J. Pharm. Pharmacol. 1981, 33: 607–610
Communicated January 21, 1981

0022–3573/81/090607–04 $02.50/0

Identification of arachidonic acid metabolites extracted from human uterus, and their effects on the isolated myometrium

G. J. SANGER*, C. N. HENSBY†**, I. F. STAMFORD, A. BENNETT, *Department of Surgery, King's College Hospital Medical School, London SE5 8RX and †Department of Clinical Pharmacology, Royal Postgraduate Medical School, London, W12 DHS, U.K.*

Normal and diseased human endometrium can form substantial amounts of prostaglandin (PG)E_2 and $F_{2\alpha}$ (Downie et al 1974; Willman et al 1976), whereas comparatively small amounts of PGE_2 occur in myometrial extracts (Willman & Collins 1978). Human uterus can also form 6-keto-$PGF_{1\alpha}$, a non-enzymic breakdown product of prostacyclin (PGI_2), from PGH_2 (Sun et al 1977) or arachidonic acid (Abel & Kelly 1980), and isolated endometrium releases 6-keto-$PGF_{1\alpha}$ and thromboxane (Tx) B_2 (Liggins et al 1980).

We have now looked for the presence of C_{20}-unsaturated fatty acid metabolites in homogenates of human endometrium or myometrium, and examined the effects of many of these compounds on strips of human myometrium.

Macroscopically normal specimens of human uterus, obtained at operation for menorrhagia (6 patients aged 33–42 years) were used. The menstrual phase was classified histologically (3 proliferative, 3 secretory: no obvious age difference between the two groups). The endometrium was scraped from the myometrium and both parts of the uterine tissue were separately cut into small pieces and washed in Krebs solution (g litre^{-1} NaCl 7·1, $CaCl_2$ $6H_2O$ 0·55, KH_2PO_4 0·16, KCl 0·35, $MgSO_4$ $7H_2O$ 0·29, $NaHCO_3$ 2·1, dextrose 1·0, equilibrated with 5% CO_2 in O_2). Weighed amounts (0·08–0·9 g or 0·4–4 g wet weight of endometrium or myometrium, respectively) were homogenized at room temperature (20 °C) in Krebs solution (0·1 g ml^{-1}, Silverson homogenizer) to stimulate synthesis of eicosanoids (prostanoids and lipoxygenase products) from released endogenous precursors (Bennett et al 1973).

After chloroform extraction (Unger et al 1971) and evaporation, samples were dissolved in dichloromethane and purified using LH20 column chromatography with dichloromethane to elute non-polar impurities. The eicosanoids were eluted with methanol, evaporated to dryness, dissolved in 10 ml double-distilled water acidified to pH 3·0 with hydrochloric acid, and percolated through an Amberlite XAD-2 column. After the column had been washed with 15 ml distilled water followed by 5 ml n-heptane, the eicosanoids were eluted with 10 ml methanol and dried, first at 40 °C under nitrogen and then under vacuum. The residue was dissolved in 200 µl methanol–chloroform (1:1 v/v) and applied as a narrow band onto a silica gel G thin-layer chromatography plate (200 × 100 × 0·2 mm Merck; FIV solvent system; Anderson 1969), next to authentic prostanoid standards. The plates were developed to 15 cm from the origin and 1 cm zones were eluted twice with 5 ml methanol which was then evaporated. Zones corresponding to arachidonic acid (AA) and 12-hydroxy-eicosatetraenoic acid (12-HETE) were pooled and the residues rechromatographed as described above, using diethyl ether–light petroleum (b.p. 40–60 °C)–acetic acid (50:50:1 by volume). This gave better separation of the zones corresponding to AA and 12-HETE which were eluted with more of the same solvent. Quantitation was not possible because no deuterated standards were available. No determination of leukotrienes was made. *O*-Methyloximes were prepared from residues dissolved in 100 µl pyridine containing methyloxime hydrochloride 5 µl ml^{-1}, and heated at 60 °–80 °C for 1 h. The pyridine was removed under vacuum for 30 min and the substances remaining were dissolved in 100 µl methanol and treated with 200 µl freshly re-distilled diazomethane. The *O*-methyl esters formed were vortexed, the samples were evaporated under nitrogen at room temperature and the treatment with methanol–diazomethane repeated. Trimethylsilyl ethers were prepared by dissolving the residues from *O*-methylester synthesis in 25 µl *NN*-bis (trimethylsilyl)-tri-fluoroacetamide (BSTFA, Sigma) and heating at 60 °C for 15 min.

Aliquots (10 µl) of standard or samples were injected into a Finnigan 9600 gas chromatograph equipped with a glass column (1·5 m × 2 mm) packed with 1% SE-30 on Supelcoport (phase separation), interfaced via a glass jet separator with a Finnigan 3200 quadrupole mass spectrometer operated by a Finnigan 6000 data system. The gas chromatograph was operated between 175 ° and 220 °C using helium (30 ml min^{-1}) as the carrier gas. Settings for the mass spectrometer were 25 eV electron energy, 10^{-7} amp V^{-1} pre-amplifier and 1700 V electron multiplier.

Each sample was scanned for the presence of C_{20}-unsaturated fatty acid metabolites, but only compounds formed from arachidonic acid were detected. If metabolites of eicosatrienoic or eicosapentaenoic acid had been present, they would have been recovered from the extracts in amounts probably at most 80 ng g^{-1} wet weight of tissue (recoveries for AA, PGE_2 and $PGF_{2\alpha}$ were about 70–90%) as judged from quantities of pure standards that can be

* Correspondence and present address: Beecham Pharmaceuticals, Harlow, Essex CM19 5AD U.K.
** Present Address: Département de Pharmacologie, Centre International De Recherches Dermatologiques, Sofia-Antipolis, 06560 Valbonne, France.

Table 1. Effects of various prostanoids on strips of human myometrium. The actions of each prostanoid are described as excitatory or inhibitory, but more detail is given in the text. Each prostanoid was tested in concentrations ranging from 0·01 or 1 ng ml^{-1} to 1 or 10 μg ml^{-1}. n = number of specimens examined.

	Myometrium with proliferative endometrium			Myometrium with secretory endometrium		
Prostanoid	Response	Approximate Threshold Concn. (ng ml^{-1})	n	Response	Approximate Threshold Concn. (ng ml^{-1})	n
PGD_2	Inhibitory	100–1000	4	Inhibitory	10–1000	3
U-46619	Excitatory	1–10	4	Excitatory	0·01–1	3
PGI_2	Inhibitory	10–100	3	Inhibitory	100–1000	3
6-keto-$PGF_{1\alpha}$	Inhibitory	10000	1	Excitatory	10000	1
	No effect	—	3	No effect	—	2
6,15-diketo-$PGF_{1\alpha}$	Inhibitory	1000–10000	3	Inhibitory	1000–10000	2
				No effect	—	1
TxB_2	Excitatory	1000	1	Excitatory	1000	2
	No effect	—	1	No effect	—	1

identified from complete scans. All the extracts contained arachidonic acid, 6-keto-$PGF_{1\alpha}$, TxB_2 and 12-HETE, but PGD_2, PGE_2, or $PGF_{2\alpha}$ were not always detected, particularly with myometrium (Fig. 1).

Willman & Collins (1978), using radioimmunoassay, found more PGE_2 in homogenates of human endometrium than of myometrium. Our g.c.-m.s. results support this and also indicate that more $PGF_{2\alpha}$ occurs in extracts of homogenized endometrium than of the myometrium. Both the endometrium and myometrium consistently yielded 6-keto-$PGF_{1\alpha}$, TxB_2 and 12-HETE, but we do not know the amounts formed by each tissue, or the contribution from vascular constituents. Abel & Kelly (1980) found that homogenates of human endometrium incubated with arachidonic acid, formed predominately $PGF_{2\alpha}$ and PGE_2, with some PGD_2. In contrast, the myometrium formed predominantly 6-keto-$PGF_{1\alpha}$, and the percentage conversion of arachidonic acid was less. The differences between these observations and our findings may arise in methodology; we did not add arachidonic acid to the homogenates. Sun et al (1977) showed that the microsomal fraction of human uterus could produce 6-keto-$PGF_{1\alpha}$. In other tissues, they found that different amounts of different types of prostanoid were formed as the substrate concentration was varied. Isolation of human uterus reduces its ability to synthesize PGE and PGF_α compounds (Jordan & Pokoly 1977) and perhaps the types of prostanoid formed vary with the amount of substrate released in the endometrium or myometrium.

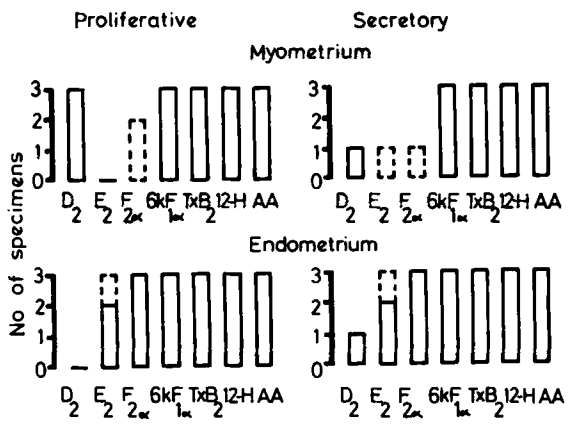

FIG. 1. Results of g.c.–m.s. analysis of eicosanoids extracted from homogenates of endometrium or myometrium removed at hysterectomy for menorrhagia. Results are given as the number of specimens in which a particular eicosanoid was detected, either as a full spectrum (essentially identical to standards; closed columns) or as a partial spectrum (where major fragmented ions corresponded to the standards, but the minor ones did not because of insufficient material or impurities; dotted columns). Those detected were PGs D_2, E_2, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ (6k$F_{1\alpha}$), TxB_2, 12-HETE (12-H) and arachidonic acid (AA).

In experiments investigating the actions of prostanoids on the myometrium, strips of myometrial body (approximately 4 × 4 × 50 mm, parallel to the uterine axis), were obtained from macroscopically normal specimens of uterus removed from patients (aged 32–47 years) for menorrhagia (8 patients) or abdominal or back pain and/or severe dysmenorrhoea (5 patients).

The strips were placed in Krebs solution 15–100 min after excision and studied immediately or after overnight storage at 4 °C (load 1 g, 10 ml Krebs solution, 37 °C, 5% CO_2 in O_2). Isotonic responses were measured using transducers and pen recorders (magnification × 8 to 16).

The drugs used were: acetylcholine perchlorate, PGD_2, PGE_2, $PGF_{2\alpha}$ tromethamine salt, (15S)-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13E-dienoic acid (U-46619), sodium PGI_2, 6-keto-$PGF_{1\alpha}$, TxB_2 and indomethacin. Concentrations are expressed as the above free acid or salts. U-46619 10 mg ml^{-1} in ethanol was diluted with 0·9% NaCl (saline) to 100 μg ml^{-1}, and freshly diluted with Krebs solution (normal or low Ca^{2+}). Sodium PGI_2 5 mg ml^{-1} in 1 M Tris buffer, was freshly diluted with 50 mM Tris buffer adjusted to pH 7·8 with 1 M HCl. All

other prostanoids were dissolved (5 or 10 mg ml^{-1}) in ethanol, and diluted with saline. The actions of prostanoids were assessed using ten-fold increments of concentrations, ranging from 0·01 or 1 ng ml^{-1} to 1 or 10 μg ml^{-1}, with 15 min contact times (Table 1). Comparisons were made with vehicle controls but the spontaneous activity of the myometrium made small changes difficult to detect. PGE_2 and $PGF_{2\alpha}$ were not investigated as they have been studied (Bygdeman 1964). The responses and sensitivities to these prostanoids were similar in myometrium from the proliferative and secretory phases. PGI_2 or PGD_2 dose-dependently inhibited the myometrium, by reducing the amplitude and number of spontaneous contractions and usually reducing the muscle tone; the effects with PGI_2 were usually more marked than with PGD_2 (Fig. 2). In general, this supports the observations of Omini et al (1978), who found PGD_2, PGE_2 and PGI_2 approximately equipotent. 6-Keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ or TxB_2 caused weak and variable responses, usually in relatively high concentrations. U-46619 at much lower concentrations than required for $PGF_{2\alpha}$ (Omini et al 1978) caused dose-dependent myometrial contraction, which was often accompanied by fewer and smaller spontaneous contractions (Fig. 2).

Karim & Adaikan (1979) showed that PGI_2 relaxed strips of myometrium from non-pregnant women, but the pregnant uterus was not affected in vitro (as strips) or in vivo with intra-uterine doses of up to 100 μg PGI_2 or 6-keto-$PGF_{1\alpha}$. In contrast, Omini et al (1979) reported that PGI_2 relaxed myometrial strips from pregnant women. Wiqvist et al (1979) reported that PGI_2, TxA_2 or PGH_2 contracted human isolated uterus, but another sample of PGI_2 caused relaxation, although it had little or no effect on uterine motility when injected intravenously into pregnant or non-pregnant women (Wilhelmsson et al 1981).

Using a low Ca^{2+}-Krebs solution ($CaCl_2.6H_2O$, 0·02–0·11 g $litre^{-1}$) to inhibit spontaneous contractions, we found that indomethacin 1 μg ml^{-1} reduced submaximal contractions to U-46619 (1–1000 ng ml^{-1}) or ACh (0·1–1 μg ml^{-1}) by 83(74–94)% and 48(28–63)% respectively (n = 7 for each), the greater effect on U-46619 being statistically significant ($P < 0·05$). Various fenamates, which can inhibit both prostanoid synthesis and action, antagonized contractions of human myometrium to U-46619 without consistently reducing those to ACh or $PGF_{2\alpha}$ (Sanger & Bennett 1979). Presumably U-46619 causes contraction of human isolated myometrium by selectively stimulating synthesis of excitatory prostanoids, or the fenamates and indomethacin block the action of U-46619.

We investigated the interactions between inhibitory PGI_2 or PGE_2 and excitatory $PGF_{2\alpha}$ or U-46619. Myometrial strips from patients hysterectomized 1–11 days after starting their last menstrual period, were suspended in low Ca^{2+}-Krebs solution. Submaximal contractions to $PGF_{2\alpha}$ (20-500 ng ml^{-1}) or U-46619 (5–300 mg ml^{-1}) were compared with approximately equal submaximal contractions to acetylcholine (ACh, 0·1–1·5 μg ml^{-1}), using 30 s contact times and 5–10 min cycle times.

The low Ca^{2+}-Krebs solution made relaxations difficult to detect, but PGI_2 1 μg ml^{-1} either slightly reduced muscle tone or had no effect. PGE_2 1 μg ml^{-1} acted similarly, except that 3 out of 10 specimens initially contracted. PGI_2 1 μg ml^{-1} reduced contractions to $PGF_{2\alpha}$ and U-46619 by 78(7–95)% and 89(57–100)% respectively ($P = 0·05$; n = 7 for each), but had no significant effect on those to ACh (6(21 to −11)% reduction; n = 14, $P = 0·39$). PGE_2 1 μg ml^{-1} reduced contractions to $PGF_{2\alpha}$, U-46619 and ACh by 83(76–97)%, 89(72–95)% and 50(17–65)% respectively. The greater effect on the prostanoids (n = 7 for each) compared with ACh (n = 14) was statistically significant ($P < 0·05$). Omini et al (1978) found similar results for PGI_2, using $PGF_{2\alpha}$ and $BaCl_2$. Some prostanoids may also preferentially antagonize responses to other prostanoids in the vasculature of canine uterus (Clark & Brody 1974), or in the longitudinal muscle of human isolated stomach (Bennett & Sanger 1980).

With normal non-pregnant women, PGE_2 causes contraction in vivo, except during menses when it causes relaxation in both normal and dysmenorrhoeic women (Bygdeman 1964; Martin et al 1978; Bygdeman et al 1979). Oral administration of PGE_2 during severe dysmenorrhoea, in which excessively high $PGF_{2\alpha}$ production occurs in the endometrium, decreases myometrial tonus and pain (Lundstrom & Bygdeman 1979); an imbalanced formation of excitatory and inhibitory prostanoids by the uterus during menstruation might therefore derange uterine function. Perhaps in normal non-dysmenorrhoeic women this balance is at least partly regulated by a selective interaction between excitatory and inhibitory prostanoids.

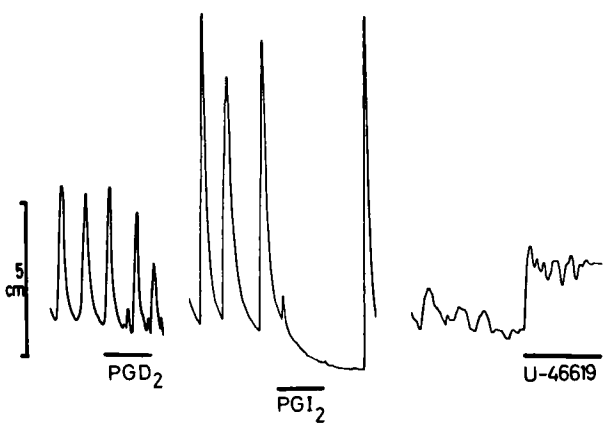

FIG 2. Effects of PGD_2, PGI_2 (1 μg ml^{-1}) or U-46619 (0·1 μg ml^{-1}) on strips of human myometrium (contact time 15 min). The responses were dose-related and reversible.

We thank the MRC for support, Upjohn Co Ltd and Wellcome Research Laboratories for prostanoids, and Miss M. A. Carroll, Miss K. Crowley and Mrs J. E. Wright for their help. Preliminary results of this paper were presented to the Physiological Society (Bennett & Sanger 1979).

REFERENCES

Abel, M. H., Kelly, R. W. (1980) Prostaglandins 18: 821–828

Anderson, N. H. (1969) J. Lipid Res., 10: 316–319

Bennett, A., Sanger, G. J. (1979) J. Physiol. (London) 292: 36–37P

Bennett, A., Sanger, G. J. (1980) Ibid. 298: 45–46P

Bennett, A., Stamford, I. F. & Unger, W. G. (1973). Ibid, 229, 349–360

Bygdeman, M. (1964) Acta Physiol. Scand. 63: Suppl, 242: 1–78

Bygdeman, M., Bremme, K., Gillespie, A., Lundstrom, V. (1979) Acta Obstet. Gynecol. Scand. Suppl. 87: 33–38

Clark, K. E., Brody, M. J. (1974) Pharmacologist 16: 197

Downie, J., Poyser, N. L., Wunderlich, M. (1974) J. Physiol. (London) 236: 465–472

Jordan, V. C., Pokoly, T. B. (1977) Obstet. Gynecol. 49: 449–453

Karim, S. M. M., Adaikan, P. G. (1979) in: Vane, J. R. & Bergstrom, S. (eds) Prostacyclin. Raven Press, New York, pp 419–433

Liggins, G. C., Campos, G. A., Roberts, C., Skinner, S. J. (1980) Ibid. 19: 461–477

Lundstrom, V., Bygdeman, M. (1979) Abstr. 4th. Int. Prostaglandin Conference. Washington D.C., pp 72.

Martin, J. N., Bygdeman, M., Eneroth, P. (1978) Acta Obstet, Gynecol. Scand. 57: 141–147

Omini, C., Pasargiklian, R., Folco, G. C., Fano, M., Berti, F. (1978) Prostaglandins 15: 1045–1054

Omini, C., Folco, G. C., Pasargiklian, R., Fano, M., Berti, F. (1979) Ibid. 17: 113–120

Sanger, G. C., Bennett, A. (1979) Br. J. Clin. Pharmac. 8: 479–482

Sun, F. F., Chapman, J. P., McGuire, J. C. (1977) Prostaglandins 14: 1055–1074

Unger, W. G., Stamford, I. F., Bennett, A. (1971) Nature (London) 233: 336–337

Wilhelmsson, L., Wikland, M., Wiqvist, N. (1981) Prostaglandins 21: 277–286

Willman, E. A., Collins, W. P. (1978) Acta Endocrinol. 87: 632–642

Willman, E. A., Collins, W. P., Clayton, S. G. (1976) Br. J. Obstet. Gynaecol. 83: 337–341

Wiqvist, N., Lindblom, B., Wilhelmsson, L. (1979) Res. Clin. Forums 1: 47–54

J. Pharm. Pharmacol. 1981, 33: 610–612
Communicated February 5, 1981

0022–3573/81/090610–03 $02.50/0

The adhesion of film coatings to tablet surfaces—a problem of stress distribution

R. C. Rowe, *ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, SK10 2TG, U.K.*

An important prerequisite of a film coating is that it should adhere strongly to the tablet core. Unfortunately only limited research has been carried out on the measurement of the adhesion of film coatings to tablet surfaces. The first method used was a peel test (Wood & Harder 1970; Nadkarni et al 1975) in which a strip of the film coating was peeled from the tablet surface using a tensile tester. To overcome the many deficiencies of the peel test, an instrument was designed to remove a known area of film coating normal to the film/tablet interface (Fisher & Rowe 1976). This method has since been used extensively to study the various factors affecting the adhesion of film coatings to tablet surfaces (Rowe 1977, 1978; Porter 1980; Fung & Parrott 1980). The results of these studies have generally been interpreted in terms of the physicochemical interaction between the film-coating polymers and tablet excipients. Recently, however, work on the bridging of the intagliations (Rowe & Forse 1980, 1981)—a practical consequence of poor film/tablet adhesion and thought to be due to the presence of high internal stress in the film coating—has highlighted the need to reappraise the results based on the stress distribution at the film/tablet interface. This has been undertaken in this report.

A film coating applied to a tablet surface is under the influence of two stresses (Fig. 1): one due to the forces of adhesion holding it to the substrate (A), and one inherent in the film (P) parallel to the tablet surface—the sum of the stress due to shrinkage of the film on evaporation of the solvent and the thermal stress due to differences in thermal expansion of the film coating and tablet substrate during changes in temperature arising out of the coating process. If the substrate was flexible, P would cause it to bend (Chow et al 1976) but since it is non-flexible this stress will cause a reaction R at the interface acting in the opposite direction to A. The relationships between P, R and t—the thickness of the film coating—are not fully understood but it is known that R will increase as both P and t increase

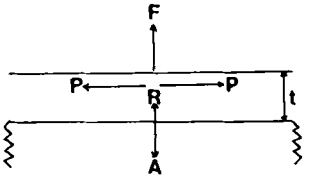

Fig. 1. A schematic diagram showing the stress distribution at the film/tablet interface.