. *Br. J. Pharm.* **62**, 414P.
- Irving, G., Jones, D.E. & Knifton, A. (1972) Progesterone concentration in the plasma of pregnant goats. *J. Endocr.* **53**, 447–452.
- Jones, D.E. & Knifton, A. (1975) Effects of female sex hormones on uterine motility and reactivity to oxytocin in goats. *Res. vet. Sci.* **18**, 288–293.
- Jones, R.L., Poyser, N.L. & Wilson, N.H. (1977) Production of 6-oxo-prostaglandin F_{1 α} by rat, guinea-pig and sheep uteri *in vitro*. *Br. J. Pharm.* **59**, 436P, abstr.
- Kelly, R.W. (1981) Prostaglandin synthesis in the male and female reproductive tract. *J. Reprod. Fert.* **62**, 293–304.
- Kennedy, T.G. & Zamecnik, J. (1978) The concentration of 6-keto PGF_{1 α} is markedly elevated at the site of blastocyst implantation in the rat. *Prostaglandins* **16**, 599–605.
- Liggins, G.C., Campos, G.A., Roberts, C.M. & Skinner, S.J. (1980) Production rates of prostaglandin F_{1 α} , 6-keto-PGFI- α and thromboxane B₂ by perfused human endometrium. *Prostaglandins* **19**, 461–477.
- Lye, S.J. & Challis, J.R.G. (1982) Inhibition by PGI₂ of myometrial activity *in vivo* in non-pregnant ovariectomized sheep. *J. Reprod. Fert.* **66**, 311–315.
- McCracken, J.A., Schramm, W. & Okulicz, W.C. (1984) Hormone receptor control of pulsatile secretion of PGF_{2 α} from the ovine uterus during luteolysis and its abrogation in early pregnancy. *Anim. Reprod. Sci.* **7**, 57–54.
- Milvae, R.A. & Hansel, W. (1980) The effects of prostacyclin (PGI₂) and 6-keto-PGF_{1 α} on bovine plasma progesterone and LH concentrations. *Prostaglandins* **20**, 641–647.
- Moncada, S., Gryglewski, R., Bunting, S. & Vane, J.R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature, Lond.* **263**, 663–665.
- Omini, C., Folco, G.C., Pasargiklian, R., Fano, M. & Berri, F. (1979) Prostacyclin (PGI₂) on pregnant human uterus. *Prostaglandins* **17**, 113–120.
- Thorburn, G.D. & Schneider, D.W. (1972) The progesterone concentration in the plasma of the goat during the oestrous cycle and pregnancy. *J. Endocr.* **52**, 23–26.
- Wikland, M., Lindblom, B., Hammarström, S. & Wijkvist, N. (1983) The effect of prostaglandin I₂ on the contractility of the term pregnant human myometrium. *Prostaglandins* **26**, 905–916.
- Williams, K.I., Dembenska-Kiec, A., Zmuda, A. & Gryglewski, R.J. (1978) Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus. *Prostaglandins* **15**, 343–350.
- Williams, K.I., El Tahir, K.E.H. & Marcinkiewicz, E. (1979) Dual action of prostacyclin (PGI₂) on the pregnant rat uterus. *Prostaglandins* **17**, 667–672.

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