Effects of prostaglandins F-2 α and E-2 on cervical extensibility in the late pregnant rat

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Summary. The extensibility of the cervix of rats was measured *in vitro* on Day 19 of pregnancy and was doubled after 2 s.c. injections of PGF-2 α (1 mg/kg) or PGE-2 (5 mg/kg) on Day 18. Progesterone (10 mg/kg) given on Day 18 had no effect alone or on the effect of PGE-2 but abolished the action of PGF-2 α . PGE-2, but not PGF-2 α , increased extensibility when given on Day 18 to rats which had been bilaterally ovariectomized on Day 16 and given oestradiol benzoate plus progesterone twice daily. High concentrations of PGF-2 α or PGE-2 had no effect on the extensibility of Day-18 pregnant rat cervix *in vitro*. It is suggested that PGF-2 α acts via the ovaries to decrease progesterone secretion and perhaps release relaxin, but PGE-2 would appear to have another site of action.

Introduction

With advancing gestation there is an increase in the extensibility of the cervix of rats (Harkness & Nightingale, 1962; Hollingsworth, Isherwood & Foster, 1979a), ewes (Fitzpatrick, 1977b; Stys, Clewell & Meschia, 1978) and women (Conrad & Ueland, 1976). The hormonal control of these changes is uncertain but could involve decreased plasma progesterone concentrations and/or increased plasma oestrogen, relaxin or prostaglandin concentrations (Fitzpatrick, 1977a, b; Hollingsworth *et al.*, 1979a). Several prostaglandins are able to soften the human cervix when used to induce abortion or labour at term and when given by a variety of routes, including intra-cervically, without necessarily inducing uterine contractions (see MacKenzie & Embrey, 1977). The site and mechanism of action of the prostaglandins in producing this effect are uncertain.

The objectives of the present experiments were to determine whether prostaglandin (PG) F-2 α and E-2 could increase cervical extensibility in the late pregnant rat and to elucidate the site and mechanism of action. As it is known that PGF-2 α can act on the ovary of the pregnant rat to decrease progesterone secretion (Fuchs, Mok & Sundaram, 1974; Buckle & Nathanielsz, 1975) this was investigated as a possible mechanism. Preliminary results have been published (Hollingsworth, Isherwood & Gallimore, 1979b).

Materials and Methods

Primigravid Sprague–Dawley rats (300–450 g), of known gestational age (day of finding copulation plug = Day 1), were housed in a daily 13-h light period (07:00–20:00 h). After treatment of the rats (see Table 1) each was killed and the cervix removed. Cervical wet weight, weight of total products of conception and numbers of live and dead fetuses per litter were routinely determined (Hollingsworth *et al.*, 1979a). Tensile properties of the isolated cervix were measured by the method of Hollingsworth & Isherwood (1977) and Hollingsworth *et al.* (1979a). Extensibility is the fractional increase in inner circumference per min after application of a load to the isolated cervix. In-vitro experiments were performed by preincubating the cervix

in Krebs–Henseleit solution containing the test drug for 30 min before a mechanical test was performed in the continued presence of the drug. Some rats were bilaterally ovariectomized on Day 16 and injected s.c. with oestradiol benzoate ($0.5 \mu\text{g/kg}$) plus progesterone (10 mg/kg) (Sigma Chemical Company) in arachis oil once on Day 16 and then twice daily. The absence of ovarian tissue was confirmed at autopsy. PGF-2 α and PGE-2 (Upjohn Ltd) were injected s.c. in saline (9 g NaCl/l) and in 0.1 M -phosphate buffer $5.5 \text{ g NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ plus $4.2 \text{ g Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O/l}$ respectively. Statistical comparisons were made using the Mann–Whitney U-test (Seigel, 1956).

Results

Prostaglandins in vivo

Cervical extensibility was measured on Day 19 of pregnancy following various treatments given s.c. twice (09:00 and 16:00 h) on Day 18. The properties of the cervix and products of conception following saline, phosphate buffer or arachis oil did not differ from each other and the data were therefore pooled to form the control group. Cervical extensibilities were approximately twice those of controls after treatment with PGF-2 α (1 mg/kg) or PGE-2 at a dose of 5 mg/kg , but PGE-2 at 1 mg/kg had no effect (Table 1). Cervical inner circumference and wet weight were also increased by PGF-2 α .

Progesterone did not alter cervical extensibility but completely antagonized the effect of PGF-2 α . The extensibility after PGF-2 α + progesterone was less than that of controls but not significantly less than that after progesterone alone. The increase in extensibility in rats given 5 mg PGE-2/kg was not antagonized by concurrent administration of progesterone.

The cervical extensibility, wet weight and inner circumference of the ovariectomized, steroid-treated rats on Day 19 were considerably less than those of intact controls, although normal fetal growth had been maintained, as found by Hollingsworth *et al.* (1979a). PGF-2 α had no effect, but PGE-2 still produced an increase in extensibility.

PGF-2 α had no effect on fetal survival when given alone or with progesterone. Of the 13 pregnant rats ovariectomized and given steroids plus PGF-2 α , 7 had fewer than 3 living fetuses per litter. Cervical extensibility in these 7 rats with mostly dead fetuses ($2.7 \pm 0.6 \text{ min}^{-1}$) did not differ from that ($3.1 \pm 0.7 \text{ min}^{-1}$) in the 6 rats with mostly living fetuses. PGE-2 (5 mg/kg) produced a few fetal deaths when given alone or when given with progesterone. The ovariectomized, steroid-treated rats given PGE-2 had normal living fetuses.

Prostaglandins in vitro

The extensibility of cervix on Day 18 pregnant rats in the presence of and after 30 min pre-incubation in PGF-2 α (10^{-5} M) was $8.5 \pm 0.6 \text{ min}^{-1}$ and in PGE-2 (10^{-5} M) was $5.5 \pm 0.6 \text{ min}^{-1}$. These values were not different from those of the respective saline ($7.2 \pm 0.5 \text{ min}^{-1}$) and ethanol ($5.9 \pm 0.9 \text{ min}^{-1}$) controls ($n = 6-9$).

Discussion

We have used an objective method to show that PGF-2 α and PGE-2 given *in vivo* can increase cervical extensibility in the late pregnant rat. This agrees with the finding of increased cervical compliance with the prostaglandin analogue, cloprostenol, in the goat (Fitzpatrick, 1977b), and the subjective observation of increased cervical softening after administration of PGF-2 α and PGE-2 in the sheep (Fitzpatrick, 1977a), PGF-2 α in the goat (Cooke, Knifton, Fitzpatrick & Ward, 1977) and various prostaglandins in women (MacKenzie & Embrey, 1977).

Table 1. Properties of the rat cervix and products of conception on Day 19 of pregnancy after various treatments given twice on Day 18

Treatment	Dose (mg/kg)	No. of rats	Cervix			Products of conception	
			Wet wt (g)	Extensibility (min ⁻¹)	Inner circumference (mm)	Weight (g)	Mean no. of fetuses alive/total
None (controls)		13	0.17 ± 0.02 ^{a,d}	8.0 ± 0.6 ^{a,d,e,f,g}	19.8 ± 1.1 ^{a,d}	37.6 ± 1.5 ^{a,d,e}	13.9 ^a /14.0
PGF-2α	1	8	0.23 ± 0.02 ^a	14.8 ± 1.9 ^{d,h}	24.9 ± 1.3 ^a	39.5 ± 2.1 ^b	13.8/14.4
PGE-2	1	8	0.17 ± 0.01	8.4 ± 1.9	21.2 ± 1.0	38.2 ± 2.5	13.5/13.5
	5	14	0.17 ± 0.01	16.4 ± 3.8 ^e	22.7 ± 1.1	30.7 ± 1.3 ^d	11.1/12.6
Progesterone	10	13	0.19 ± 0.01 ^e	7.5 ± 1.1 ⁱ	18.6 ± 1.4 ^b	33.8 ± 1.4	12.2/12.3
Progesterone + PGF-2α	10	7	0.19 ± 0.01	4.6 ± 0.7 ^{f,h}	23.6 ± 2.7	31.8 ± 1.6 ^{a,b}	12.3/12.4
Progesterone + PGE-2	10	10	0.14 ± 0.01 ^c	15.1 ± 2.5 ^{a,i}	22.0 ± 1.9 ^b	31.9 ± 3.4 ^e	10.2 ^a /13.5
	5						
Ovariectomy*		14	0.10 ± 0.01 ^d	2.8 ± 0.5 ^{a,j}	11.8 ± 0.7 ^{d,c}	35.7 ± 1.6 ^f	12.3 ^d /12.6
Ovariectomy* + PGF-2α	1	13	0.12 ± 0.01	2.9 ± 0.5	12.5 ± 0.7	27.2 ± 1.6 ^f	4.9 ^d /12.2
Ovariectomy* + PGE-2	5	7	0.09 ± 0.01	6.6 ± 0.9 ^j	15.3 ± 1.3 ^c	39.0 ± 2.1	14.7/14.7

Values are mean ± s.e.m.

Values within columns with the same superscripts were compared and are significantly different (a, b, c 2P < 0.05; d, e, f, g, h, i, j, 2P < 0.01).

* On Day 16 and twice daily treatment with 0.5 µg oestradiol benzoate and 10 mg progesterone/kg.

As PGE-2 was able to increase cervical extensibility in ovariectomized, steroid-treated rats an extraovarian site of action must be considered. This effect of PGE-2 was not antagonized by progesterone, suggesting that PGE-2 was not acting by producing a fall in progesterone secretion. PGE-2 could be acting directly on the cervical connective tissue. Fitzpatrick (1977a) has described local areas of softening of the sheep cervix, adjacent to the cannula tip, following intra-cervical infusions of PGF-2 α or PGE-2. Conrad & Ueland (1976) observed increased stretch modulus of isolated human cervical strips within 10 min of incubation with high concentrations (10^{-6} g/ml) of PGE-2. The lack of effect of high concentrations of PGE-2 *in vitro* on the extensibility of the rat cervix does not support a direct action in this species but it is possible that the period of incubation was too short.

The mechanism(s) of the fetotoxicity with PGF-2 α and PGE-2 cannot be deduced from the present results but may include increased uterine motility. The lack of correlation between fetotoxicity and increased cervical extensibility suggests that the latter is not related to greater uterine motility.

The lack of effect of PGF-2 α in ovariectomized rats suggests an intermediate, ovarian site of action for this PG. It is well established that PGF-2 α , in the dose range used and at this time of pregnancy in the rat, will result in a rapid and sustained fall in plasma progesterone concentrations (Fuchs *et al.*, 1974; Buckle & Nathanielsz, 1975). For this reason, treatments were given on Day 18 of pregnancy. As the increase in cervical extensibility following PGF-2 α was antagonized by progesterone it is suggested that the cervical action of PGF-2 α involves a decrease in ovarian progesterone secretion. This may not be the only mechanism as progesterone itself did not significantly decrease extensibility and a progesterone-withdrawal treatment did not increase extensibility (Hollingsworth *et al.*, 1979a). Hollingsworth *et al.* (1979a) also showed that relaxin could increase cervical extensibility in ovariectomized, steroid-treated, late pregnant rats. Release of relaxin could, therefore, be an additional intermediate step in the increased cervical extensibility due to PGF-2 α in the intact rat. Relaxin is stored in rat ovaries and the highest concentration, as determined by bioassay, was in the corpora lutea (Anderson & Long, 1978). Immunochemical techniques suggest that relaxin is stored in the same cells that synthesize progesterone in pig corpora lutea (Larkin, Fields & Oliver, 1977; Kendall, Plopper & Bryant-Greenwood, 1978) and in the late pregnant pig, PGF-2 α produces a rapid release of relaxin (Sherwood, Chang, BeVier, Diehl & Dziuk, 1976). Fuchs & Mok (1974) have described histological degeneration of luteal cells after PGF-2 α treatment of late pregnant rats and therefore release of relaxin might be anticipated. Further investigation of the hypothesis of the involvement of decreased progesterone secretion and increased relaxin release in the increased cervical extensibility with PGF-2 α will require measurement of plasma hormone concentrations.

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