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METABOLITES OF ARACHIDONIC ACID FORMED BY HUMAN GASTROINTESTINAL TISSUES AND THEIR ACTIONS ON THE MUSCLE LAYERS

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1 Gas chromatography-mass spectrometry demonstrated the presence of arachidonic acid (AA), 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 (TxB_2) in all extracts of homogenized muscle or mucosa from human stomach, terminal ileum or sigmoid colon. Prostaglandin D_2 (PGD_2), PGE_2 or $PGF_{2\alpha}$ were usually found more often in the mucosal extracts. The 12-hydroxy-derivative of AA (12-HETE) was detected in all extracts of the colon but in only some of the other tissues.

2 Most prostanoids tested contracted the longitudinal muscle, the order of potency being U-46619 (an epoxymethano analogue of PGH_2) > PGE_2 > $PGF_{2\alpha}$ > PGD_2; PGI_2 usually caused relaxation, whereas its breakdown products or TxB_2 had weak and variable effects.

3 U-46619 or, less potently, $PGF_{2\alpha}$ contracted the circular muscle, whereas PGI_2 and usually PGE_2 caused relaxation. PGD_2, 6-keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ or TxB_2 usually had little or no effect.

4 PGI_2 antagonized contractions to some excitatory prostanoids, without greatly affecting contractions to acetylcholine.

5 For both muscle layers there was a gradient in sensitivity to prostanoids along the gastrointestinal tract. The sensitivities were stomach > distal ileum > sigmoid colon.

6 The results are discussed in relation to gastrointestinal physiology and pathophysiology.

Introduction

Many prostaglandins and related substances potently affect the gastrointestinal tract and may have various roles in its normal or deranged function. In particular, prostaglandins are implicated as contributory factors in various gastrointestinal disorders including inflammatory conditions and many types of diarrhoea (see Bennett, 1978). It is therefore important to investigate the types of compound formed from eicosatrienoic, eicosatetraenoic (arachidonic) and eicosapentaenoic acids, the C20-unsaturated fatty acid precursors of prostaglandins, and to study their actions on the human gut. Most previous studies have concerned prostaglandin E (PGE) and PGF_α compounds (see Bennett, Stamford & Stockley, 1977), but we have now extended the range to include other related metabolites. Preliminary results were described to the Physiological Society (Bennett & Sanger, 1980).

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Methods

Specimens of human stomach, duodenum, terminal ileum, transverse or sigmoid colon were obtained at surgery for benign or malignant disease. Samples were taken at least 6 cm away from any macroscopic lesion, and were macroscopically normal. The mesentery and fat were cut off, and the muscle layers and serosa were separated from the mucosal layers by dividing along the submucosal plexus.

Gas chromatographic-mass spectrometric (g.c.-m.s.) analysis

The fresh muscle or mucosal layers were cut into small pieces and washed in Krebs solution. Weighed amounts were homogenized at room temperature in Krebs solution (0.1 g/ml; 30 s; Silverson homogenizer) to obtain new synthesis of eicosanoids (i.e. prostanoids and lipoxygenase products) from released endogenous precursors (Bennett, Stamford & Unger, 1973). Following chloroform extraction (Unger, Stamford & Bennett, 1971) and evaporation, samples were dissolved in dichloromethane and purified by LH20 column chromatography. Elution was carried out first with dichloromethane to remove

non-polar impurities, and then with methanol to elute the eicosanoids. Each eluate was evaporated to dryness, dissolved in 10 ml twice-distilled water acidified to pH 3.0 with HCl and percolated through an Amberlite XAD-2 column. Following washes first with 15 ml distilled water and then with 5 ml *n*-heptane, the eicosanoids were eluted with 10 ml methanol which was evaporated at 40°C under nitrogen and then desiccated under vacuum. The residue was dissolved in 200 μl methanol:chloroform (1:1, v/v) and applied as a narrow band on a silica gel G thin-layer chromatography plate (200 × 100 × 0.2 mm Merck; FIV solvent system; Anderson, 1969). Authentic prostanoid standards were applied next to each biological sample. 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 authentic arachidonic acid (AA) and 12-hydroxy-eicosatetraenoic acid (12-HETE) were pooled and the residues re-chromatographed as in the above thin-layer system with diethyl ether:petroleum spirit:acetic acid (50:50:1 by volume). This gave good separation of AA and 12-HETE which were eluted as described above. No search was made for leukotrienes (Samuelsson, Borgeat, Hammarström & Murphy, 1979).

Chemical derivatization was as follows: (a) O-Methyloxime. Residues from prostanoid zones were dissolved in 100 μl pyridine containing methyloxime hydrochloride 5 mg/ml, and heated at 60–80°C for 1 h. The pyridine was removed under vacuum for 30 min. (b) Methylesters. The residues from (a) above were dissolved in 100 μl methanol and treated with 200 μl freshly re-distilled diazomethane. After vortexing, the samples were evaporated under nitrogen at room temperature and the procedure repeated. (c) Trimethylsilyl ether. Vacuum-desiccated residues from (b) were dissolved in 25 μl N,N-bis (trimethylsilyl)-tri-fluoroacetamide (BSTFA:Sigma) and heated at 60°C for 15 min.

G.c.-m.s. was performed by injecting 10 μl aliquots of standards or samples 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). The chromatograph was interfaced via a glass jet separator with a Finnigan 3200 quadrupole mass spectrometer, and the system was operated using a Finnigan 6000 data system. The temperature of the gas chromatograph was 175° to 220°C and helium (30 ml/min) was the carrier gas. Settings for the mass spectrometer were 25 eV electron energy, 10^{-7} A/V pre-amplifier and 1700 V electron multiplier. Results for each eicosanoid are expressed as the number of samples for which there was a full mass spectrum (i.e. essentially identical to the eicosanoid standard) or a partial spectrum (i.e. where the major fragmented ions corresponding to the standards were found, but the minor fragments were not detected, due either to insufficient material or to impure samples).

Muscle strips

Tissue was used either immediately or after overnight storage at 4°C in Krebs solution (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 and dextrose 1.0 g/l) equilibrated with 5% CO_2 in O_2. Muscle strips approximately 4 mm wide and 3 cm long were cut parallel to the longitudinal or circular muscle fibres (the taenia were used as colonic longitudinal muscle).

Each strip was suspended under 1 g load in 10 ml Krebs solution (37°C, 5% CO_2 in O_2), and responses, magnified 6–18 times, were registered with an isotonic transducer. Results are expressed as ranges, or medians with semi-quartile ranges in parentheses and analysed using the Mann Whitney U-test.

Drugs

The following drugs were used: arachidonic acid, 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-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$, TxB_2, acetylcholine perchlorate and indomethacin. Concentrations are expressed as these free acids or salts. U-46619

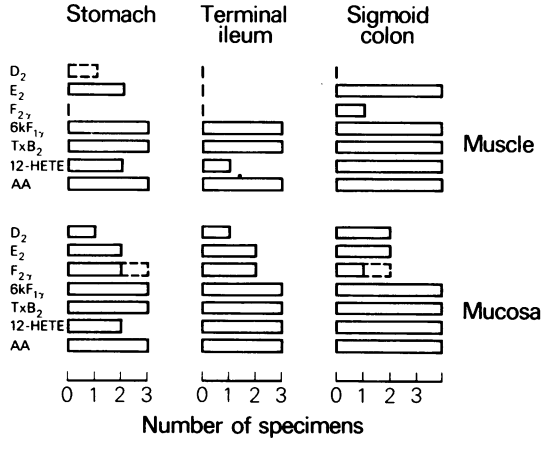

Figure 1 G.c.-m.s. analysis of eicosanoids present in homogenates of human gastrointestinal muscle or mucosa. Results are given as the number of specimens in which a particular eicosanoid was detected, either as a full spectrum (columns with continuous outline) or as a partial spectrum (columns with broken outline). Those detected were prostaglandins D_2, E_2, $F_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ ($6kF_{1\alpha}$), thromboxane B_2 (TxB_2), 12-HETE and arachidonic acid (AA).

10 mg/ml in ethanol was diluted to 100 μg/ml with 0.9% w/v NaCl solution (saline) and further dilutions were freshly prepared from this by diluting with Krebs solution. Sodium PGI_2 was prepared as a 5 mg/ml solution in 1 M Tris buffer, and solutions freshly prepared from this with 50 mM Tris buffer adjusted to pH 7.8 with 1 M HCl. All other prostanoids were prepared as 5 or 10 mg/ml solutions in ethanol and dilutions made with saline. All the organic solvents were Analar grade and the dichloromethane was re-distilled in glass.

Results

Gas chromatograph-mass spectrometry

For this study, tissue from 10 patients (five male, five female) aged 25–80 (median 63) years was obtained from specimens resected for cancer, pyloric ulceration, Crohn's disease or neoplastic changes with abscess. The efficiencies of recovery for arachidonic acid, $PGF_{2\alpha}$ and PGE_2 were 70–90%.

Only compounds formed from arachidonic acid were detected. If metabolites of eicosatrienoic or eicosapentaenoic acid were present, their amounts recovered from the extracts were probably at most 80 ng/g wet weight tissue, because relatively large amounts of prostanoids were needed for full mass spectra. We could not make quantitative measurements because deuterated standards were not available. All the extracts of muscle or mucosa contained detected amounts (>80 ng/g) of arachidonic acid, 6-keto-$PGF_{1\alpha}$ (a degradation product of PGI_2), and TxB_2 (a degradation product of TxA_2) but other prostanoids and 12-HETE were detected in only some samples (Figure 1). In mucosa, PGD_2, PGE_2 and $PGF_{2\alpha}$ were found more often than in the muscle, particularly in the terminal ileum; an exception is the colon where PGE_2 was found in all extracts of muscle

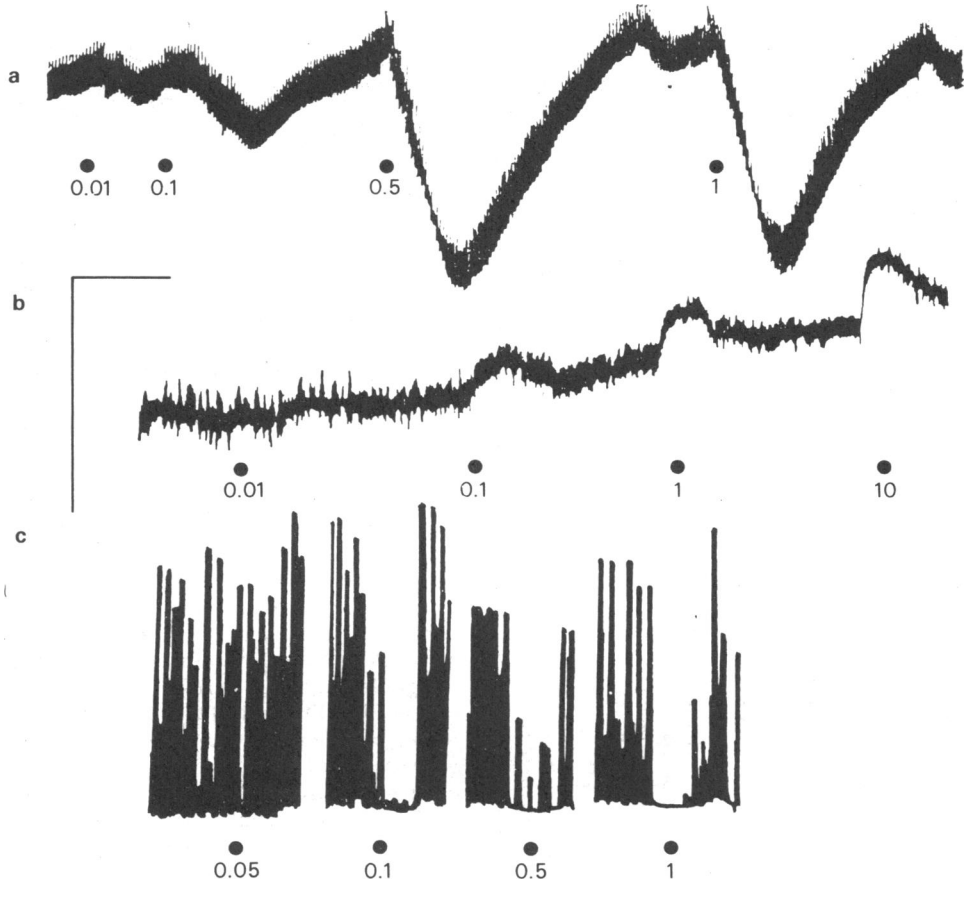

Figure 2 Effects of prostacyclin (PGI_2) on strips of longitudinal muscle from human stomach (a), terminal ileum (b) and sigmoid colon (c). Concentrations are ng/ml bathing fluid. Contact time 10 min. Horizontal bar: (b and c) 10 min, (a) 20 min. Vertical bar, 5 cm.

Table 1 Effects of prostanoids on longitudinal muscle strips from human stomach, terminal ileum or sigmoid colon

	Stomach				*Terminal ileum*			*Sigmoid colon*		
		Threshold			*Threshold*			*Threshold*		
Prostanoid	*Response*	(ng/ml)	n	*Response*	(ng/ml)	n	*Response*	(ng/ml)	n
PGD_2	Weak contraction	20 –1000	3	Contraction	100 –2000	3	Contraction	10–1000	3
PGE_2	Contraction	0.5 –10	3	Contraction	1	2	Contraction	10–100	3
$PGF_{2\alpha}$	Contraction	1 –10	3	Contraction	10	3	Contraction	100–1000	3
U-46619	Contraction	0.0001–1	4	Contraction	0.001–5	3	Contraction	1–10	3
PGI_2	Relaxation	50 –200	3	Weak relaxation	10000	1	Relaxation	1–10	4
				Weak contraction	10 –1000	2			
6-keto-$PGF_{1\alpha}$	Weak contraction	100	3	Weak contraction	10000	1	Weak contraction or relaxation	1000–10000	3
6,15-diketo-$PGF_{1\alpha}$	Weak contraction or relaxation	10000	3	Contraction	100	1	Weak contraction	1000	2
				No effect	—	1			
TxB_2	Contraction	1000	1	Weak	100	2	Weak contraction	1000	2
	No effect	—	2	contraction					
							No effect	—	1

Where weak contraction or relaxation are indicated, this may often mean only a small change in spontaneous activity, compared with vehicle controls. Each prostanoid was tested in concentrations ranging from at least 1 ng/ml to 1–10 μg/ml, with 10 min contact times. *n* = number of specimens examined. In addition, in 1 specimen each of jejunum or transverse colon, PGI_2 caused dose-dependent relaxation in a concentration as low as 0.1 μg/ml.

but in only half of the mucosal samples. The lipoxygenase product 12-HETE occurred in all extracts of colonic muscle and mucosa but in only some from stomach or terminal ileum.

Effects of prostanoids on gastrointestinal muscle

Studies were made on muscle strips from 27 patients (18 male, nine female) aged 25–80 (median 64) years. Most of the specimens were resected for cancer of the stomach, colon or rectum, or for peptic ulceration. The numbers of experiments were too small to allow a determination of how disease, age or sex may affect the response. The detailed results of approximate threshold concentrations and types of response are presented in Tables 1 and 2.

Longitudinal muscle: PGD_2, PGE_2, $PGF_{2\alpha}$ and the PGH_2 analogue U-46619 dose-dependently contracted all specimens (stomach, terminal ileum and sigmoid colon). PGI_2 dose-dependently relaxed gastric, jejunal and colonic specimens, but usually caused weak contractions of terminal ileum (Figure 2). Responses of tissues from all regions to 6-keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ or TxB_2 were generally weak and variable (Table 1).

In general the stomach was the most sensitive to prostanoids and the sigmoid colon the least sensitive. U-46619 was the most potent excitatory prostanoid, particularly in gastric muscle which contracted to concentrations as low as 0.1 pg/ml–1 ng/ml (Table 1). In all tissues, high concentrations of U-46619 caused a long-lasting contraction which was difficult to wash out. Gastric muscle responded more than the other tissues to PGI_2 but the greater relaxation of stomach muscle, compared with taenia coli (Figure 2) could be due, at least partly, to a higher stomach muscle tone.

In order to investigate the interaction of PGI_2 with prostanoids that potently contract stomach longitudinal muscle, submaximally effective concentrations of acetylcholine (ACh), PGE_2, $PGF_{2\alpha}$ and U46619 were chosen from dose-response curves (10 min cycle time; 30 s contact time). In any one experiment, consistent submaximal contractions were obtained with ACh (0.05–1 μg/ml) and either PGE_2 (3 ng/ml–1 μg/ml), $PGF_{2\alpha}$ (0.1–1 μg/ml) or U-46619 (5–100 ng/ml). PGI_2 1 μg/ml was then added to the bathing solution and new consistent responses were obtained to the same doses of ACh and the excitatory prostanoid, replacing PGI_2 after washout of each dose of excitatory substance.

In three specimens of stomach circular muscle, the effects of PGI_2 1 μg/ml were examined against submaximal contractions to ACh (0.2–10 μg/ml) or U-46619 (20–200 ng/ml), as described for the longitudinal muscle. PGI_2 reduced contractions to U-

Table 2 Effects of prostanoids on circular muscle strips from human stomach or sigmoid colon

	Stomach			*Sigmoid colon*		
Prostanoid	*Response*	*Threshold (ng/ml)*	n	*Response*	*Threshold (ng/ml)*	n
PGD_2	Contraction	100 -1000	2	Contraction	10	1
	Contraction preceded by weak relaxation at 100 ng/ml	1000	1	No effect	—	2
	No effect	—	1			
PGE_2	Contraction	1 -100	2	Contraction	10	1
	Relaxation	1000	2	Relaxation followed by contractions at higher concentrations	1	1
				Relaxation	1	1
$PGF_{2\alpha}$	Contraction	1 -100	3	Contraction	10	1
	No effect	—	1	Relaxation at 10-100 ng/ml followed by contraction	1000-10000	2
U-46619	Contraction	0.1-1	4	Contraction	10	3
PGI_2	Relaxation	0.1-100	3	Relaxation	1-10	3
6-keto-$PGF_{1\alpha}$	No effect	—	2	No effect	—	2
6,15-diketo-$PGF_{1\alpha}$	No effect	—	2	No effect	—	2
TxB_2	Contraction	1000	2	Weak contraction	10000	2
	No effect	—	1	No effect	—	1

Each prostanoid was tested in concentrations ranging from at least 1 ng/ml to 1-10 μg/ml, with 10 min contact times. n = number of specimens examined.

46619 or ACh by 50-76% and 4-32% respectively, the effect always being greater on U-46619 than on ACh.

PGI_2 lowered muscle tone, and this probably explains why contractions to U-46619 or ACh tended to increase (by 14(7 to 29)% and 6(6 to -26)% respectively; $n = 6$ and 19). In contrast, PGI_2 reduced contractions to PGE_2 or $PGF_{2\alpha}$ respectively by 34(15-81)% and 51(20-79)%; $n = 7$ and 6; $P < 0.01$ compared with the effect on ACh or U-46619.

In three other similar experiments (stomach 2, colon 1), incubation with indomethacin 1 μg/ml for at least 30 min did not greatly affect submaximal contractions to U-46619 or ACh, which were respectively 86-122% and 100-102% of control responses. Indomethacin reduced the muscle tone in one specimen of stomach but had no effect in the other tissues.

Circular muscle: U-46619 was the only prostanoid that consistently contracted the circular muscle of stomach or colon. As with the longitudinal muscle, the stomach was more responsive; U-46619 caused a dose-dependent contraction with concentrations as low as 0.1-1 ng/ml (Table 2). In the colon the amplitude of contraction with U-46619 was small and not clearly dose-dependent.

PGI_2 was the only prostanoid that consistently relaxed stomach and colonic muscle, both of which showed similar sensitivity (Table 2). In tissues from either region PGD_2 or $PGF_{2\alpha}$ usually caused contraction, sometimes preceded by a relaxation. PGE_2 usually caused relaxation of the colon, and relaxed the two gastric specimens resected from, or near to, the pylorus whereas two specimens which were probably from the upper portion of the stomach contracted to PGE_2. In most cases, 6-keto-$PGF_{1\alpha}$ or 6,15-diketo-$PGF_{1\alpha}$ had no effect on gastric or colonic circular muscle, but TxB_2 sometimes caused weak contraction (Table 2).

Discussion

Bennett *et al.* (1977) bioassayed the prostaglandin-like material (PG-lm) extracted from human gastrointestinal homogenates prepared as in the present work. With the stomach and terminal ileum more PG-lm was found in extracts of mucosa than of the muscle, whereas the reverse was true for the sigmoid colon. These bioassay results probably reflect mainly PGE_2 and $PGF_{2\alpha}$, with little contribution from 6-keto-$PGF_{1\alpha}$ or TxB_2; the rat stomach bioassay technique they used is sensitive to PGE_2 whereas $PGF_{2\alpha}$,

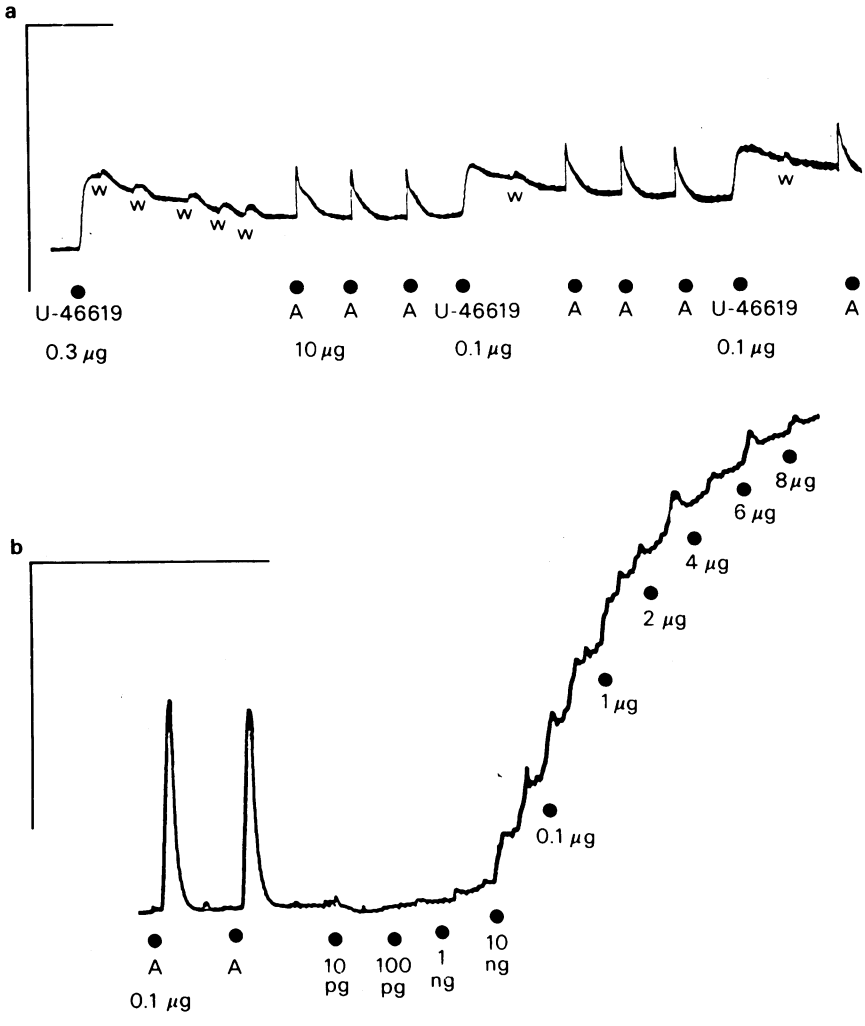

Figure 3 Tracings obtained from specimens of human stomach, longitudinal muscle. (a) The analogue U-46619 potently and reproducibly contracted the tissue, with a slow recovery after washout. Concentrations are /ml bathing fluid. Contact times for U-46619 and acetylcholine (A) were 30 s. Additional washouts are indicated by w. The contraction to acetylcholine 50 μg/ml was a little bigger than that to U-46619 0.3 μg/ml (not shown). (b) Cumulative dose-response curve to U-46619, with doses added at 2 min intervals. Vertical bar 5cm. Horizontal bar 10 min for (a) and (b), except that the chart speed was 3 times faster for acetylcholine (A) in (b).

PGD_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 are respectively about 9, 55, 240 and 440 times less potent (see Bennett, Jarosik, Sanger & Wilson, 1980a). In the present study g.c.-m.s. detected PGE_2 and $PGF_{2\alpha}$ more frequently in homogenates of stomach and ileum mucosa than in colonic mucosa, and PGE_2 was always present in extracts of sigmoid colonic muscle. This could explain the different amounts of bioassayed PG-lm reported by Bennett *et al.* (1977). Peskar, Seyberth & Peskar (1980) found that microsomal fractions or whole cell preparations of human gastric mucosa formed more PGE_2 than 6-keto-$PGF_{1\alpha}$, as measured by radioimmunoassay. However, the two mass spectrometric analyses reported by Bennett *et al.* (1977) indicate that 6-keto-$PGF_{1\alpha}$ was the most abundant prostanoid extracted from gastric mucosa. Although our present results with g.c.-m.s. are at best only semi-quantitative and the purification technique was different from that used in 1977, they indicate that human gastrointestinal muscle or mucosa may be able to form more PGI_2 and TxA_2 than PGE_2. Gut homogenates from vari-

ous animals also contain 6-keto-$PGF_{1\alpha}$ and TxB_2 which are presumably formed from PGI_2 and TxA_2 respectively (Pace-Asciak & Wolfe, 1971; Pace-Asciak, 1976; Ali, Zamecnik, Gerskus, Stoessl, Barnet & McDonald, 1977; Moncada, Salmon, Vane & Whittle, 1978).

The lipoxygenase product 12-HETE was detected in the muscle and mucosa of all the specimens of sigmoid colon studied, but was found only in some homogenates of stomach or terminal ileum. The actions of such compounds of the human gut are not known but this distribution might reflect regional differences in the contribution of lipoxygenase products to gastrointestinal functions or disorders. In other tissues 12-HPETE, the hydroperoxy precursor of 12-HETE, may modulate arachidonic acid metabolism (Moncada, Gryglewski, Bunting & Vane, 1976; Hammarström & Falardean, 1977; Ham, Egan, Soderman, Gale & Kuehl, 1979; Siegel, McConnel, Abrahams, Porter & Cuatrecasas, 1979), or activate guanylate cyclase (Graff, Stephenson, Glass, Haddox & Goldberg, 1978).

Although various eicosanoids can be produced by human gastrointestinal tissues, the significance and relative importance of the different pathways of arachidonic acid metabolism is still far from clear. At least some of the TxB_2, 6-keto-$PGF_{1\alpha}$ and 12-HETE must have been formed by the platelets or blood vessels in the tissue, although the regional differences in 12-HETE formation suggest an ability of some gastrointestinal tissues to form this compound. Prostanoid metabolism may also be altered by factors which include the disease for which the specimen was resected, medication, diet, anaesthesia, muscle activity, and various endogenous substances which can affect eicosanoid metabolism. No sex or age differences were evident in the types of prostanoid formed, but our numbers of tissues were too small for a valid comparison. Sinzinger, Silberbauer, Winter & Seyfried (1978) found no sex or age differences in the ability of normal human rectal mucosa to synthesize PGI_2.

The actions of PGE_2 and $PGF_{2\alpha}$ in this study were similar to those found previously for human tissues (Bennett, Murray & Wyllie, 1968; Fleshler & Bennett, 1969; Bennett & Fleshler, 1970; Bennett & Posner, 1971; Adaikan & Karim, 1976; Bennett & Stockley, 1975; 1977). PGA or PGB compounds were not studied, but others have shown that those prostanoids contract the longitudinal muscle of human stomach, ileum and colon (Schuster & Vanasin, 1971; Adaikan & Karim, 1976).

The epoxymethano analogue of PGH_2, U-44619, was the most potent agonist of both longitudinal and circular muscle in our studies. U-46619 may have complex actions and the extent to which this compound acts on receptors for PGH_2 or TxA_2 is not clear (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980a). In blood platelets or in human isolated myometrium, U-46619 may act in part by stimulating prostanoid synthesis (Malmsten, 1977; Sanger, Hensby, Stamford & Bennett, 1981), but our experiments with indomethacin and U-46619 on the longitudinal muscle argue against a similar action in human gut muscle. Similarly, indomethacin did not greatly reduce contractions of rat stomach muscle to epoxymethano analogues of PGH_2 (Bennett *et al.*, 1980a).

The potent effect of U-46619 on human gut muscle contrasts with its lack of effect on adenylate cyclase in homogenates of human gastric mucosa, whereas similar concentrations of another PGH_2 epoxymethano analogue (U-44069) caused activation (Simon & Kather, 1980). In several other tissues including guinea-pig ileum, rat stomach and colon, both of these compounds are approximately equipotent in causing muscle contraction (Chijimatsu, Van Nguyen & Said, 1977; Bennett, Jarosik & Wilson, 1978; Bennett, Pratt & Sanger, 1980b), but we have not examined U-44069 on human gastrointestinal muscle.

Of all the prostanoids tested only PGI_2 consistently relaxed the longitudinal muscle of human stomach or colon, although with PGE_1 a relaxation sometimes preceded the contraction (Oasti, Omuro, Toyosaka, Kuwata, Miyomoto & Okamoto, 1973), and PGE_2 sometimes inhibited ACh-induced contractions (Bennett *et al.*, 1968; Crofts, Stockley & Johnson, 1979). In addition to causing relaxation, PGI_2 1 μg/ml reduced contractions of the stomach longitudinal muscle to PGE_2 or $PGF_{2\alpha}$ with little effect on U-46619 or ACh. Similarly PGI_2 or PGE_2 relaxed strips of human myometrium and preferentially reduced contractions to prostanoids (Bennett & Sanger, 1979). Thus prostanoids which cause muscle relaxation may act, at least in part, by preferentially antagonizing contractions to certain endogenous prostanoids.

The importance of PGI_2 in gastrointestinal motility is not understood, but those prostanoids which contribute to the tone of human gastrointestinal longitudinal muscle seem to be mainly excitatory, since inhibitors of PG synthesis usually reduce the tone (Bennett & Stockley, 1977; Burleigh, 1977; Bennett *et al.*, 1980b). Furthermore, the role of PGI_2 might vary in different regions. The ileal muscle contrasts with gastric and colonic muscle in its weak contractions to PGI_2 and in the failure to yield detected amounts of PGD_2, PGE_2 or $PGF_{2\alpha}$.

The sensitivity to excitatory prostanoids in the longitudinal muscle was usually greatest for the stomach and least for the colon. Factors such as the type of disease and extent of the inflammation may contribute to small differences in responses of human

tissues to prostanoids (Hughes, Kadowitz, Hyman, Ray & Joiner, 1976; Crofts *et al.*, 1979), but these seem unlikely to explain large regional differences in sensitivity. Similar differences in sensitivity occur with $PGF_{2\alpha}$ in the normal human ileum and colon *in vivo* (Cummings, Newman, Misiewicz, Milton-Thompson & Billings, 1973; Hunt, Dilawari & Misiewicz, 1975), and with PGE_2, $PGF_{2\alpha}$ or PGI_2 in rat isolated stomach and colon (Gilmore, Vane & Wyllie, 1968; Omini, Moncada & Vane, 1977). Various other gradients occur in the human gut, such as biochemical and electrical activities (Alvarez, 1948), the muscle response to gastrin (Waller & Misiewicz, 1979) and innervation (Bennett & Stockley, 1975). The gradient of longitudinal muscle sensitivity to agonist prostanoids may therefore reflect and contribute to regional differences in gastrointestinal motility *in vivo*. Prostanoids also exert regional differences in mucosal effects; PGD_2, PGE_2 or PGI_2 equipotently activated adenylate cyclase in homogenates of human colonic mucosa, but PGD_2, unlike PGE_2 or PGI_2, had no effect in gastric mucosa (Simon & Kather, 1979).

With circular muscle, as with the longitudinal muscle, U-46619 was most potent in causing contractions and PGI_2 was the only prostanoid that consistently caused muscle relaxation. PGE_2 usually relaxed the colon, but the gastric response to PGE_2 varied, perhaps depending on the region examined. Since inhibitors of prostaglandin synthesis can increase spontaneous activity and tone in circular muscle (Bennett & Stockley, 1977; Burleigh, 1977; Bennett *et al.*, 1980b), perhaps PGI_2 and PGE_2 normally exert an inhibitory function. In this respect it is of interest that, unlike stomach longitudinal muscle, PGI_2 1 μg/ml reduced contractions of stomach circular muscle to U-46619 more than those to ACh. The sensitivity of U-46619 to antagonism by PGI_2 can therefore vary with the muscle layer. A gradient in sensitivity to U-46619 may also exist for the circular muscle; low concentrations of U-46619 markedly contracted gastric circular muscle but the effect in the colon was weak and not clearly dose-dependent.

Many of the results on human gastrointestinal muscle are similar to those in tissues from laboratory animals, but some important differences exist. In the human gut, U-46619 is the most potent agonist, compared with PGE_2 in the rat or guinea-pig (Bennett *et al.*, 1980a,b; Coleman, Humphrey, Kennedy, Levy & Lumley, 1980b; Sanger & Bennett, 1980). PGI_2 relaxed the longitudinal muscle of human stomach and colon, but in the stomach of the rabbit or rat all prostanoids so far tested caused contraction, although PGI_2 may sometimes reduce spontaneous muscle activity in rat colon (Horton & Jones, 1969; 1974; Hong, 1974; Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975; Bunting, Moncada & Vane, 1976; Omini *et al.*, 1977; Whittle, Mugridge & Moncada, 1979; Bennett *et al.*, 1980a). In addition, PGI_2 consistently relaxed the circular muscle of human stomach or colon, contrasting with the guinea-pig where PGE_2 was the only prostanoid which consistently relaxed intestinal circular muscle (Sanger & Bennett, 1980).

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