Br. J. Pharmac. (1975), 55, 515-518

PROSTAGLANDINS IN PYOMETRIAL FLUID FROM THE COW, BITCH AND FERRET

R.B. HEAP
A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

N.L. POYSER
Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

1 Pyometra is a disorder of the uterus usually associated with bacterial infection plus obstruction.

2 Large quantities of fluid often collect in the uterus during this condition.

3 Pyometrial fluid obtained from three species was found to contain prostaglandin $F_{2\alpha}$, usually in large quantities.

4 Prostaglandin E_2 was present in smaller quantities in five of the six samples.

5 These findings are discussed in relation to the known occurrence of prostaglandins in inflammatory fluid, and to the problem of infertility.

Introduction

Pyometra is characterized by a progressive collection of pus in the cavity of the uterus and the persistence of functional luteal tissue in the ovary. It is a pathological and inflammatory condition usually associated with uterine infection and infertility. The pus may be watery in nature and present in large quantities. In the majority of cases the cervix is closed, but animals have been seen in which there was a purulent discharge (Arthur, 1964). In the bitch, the so-called 'closed' condition, where fluid accumulates in the uterus, is accompanied by cystic hyperplasia of the endometrium, vaginal discharge, vulval oedema and uterine infection (Dow, 1957).

Inflammatory fluid from several animals obtained from various sites has been found to contain prostaglandins. They are usually prostaglandins of the E series and, where identification has been taken further, prostaglandin E_2 mainly is present (Willis, 1970; Greaves, Søndergaard & McDonald-Gibson, 1971; Änggård & Jonsson, 1971; Eakins, Whitelocke, Perkins, Bennett & Unger, 1972; Greenwood & Kerry, 1975). Uterine tissue is known to produce and release prostaglandins in several species and prostaglandin $F_{2\alpha}$ is the main prostaglandin formed (see Poyser, 1973, 1974a,b for references). The endotoxin from *Salmonella* enteritis has been found to increase the endometrial levels of prostaglandin fourfold when injected into pregnant mice on day 16. Furthermore, abortion occurred following such treatment. Previous treatment with indomethacin prevented the abortifacient action of the endotoxin, though it did not prevent abortion due to administered prostaglandin $F_{2\alpha}$ (Skarnes & Harper, 1972). The study indicated that prostaglandin released from the uterus in response to bacterial infection could adversely affect fertility in animals.

In the present study, pyometrial fluid has been obtained from the cow, bitch and ferret uterus to study the nature and concentrations of prostaglandins present.

Methods

Pyometrial fluid was obtained during clinical investigations of infertility in three cows, two bitches and one ferret. The fluid was recovered by aspiration from the uterus at slaughter or laparotomy. It was stored at -20° C before assay. After thawing, the pH of the fluid was recorded and lowered to pH 4.5 by the addition of 1 N or 0.1 N HCl. Prostaglandins were extracted by solvents, purified by silicic acid column chromatography and assayed on the rat fundal strip by methods previously described (Blatchley, Donovan, Horton & Poyser, 1972). Following assay, the prostaglandin E and F extracts obtained from the bitch pyometrial fluid were further purified by thin-layer chromatography using the AI solvent system of Gréen & Samuelsson (1964)

and performed as described by Downie, Poyser & Wunderlich (1974).

The prostaglandins extracted from the cow, bitch and ferret pyometrial fluid were then identified by combined gas chromatography and mass spectrometry (g.c.-m.s.) following the procedure of Thompson, Los & Horton (1970). Prostaglandins of the F series were first converted to the methyl ester, trimethylsilyl ether (Me-TMS) and identified in the manner outlined previously (Blatchley *et al.*, 1972). Prostaglandins of the E series were converted to the corresponding prostaglandin B compounds, reacted to form the Me-TMS derivatives, and then finally identified, by procedures described elsewhere (Downie *et al.*, 1974).

Prostaglandin F levels in ferret pyometrial fluid were also measured by radioimmunoassay (R.I.A.). A 100 μl portion of the fluid was diluted with an equal volume of 5% formic acid and extracted twice with 500 μl ethyl acetate. The organic phase extract was evaporated to dryness and the residue taken up in 200 μl tris-HCl buffer, pH 7.2. The concentration of prostaglandin F compounds was determined by the addition of 50 μl antiserum raised in a goat against prostaglandin $F_{2\alpha}$ conjugated to bovine serum albumin (titre used 1/500). This was followed by the addition of 50 μl buffer containing approximately 1000 ct/min [^{3}H]-prostaglandin $F_{1\alpha}$ (50 Ci/mmol) with incubation for 4 h at 4°C. The antibody-bound radioactivity was separated from unbound label by the addition of 300 μl saturated ammonium sulphate and 100 μl bovine gamma globulins (10 mg/ml buffer). The precipitate was separated by centrifugation and its radioactivity was determined by liquid scintillation spectrometry. The antiserum revealed a high cross-reaction with prostaglandin $F_{2\alpha}$ (100%) and prostaglandin $F_{1\alpha}$ (53.6%), but low cross-reactions with prostaglandins A_1, A_2, B_1, B_2, E_1, E_2, E_3 (all less than 0.04%).

Results

The results obtained in three cows, two bitches and one ferret are summarized in Table 1. Samples of pyometrial fluid were obtained from six uteri in amounts ranging from 17 to 110 ml. The fluid varied in consistency and nature, and was generally dark in colour. One sample of fluid from a bitch was quite viscous. The pyometrial sample obtained from a ferret had a thick consistency and a pungent odour. The pH of the fluid tended to be neutral or slightly alkaline, although the viscous sample from a bitch was slightly acid. The nature of the infection in the uterus was not determined and the cyclic activity and reproductive capabilities of the animals was not known except for one cow, which had a twin pregnancy.

The fluid from all six animals contained large quantities of prostaglandins. In all instances the prostaglandin F extracted was identified by g.c.-m.s. as being prostaglandin $F_{2\alpha}$. The prostaglandin E extracts consisted of predominantly prostaglandin E_2, though the extracts from two cow fluids were found to contain 5% to 10% prostaglandin E_1 as indicated by g.c.-m.s. Unfortunately, in two instances (one extract each from the cow and bitch), the amount of prostaglandin E-like material present was too small to obtain conclusive evidence of its identity by g.c.-m.s.

The levels of prostaglandin $F_{2\alpha}$ in the samples of cow and bitch pyometrial fluid, as measured by bioassay and uncorrected for procedural losses, ranged from 32 to 256 ng/ml. The level of prostaglandin E_2 was lower than the level of prostaglandin $F_{2\alpha}$ in each sample, and the levels varied from 6 to 85 ng/ml. The ratio of prostaglandin $F_{2\alpha}$ to E_2 ranged from 1.7 to 23.2. The concentration of prostaglandin $F_{2\alpha}$ in the pyometrial fluid of the ferret was 5.4 ng/ml, as determined by bioassay, and 10 ng/ml as measured by RIA. This difference is mainly attributable to

Table 1. Levels of prostaglandins in pyometrial fluid from the cow, bitch and ferret.

Species	*Sample No.*	*pH of fluid*	*Level of prostaglandin (ng/ml)* $PGF_{2\alpha}$	PGE_2	*Ratio of* $PGF_{2\alpha} : PGE_2$
Cow	1	7.0	142	85⁺	1.7
	2	7.4	197	27⁺	7.3
	3	–	32	6*	5.3
Bitch	4	5.6	256	11*	23.2
	5	7.2	117	56	2.1
Ferret	6	8.0	5.4	<0.5	–

⁺ Contained 5 to 10% prostaglandin E_1 as indicated by combined gas chromatography and mass spectrometry (g.c.-m.s.).

* Amounts present too low for positive identification by g.c.-m.s.

procedural losses which occurred during the former method of determination. Prostaglandin E was not detectable (<0.5 ng/ml prostaglandin E_2) in the ferret pyometrial fluid, as measured by bioassay.

Discussion

This investigation has shown that pyometrial fluid obtained from the uterine lumen of both the cow and bitch contains high concentrations of prostaglandins $F_{2\alpha}$ and E_2. Prostaglandin $F_{2\alpha}$ was also found in pyometrial fluid from the ferret. Small amounts of prostaglandin E_1 were detectable in two samples of cow fluid. Thus, inflammatory fluid obtained from the uterus appears to contain prostaglandin $F_{2\alpha}$ predominantly, unlike inflammatory fluid from other sites which contains mainly prostaglandins of the E series.

In inflammation it is probable that tissue breakdown and the coalescence of cell contents results in a non-specific production of prostaglandins. In guinea-pigs and rats the uterus synthesizes mainly prostaglandin $F_{2\alpha}$ (Poyser, 1972; Williams, Sneddon & Harney, 1974), though it is not yet known whether this is true in the bitch, cow and ferret. It would appear, therefore, that inflammatory fluid contains significant amounts of the prostaglandin which the tissue is capable of producing, prostaglandin $F_{2\alpha}$ in the case of the uterus.

Pyometrial fluid from the cow and bitch was not lacking prostaglandin E_2, though the stimulus of prostaglandin production appeared incapable of directing synthesis towards producing predominantly prostaglandin E_2, the 'active' prostaglandin in inflammation (Beitch & Eakins, 1969; Crunkhorn & Willis, 1971). This is in contrast to the sheep where, under experimental physiological conditions, a sterile fluid containing only prostaglandin $F_{2\alpha}$ (the 'active' uterine prostaglandin in reproduction) collects in the uterus (Harrison, Heap, Horton & Poyser, 1972).

One cannot overlook the possibility that prostaglandins present in pyometrial fluid are formed in the uterus by cells other than uterine tissue cells themselves. For example, the bacteria may be synthesizing and releasing the prostaglandins. In addition Higgs, McCall & Youlten (1975) have found phagocytosing polymorphonuclear leucocytes from the rabbit produce prostaglandins, though prostaglandin E_1 is the main one formed. It is likely that the majority of prostaglandins in pyometrial fluid are derived from the uterine tissue, though the prostaglandin E_1 found in two samples of cow pyometrial fluid could possibly have come from polymorphs.

It seems unlikely that uterine prostaglandins are the cause of infertility in animals suffering from pyometra. However, they may have a contributory role. Prostaglandin $F_{2\alpha}$ is released towards the end of the oestrous cycle in the cow, and it probably acts to cause regression of the corpus luteum in the ovary and thereby regulates oestrous cycle length (Nancarrow, Buckmaster, Chamley, Cox, Cumming, Cummins, Drinan, Findlay, Goding, Restall, Schneider & Thorburn, 1973). The present findings in the cow do present a paradox, since in this species the corpus luteum is sensitive to the luteolytic properties of prostaglandin $F_{2\alpha}$ and its analogues, yet in the condition of pyometra the life of the corpus luteum may be prolonged even though the luminal fluid in the uterus contains large amounts of prostaglandin $F_{2\alpha}$. This condition is similar to that produced experimentally in sheep by repeated daily doses of progesterone, which leads to a hydrometra rich in prostaglandin $F_{2\alpha}$. The prostaglandin $F_{2\alpha}$, is not released into uterine vein blood in significant amounts in the absence of oestrogen stimulation (Harrison & Heap, 1975). The general involvement of uterine prostaglandins in the control of fertility in primate and many sub-primate species is now under investigation.

This work was supported by a grant from the M.R.C. Authentic prostaglandins and prostaglandin $F_{2\alpha}$ antiserum were kindly supplied by the Upjohn Company, Kalamazoo, Michigan, U.S.A., and pyometrial samples by Mr A.D. Forteath of the Milk Marketing Board, Exeter, Devon, Mr J. Hammond Jr., Department of Applied Biology, University of Cambridge and Mr J. Hadley, The Royal Veterinary College, Potters Bar, Herts.

References

ÄNGGÅRD, E. & JONSSON, C.-E. (1971). Efflux of prostaglandins in lymph from scalded tissue. *Acta physiol scand.*, 81, 440-447.

ARTHUR, G.H. (1964). In Wright's *Veterinary Obstetrics*, 3rd ed., pp. 421-425. London: Ballière, Tindall & Cox.

BEITCH, B.R. & EAKINS, K.E. (1969). The effects of prostaglandins on the intraocular pressure of the rabbit. *Br. J. Pharmac.*, 37, 158-167.

BLATCHLEY, F.R., DONOVAN, B.T., HORTON, E.W. & POYSER, N.L. (1972). The release of prostaglandins and progestin into the utero-ovarian

venous blood of guinea-pigs during the oestrous cycle and following oestrogen treatment. *J. Physiol.*, **223**, 69-88.

CRUNKHORN, P. & WILLIS, A.L. (1971). Interaction between prostaglandin E and F given intradermally in the rat. *Br. J. Pharmac.*, **41**, 507-512.

DOW, C. (1957). The cystic hyperplasia-pyometra complex in the bitch. *Vet. Rec.*, **69**, 1409-1414.

DOWNIE, J., POYSER, N.L. & WUNDERLICH, M. (1974). Levels of prostaglandins in human endometrium during the normal menstrual cycle. *J. Physiol.*, **236**, 465-472.

EAKINS, K.E., WHITELOCKE, R.A.F., PERKINS, E.S., BENNETT, A. & UNGER, W.G. (1972). Release of prostaglandins in ocular inflammation in the rabbit. *Nature, (New Biol.)*, **239**, 248-249.

GREAVES, M.W., SØNDERGAARD, J. & McDONALD-GIBSON, W. (1971). Recovery of prostaglandins in human cutaneous inflammation. *Br. med. J.*, ii, 258-260.

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX. Thin-layer chromatography of the prostaglandins. *J. Lipid Res.*, **5**, 117-120.

GREENWOOD, B. & KERRY, P.J. (1975). Prostaglandin production by a mild inflammatory lesion in sheep. *Br. J. Pharmac.*, **53**, 157-162.

HARRISON, F.A. & HEAP, R.B. (1975). Prostaglandin secretion by the autotransplanted uterus in sheep. *J. Endocr.*, **64**, 13-14P.

HARRISON, F.A., HEAP, R.B., HORTON, E.W. & POYSER, N.L. (1972). Identification of prostaglandin $F_{2\alpha}$ in uterine fluid from the non-pregnant sheep with an autotransplanted ovary. *J. Endocr.*, **53**, 215-222.

HIGGS, G.A., McCALL, E. & YOULTEN, L.J.F. (1975). A chemotactic role for prostaglandins released from polymorphonuclear leucocytes during phagocytosis. *Br. J. Pharmac.*, **53**, 539-546.

NANCARROW, C.D., BUCKMASTER, J., CHAMLEY, W., COX, R.I., CUMMING, I.A., CUMMINS, L., DRINAN, J.P., FINDLAY, J.K., GODING, J.R., RESTALL, B.J., SCHNEIDER, W. & THORBURN, G.D. (1973). Hormonal changes around oestrus in the cow. *J. Reprod. Fert.*, **32**, 320-321.

POYSER, N.L. (1972). Production of prostaglandins by the guinea-pig uterus. *J. Endocr.*, **54**, 147-159.

POYSER, N.L. (1973). The physiology of prostaglandins. In *Clinics in Endocrinology and Metabolism, 2 (no. 3), Reproductive Endocrinology and World Population* (ed. Loraine, J.A.), pp. 393-410. London: Saunders & Company.

POYSER, N.L. (1974a). The significance of prostaglandin production by the uterus. *Pol. J. Pharmac. Pharm.*, **26**, 189-203.

POYSER, N.L. (1947b). Some aspects of prostaglandins in reproduction. *Biochem. soc. trans.*, **2**, 1196-1200.

SKARNES, R.C. & HARPER, M.J.K. (1972). Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. *Prostaglandins*, **1**, 191-203.

THOMPSON, C.J., LOS, M. & HORTON, E.W. (1970). The separation, identification and estimation of prostaglandins in nanogram quantities by combined gas chromatography–mass spectrometry. *Life Sci.*, **9**, 983-988.

WILLIAMS, K.I., SNEDDON, J.M. & HARNEY, P.J. (1974). Prostaglandin production by the pregnant rat uterus *in vitro* and its relevance to parturition. *Pol. J. Pharmac. Pharm.*, **26**, 207-215.

WILLIS, A.L. (1970). Identification of prostaglandin E_2 in rat inflammatory exudate. *Pharmac. Res. Comm.*, **2**, 297-304.

(Received May 1, 1975.
Revised June 26, 1975.)