Br. J. Pharmac. (1980), **71**, 169–175

ANTAGONISM OF PROSTANOID-INDUCED CONTRACTIONS OF RAT GASTRIC FUNDUS MUSCLE BY SC-19220, SODIUM MECLOFENAMATE, INDOMETHACIN OR TRIMETHOQUINOL

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1 The effects of SC-19220, sodium meclofenamate, indomethacin or trimethoquinol were studied on contractions of the rat stomach longitudinal muscle to prostaglandin D_2 (PGD_2), PGE_2, $PGF_{2\alpha}$, PGH_2, epoxymethano PGH_2 analogues, PGI_2, 6-keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ and thromboxane B_2. All the drugs reduced contractions to all the prostanoids, but the degree of reduction differed widely. Selectivity of blockade was assessed by comparison with acetylcholine (ACh).

2 With SC-19220 5 μg/ml the effect on thromboxane B_2, PGD_2 or PGH_2 and its epoxymethano analogues was not significantly different from the small effect on ACh, but the other prostanoids were blocked to greater extents.

3 The effect of the cyclo-oxygenase inhibitor sodium meclofenamate, 1 or 2 μg/ml, on 6,15-diketo-$PGF_{1\alpha}$ or thromboxane B_2 was similar to the small antagonism of ACh, whereas the other prostanoids were blocked to greater extents. Indomethacin, 1 μg/ml, also reduced contractions to the prostanoids, but antagonism of the PGH_2 epoxymethano analogues was considerably less than with meclofenamate.

4 The β-adrenoceptor stimulant trimethoquinol, 50 ng/ml, was the most potent prostanoid antagonist tested; all the prostanoids except PGE_2 were antagonized more than ACh.

Introduction

All prostanoids so far tested contract the longitudinal muscle of rat gastric fundus. The prostaglandin antagonist SC-19220 (Sanner, 1969) blocks contraction of this tissue to prostaglandin E_2 (PGE_2) or $PGF_{2\alpha}$ with little effect on contractions to acetylcholine (Bennett & Posner, 1971). Of the other available antagonists, meclofenamate (Collier & Sweatman, 1968) and trimethoquinol (MacIntyre & Willis, 1978) are the most potent, but they block only some prostanoids in some smooth muscles. Since meclofenamate and trimethoquinol have not been tested previously on rat gastric fundus, we have compared then with SC-19220 for prostanoid antagonism. Some of these results have been presented to the British Pharmacological Society (Bennett, Jarosik & Wilson, 1978; Bennett & Sanger, 1979).

Methods

Adult Wistar rats of either sex were stunned and bled. Strips of gastric fundus approx. 20 mm long and 3 mm wide were cut parallel to the longitudinal muscle fibres, one from each side of the greater curvature, and suspended under a 1 g load in 10 ml organ baths containing 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). The solution was maintained at 37°C and bubbled with 5% CO_2 in O_2. Isotonic muscle contractions were recorded with transducers and pen recorders.

Drugs used were: PGD_2, PGE_2, $PGF_{2\alpha}$ tromethamine salt, PGH_2 (15S)-hydroxy-9α,11α and (15S)-hydroxy-11α,9α(epoxymethano prosta-15Z,13E-dienoic acids (U-44069 and U-46619 respectively), sodium prostacyclin (PGI_2), 6-keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$, thromboxane B_2 (TxB_2), acetylcholine perchlorate (ACh), 1-acetyl-2-(8-chloro-10,11-dihydrobenz (b,f) (1, 4) oxazepine-10-carbonyl)hydrazine (SC-19220), sodium meclofenamate, indomethacin and trimethoquinol. All concentrations refer to the acid or salt listed above.

Sodium PGI_2 was dissolved in 1 M Tris buffer and freshly diluted further with 50 mM Tris buffer, adjusted to pH 7.8 with HCl. U-44069 and U-46619

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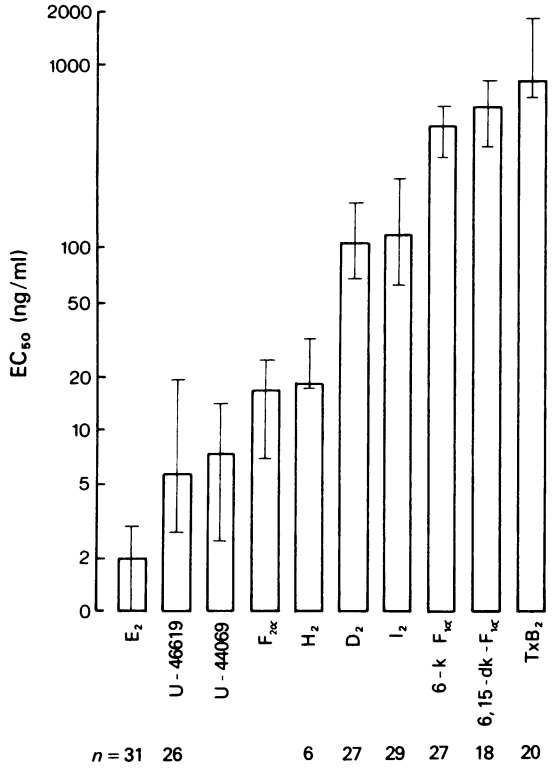

Figure 1 Potencies of prostanoids in causing contraction of rat gastric fundus longitudinal muscle. Results were calculated as the concentration of prostanoids required to produce a 50% of maximum response (EC_{50}). The columns are medians, and the vertical bars are semiquartile ranges on a logarithmic scale. n = number of experiments.

were dissolved in ethanol (10 mg/ml), diluted to 1 or 0.1 mg/ml with 0.15 M NaCl and then further diluted with Krebs solution. PGH_2 was administered in dry acetone with a microsyringe. Other prostanoids were dissolved in ethanol (5 or 10 mg/ml) and diluted with 0.15 M NaCl. SC-19220 was dissolved in polyethylene glycol 400. Acetylcholine, sodium meclofenamate or trimethoquinol (prepared daily) were dissolved in 0.15 M NaCl. Indomethacin was dissolved in ethanol (10 mg/ml) and diluted with Krebs solution.

In each experiment, cumulative dose-response curves were obtained to one prostanoid and usually to ACh, with 2 min intervals between each addition. An antagonist was then added to the bathing solution and after at least 30 min the dose-response curves were repeated. Only one antagonist was tested on each tissue. Measurements were made of the maximum response, and of the agonist concentrations required to give a contraction of 50% of maximum (EC_{50}). Results are given as medians with semiquartile ranges in parentheses, and analyzed using the Wilcoxon matched-pairs test or the Mann-Whitney U-test.

Results

All the prostanoids contracted the longitudinal muscle of rat gastric fundus. On the basis of EC_{50} values, PGE_2 was most potent and TxB_2 was least potent (Figure 1).

The drugs tested as prostanoid antagonists usually caused a marked reduction of the muscle tone which probably explained, at least in part, the subsequent increase in the amplitudes of maximum contraction (Table 1).

Measurements of the EC_{50} values showed that SC-19220 (5 μg/ml) antagonized submaximal contractions to PGE_2, $PGF_{2\alpha}$, PGI_2, 6-keto-$PGF_{1\alpha}$ and 6,15-diketo-$PGF_{1\alpha}$ but in contrast, contractions to PGD_2, TxB_2, PGH_2 or its epoxymethano analogues were not reduced more than the small extent seen with ACh (Figure 2).

Sodium meclofenamate (1 μg/ml) reduced contractions to all the prostanoids but the inhibition of 6,15-diketo-$PGF_{1\alpha}$ and TxB_2 was equivalent to the small effect on ACh (Figure 3). Meclofenamate (2 μg/ml) was tested against most of the prostanoids. Antagonism of PGE_2 or PGI_2 was greater than with 1 μg/ml meclofenamate and a similar tendency occurred with $PGF_{2\alpha}$ and U-46619 (Figure 4).

Indomethacin (1 μg/ml) was tested only against those prostanoids most antagonized by meclofenamate, and it too reduced the contractions to PGD_2, PGI_2 or the PGH_2 analogues more than ACh. However, the blockade of the PGH_2 analogues was substantially less than with sodium meclofenamate, even though the block of ACh was slightly greater with indomethacin (Figure 4).

Trimethoquinol was by far the most potent antagonist tested; 50 ng/ml reduced contractions to all the substances and, except for PGE_2, the effect on the prostanoids was much greater than on ACh (Figure 5).

Discussion

All prostanoids studied so far, including PGE_1, $PGF_{1\alpha}$, PGA_1, PGA_2, PGG_2 and TxA_2, contract the longitudinal muscle of rat gastric fundus (Horton & Jones, 1969; 1974; Hong, 1974; Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975; Bunting, Moncada & Vane, 1976; Omini, Moncada & Vane, 1977). In the rabbit stomach longitudinal muscle, prostanoids also consistently cause contraction

Table 1 Effect of SC-19220, trimethoquinol, sodium meclofenamate and indomethacin on the maximal responses to acetylcholine (ACh) and various prostanoids

Drug	*ACh*	*PGD_2*	*PGE_2*	*$PGF_{2\alpha}$*	*PGH_2*	*U-46619*	*U-44069*	*PGI_2*	*6-keto $PGF_{1\alpha}$*	*6,15-diketo $PGF_{1\alpha}$*	*txB_2*
SC-19220	108	115	127**	113	80**	119	92*	126	106	91**	94
5 μg/ml	(98–119)	(95–132)	(116–167)	(103–120)	(71–92)	(96–131)	(87–102)	(97–138)	(87–121)	(70–98)	(84–108)
Trimethoquinol	128	122	125**	110*	Not	128	103*	113	138**	84**	113
50 ng/ml	(111–157)	(117–128)	(120–141)	(106–114)	tested	(118–138)	(82–122)	(111–146)	(100–182)	(68–110)	(107–151)
Sodium meclofenamate	117	163	109	115	Not	163	120	106	166*	114	144
1 μg/ml	(102–130)	(93–180)	(107–159)	(105–123)	tested	(98–131)	(115–156)	(97–130)	(155–192)	(87–129)	(65–173)
Sodium meclofenamate	143	134	112	103*	Not	124	134	108	143	Not	Not
2 μg/ml	(109–164)	(97–213)	(102–130)	(90–114)	tested	(102–133)	(124–159)	(99–138)	(129–194)	tested	tested
Indomethacin	142	144	Not	Not	Not	124	150	177*	Not	Not	Not
1 μg/ml	(114–160)	(115–165)	tested	tested	tested	(115–151)	(138–193)	(151–220)	tested	tested	tested

Results are expressed as median % control with semi-quartile ranges in parentheses. *$P < 0.05$; ** < 0.01 compared to ACh

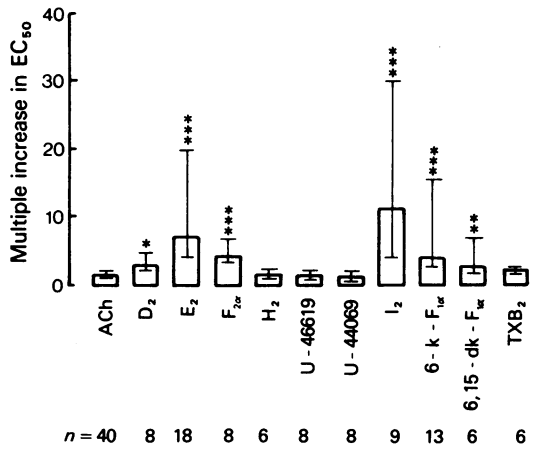

Figure 2 SC-19220 (5 μg/ml) preferentially antagonized contractions of rat stomach fundus longitudinal muscle to some prostanoids. Results are expressed as the multiple increase in EC_{50} after addition of SC-19220, and are given as medians and semiquartile ranges. The effect of SC-19220 on each prostanoid is compared with that on acetylcholine (ACh); $*P < 0.1$, $**P < 0.05$, $***P < 0.01$; n = number of experiments.

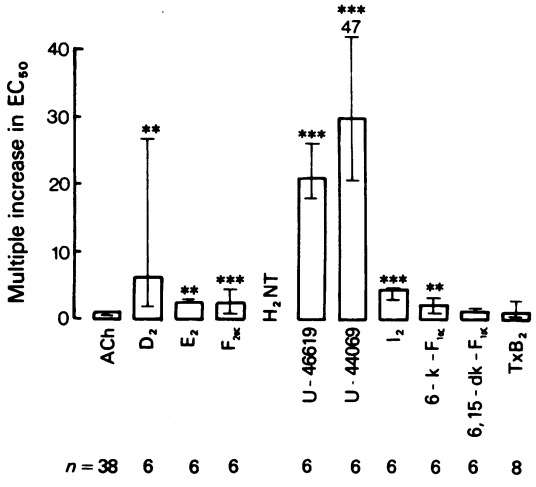

Figure 3 Sodium meclofenamate (1 μg/ml) preferentially antagonized contractions of rat stomach fundus longitudinal muscle to some prostanoids (prostaglandin H_2 (H_2) was not tested, NT). Results are expressed as for Figure 2.

(Whittle, Mugridge & Moncada, 1979), whereas in the longitudinal muscle of human isolated stomach, PGI_2 caused relaxation but other prostanoids caused contraction (Bennett & Sanger, 1980).

The potencies of PGD_2, PGE_2, $PGF_{2\alpha}$ and PGH_2 differed by about the extents reported by Horton & Jones (1974) and Bunting *et al.* (1976). This might partly reflect the activation of one receptor type by prostanoids of different affinities or efficacies. However, the differences in antagonism by various drugs may mean that rat gastric fundus contains more than one type of prostanoid receptor.

We have confirmed that SC-19220 (5 μg/ml) antagonizes PGE_2 and $PGF_{2\alpha}$ more than ACh (Bennett & Posner, 1971). In addition, we have shown that SC-19220 greatly reduces responses to PGI_2, 6-keto-$PGF_{1\alpha}$ and 6,15-diketo-$PGF_{1\alpha}$, but the effect on PGH_2, its epoxymethano analogues, or TxB_2 is similar to the small effect on ACh; the tendency for a preferential antagonism of PGD_2 by SC-19220 was not statistically significant. Since similar concentrations of SC-19220 block contraction of the rat gastric fundus to arachidonic acid (Splawinski, Nies, Sweatman & Oates, 1973), PGH_2 formed from arachidonic acid seems unlikely to contribute substantially to the contraction unless SC-19220 blocks endogenous PGH_2 more than exogenous PGH_2. Sodium meclofenamate and indomethacin are drugs which may be given to man, although meclofenamate has not yet been marketed. Both inhibit fatty acid cyclooxygenase (Flower, 1974), but in addition meclofenamic acid potently antagonizes certain prostanoid responses, as first shown by Collier & Sweatmen (1968).

In our experiments, sodium meclofenamate or indomethacin reduced the contractions to some prostanoids significantly more than responses to ACh. Similar concentrations of indomethacin also reduced submaximal contractions to PGE_1 in gerbil colon (Tolman & Partridge, 1979), but much higher amounts were needed for preferential antagonism of contractions to prostanoids in guinea-pig ileum (Sorrentino, Capasso & Di Rosa, 1972; Lembeck & Juan, 1974; Famaey, Fontaine & Reuse, 1977). There are many possible explanations for our results with indomethacin. Endogenous prostanoids might potentiate responses to exogenous agonists (particularly other prostanoids), and indomethacin may merely act by inhibiting endogenous prostanoid synthesis. Meclofenamate could also act in this way, but its greater block of PGH_2 epoxymethano analogues suggests a preferential effect on endoperoxide responses (Sanger & Bennett, 1979) or, if the analogues are thromboxane-like (Coleman, Humphrey, Kennedy, Levy & Lumley 1980), on thromboxane A_2 receptors.

Also in the rat, sodium meclofenamate (and flufenamate or mefenamate) antagonized the stimulation of gastric secretion induced by $PGF_{2\alpha}$, but not the inhibitory effect of PGE_2 (Karpaanen & Puurunen, 1976). Sodium flufenamate antagonized increases in rat mesenteric vasoconstriction induced by PGE_2 or epoxymethano PGH_2 in the presence of noradrenaline (Bennett, Carroll & Sanger, 1979). Fenamates

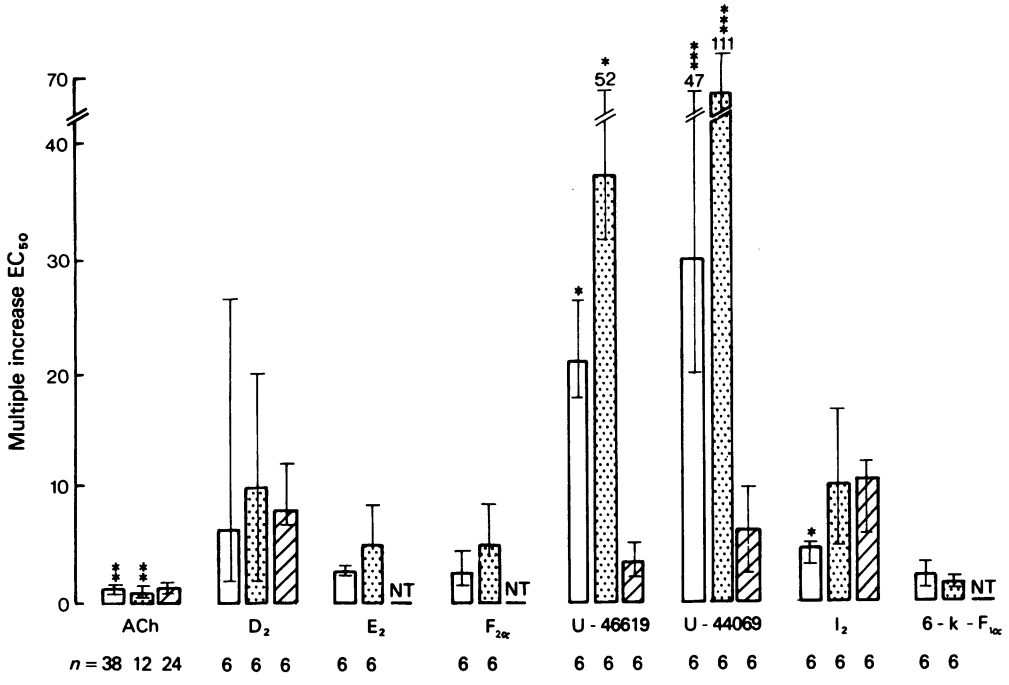

Figure 4 Preferential antagonism by meclofenamate of contractions of rat stomach fundus longitudinal muscle to epoxymethano analogues of prostaglandin H_2 (H_2). Results are expressed as in Figure 2, for meclofenamate 1 μg/ml (open columns), 2 μg/ml (stippled columns) and indomethacin 1 μg/ml (hatched columns). The effects of meclofenamate and indomethacin are compared on response to acetylcholine (ACh) and certain prostanoids (*$P < 0.1$, **$P < 0.05$, ***$P < 0.01$). With 2 μg/ml meclofenamate, antagonism of PGE_2 or PGI_2 was greater than with 1 μg/ml meclofenamate ($P < 0.007$ and <0.038 respectively), and a similar tendency occurred with $PGF_{2\alpha}$ and U-46619 ($P = 0.055$ and 0.078 respectively). There was no significant difference between the effects of the two meclofenamate concentrations on the other prostanoids tested ($P > 0.1$).

also preferentially antagonize contractions of gerbil, guinea-pig or human gastro-intestinal muscle to certain prostaglandins (Tolman & Partridge, 1975; Famaey *et al.*, 1977; Bennett, Pratt & Sanger, 1980). In human myometrium the drugs did not consistently antagonize submaximal contractions to ACh or $PGF_{2\alpha}$ but greatly reduced those to the PGH_2 analogue, U-46619 (Sanger & Bennett, 1979). Since indomethacin (1 μg/ml) caused a similar block of myometrial contractions to U-46619 (unpublished), epoxymethano analogues of PGH_2 may cause contraction by stimulating prostanoid synthesis, as seems to occur in platelets (Malmsten, 1977). In rat stomach meclofenamate is more effective than indomethacin in blocking contractions to PGH_2 analogues, and can probably antagonize the analogue-induced contractions directly.

Trimethoquinol, a β-adrenoceptor stimulant used clinically in some countries as a bronchodilator (Yamamura & Kishmoto, 1968), antagonized PGH_2-induced platelet aggregation and contraction of rabbit aorta; with TxA_2 the drug was a less effective antagonist in platelets and was ineffective against aortic contractions (MacIntyre & Willis, 1978). We have found that trimethoquinol (50 ng/ml) greatly reduced contraction of the rat stomach muscle to all the prostanoids except PGE_2. The effects on PGE_2 and PGE_1 may differ, although both are reported to have similar binding sites in this tissue (Miller & Magee, 1973), since in subsequent experiments trimethoquinol (50 ng/ml) antagonized PGE_1-induced contractions of the rat stomach (dose-ratio, 11.5 (7.8-13.6)). Propranolol (2 μg/ml) prevented or substantially reduced the preferential (compared with ACh) antagonism of PGE_1, $PGF_{2\alpha}$ or PGI_2 by trimethoquinol (Lacey & Sanger, 1980). These results with PGE_1 are similar to those in which the β-adrenoceptor stimulant, isoxsuprine (0.1 to 1 μg/ml) reduced the fundic contraction to PGE_1 without greatly affecting the response to ACh (Coceani & Wolfe, 1966; Wolfe, Coceani & Pace-Asciak, 1967). However, in our experiments isoxsuprine (0.5 μg/ml)

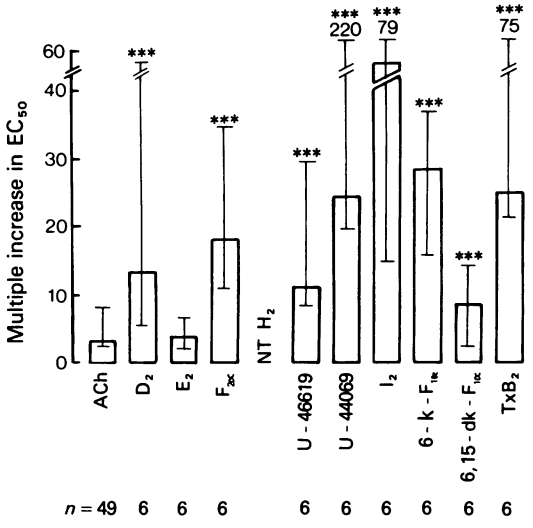

Figure 5 Trimethoquinol (50 ng/ml) preferentially antagonized contractions of rat stomach fundus longitudinal muscle to various prostanoids (prostaglandin H_2 (H_2) was not tested). Results are expressed as for Figure 2.

produced only weak selective inhibition of contractions to PGE_1 so making the tendency for a differential effect on PGE_1 and PGE_2 difficult to detect (Lacey & Sanger, 1980).

Differentiation of prostanoid receptors in the gut with antagonist drugs

Since PGF compounds contracted gut circular muscle, whereas PGE compounds usually caused relaxation, Bennett & Posner (1971) suggested that these receptors for PGE and PGF are different. Furthermore, because in tissues such as guinea-pig colon, the prostaglandin antagonists SC-19220 or polyphloretin phosphate blocked contraction of the longitudinal muscle to PGE compounds but not PGE-induced relaxation of the circular muscle, they suggested that at least two types of PGE receptor occur. The ability of the antagonists to block prostaglandin-induced contractions varied with the species, indicating yet other differences in the receptors. Additional evidence of receptor differences between species is the varying ability of certain fenamates to antagonize PGE_2- or $PGF_{2\alpha}$-induced contractions of human gastrointestinal longitudinal muscle, and the varying antagonism by meclofenamate of contractions to PGD_2, PGE_2 or $PGF_{2\alpha}$ in guinea-pig intestinal muscle (Bennett *et al.*, 1980). There are also regional differences in prostanoid receptor type or distribution in the circular muscle of guinea-pig ileum and colon (Bennett & Sanger, 1978; 1980).

The present studies indicate that several types of prostaglandin receptor may be present in rat gastric fundus. Thus PGE_2 may act on receptors different from those activated by the PGH_2 analogues, since SC-19220, meclofenamate or trimethoquinol antagonized these compounds to different extents. However, some of the antagonists we used have other well-known actions which may be important in reducing the responses to prostanoids. Apart from inhibition of endogenous prostanoid synthesis with meclofenamate and indomethacin, and β-adrenoceptor stimulation with trimethoquinol, the drugs have other potentially important actions. These include an effect on tissue calcium binding with meclofenamate and indomethacin (Northover, 1973), and papaverine-like activity with high concentrations of trimethoquinol (see Hanna & Eyre, 1979). The extent to which blockade of contractions is due to interaction with various prostanoid receptors is therefore not known, but it seems likely that rat gastric fundus contains a multiplicity of prostanoid receptor types. Other authors have reached this conclusion with regard to lung tissue (Gardiner & Collier, 1980).

We thank Mr E.V. Bagshaw for assistance, the MRC and Wellcome Trust for support, the Upjohn Co. Ltd and Wellcome Research for prostanoids, Warner-Lambert for meclofenamate, Roche Products Ltd for trimethoquinol and G. D. Searle & Co. for SC-19220.

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(Received November 26, 1979.
Revised March 20, 1980.)