Studies on prostaglandin antagonists

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Summary

1. Three prostaglandin antagonists have been examined for their ability to block PGE_2 and $PGF_{2\alpha}$ on human, guinea-pig and isolated rat gastrointestinal muscle.

2. 7-*oxa*-13-Prostynoic acid was either a non-selective antagonist, or was ineffective on the tissues studied; it had marked spasmogenic activity on the rat fundus.

3. 1-Acetyl-2-(8-chloro-10,11-dihydrodibenz (b,f)(1,4) oxazepine-10-carbonyl) hydrazine selectively antagonized the excitatory effects of PGE_2 and $PGF_{2\alpha}$ in guinea-pig and rat tissues, but not in human muscle.

4. Polyphloretin phosphate selectively antagonized the excitatory effects of prostaglandins in both human and guinea-pig muscle preparations, but it caused stimulation of the rat fundus.

5. All the antagonists lowered the tone in many tissues. They also reduced contractions caused by potassium.

6. None of the compounds blocked the inhibitory effect of PGE_2 on intestinal circular muscle.

7. The implication of these results on the nature of prostaglandin receptors, and the value of each compound as a prostaglandin antagonist are discussed.

Introduction

A prostaglandin antagonist would be a valuable tool in determining the involvement of prostaglandins in physiological and pathological processes, and it might also be of therapeutic value. Fried, Santhanakrishnan, Himizu, Lin, Ford, Rubin & Grigas (1969) synthesized several compounds, structurally related to the prostaglandins which had various degrees of antagonist activity against PGE_1 on the longitudinal muscle of guinea-pig ileum, rabbit ileum and jird colon. The substance 7-*oxa*-13-prostynoic acid (compound 19396, Fig. 1) was reported to be relatively specific in these tissues, but Flack (1970) considered this to be true only in the jird colon. Sanner (1969) showed that the substance 1-acetyl-2-(8-chloro-10,11-dihydrodibenz (b,f)(1,4) oxazepine-10-carbonyl) hydrazine (SC-19220, Fig. 1) specifically inhibited the contractions produced by PGE_2 on guinea-pig ileum, but tests on other tissues or with other prostaglandins have not been reported. Eakins, Karim & Miller (1970) found that polyphloretin phosphate (PPP) antagonized PGE_2- and $PGF_{2\alpha}$-induced contractions of the isolated longitudinal muscle of the jird colon, rabbit jejunum and rabbit uterus; the responses to several other agonists were unaffected. Lastly, fenamates were found to be of potential value as prostaglandin antagonists since

they inhibited $PGF_{2\alpha}$ on human isolated bronchial muscle (Collier & Sweatman, 1968). We report studies of the activities and selectivities of compound 19396, SC-19220 and PPP against PGE_2 and $PGF_{2\alpha}$ on human, guinea-pig and rat isolated gastrointestinal smooth muscle.

Methods

Human gut muscle was obtained from operation specimens resected for a variety of diseases. The material was obtained immediately after surgery and was studied the same day, or on the following day after storage at 4° C. The mucosa and sub-mucosa were removed and strips of muscle 2–3 cm long and 2–3 mm wide were cut parallel to the longitudinal or circular layers. Smooth muscle preparations from guinea-pigs and rats were obtained after the animals were stunned and bled. Whole segments of intestine 2–3 cm long were used to study the longitudinal muscle, whereas the circular muscle preparations (guinea-pig colon 6–15 cm from the anus) were obtained by cutting a shallow spiral. Strips of rat fundus were prepared according to Vane (1957). The preparations were suspended under a load of 0·5–1 g in Krebs solution at 37° C, bubbled with 5% CO_2 in O_2. The movements of the muscle were recorded on a kymograph by an isotonic frontal writing lever with a magnification of 8–12. Because the antagonists often caused a reduction in tone, the relaxant effects of the prostaglandins were usually assessed by the inhibi-
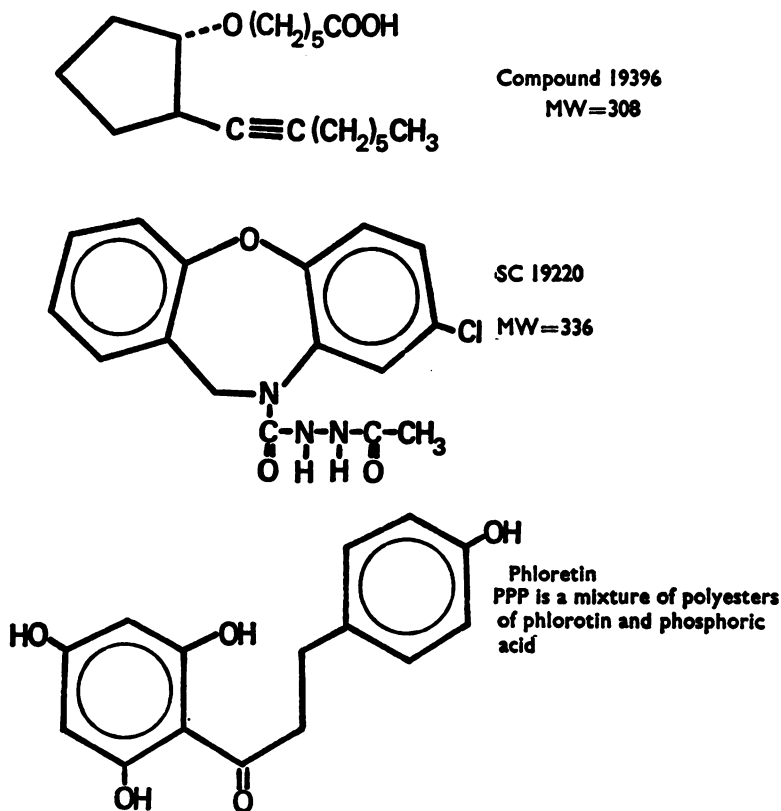

FIG. 1. Structures of the prostaglandin antagonists used.

tion of responses to potassium chloride (10^{-2} to 3×10^{-2}M) or to acetylcholine. The specificity of prostaglandin block was determined by measuring the effect on contractions produced by a constant dose of acetylcholine or, sometimes, of other agonists.

Drugs

The drugs used were 1-acetyl-2-(8-chloro-10,11-dihydrodibenz (b,f)(1,4) oxazepine-10-carbonyl) hydrazine (SC-19220), acetylcholine chloride, histamine hydrogen tartrate, 5-hydroxytryptamine creatinine sulphate (5-HT), nicotine hydrogen tartrate, 7-*oxa*-13-prostynoic acid (compound 19396), polyphloretin phosphate (PPP), potassium chloride and prostaglandins E_2 and $F_{2\alpha}$ (PGE$_2$ and PGF$_{2\alpha}$).

SC-19220 was dissolved either in propylene glycol (1 mg/ml) or ethyl alcohol (10 mg/ml). The prostaglandins and compound 19396 were dissolved in ethyl alcohol (10 mg/ml) and diluted to 1 mg/ml with 0·9% w/v NaCl containing sodium carbonate (0·2 mg/ml). PPP was dissolved in water (100 mg/ml) and the pH adjusted to 7·0 with sodium carbonate. All further dilutions were made with Krebs solution. PPP is a mixture of polymers, and its bath concentration is expressed in μg/ml. All other concentrations are molar.

Results

The selectivities of compound 19396, SC-19220 and PPP in blocking the responses of various isolated smooth muscle preparations to PGE$_2$ and PGF$_{2\alpha}$ have been examined. For each region of the human gastrointestinal tract studied, two to five experiments were made with each antagonist against PGE$_2$ and PGF$_{2\alpha}$ on different specimens. Similarly, three to nine experiments were made on guinea-pig and rat tissues. The responses to prostaglandins in most of these tissues (Table 1) have been described previously (Bennett, Eley & Scholes, 1968; Bennett, Murray & Wyllie, 1968; Fleshler & Bennett, 1969; Bennett, 1970; Bennett & Fleshler, 1969, 1970). There have been no previous reports of the effects of PGE$_2$ and PGF$_{2\alpha}$ on the human ascending colon, and the responses of human stomach muscle to PGF$_{2\alpha}$ have been referred to only as unpublished data (Bennett & Fleshler, 1970). Since the longitudinal muscle of the gut is usually contracted by PGE$_2$, whereas the circular muscle is inhibited (Table 1), both layers of the gut have been examined. The results with each antagonist are recorded in separate sections, which are subdivided according to each species studied. A summary of the results is presented in Table 2.

TABLE 1. *Responses of the gastrointestinal preparations used to PGE$_2$ and PGF$_2\alpha$, together with approximate bath concentrations and contact times*

Muscle layer	Species	Prostaglandin	Bath concentration ($\times 10^{-8}$M)	Contact time (min)	Response
Longitudinal	Man	E$_2$	5–50	2–5	↑
		F$_{2\alpha}$	5–50	2–5	↑
	Guinea-pig	E$_2$	0·5–5	1–3	↑
		F$_{2\alpha}$	5–50		↑
	Rat	E$_2$	0·1–1	1·5–2	↑
		F$_{2\alpha}$	1–10	1·5–2	↑
Circular	Man	E$_2$	5–100	2–8	↓
		F$_{2\alpha}$	10–100	2–5	↓ ↑ *
	Guinea-pig	E$_2$	5–50	2–5	↓
		F$_{2\alpha}$	10–100	2–5	↑

↑, Contraction; ↓, inhibition. *Some strips of ascending colon are relaxed by PGF$_{2\alpha}$.

Compound 19396

This substance did not block prostaglandins selectively in any tissue. It produced dose dependent contractions of the rat stomach.

Human (longitudinal muscle of gastric body and terminal ileum ; circular muscle of gastric body and sigmoid colon). Compound 19396 (at least $5·5 \times 10^{-5}$M in each experiment, and up to 3×10^{-4}M in some instances) either had little or no effect on responses to prostaglandins, or at the higher concentrations it depressed the responses to acetylcholine as well as to prostaglandins (Fig. 2). Compound 19396 usually reduced the tone of intestinal preparations but increased the tone of gastric circular muscle strips, whilst that of longitudinal gastric muscle was virtually unaffected.

Guinea-pig (longitudinal muscle of ileum ; circular colonic muscle). Compound 19396 (2 to 8×10^{-5}M) inhibited contractions of the longitudinal muscle of the ileum to acetylcholine, nicotine, histamine and PGE_2. The colonic circular muscle which, in contrast to the longitudinal muscle of the ileum, has a resting tone, was relaxed

TABLE 2. *The selectivity of compound* 19396, SC-19220 *and PPP in antagonizing contractions produced by* PGE_2 *and* $PGF_{2\alpha}$ *in different tissues*

Muscle layer	Prostaglandin	Species	Tissue	19396	SC-19220	PPP
Longitudinal	E_2 and $F_{2\alpha}$	Man	Gastric body	×	×	√
			Ileum	×	×	
			Sigmoid			
			Taenia Coli	×	×	√
		Guinea-pig	Ileum	×	√	√
			Colon			
		Rat	Fundus	Stimulates	√	Stimulates
Circular	$F_{2\alpha}$	Man	Gastric body	×	×	√
			Ascending colon			√
			Sigmoid colon		×	√
		Guinea-pig	Colon	×	√	√

The inhibitory effects of PGE_2 on circular muscle (not shown) were not antagonized by any drug. ×, Not selective; √, selective.
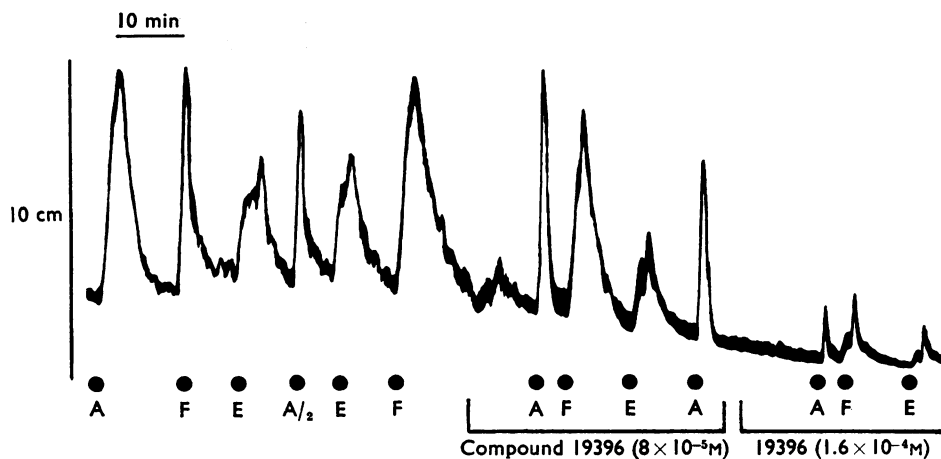

FIG. 2. Contractions of longitudinal muscle from human terminal ileum. Compound 19396 depressed responses to PGE_2, 5×10^{-7}M, (E), $PGF_{2\alpha}$, 7×10^{-8}M, (F) and acetylcholine, 10^{-6}M, (A).

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by compound 19396 (2 to 8×10^{-5}M). The highest concentration of antagonist (8×10^{-5}M) reduced the contractions produced by acetylcholine, $PGF_{2\alpha}$ and potassium. The effects on tone and the responses to agonists were partly due to the ethanol used in preparing the solution. A slight degree of selectivity was seen at lower concentrations (compound 19396, 2 to 4×10^{-5}M): in three of four experiments the responses to $PGF_{2\alpha}$ were reduced by 20–50% without change in the response to acetylcholine. There was no selective blockade of the inhibitory effects of PGE_2 on maintained submaximal contractions to KCl.

Rat (stomach strip). Compound 19396 (3×10^{-6} to 8×10^{-5}M) caused dose-dependent contractions of the rat fundus (Fig. 3). When the compound was kept in contact with the tissue (up to 30 min) the contraction was maintained. Neither PGE_2 nor $PGF_{2\alpha}$ was antagonized when added in the presence of a submaximal stimulating concentration of compound 19396 or after its removal from the bath when the tissue had relaxed (Fig. 3).

Compound SC-19220

This substance specifically antagonized the excitatory effects of PGE_2 and $PGF_{2\alpha}$ in guinea-pig and rat tissues, but not in human tissues. It did not antagonize the inhibitory effects of PGE_2.

Human (longitudinal muscle of gastric body, terminal ileum and sigmoid colon ; circular muscle of gastric body). SC-19220 ($7 \cdot 5 \times 10^{-6}$M to $1 \cdot 5 \times 10^{-4}$M) either had little or no effect on contractions produced by PGE_2 and $PGF_{2\alpha}$, or it reduced the responses to both acetylcholine and the prostaglandins ; this non-selective inhibition increased with the dose. In only one experiment (longitudinal ileum, SC-19220
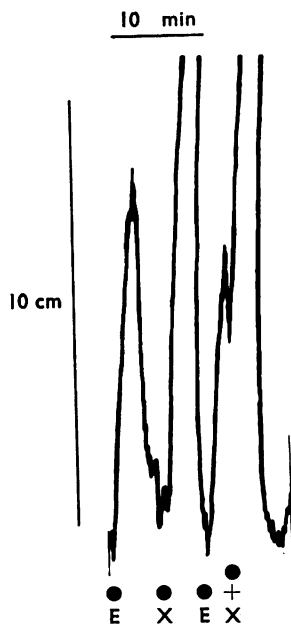

FIG. 3. Contractions of rat fundus produced by PGE_2, $1 \cdot 5 \times 10^{-9}$M (E), and compound 19396, 3×10^{-5}M (X). When compound 19396 was washed out of the bath and the tissues had relaxed, PGE_2 still produced a large contraction. The + sign shows that the last addition of compound 19396 was made while prostaglandin E_2 was kept in the bath.

7.5×10^{-5}M) was there any evidence of a preferential block ; the effect of PGE$_2$ was almost abolished, whereas responses to acetylcholine and PGF$_{2\alpha}$ were reduced by about 60%. SC-19220 reduced the tone of the muscle strips, and with large amounts of the antagonist the solvent propylene glycol used in preparing the solution added to this lowering of tone and to the general depression of responses to agonists.

Guinea-pig (longitudinal muscle of the ileum ; circular colonic muscle). Cumulative log dose-response curves to PGE$_2$ and to PGF$_{2\alpha}$ on the longitudinal muscle of the ileum were shifted to the right in parallel by SC-19220 5×10^{-6} to 10^{-5}M ; Fig. 4). With concentrations of antagonist up to 10^{-5}M responses to acetylcholine and the maximum contractions to the prostaglandins were constant. Straight lines were obtained for plots of $-$log concentrations of SC-19220 against log (dose ratio-1), and the pA$_2$ of SC-19220 against PGE$_2$ and PGF$_{2\alpha}$ was 5·6 and 5·5, respectively. When the drug was washed out of the bath after being in contact with the tissue for 15 min, responses to prostaglandins returned to normal in 10 minutes. A longer exposure to the antagonist prolonged this reversal.

The contractions of the colonic circular muscle to PGF$_{2\alpha}$ were inhibited by SC-19220 (10^{-5}M). Responses to acetylcholine were unaltered or, possibly because SC-19220 lowered the tone of the muscle, were even larger. The degree of antagonism was therefore probably greater than calculated, since reduction of the tone with a lower concentration of SC-19220 (5×10^{-6}M) increased the response to PGF$_{2\alpha}$. The antagonist also reduced the contractions to KCl, so that up to twice the amount of the salt was required to elicit contractions equal to control responses. SC-19220 did not reduce the inhibition of KCl responses by PGE$_2$.

Rat (stomach strip). The tone of rat fundus preparations was reduced by SC-19220 in a dose-dependent manner (Fig. 5). SC-19220 (5×10^{-5}M) also blocked the responses to PGE$_2$ and PGF$_{2\alpha}$ and the cumulative log dose-response curves were shifted to the right in parallel. The contractions produced by acetylcholine were increased, presumably because tone was reduced, and again the degree of prostaglandin antagonism was probably greater than apparent. The tone of the fundus could be greatly reduced by frequent and regular dosing with prostaglandin and by increasing the load. Under these conditions SC-19220 did not further reduce the tone and responses to prostaglandins were inhibited with no alteration of responses to acetylcholine.
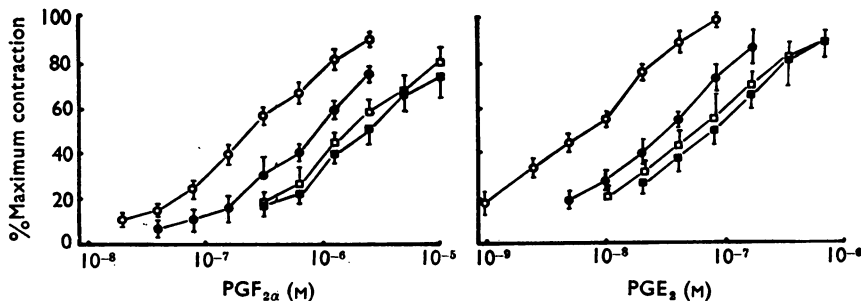

FIG. 4. Cumulative dose-response curves for PGF$_{2\alpha}$ (left hand graph) and PGE$_2$ (right hand graph) in the presence of different concentrations of SC-19220. $\bigcirc$, $\bullet$, $\square$ and $\blacksquare$ represent 0, 5×10^{-6}M, 7.5×10^{-6}M and 10^{-5}M SC-19220, respectively. The vertical bars show the standard errors of the mean.

Polyphloretin phosphate (PPP)

PPP antagonized the excitatory effects of prostaglandins on most of the tissues studied, but caused stimulation of the rat fundus. High concentrations were required to block the effects of prostaglandins on human gastrointestinal muscle. In general, there was no antagonism of inhibitory responses to PGE_2.

Human (longitudinal and circular muscle of gastric body and sigmoid colon ; circular muscle of ascending colon). The tone and spontaneous activity of all tissues were reduced gradually over a period of approximately 2 h by PPP in concentrations of 100–1,200 μg/ml, and in some cases by as little as 25 μg/ml. At the lower concentrations the contractions to PGE_2 and $PGF_{2\alpha}$ were often increased, probably because of the reduction of tone. However, at 600 μg/ml, PPP inhibited the prostaglandins without affecting responses to acetylcholine, and up to 20-fold increases in the concentration of the prostaglandins produced only small or moderate responses. Only with the highest concentration of PPP used (1,200 μg/ml) were the responses to acetylcholine slightly reduced. As with the effect on tone, the blockade took up to 2 h to equilibrate ; a similar period was required to reverse these effects after washing out the drug.

The inhibitory responses of the circular muscle of gastric body and of sigmoid colon to PGE_2 (assessed by the reduction of submaximal contractions to constant doses of acetylcholine), were not blocked by PPP (250–1,200 μg/ml). Indeed, it was sometimes found that at the highest concentration of PPP the PGE_2 inhibition of acetylcholine contractions was greatly prolonged (tested up to 40 min).

As seen in Table 1, the responses of the circular muscle of the ascending colon to $PGF_{2\alpha}$ were variable, and unlike almost all other gastrointestinal muscle studied so
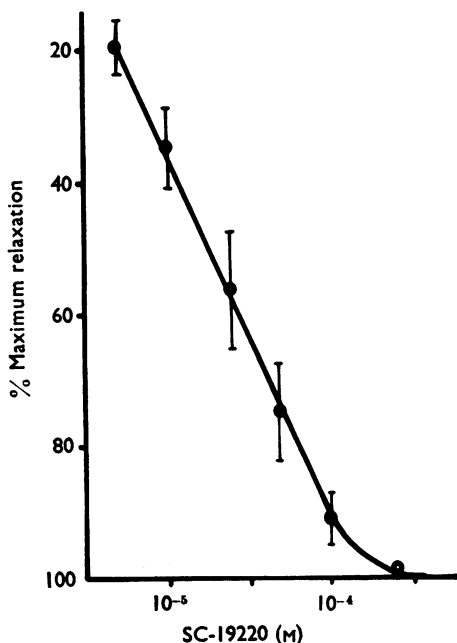

Fig. 5. The reduction of tone of the rat fundus by SC-19220. The vertical bars show the standard errors of the mean.

far, inhibitory responses to $PGF_{2\alpha}$ sometimes occurred. In contrast to PGE_2, the inhibitory responses to $PGF_{2\alpha}$ were prevented in each case by PPP (250 $\mu g/ml$; Fig. 6).

Guinea-pig (longitudinal and circular colonic muscle). The results were qualitatively similar to those obtained with the human tissues. Tone was generally reduced, but in a few instances this was preceded by a small increase. The contractile responses to the prostaglandins were inhibited by PPP (50–300 $\mu g/ml$ for longitudinal muscle, and 200–300 $\mu g/ml$ for circular muscle), whereas responses to acetylcholine were either unaffected or increased when the tone was lowered. As with compound 19396 and SC-19220, the responses to KCl were usually reduced. In only two of seven experiments was there any indication of an antagonism of the inhibitory effects of PGE_2.

Rat (stomach strip). PPP (20–100 $\mu g/ml$) caused small dose-dependent contractions of the rat fundus which were maintained when the drug was kept in contact with the tissue. However, the maximal responses obtained with PPP were small compared with those to prostaglandins or 5-HT. Contractions to prostaglandins or 5-HT superimposed on the PPP-induced contractions were either unaffected or,
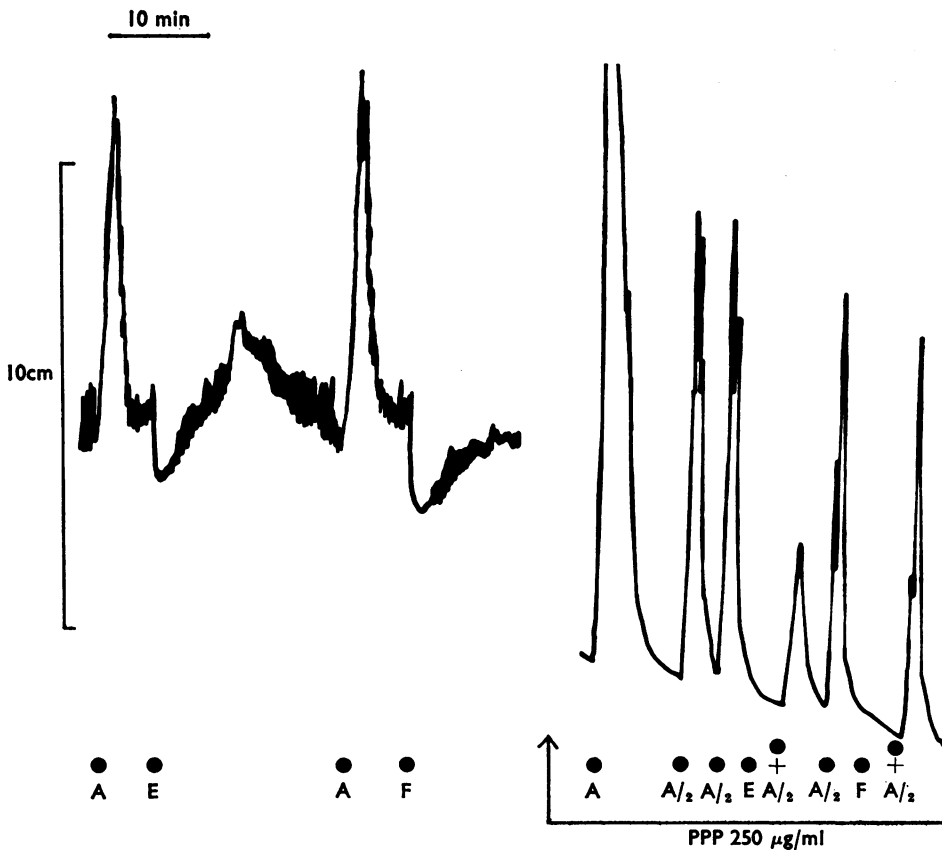

FIG. 6. The inhibitory effects of PGE_2, $10^{-6}M$ (E), and $PGF_{2\alpha}$, $7 \times 10^{-7}M$ (F), on the circular muscle of human ascending colon. PPP (250 $\mu g/ml$) lowered the tone so that the inhibitory effect of PGE_2 was demonstrated by the reduction of responses to acetylcholine $5 \times 10^{-7}M$ (A). The inhibitory effect of $PGF_{2\alpha}$ only was blocked. The + sign shows that the addition of acetylcholine was made while the preceding dose of prostaglandin was kept in the bath.

perhaps because of the increased tone, were slightly reduced. Higher concentrations of PPP (200–2,000 μg/ml) lowered the tone of the rat fundus and reduced the effects of 5-HT as well as PGE_2 and $PGF_{2\alpha}$.

Discussion

The three compounds tested for their activities as prostaglandin antagonists had varying effects on different tissues. Where a block did occur, this could not have been caused by chemical interaction with prostaglandins since there were several tissues in which no antagonism was observed. Absence of antagonism (for example with SC-19220 and compound 19396 on human tissues) might be attributable to insufficient drug concentrations. Larger amounts could not be tested because of the general depressant effects of the organic solvents. This emphasizes the need for more water soluble and more potent antagonists.

The experiments with compound 19396 are essentially an extension of the studies of Fried *et al.* (1969) and those of Flack (1970). Some of the tissues used in our studies were different, and we studied PGE_2 and $PGF_{2\alpha}$ instead of PGE_1. In addition to finding that compound 19396 is a non-selective antagonist in many animal tissues (jird colon is the only known exception ; Flack 1970), it appears to be almost ineffective on human gut muscle. The ability of the compound to excite rat fundus and the circular muscle from the body of the human stomach disagrees with the conclusions of Fried *et al.* (1969) that only analogues with a C15-OH group have agonist activity.

Compound SC-19220 and PPP, on the other hand, were fairly selective antagonists for PGE_2 and $PGF_{2\alpha}$ in a variety of tissues. These results agree with and extend the findings of Sanner (1969), Eakins *et al.* (1970) and Eakins (personal communication). The competitive nature of the antagonism of PGE_2 and $PGF_{2\alpha}$ with SC-19220 in guinea-pig ileum and in rat fundus is suggested by the shift of the log dose-response curves in parallel to the right. Furthermore, the data from guinea-pig ileum produce straight lines on plotting $-$log concentration of SC-19220 against log (dose ratio$-$1). Arunlakshana & Schild (1959) pointed out that if two agonists act on the same receptors they can be expected to be antagonized by the same antagonist. If the blockade is competitive, the same concentration of the antagonist will be effective in each case. Similar dose ratios were obtained at the 50% maximal response for both prostaglandins with each concentration of SC-19220, and almost identical pA_2 values were produced. It is therefore possible that in the longitudinal muscle of the guinea-pig ileum, PGE_2 and $PGF_{2\alpha}$ act at a common receptor site.

By contrast, in circular muscle where PGE_2 causes relaxation and $PGF_{2\alpha}$ produces contraction, the receptor sites for the two prostaglandins must be different. Furthermore, the inhibitory receptors for PGE_2 in circular muscle appear to be different from the excitatory receptors for PGE_2 in the longitudinal muscle, since only the latter are susceptible to block by SC-19220 and PPP. Lastly, with regard to differentiation of receptors, the inhibitory effect of $PGF_{2\alpha}$ in the circular muscle of human ascending colon appears to involve a different site from that at which PGE_2 produces relaxations since only $PGF_{2\alpha}$ is blocked by PPP.

All three compounds had two properties in common. First, they reduced the contractions to KCl in concentrations which did not inhibit the effect of acetylcholine. The reason for this is not apparent, but it is interesting that there have been

several reports that the responsiveness of tissues to prostaglandins are altered by changes in K^+ concentration. Thus the inhibitory effect of prostaglandins on human isolated myometrium is potentiated by a reduction in the extracellular K^+ concentration (Bygdeman & Eliasson, 1963), and the response of rat fundus to PGE_1 is greater when the concentration of potassium in the bathing fluid is increased (Coceani & Wolfe, 1966). Second, the antagonists lowered the tone of most tissues. One possible explanation of this phenomenon is that prostaglandins might contribute to the maintenance of tone within some smooth muscles. Such a view is consistent with the correlation between prostaglandin release and the degree of tone in bovine isolated sphincter pupillae (Posner, 1970). However, in some gut preparations the tone was lowered without any reduction in response to prostaglandins. Furthermore, both PGE_2 and $PGF_{2\alpha}$ may inhibit human colonic circular muscle, so that if these substances are released intrinsically their antagonism would be expected to raise the tone.

The variation in potency of the antagonists in different tissues is interesting but poses several problems. At the simplest level, the right antagonist must be chosen for each tissue, and compounds intended for clinical application should be screened on human material, at least until a suitable animal model is found. Although it is clear that none of the substances used in this study is a universally satisfactory prostaglandin antagonist, for certain experimental purposes both SC-19220 and PPP are useful drugs.

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