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PINANE THROMBOXANE A_2 ANALOGUES ARE NON-SELECTIVE PROSTANOID ANTAGONISTS IN RAT AND HUMAN STOMACH MUSCLE

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1 Pinane thromboxane A_2 ($PTxA_2$) and its epi-OH isomer were studied on rat and human stomach longitudinal muscle.

2 $PTxA_2$ (0.5μg/ml) usually caused a slight contraction of rat gastric fundus. Contractions to PGE_2, $PGF_{2\alpha}$, PGI_2 and expoxymethano analogues of PGH_2 (U-46619 and U-44069) were substantially inhibited, whereas those to PGD_2 and acetylcholine were only slightly reduced.

3 In human stomach, $PTxA_2$ 0.5 μg/ml rarely stimulated the muscle. Contractions to PGE_2, $PGF_{2\alpha}$ and U-46619 were antagonized, with little effect on those to acetylcholine.

4 epi-$PTxA_2$ (0.5 μg/ml) did not affect rat gastric tone. It was moderately potent against PGI_2 on rat gastric fundus, but was less effective than $PTxA_2$ against U-44069.

Introduction

The pinane analogue of thromboxane A_2 ($PTxA_2$; Figure 1) antagonizes vasoconstriction caused by azo or epoxymethano analogues of prostaglandin H_2 (PGH_2), but its potency may vary with the blood vessel used (Nicolaou, Magolda, Aharony, Smith & Lefer, 1979; Aharony, Smith, Lefer, Magolda & Nicolaou, 1980; Ansell, Caton, Palfreyman, Stuttle, Tuffin & Walker, 1980). This finding is consistent with the expectation that $PTxA_2$ might act on thromboxane receptors, since the analogues of PGH_2 mimic the action of thromboxane A_2 (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980). Evidence of selectivity was provided by the failure of the analogue to antagonize inhibition of platelet aggregation caused by PGD_2 or PGI_2, in contrast to $PTxA_2$'s antagonism of platelet aggregation caused by PGH_2 analogues (Nicolaou *et al.*, 1979; Aharony *et al.*, 1980).

We have now tested $PTxA_2$ and its epi-OH stereoisomer (epi-$PTxA_2$; Figure 1) as prostanoid antagonists in longitudinal muscle strips of rat and human stomach. All the prostanoids tested contract these tissues, except for PGI_2 which relaxes the longitudinal muscle of human stomach (see Bennett, Jarosik, Sanger & Wilson, 1980; Bennett & Sanger, 1980; Bennett, Hensby, Sanger & Stamford, 1981). The results show that although the pinane thromboxane analogues antagonize substances thought to be thromboxane mimetics, they also block responses to other prostanoids.

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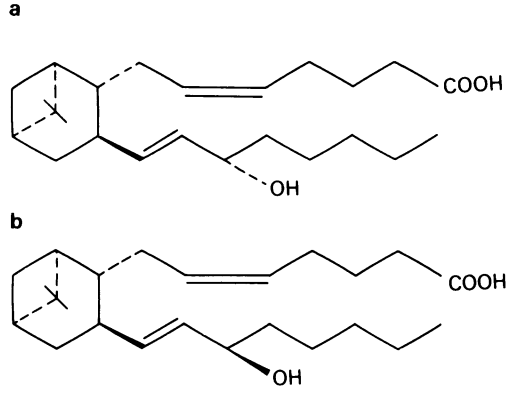

Figure 1 Structures of the pinane thromboxane A_2 ($PTxA_2$) isomers. (a) $PTxA_2$; (b) epi-$PTxA_2$, which are respectively 7((1S, 2R, 3R, 5S)-(3-(3S, 3-hydroxyoct-*trans*-1-enyl)-6,6-dimethyl-bicyclo (3.1.1) hept-2-yl) hept-5-*cis*-enoic acid, and 7((1S, 2R, 3R, 5S)-(3-(3R, 3-hydroxyoct-*trans*-1-enyl)-6,6-dimethylbicyclo (3.1.1) hept-2-yl) hept-5-*cis*-enoic acid.

Methods

Rat stomach

Adult Wistar rats of either sex were stunned and bled. Strips of gastric fundus approximately 2 cm long and 3 mm wide were cut parallel to the longitudinal muscle fibres, one from each side of the greater curvature. These were suspended under a 1 g load in 10 ml tissue baths containing Krebs solution (NaCl 7.1, $CaCl_2$ $6H_2O$ 0.55, KH_2PO_4 0.16, KCl 0.35,

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$MgSO_4$ $7H_2O$ 0.29, $NaHCO_3$ 2.1 and dextrose 1.0 g l^{-1}). 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, using magnifications of 8–16.

In each experiment, cumulative dose-response curves were obtained to one prostanoid and to acetylcholine (ACh), with 2 min intervals between each cumulative addition. A $PTxA_2$ isomer was then added to the bathing solution, and at 10 min intervals submaximal contractions to a single dose of ACh were obtained (30 s contact; pinane analogue replaced after each washout). After approximately 1 h, a time chosen arbitrarily to allow equilibration of the analogue with the tissue so that the effect would be near-maximal during each cumulative dose-response determination, responses to each agonist were redetermined. Measurements were made of the maximum response and of the concentration of agonist required to give a contraction 50% of maximum (EC_{50}).

Human stomach

Macroscopically normal specimens of human stomach, at least 6 cm from any lesion, were obtained at operation for benign or malignant disease. The mucosa and submucosa were cut away, and strips 2 to 3 cm long and 4 to 5 mm wide were cut parallel to the longitudinal muscle fibres. 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.

Human stomach strips were suspended in tissue baths, as described for the rat stomach. After obtaining rough dose-response curves to ACh and to one prostanoid, doses were chosen which gave approximately equal consistent submaximal responses for each substance. Contact times were 30 s; cycle times were 10 min except when repeated washing for up to 30 min was necessary to restore resting tone after a dose of prostanoid. $PTxA_2$ was then added to the bath and consistent responses again obtained to ACh, and the prostanoid was then tested. The interval between adding $PTxA_2$ and the prostanoid was 30–40 min. Contractions or relaxations were expressed as a percentage of pre-$PTxA_2$ controls (mean of two or three preceding responses). In addition, the degree of block was assessed where possible by determining the increase in the dose of agonist needed to restore the response (dose-ratio).

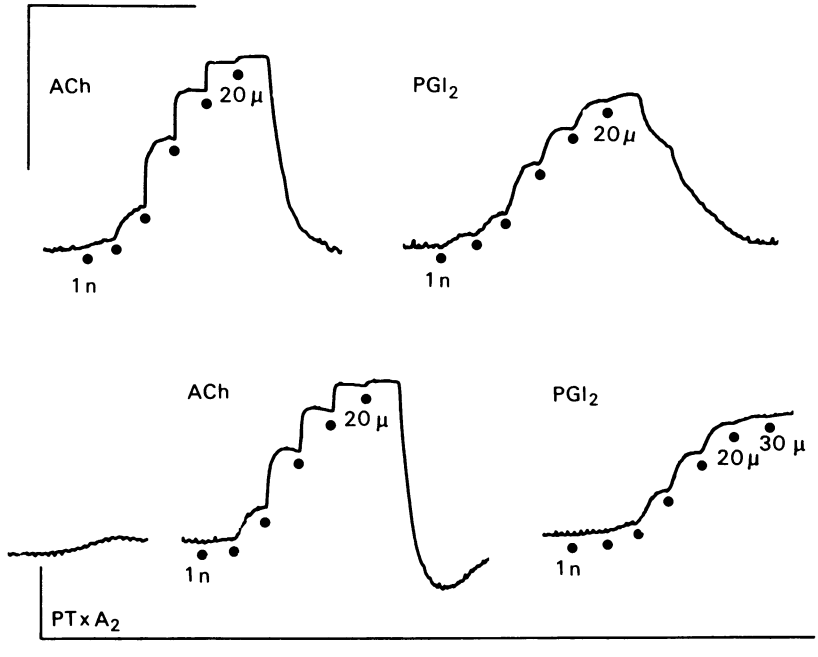

Figure 2 Cumulative dose-responses of rat gastric fundus to acetylcholine (ACh) and prostacyclin (PGI_2) alone (top traces) or in the presence of pinane thromboxane A_2 ($PTxA_2$) 0.5 μg ml^{-1} (bottom traces). The concentrations of agonists were increased 10 fold at each dot, unless shown otherwise; n and μ represent ng and μg ml^{-1} bathing fluid. $PTxA_2$ caused a slight contraction of rat gastric fundus. It reduced the response to PGI_2 but had little effect on ACh. Horizontal bar 5 min; vertical bar 5 cm.

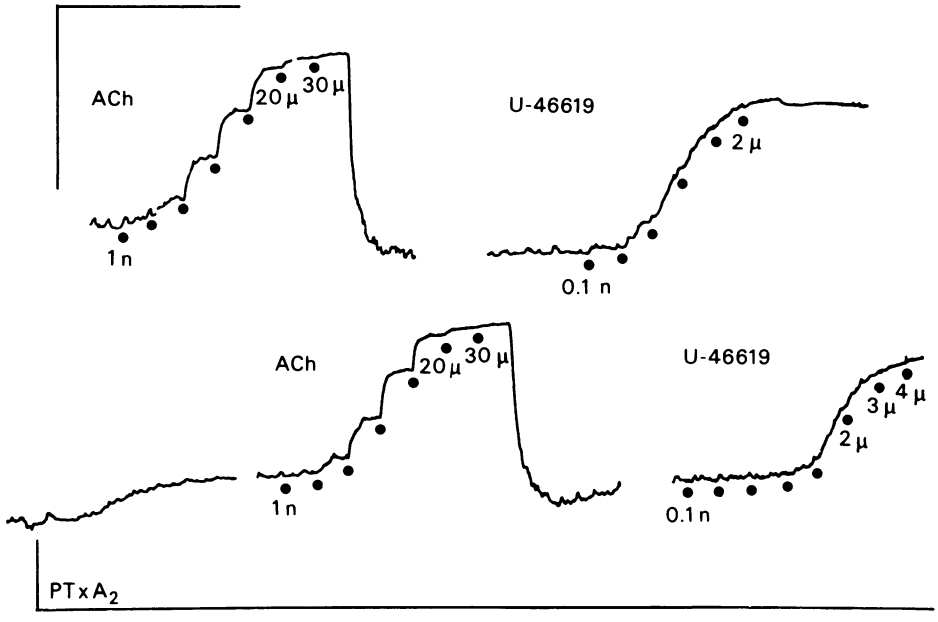

Figure 3 Cumulative dose-responses of rat gastric fundus to acetylcholine (ACh) and U-46619 alone (top traces) or in the presence of pinane tromboxane A_2 ($PTxA_2$) 0.5 $\mu g\,ml^{-1}$ (bottom traces). The concentrations of agonists were increased 10 fold at each dot, unless shown otherwise; n and μ represent ng and $\mu g\,ml^{-1}$ bathing fluid. $PTxA_2$ caused a slight contraction of rat gastric fundus. It reduced the response to U-46619 but had little effect on ACh. Horizontal bar 5 min; vertical bar 5 cm.

Drugs

The following were used: acetylcholine perchlorate, PGD_2, PGE_2, $PGF_{2\alpha}$ tromethamine salt, sodium PGI_2, (15S)-hydroxy-9α, 11α and (15S)-hydroxy-11α, 9α (epoxymethano) prosta- 5Z, 13E-dienoic acids (U-44069 and U-46619 respectively), $PTxA_2$ and epi-$PTxA_2$ (May and Baker Ltd, see Figure 1). All concentrations refer to the acid or salt listed above.

Sodium PGI_2 was dissolved in 1M Tris buffer (5 mg PGI_2 ml^{-1}) and freshly diluted further with 50 mM Tris buffer adjusted to pH 7.9 with 1M HCl. U-44069 and U-46619 were dissolved in ethanol (10 mg ml^{-1}), diluted to 0.1 mg ml^{-1} with 0.9% w/v NaCl solution (saline) and then further diluted with Krebs solution. Other prostanoids were dissolved in ethanol (5 or 10 mg ml^{-1}) and diluted with saline. $PTxA_2$ isomers were dissolved in ethanol (5 mg ml^{-1}) and diluted with saline. Acetylcholine was dissolved in saline.

Vehicle controls had little or no effect on muscle tone, except that those for high concentrations of PGI_2 usually caused weak contraction of rat stomach. Results are given as medians with ranges or semi-quartile ranges in parentheses, and analysed using the Wilcoxon matched-pairs test or the Mann-Whitney U-test.

Results

Rat stomach

The concentrations of $PTxA_2$ chosen for these experiments (0.5$\mu g\,ml^{-1}$) was reported by Nicolaou *et al.* (1979) to be well below the concentration required for inhibition of thromboxane synthesis in washed rabbit platelets (3.7–37 $\mu g\,ml^{-1}$). $PTxA_2$ 0.5 $\mu g\,ml^{-1}$ usually caused a weak contraction of rat stomach muscle which was slow in onset but sustained while $PTxA_2$ was present (Figures 2 and 3). In two experiments, incubation for at least 90 min with hyoscine, mepyramine, methysergide, phenoxybenzamine, and pronethalol (0.2, 0.2, 0.1, 0.5 and 1 $\mu g\,ml^{-1}$ respectively) slightly reduced the muscle tone but did not greatly affect the contraction to $PTxA_2$(0.5 $\mu g\,ml^{-1}$ for 10 min).

ACh, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, U-46619 and U-44069 each caused muscle contraction. Their respective median EC_{50} values were 39, 270, 1.1, 21, 19, 9.6 and 16 ng/ml, in broad agreement with our earlier findings (Bennett *et al.*, 1980).

In the presence of $PTxA_2$ (0.5 $\mu g\,ml^{-1}$) the maximum contraction to ACh or the prostanoids was less, probably because of the additional contraction to $PTxA_2$, since the overall maximum muscle shortening was unaffected. Contractions to PGE_2, $PGF_{2\alpha}$,

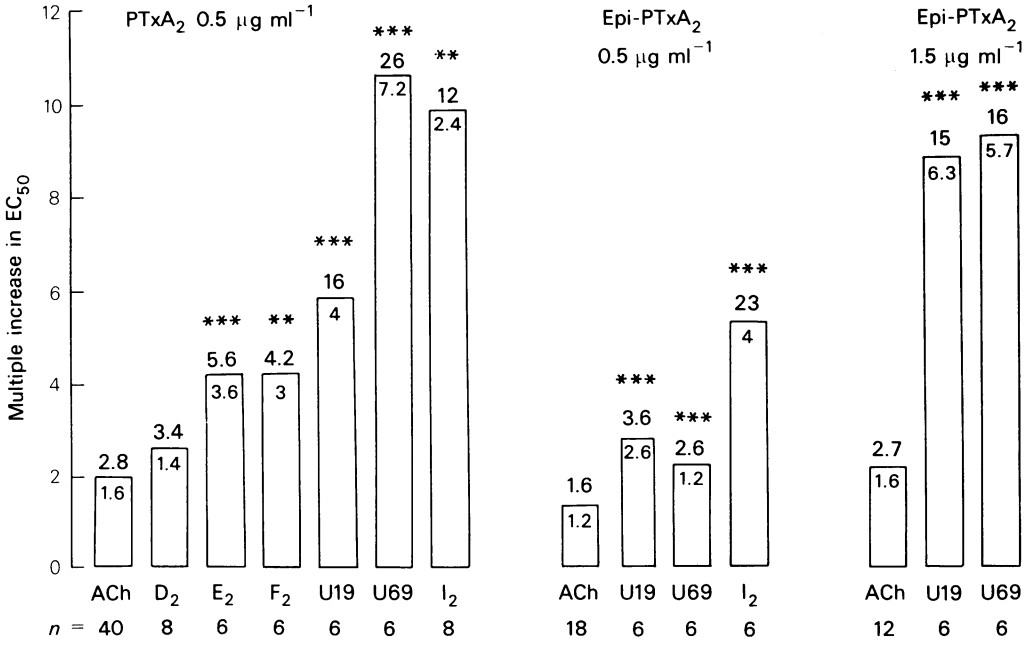

Figure 4 Antagonism by pinane thromboxane A_2 ($PTxA_2$) or epi-$PTxA_2$ of submaximal contractions to prostanoids in rat isolated stomach muscle. Results are expressed as the multiple increased in EC_{50} after addition of a pinane isomer, and are given as columns representing medians, with semiquartile ranges shown as numbers above and below the medians, D_2, E_2, F_2, I_2, U19 and U69 are prostaglandins D_2, E_2, $F_{2\alpha}$, U-46619 and U-44069 respectively. The effect of the isomers on each prostanoid are compared with that on acetylcholine (ACh); $^{**}P<0.05$; $^{***}P<0.01$; n = number of experiments.

PGI_2 and the epoxymethano analogues of PGH_2 were inhibited with $PTxA_2$, as shown for PGI_2 and U-46619 in Figures 2 and 3, and by the increase in EC_{50} values (Figure 4). In contrast, contractions to PGD_2 were not reduced more than the small extent seen with ACh (Figure 4).

Epi-$PTxA_2$ (0.5 or 1.5 $\mu g\,ml^{-1}$) did not affect the tone of rat isolated gastric fundus. The only prostanoids tested were PGI_2 and the epoxymethano analogues of PGH_2, since they were most susceptible to $PTxA_2$. Contractions to them were antagonized by epi-$PTxA_2$ more than were contractions to ACh (Figure 2). Overall, the epi-isomer was less effective than $PTxA_2$, but this was statistically significant ($P<0.05$) only with ACh and U-44069. Raising the concentration of epi-$PTxA_2$ to 1.5 $\mu g\,ml^{-1}$ increased the antagonism to the PGH_2 analogues and, to some extent, of ACh (Figure 4).

Human stomach

The effects of $PTxA_2$ were studied on 10 longitudinal stomach muscle strips, taken from 4 patients (3 with cancer and one with persistent prepyloric ulcer; 3 female, 1 male; 62–67 years). $PTxA_2$ 0.5 $\mu g\,ml^{-1}$ usually had no effect on muscle tone, except for a slow increase in 2 out of 4 strips from one patient.

$PTxA_2$ 0.5 $\mu g\,ml^{-1}$ had little effect on submaximal contractions to ACh (1 to 20 $\mu g\,ml^{-1}$), but somewhat reduced contractions to PGE_2 (0.5 or 1 $\mu g\,ml^{-1}$), $PGF_{2\alpha}$ (1 or 5 $\mu g\,ml^{-1}$) or U-46619 (0.01 to 1 $\mu g\,ml^{-1}$); relaxations to PGI_2 (1 or 2 $\mu g\,ml^{-1}$) were not greatly altered. Figure 5 shows the results for ACh, U-46619 and $PGF_{2\alpha}$, and Table 1 shows all the results. The effect of $PTxA_2$ on contractions to PGD_2 was not studied, since the longitudinal muscle of human isolated stomach usually contracts only weakly to this prostanoid (Bennett *et al.*, 1981). No studies were made with epi-$PTxA_2$.

Discussion

Nicolaou *et al.* (1979) and Aharony *et al.* (1980) found that $PTxA_2$ potently antagonized constriction of cat coronary artery and aggregation of human platelets induced by PGH_2 analogues. Since the drug did not affect the inhibition of platelet aggregation by PGD_2 or PGI_2, this might reflect the finding in smooth muscle that antagonists of prostaglandin excitatory effects do not block inhibitory effects (Bennett, 1974; Sanner & Eakins, 1976). The potency of

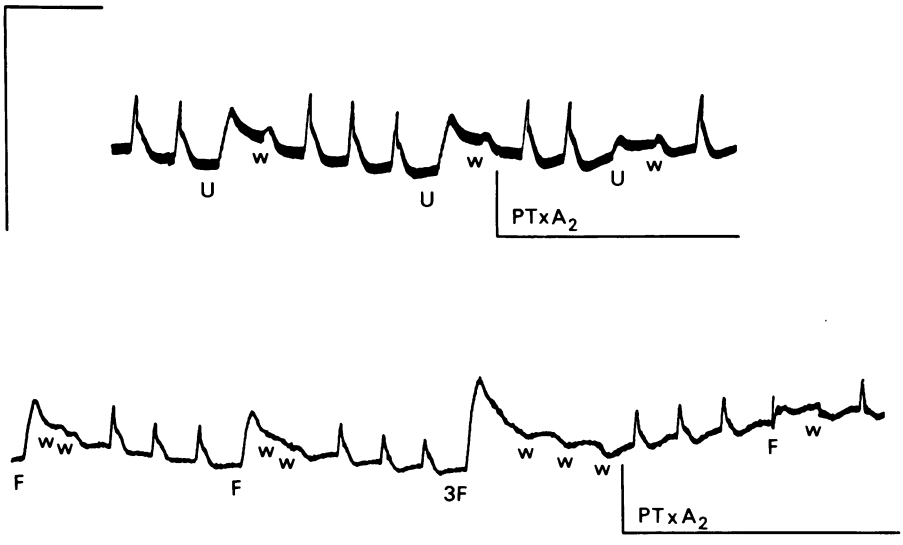

Figure 5 Responses of human gastric longitudinal muscle. Top trace U-46619 (U, 0.1 $\mu g\,ml^{-1}$). Bottom trace prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, F, 5 $\mu g\,ml^{-1}$), Both traces, unlabelled contractions are ACh 10 $\mu g\,ml^{-1}$; w = drug washed from bath. $PTxA_2$ 0.5 $\mu g\,ml^{-1}$ reduced responses to U-46619 and $PGF_{2\alpha}$, with little effect on those to acetylcholine. Horizontal bar 30 min; vertical bar 5 cm.

$PTxA_2$ and its epi-isomer may vary with the vascular muscle or the agonist used. Ansell *et al.* (1980) obtained a slight stimulation of rabbit aorta and mesenteric artery, and a weak antagonism of the TxA_2-like material released from guinea-pig lung. In these arterial tissues both thromboxane analogues were partial agonists, but we obtained weak excitation only with $PTxA_2$, whereas both isomers inhibited contractions to various prostanoids.

Epoxymethano analogues of PGH_2 are thought to act on thromboxane receptors (Coleman *et al.*, 1980), and $PTxA_2$ 0.5 $\mu g\,ml^{-1}$ did antagonize submaximal contractions of rat stomach to these analogues. However, the effect was not selective, since contractions to PGE_2, $PGF_{2\alpha}$, PGI_2 and, to a lesser extent ACh, were also reduced. The findings with the longitudinal muscle of human stomach were similar to those with rat stomach, except that PGI_2 relaxes the human tissue and this response seemed unaffected by $PTxA_2$.

Other drugs besides $PTxA_2$ have been reported to antagonize aggregation of blood platelets with arachidonic acid, PGH_2 or analogues of PGH_2, but so far there are no data on antagonism of other prostanoids which cause excitatory responses in other tissues. These antagonists include monocylic prostaglandin endoperoxides (Menzel, Roycroft, Nixon, Isaac & Porter, 1977), 13-aza-prostanoic acid (Le Breton, Venton, Enke & Halushka, 1979), carbocyclic TxA_2 (Lefer, Smith, Araki, Smith, Aharony, Claremon, Magolda & Nicolaou, 1980) and 7-oxabicyclo (2.2.1) heptane prostaglandin analogues (Sprague, Heikes, Harris & Greenberg, 1980). Of these compounds, only 13-aza-prostanoic acid was tested against PGE_1- or PGI_2-induced inhibition of platelet aggregation, where it was ineffective as an

Table 1 Effects of pinane thromboxane A_2 on the reaction of human stomach to acetylcholine (ACh) and prostanoids

	Response	*% control*	n	*Dose-ratio*
ACh	Contraction	93(56–110)	8	–
PGE_2	Contraction	74(70–78)	2	4
$PGF_{2\alpha}$	Contraction	34(31–36)	2	>10
U-46619	Contraction	32(25–63)	4	>10
PGI_2	Relaxation	93(86–100)	2	–

$PTxA_2$ (0.5 $\mu g\,ml^{-1}$) had little effect on submaximal contraction to ACh or relaxation to PGI_2, but inhibited contractions to the other prostanoids. % control shows medians with the ranges in parentheses. n = number of experiments. The dose-ratios are results of single experiments.

antagonist (Le Breton *et al.*, 1979).

Nicolaou *et al.* (1979) found that epi-$PTxA_2$ was less potent than $PTxA_2$ as an antagonist of platelet aggregation. We obtained a similar potency relationship in rat stomach but, unlike $PTxA_2$, the epi-compound had the advantage of not causing contraction, perhaps due to the unnatural 15-hydroxy configuration (Fried, Santhanakrishnan, Himizu, Lin, Ford, Rubin & Grigas, 1969; Tolman, Partridge & Barris, 1977; Birnbaum & Tolman, 1979). Since the block of contractions to PGI_2 in rat stomach by both pinane isomers was substantial, and evidence with the prostaglandin antagonist SC-19220 suggests that PGI_2 and PGH_2 analogues act at different receptors in rat fundus (Bennett *et al.*, 1980), neither $PTxA_2$ nor epi-$PTxA_2$ are selective thromboxane antagonists. However, $PTxA_2$ and epi-$PTxA_2$ do not block responses to all excitatory prostanoids, as shown by the weak effect on PGD_2.

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