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ANTAGONISM BY FENAMATES OF PROSTAGLANDIN ACTION IN GUINEA-PIG AND HUMAN ALIMENTARY MUSCLE

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1 Low concentrations of meclofenamate, flufenamate or mefenamate had little effect on contractions in response to acetylcholine in any tissue studied.

2 Sodium meclofenamate potently antagonized contractions of guinea-pig ileum longitudinal muscle to prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ or PGD_2.

3 In guinea-pig colonic longitudinal muscle, contractions to PGE_2 were reduced by sodium meclofenamate, but contractions of the longitudinal or circular muscle to $PGF_{2\alpha}$ or PGD_2 were less effectively inhibited.

4 In human gastrointestinal longitudinal muscle, sodium meclofenamate or flufenamate potently inhibited contractions to $PGF_{2\alpha}$, but not to PGE_2.

5 Sodium mefenamate or mefenamic acid, even in high concentrations, had little effect on contractions to $PGF_{2\alpha}$, but tended to inhibit PGE_2-induced contractions of human gastrointestinal longitudinal muscle.

6 The therapeutic advantages of prostaglandin synthesis inhibitors which also antagonize responses to certain prostaglandins are discussed.

Introduction

Mefenamic and flufenamic acids are used clinically to treat pain and inflammatory joint disease. Meclofenamic acid, a similar drug, has been given to man but has not been marketed. The potent ability of these compounds to inhibit prostaglandin synthesis through an action on cyclo-oxygenase (see Flower, 1974) is probably of major importance in their therapeutic action. However, meclofenamate or flufenamate can also selectively block contractions to prostaglandins in some tissues. For example, meclofenamate inhibits prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced contractions of human isolated bronchial muscle, without affecting contractions to histamine (Collier & Sweatman, 1968). Since therapeutic doses of non-steroidal anti-inflammatory drugs do not completely block cyclo-oxygenase (Samuelsson, 1973), the ability to antagonize the action of certain residual prostaglandins may also be of therapeutic importance. With regard to the gut, blockade of prostaglandin action by fenamates may contribute to the relief of gastrointestinal disturbances involving prostaglandins. The present experiments examine the effects of some fenamates on prostaglandin-induced contractions of guinea-pig longitudinal and circular intestinal muscle and human longitudinal gastrointestinal muscle. There are no previous studies on fenamates with regard to PGD_2, guinea-pig colon, the circular muscle of guinea-pig intestine or to human gastrointestinal muscle.

Methods

Adult male albino guinea-pigs were stunned and bled. Segments 2 to 3 cm long, and spiral strips 2 to 3 mm wide and 2 to 3 cm long, were cut from the distal ileum and colon to determine longitudinal and circular muscle responses respectively.

Macroscopically normal specimens of human stomach, terminal ileum and colon (taenia coli), at least 6 cm from any lesion, were obtained at operation for benign or malignant gastrointestinal disease. They were placed in Krebs solution equilibrated with 5% CO_2 in O_2 and studied the same day or after overnight storage at 4°C.

The mucosa and submucosa were cut away and strips (4 to 5 mm wide, 2 to 3 cm long) were cut parallel to the longitudinal muscle fibres. Each strip was suspended under a load of 1 g in a 10 ml organ bath 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) maintained at 37°C and bubbled with 5% CO_2 in O_2. Isotonic responses were measured with trans-

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ducers and pen recorders. After obtaining rough dose-response curves to acetylcholine (ACh), PGD_2, PGE_2 or $PGF_{2\alpha}$, doses were chosen which gave approximately equal consistent submaximal contractions. Contact times were usually 30 s in guinea-pig intestine and 1 min in human preparations. Cycle times were constant in each experiment, but varied with the tissue and prostaglandin, being 3 to 10 min with guinea-pig intestine, and usually 15 min (10 to 40 min) with human tissues. A fenamate was then added to the bath and consistent contractions again obtained to the agonists. Contraction heights were expressed as a percentage of pre-fenamate controls (mean of two or three responses). Only one fenamate was tested on each preparation.

Drugs used were acetylcholine perchlorate, PGD_2, PGE_2, $PGF_{2\alpha}$ tromethamine salt, mefenamic acid, sodium mefenamate, sodium meclofenamate and sodium flufenamate. Concentrations are expressed as these free acids or salts. Mefenamic acid 10 mg/ml was dissolved in ethanol and diluted with Krebs solution. Sodium mefenamate, meclofenamate or flufenamate 5 mg/ml were prepared in 0.9% w/v NaCl solution (saline).

The degree of block was assessed in several human preparations by determining the increase in the dose of agonist needed to restore the contraction heights (dose-ratio). Results are expressed as medians and semiquartile ranges, and analysed by the Wilcoxon matched-pairs test or the Mann-Whitney U test.

Results

Guinea-pig intestine

Muscle tone and spontaneous activity The effects of meclofenamate on spontaneous activity differed in the ileum and colon; the maximum response was usually obtained within about 10 min. In the ileum, sodium meclofenamate 1 μg/ml often markedly increased the spontaneous activity of the longitudinal muscle and circular muscle. Further studies on the longitudinal muscle showed that this effect lasted several hours after washing out the drug, and was not completely removed even after repeated washings. By contrast, with colonic muscle 1 μg/ml meclofenamate had no effect on either muscle layer or reduced spontaneous activity and/or muscle tone (Table 1).

Drug-induced muscle contractions The effect of meclofenamate on the prostaglandin-induced responses were measured after a 15 to 20 min contact time. In guinea-pig ileal longitudinal muscle, sodium meclofenamate 1 or 2.5 μg/ml produced a dose-related inhibition of contractions to PGD_2, PGE_2 or $PGF_{2\alpha}$, with little effect on contractions to ACh (Figure 1).

Table 1 Effect of fenamates on muscle activity

Fenamate	*Concentration (μg/ml)*	*Muscle*	*Activity Unaffected*	*Reduced*	*Increased*
Guinea-pig					
Sodium meclofenamate	1	Ileum longitudinal	13	0	18
	1	Ileum circular	0	2	6
	1	Colon longitudinal	5	3	0
	1	Colon circular	1	7	0
Human longitudinal muscle					
Sodium meclofenamate	1 and 5	Stomach	0	7	0
	1 and 5	Ileum	2	1	0
	1 and 5	Taenia coli	2	3	0
Sodium flufenamate	2 and 5	Stomach	0	1	1
	2 and 5	Ileum	1	2	0
	2	Taenia coli	0	2	0
Sodium mefenamate plus mefenamic acid	20, 50 and 100	Stomach	0	8	0
	20 and 100	Ileum	0	1	1
	20 and 50	Taenia coli	2	3	0

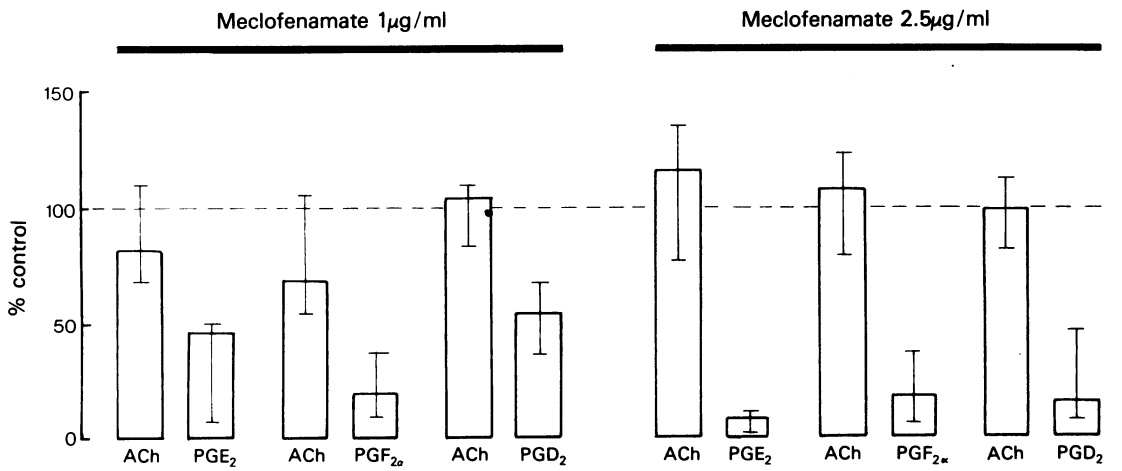

Figure 1 Antagonism by meclofenamate of prostaglandin-induced contractions in guinea-pig ileum longitudinal muscle. Results are expressed as a percentage of control; the columns represent medians and the bars semiquartile ranges. Compared to acetylcholine (ACh), contractions to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGE_2 or PGD_2 were selectively antagonized by meclofenamate ($P \leq 0.01$; $n = 10$ for 1 μg/ml meclofenamate, $n = 8$ for 2.5 μg/ml).

In colonic longitudinal muscle, sodium meclofenamate often increased the contractions to ACh. This was at least partly due to the lowering of muscle tone in some preparations, but the maximum shortening of the muscle did not seem to be affected. Meclofenamate 2 or 10 μg/ml reduced contractions to PGE_2 or PGD_2 without blocking responses to ACh. The tendency to reduce contractions to $PGF_{2\alpha}$ was not statistically significant with these meclofenamate concentrations, and the lower concentration of 1 μg/ml did not significantly affect contractions to PGD_2, PGE_2 or $PGF_{2\alpha}$ (Table 2).

With regard to the circular muscle, only $PGF_{2\alpha}$ and PGD_2 were studied on guinea-pig colon. Ileal circular muscle strips do not contract to these prostaglandins, and PGE_2 inhibits contractions of guinea-pig ileal and colonic circular muscle (see Bennett & Sanger, 1978). Compared with ACh, contractions of colonic circular muscle to PGD_2 were not selectively reduced by meclofenamate 1, 2 or 10 μg/ml, and those to $PGF_{2\alpha}$ were selectively reduced only with 10 μg/ml meclofenamate (Table 2).

Human gastrointestinal longitudinal muscle

The fenamates usually reduced gastrointestinal longitudinal muscle tone and spontaneous activity, particularly in specimens of stomach (Table 1). There did

Table 2 Effect of sodium meclofenamate on prostaglandin-induced contractions of guinea-pig colonic muscle

Concn of meclofenamate (μg/ml)	*ACh*	*PGD_2*	*ACh*	*PGE_2*	*ACh*	*$PGF_{2\alpha}$*
Longitudinal muscle						
1	124(94-128)	117(109-124)	124(115-138)	87(76-110)	107(103-132)	98(71-141)
2	112(92-123)	108(106-110)	120(115-131)	73(68-113)*	110(101-132)	74(58-129)
10	110(83-123)	14(8-33)*	121(112-142)	42(18-62)*	115(113-172)	41(24-106)
Circular muscle						
1	83(75-121)	69(61-111)	Not tested		110(92-123)	97(82-129)
2	98(88-112)	119(55-123)	Not tested		112(105-117)	97(68-118)
10	97(91-109)	58(33-95)	Not tested		103(70-129)	46(25-103)*

Results are expressed as a median percentage of control, with semiquartile ranges in parentheses. *$P \leq 0.05$, compared with acetylcholine (ACh), $n = 6$ for each.

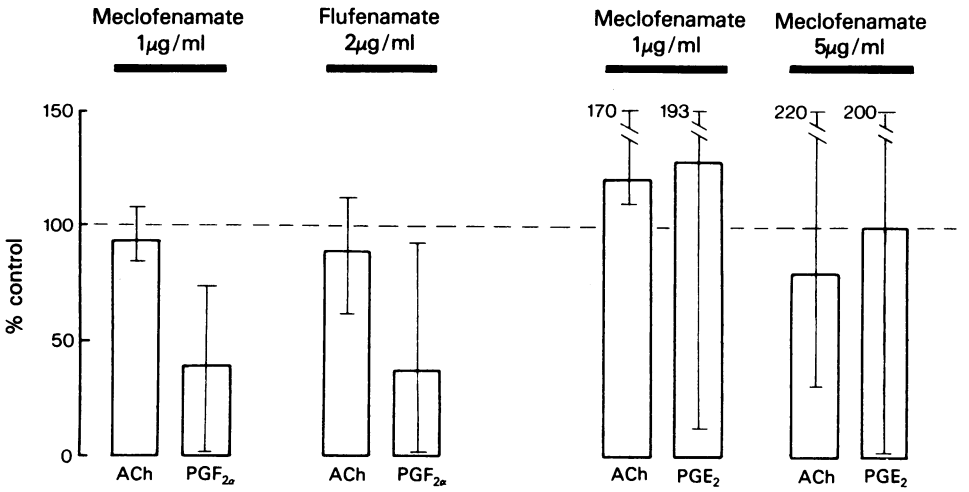

Figure 2 Effects of meclofenamate or flufenamate on prostaglandin-induced contractions in human gastrointestinal longitudinal muscle. Results are expressed as a percentage of control; the columns represent medians and the bars semiquartile ranges. Compared to acetylcholine (ACh), contractions to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were selectively antagonized with 1 μg/ml meclofenamate ($P = 0.05$; $n = 9$), and were reduced with 2 μg/ml flufenamate ($n = 5$). Meclofenamate 1 or 5 μg/ml did not selectively affect PGE_2-induced contractions ($n = 6$ and 7 respectively; $P > 0.05$ for each).

not seem to be any regional differences in the ability of these drugs to reduce prostaglandin-stimulated contractions, so for each drug the results of all the tissues are combined; antagonism with the lowest concentrations of fenamate was determined after at least 40 min contact time.

Sodium meclofenamate Sodium meclofenamate 1 μg/ml reduced longitudinal muscle contractions to $PGF_{2\alpha}$ (dose-ratio approx. 10, $n = 3$; Figure 2), and 2, 5 or 20 μg/ml meclofenamate prevented contractions to the standard concentration of $PGF_{2\alpha}$ (dose-ratios >10 to >50, $n = 4$). Contractions to ACh or PGE_2 were often increased with 1 or 5 μg/ml meclofenamate, probably due at least partly to the fall in muscle tone (Figure 2).

Sodium flufenamate Sodium flufenamate 2 μg/ml reduced contractions to $PGF_{2\alpha}$ (dose-ratio >10, $n = 4$), with little effect on those to ACh (Figure 2). Contractions to PGE_2 were not selectively blocked with 5, 10 or 50 μg/ml flufenamate (3, 2 and 2 experiments respectively).

Sodium mefenamate and mefenamic acid Sodium mefenamate 20 or 50 μg/ml often increased contractions to ACh or $PGF_{2\alpha}$ (probably due to the fall in muscle tone), but tended to reduce contractions to PGE_2 (Table 3).

Mefenamic acid, 50 or 100 μg/ml, produced results similar to those with sodium mefenamate on responses to ACh, $PGF_{2\alpha}$ or PGE_2. By combining these results with the sodium mefenamate data, the probability of a selective reduction of contractions to PGE_2 was increased (Table 3).

Discussion

The reduction by fenamates of tone and spontaneous activity of human stomach, ileum and colonic longitudinal muscle is similar to the effect of indomethacin on the intestine (Bennett & Stockley, 1977; Burleigh, 1977). Thus, endogenous prostanoid synthesis may contribute to the maintenance of muscle tone. However, in the longitudinal muscle of guinea-pig ileum the stimulation of activity by sodium meclofenamate contrasts with reduction produced by other inhibitors of prostaglandin synthesis (Davison, Ramwell & Willis, 1972; Botting & Salzmann, 1974), and in circular muscle strips the reduction of tone contrasts with a tendency for indomethacin to increase spontaneous activity (Bennett, Eley & Stockley, 1975, and unpublished). The reasons for these differences are not clear.

The selective block of $PGF_{2\alpha}$-induced contractions of human gastrointestinal longitudinal muscle by sodium meclofenamate or flufenamate occurred with concentrations (1 and 2 μg/ml respectively) within therapeutic plasma levels. Glazko (1967) reported that in normal subjects a single oral dose of 200 mg flu-

fenamic acid produced a blood level of approximately 10 μg/ml, but he did not state if this represented 'free' or albumin-bound drug. In contrast, mefenamic acid or its sodium salt did not selectively affect $PGF_{2\alpha}$-induced contractions, and the tendency for high concentrations (50 μg/ml) to reduce contractions to PGE_2 seems therapeutically unimportant since this amount exceeds the maximum plasma concentration of 'free' drug (approximately 10 μg/ml) following a single oral dose of 1 g mefenamic acid (Glazko, 1967).

In human gut, the differential effect of fenamates on PGE_2- and $PGF_{2\alpha}$-induced contractions may indicate different types of prostaglandin receptor. This contrasts with the blockade by meclofenamate of contractions to PGD_2, PGE_2 or $PGF_{2\alpha}$ in the longitudinal muscle of guinea-pig ileum. The finding that meclofenamate poorly antagonizes PGD_2 or $PGF_{2\alpha}$ in guinea-pig colonic circular muscle is consistent with the suggestion that in this tissue PGD_2 and $PGF_{2\alpha}$ act on similar receptors (Bennett & Sanger, 1978).

In other experiments on longitudinal intestinal muscle, high concentrations of flufenamic or mefenamic acids (40 μg/ml) reduced contractions of guinea-pig ileum to PGE_1 or $PGF_{2\alpha}$ more than those to ACh (Famaey, Fontaine & Reuse, 1977), and 3 to 25 μg/ml mefenamic acid antagonized contractions of gerbil colon to PGE_1 (Tolman & Partridge, 1975). Sodium meclofenamate, flufenamate or mefenamic acid also antagonized rat gastric acid secretion induced by $PGF_{2\alpha}$, but did not affect PGE_2-induced inhibition of gastric acid secretion (Karpaanen & Puurunen, 1976).

In non-gastrointestinal tissues, the fenamates show varying abilities to block prostaglandins. Meclofenamate or flufenamate antagonized $PGF_{2\alpha}$-induced contractions of human isolated bronchial muscle (Collier & Sweatman, 1968), but relaxations to PGE_1 or PGE_2 were not affected. Meclofenamate, flufenamate or mefenamate exerted a similar effect in guinea-pig tracheal muscle (Panczenko, Grodzinska & Gryglewski, 1975; Lo & Karim, 1977). In various blood vessels, some fenamates inhibited certain prostaglandin-induced contractions (Levy & Lindner, 1971; Burka & Eyre, 1974) or potentiation of noradrenaline-induced vasoconstriction (Bennett, Carroll & Sanger, 1979). By contrast, meclofenamate did not consistently antagonize $PGF_{2\alpha}$-induced contractions of rat, rabbit or human myometrium (Levy & Lindner, 1971; Vane & Williams, 1973; Sanger & Bennett, 1979), although sodium mefenamate, flufenamate or meclofenamate selectively antagonized contractions of human isolated myometrium to an epoxymethano analogue of PGH_2 (Sanger & Bennett, 1979).

Antagonism by meclofenamate or flufenamate of contractions to $PGF_{2\alpha}$ in the human gut may help to explain some therapeutic effects. High $PGF_{2\alpha}$ plasma concentrations are associated with dysmenorrhoea (Pickles, Hall, Best & Smith, 1965) and prostaglandins might reach the gut through the blood stream or by local diffusion. Since prostaglandin synthesis is not entirely inhibited by therapeutic doses of aspirin-like drugs (Samuelsson, 1973), blockade by fenamates of the action of $PGF_{2\alpha}$ in the gut could contribute to the reduction of gastrointestinal disturbances by these drugs in dysmenorrhoea (see Sanger & Bennett, 1979). The fenamates may therefore also be of value in treating other gastrointestinal disturbances involving prostaglandins.

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Table 3 Effect of sodium mefenamate and mefenamic acid on prostaglandin-induced contractions of human gastrointestinal longitudinal muscle

Concentration of fenamate (μg/ml)	*ACh*	*PGE_2*	P	*ACh*	*$PGF_{2\alpha}$*	P
Sodium mefenamate						
20	100(64-116)	78(64-94)	0.76 $n = 6$	113(62-137)	133(70-227)	— $n = 5$
50	106(33-148)	47(21-117)	0.17 $n = 6$	83(36-173)	58(5-137)	— $n = 5$
Sodium mefenamate or mefenamic acid (combined data)						
50	102(63-118)	27(21-93)	0.07 $n = 10$	62(49-118)	70(12-210)	0.23 $n = 8$
50 + 100	70(27-110)	21(0-67)	0.05 $n = 16$	61(17-113)	45(7-173)	0.31 $n = 11$

Results are expressed as a median percentage of control, with semiquartile ranges in parentheses, and the effect on each prostaglandin is compared with that on acetylcholine (ACh) (Wilcoxon).

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