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Stimulation and inhibition of prostacyclin formation in the gastric mucosa and ileum *in vitro* by anti-inflammatory agents

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1 Homogenates of rat gastric mucosa, forestomach and ileum and dog gastric mucosa reproducibly generated prostacyclin from endogenous substrate.

2 Prostacyclin formation was inhibited by pre-incubation with indomethacin, flurbiprofen, naproxen, ketoprofen or meclofenamate (0.1–10 μM).

3 BW755C (3-amino-1[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) stimulated prostacyclin production in the rat gastric mucosa and ileum with inhibition occurring only at high concentrations (> 200 μM). The stimulation of prostacyclin production by BW755C in rat forestomach homogenates was less pronounced, with inhibition at concentrations > 20 μM.

4 BW755C thus exhibits differential activity on prostacyclin production from different gastric tissues *in vitro*.

5 The antioxidant-lipoxygenase inhibitor, nordihydroguiaretic acid (NDGA, 3–15 μM) likewise augmented rat mucosal prostacyclin formation.

6 Paracetamol stimulated and, at higher concentrations, inhibited prostacyclin formation (> 1 mM), and had comparable activity in both rat gastric tissues.

7 The ability of NDGA and BW755C to enhance prostacyclin generation may reflect the removal of a modulating influence of lipoxygenase products on prostacyclin formation, the diversion of substrate to the cyclo-oxygenase pathway, or free-radical scavenging.

Introduction

The inhibition of cyclo-oxygenase (Vane, 1971) in the gastric mucosa is an important mechanism underlying gastric damage induced by non-steroid anti-inflammatory agents (Main & Whittle, 1974). In the development of novel anti-inflammatory drugs, it is therefore important to determine their actions on gastric mucosal cyclo-oxygenase activity. In doses that reduce prostaglandin levels in inflammatory exudates, aspirin, indomethacin, naproxen or flurbiprofen administered by the oral or parenteral route reduce prostacyclin formation in the gastric mucosa and lead to gastric erosions (Whittle, Higgs, Eakins, Moncada & Vane, 1980). In contrast, the recently described dual cyclo-oxygenase-lipoxygenase inhibitor, BW755C (3-amino-1[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline; Higgs, Flower & Vane, 1979) which reduced prostaglandin levels in inflammatory exudates, failed to inhibit gastric and ileal prostacyclin formation and to cause gastrointestinal damage (Whittle *et al.*, 1980; Whittle, 1981a). We have now compared the actions of BW755C with other non-steroid anti-inflammatory agents on prostacyclin production from endogenous substrate by gastrointestinal tissue *in vitro*.

A preliminary account of this work was presented to the British Pharmacological Society (Boughton-Smith & Whittle, 1981).

Methods

Generation of prostacyclin from endogenous substrate

(a) *Preparation of gastric mucosal, forestomach and ileal homogenates* Male Wistar rats (200–300 g body weight) were killed by cervical dislocation, the stomachs removed and opened along the greater curvature. After flushing out any contents, the

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stomachs were rinsed in ice-cold 50 mM Tris buffer (pH 8.4 at 0°C). The gastric mucosa was separated from its underlying muscle layer and stored on ice. The muscular forestomach tissue was cut away from the body of the stomach along the limiting ridge. Segments of the acid-secreting fundic area from gastric mucosa rinsed free of debris were also taken from pentobarbitone anaesthetized beagles of either sex (8–12 kg) and the mucosal layer cut away from underlying muscle using fine scissors. Segments of upper ileum (3–6 cm from the caecum) from the rat were freed from adherent mesentery and rinsed in ice cold Tris buffer. The tissues were weighed, chopped with scissors and homogenized for 2 min in ice-cold Tris buffer, using a Silverson 'Verso' blender (half maximum speed) to give a 20% w/v tissue preparation (0.2 g wet tissue ml^{-1}).

It should be noted that in some previous studies on prostaglandin formation by the rat stomach, the term 'fundus' was applied to the muscular forestomach region. Since in other species, the term 'fundus' is used by gastroenterologists to denote the acid-secreting area of the mucosa, the term forestomach is used in this paper.

(b) *Generation and bioassay of prostacyclin from mucosal homogenates* Aliquots of the stock tissue preparation (0.5 ml) were diluted with Tris buffer (0.5 ml) containing $CaCl_2$ (5 mM) or Ca^{2+} Tris containing the drug under study, incubated for 10 min at 22°C and then centrifuged for 20 s in an Eppendorf bench centrifuge (max speed 10,000 *g*). The tissue preparation was washed by resuspending in Ca^{2+} Tris (1 ml) or drug solution and after centrifugation, the tissue was resuspended in Ca^{2+} Tris or drug solution (0.5 ml). The suspension was then vortexed for 30 s and finally centrifuged at 10,000 *g* for 30 s and the supernatant was collected on ice for prostacyclin assay.

Prostacyclin is a potent inhibitor of platelet aggregation (Moncada, Gryglewski, Bunting & Vane, 1976), and this property has been utilized for its bioassay (Whittle, 1981b). Aliquots of the supernatant (5–50 μl) were immediately tested for their ability to inhibit platelet aggregation and assayed against authentic prostacyclin. Dose-response curves to authentic prostacyclin were determined at regular intervals throughout the 2 h assay period used in these studies. In addition, a control and test sample of homogenate were always incubated and assayed simultaneously to minimize variation and the prostacyclin levels expressed as a percentage of the paired control.

Aggregation of human platelets (0.5 ml of citrated human platelet-rich-plasma) was induced by ADP (4–6 μM) in doses sufficient to cause full aggregation, and monitored in a Payton dual-channel aggregometer. The identity of the platelet-inhibitory activity as prostacyclin was confirmed by several tests. The activity, like prostacyclin could inhibit platelet aggregation in plasma from several species and was destroyed by incubation at 37°C for 15 min at pH 7.4, or by a 30 s incubation at pH 3. The anti-aggregating activity was also abolished by a 1 min pre-incubation of the extract with a specific antiserum which binds and hence inactivates prostacyclin. None of the non-steroid anti-inflammatory drugs, nordihydroguiaretic acid (NDGA) or Ca^{2+}-Tris in the concentrations transferred to the assay cuvette, had any action on the control aggregation to ADP or on the anti-aggregating activity of prostacyclin (0.1–1 ng ml^{-1}) in human platelet-rich plasma (PRP), when tested routinely during the assay.

Confirmatory experiments were kindly carried out by Dr J.A. Salmon (Prostaglandin Research, W.R.L.) using radioimmunoassay to determine the levels of 6-oxo-$PGF_{1\alpha}$ (the stable hydrolysis product of prostacyclin) and prostaglandin E_2 (PGE_2) in the rat gastric homogenate supernatants. Initial experiments indicated that the levels of each prostanoid, assayed either directly following dilution of the sample, or following extraction (Salmon, 1978) were comparable, and thus in subsequent assays the extraction was omitted. The cross-reactivities of the antibodies used, which were raised in rabbits, are given elsewhere (Salmon, 1978). The sensitivities of the assays for 6-oxo-$PGF_{1\alpha}$ and PGE_2 were 25 pg and 10 pg respectively, the intra-assay coefficients of variation of the assays were 13% and 10% respectively, while the accuracy of the assay (adding known quantities of prostanoids) for PGE_2 was comparable to that reported for 6-oxo-$PGF_{1\alpha}$ (Salmon, 1978).

Materials

Acetylsalicylic acid, paracetamol (Wellcome Research Laboratories), indomethacin (Sigma Chemical Co.), naproxen (Syntex), flurbiprofen (Boots Ltd), ketoprofen (May & Baker Ltd) were dissolved in 5% $NaHCO_3$ (w/v) and diluted with 50 mM Tris buffer. Sodium meclofenamate (Parke-Davis) and sodium salicylate (BDH) were dissolved in distilled water. Nordihydroguiaretic acid (Sigma) was dissolved in ethanol and diluted with Tris buffer. BW755C (3-amino-1[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) as the hydrochloride (synthesized by Drs F.C. Copp and C.V. Denyer of the Chemical Research Laboratories at the Wellcome Research Laboratories, Beckenham) was dissolved in distilled water. Prostacyclin, as the sodium salt was dissolved freshly in 1M Tris buffer (pH 9.6 at 4°C) and diluted when required with 50 mM Tris buffer (pH 8.4 at 4°C).

Statistical analysis

Prostacyclin generation, measured by bioassay, was calculated as a percentage of its paired control and shown as the mean ± s.e.mean of (n) values. Significance of the difference between the means was assessed by Student's t test for paired data. $P < 0.05$ was taken as significant.

Results

Formation of prostacyclin from endogenous substrate

Homogenates of rat gastric mucosa, rat forestomach and dog gastric mucosa generated, from endogenous substrate, an anti-aggregating activity which was characterized as prostacyclin. The amount of prostacyclin generated by the resuspending-vortexing technique in homogenates of rat forestomach (361 ± 35 ng/g of tissue, from 5 preparations) was greater ($P < 0.001$) than that formed by the gastric mucosal homogenates from rat (69 ± 8 ng/g, from 10 preparations) and dog (60 ± 7 ng/g, from 4 preparations) and from rat ileum (80 ± 7 ng/g, $n = 8$).

The levels of prostacyclin formed by the crude mucosal homogenate are similar to those formed by chopped rat gastric mucosal strips (99 ± 8 ng/g tissue, $n = 30$) and also by a 'whole-cell' preparation from minced tissue (70 ± 10 ng/g tissue, $n = 5$) following similar vortex-incubation procedures.

The resuspension procedure following pre-incubation was found sufficient to remove the substances having pro-aggregating activities which would interfere with the platelet bioassay. The levels of prostacyclin detected in the final supernatant were generated during the final resuspension and 30 s vortex procedure; supernatants from non-resuspended, non-vortexed homogenate tissue contained only low levels of prostacyclin-like activity. Although resuspension of the tissue provided sufficient agitation to initiate prostacyclin formation, the 30 s vortex procedure was used to ensure a uniform stimulus. Preliminary experiments indicated that greater levels of prostacyclin-like activity were generated in calcium-containing Tris buffer and therefore buffer containing 5 mM Ca^{2+} was used routinely. The calculated limits of error ($P = 0.05$) for prostacyclin production in control samples were ±9.5% for rat mucosa, ±11.2% for forestomach and ±12.4% for ileal homogenates.

To investigate whether the level of prostaglandins detected in the final supernatant could reflect the bound or transferred prostaglandins which had been formed during the original tissue preparation, [^{3}H]-PGI_2 (0.5 μCi; 10 Ci mmol^{-1}) or [^{3}H]-PGE_2 (0.5 μCi, 160 Ci mmol^{-1}-) was incubated with the rat gastric mucosal homogenate for 10 min before the resuspension procedure. The final supernatant contained only 1.9 ± 0.1% and 1.6 ± 0.1% ($n = 4$) of the total added radioactivity from [^{3}H]-PGI_2 and [^{3}H]-PGE_2 respectively, while 0.8 ± 0.07% and 0.4 ± 0.04% of the radioactivity was associated with the residual tissue. Pre-incubation (10 min) of the homogenate with indomethacin (10 μM) did not significantly alter the levels of radioactivity in the final supernatant, but did reduce the formation of prostacyclin. These data suggest that the levels of prostacyclin detected by bioassay and radioimmunoassay (RIA) are formed

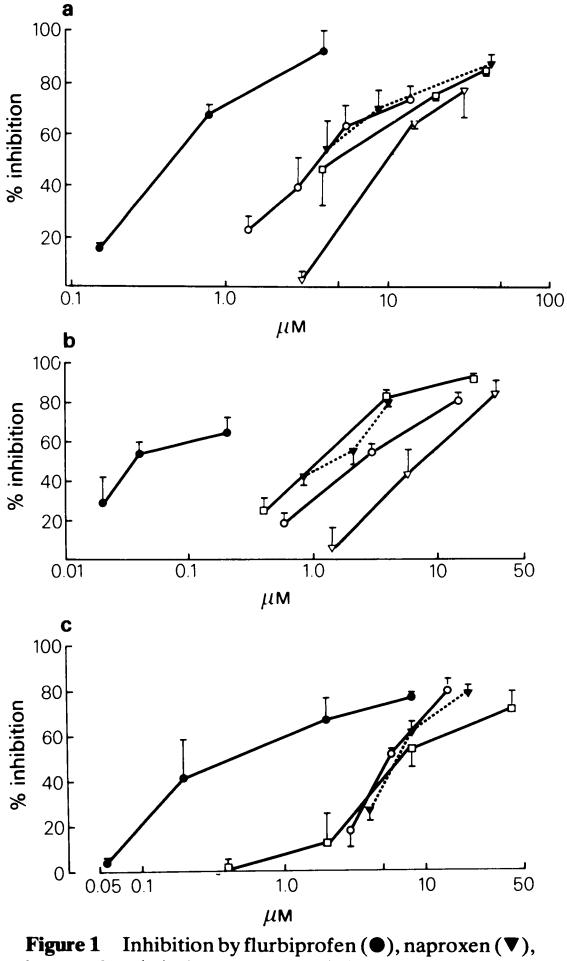

Figure 1 Inhibition by flurbiprofen (●), naproxen (▼), ketoprofen (□), indomethacin (○) or meclofenamate (▽), of prostacyclin formation from endogenous substrate in homogenates of (a) rat gastric mucosa, (b) rat forestomach and (c) rat ileum. Results, expressed as the % inhibition from control values following 10 min pre-incubation (at 22°C) with each anti-inflammatory agent are the mean of 5–10 experiments for each value; s.e.means indicated by vertical lines.

during the final resuspension-vortex procedure and not the consequence of the release of bound material.

Using RIA techniques (Salmon, 1978) to assay prostanoids in the supernatant from rat gastric mucosal homogenates, the levels of 6-oxo-$PGF_{1\alpha}$ (the hydrolysis product of prostacyclin) were 48 ± 8 ng/g of tissue and of PGE_2, 13 ± 1 ng/g of tissue ($n=12$).

Actions of anti-inflammatory compounds on the formation of prostacyclin from gastric tissue

(a) *Effects of non-steroid anti-inflammatory agents* Incubation (10 min at room temperature) with either flurbiprofen (0.2–2 μM), naproxen, ketoprofen, indomethacin or meclofenamate (1–50 μM) caused a dose-dependent reduction in the levels of prostacyclin generated from the rat gastric mucosal homogenates (Figure 1). The ID_{50} values (doses causing 50% inhibition) are shown in Table 1. Increasing the incubation period from 10 to 30 min or incubation at 37°C did not increase the inhibitory activity of these compounds. Furthermore, with only a 2 min period of pre-incubation, the inhibitory activity of these drugs was close to that observed following 10 min incubation. Incubation of the gastric homogenate with lower concentrations (0.01–0.5 μM) of these agents had no significant effect on prostacyclin production (3 experiments). Incubation of homogenates and dilution of the compounds with Ca^{2+}-free buffer did not alter the ID_{50} values of the anti-inflammatory agents. In the concentrations used, the weak cyclo-oxygenase inhibitor acetylsalicylic acid (3.3–330 μM) did not significantly reduce prostacyclin formation from mucosal homogenates (4 experiments). Sodium salicylate (6.25–3125 μM) likewise had no significant action on prostacyclin formation (4 experiments).

Prostacyclin formation from homogenates of rat forestomach was also dose-dependently inhibited by flurbiprofen, naproxen, ketoprofen, meclofenamate and indomethacin (Figure 1) following 10 min pre-incubation at 22°C; the ID_{50} values are shown in Table 1.

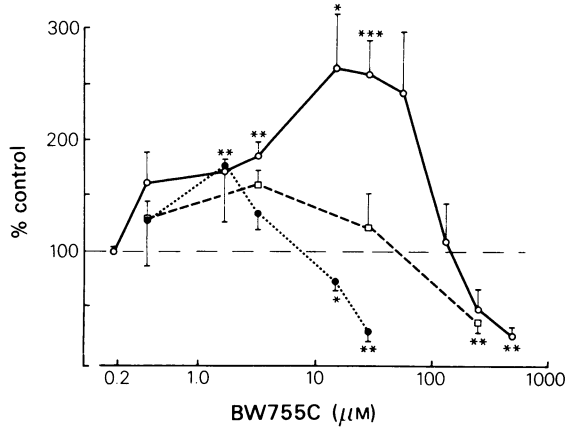

Figure 2 Stimulation and inhibition of prostacyclin formation from endogenous substrate in homogenates of rat gastric mucosa (○), forestomach (●) and ileum (□) by BW755C. Results, expressed as % of the control value following 10 min pre-incubation (22°C) with BW755C, are mean of 5–15 experiments indicated by vertical lines The control level of prostacyclin formation is given in the text. The level of statistically significant difference from controls is shown as $*P<0.05$; $**P<0.01$; $***P<0.001$.

Indomethacin likewise inhibited prostacyclin formation from endogenous substrate in dog gastric mucosal homogenates following 10 min pre-incubation at 22°C. The ID_{50} of 4.2 μM, (3 experiments) was similar to that found in the rat mucosa.

(b) *Effect of BW755C and NDGA* At low concentrations, BW755C stimulated prostacyclin formation from endogenous precursors in both rat gastric tissue

Table 1 Inhibition of prostacyclin formation from endogenous substrate in homogenates of rat gastric mucosa, forestomach and ileum by anti-inflammatory agents *in vitro*

	ID_{50}(μM)		
	Mucosa	*Ileum*	*Forestomach*
Indomethacin	3.9	5.8	2.2
Flurbiprofen	0.5	0.4	0.1
Naproxen	3.9	6.3	1.4
Ketoprofen	5.5	7.3	1.2
Meclofenamate	11.3	—	7.3
Paracetamol*	1325	>1000	1655
BW755C*	380	265	26

Results are shown as the ID_{50} values (dose causing 50% inhibition) following 10 min pre-incubation *in vitro*. Values are from at least a 3-point dose-response study in at least 3 experiments for each compound; *represents biphasic dose-response curves with stimulation of prostacyclin formation at lower concentrations.

homogenates (Figure 2). In mucosal homogenates a significant enhancement of prostacyclin formation occurred over a wide range of concentrations with the maximum enhancement being 267 ± 50% of control ($n = 6$; $P < 0.05$) with BW755C (19 μM). However, higher concentrations of BW755C (100–500 μM) significantly inhibited prostacyclin formation (ID_{50} 380 μM).

The biphasic dose-response relationship on prostacyclin formation with BW755C was also seen in homogenates of rat forestomach homogenates but was less pronounced (Figure 2). Stimulation of prostacyclin formation (178 ± 8% of control, $n = 3$; $P < 0.05$) occurred with BW755C (2 μM), but inhibition occurred at much lower concentrations (19–38 μM) than in mucosal homogenates, (ID_{50} 26 μM).

In a confirmatory experiment RIA techniques were used to determine the concentrations of prostaglandins in pooled supernatants from 3 different mucosal homogenates. Indomethacin (5.6 μM) reduced the levels of 6-oxo-$PGF_{1\alpha}$ by 51% and levels of PGE_2 by 54%. Likewise, flurbiprofen (0.8 μM) reduced 6-oxo-$PGF_{1\alpha}$ and PGE_2 concentrations by 57 and 49% respectively. Naproxen (8.7 μM) reduced 6-oxo-$PGF_{1\alpha}$ and PGE_2 concentrations by 45 and 46% respectively. In contrast, BW755C (38 μM) elevated the levels of 6-oxo-$PGF_{1\alpha}$ and PGE_2 to 370 and 194% control, respectively. With higher concentrations of BW755C (380 μM), 6-oxo-$PGF_{1\alpha}$ and PGE_2 were inhibited by 39 and 51% respectively.

NDGA (3–15 μM) significantly ($P < 0.05$) stimulated prostacyclin production in rat gastric mucosal homogenates; NDGA (3 μM) increased prostacyclin levels to 145 ± 9% of control; ($n = 4$; $P < 0.01$). At higher concentrations, NDGA (100–200 μM) significantly depressed prostacyclin levels with an ID_{50} of 150 μM ($P < 0.01$; 4 experiments).

(c) *Effects of paracetamol* A biphasic dose-response relationship for prostacyclin formation from endogenous precursor was also seen when paracetamol was incubated with rat gastric homogenates. In contrast to BW755C, the mucosal and forestomach homogenates had the same sensitivity to both the stimulatory and inhibitory effects of paracetamol (Figure 3).

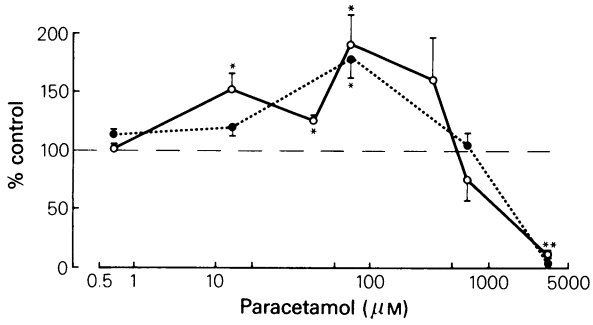

Figure 3 Stimulation and inhibition of prostacyclin formation from endogenous substrate in homogenates of rat gastric mucosa (○) and forestomach (●) by paracetamol. Results, expressed as % of the control value following 10 min pre-incubation (at 22°C) with paracetamol, are mean of 5–15 experiments for each value; s.e.means indicated by vertical lines. The control level of prostacyclin formation is given in the text. The level of statistically significant difference from control is shown as $*P < 0.05$; $**P < 0.01$.

Actions of anti-inflammatory agents in homogenates of rat ileum

A significant dose-dependent inhibition ($P < 0.05$ for each) of prostacyclin formation from endogenous substrate in homogenates of rat ileum was observed following 10 min pre-incubation at 22°C with flurbiprofen (0.2–8.0 μM), naproxen (4–20 μM) ketoprofen (5–40 μM) and indomethacin (4–12 μM), as shown in Figure 1.

BW755C (3.8 μM) significantly ($P < 0.05$) enhanced ileal prostacyclin formation (Figure 2) but inhibited at higher concentrations (100–500 μM) with an ID_{50} of 265 μM (Table 1). Paracetamol (6.6–66 μM) likewise significantly enhanced prostacyclin formation, paracetamol (66 μM) increased prostacyclin levels to 174 ± 11% of control ($n = 5$; $P < 0.01$) comparable to its effects on rat gastric tissues. Inhibition of prostacyclin formation was not observed with the highest concentration of paracetamol (1000 μM) investigated.

Discussion

Using a preparative and incubation procedure, homogenates of rat gastric mucosa, rat forestomach and ileum and dog gastric mucosa were found to generate reproducibly prostacyclin from endogenous substrate. Prostacyclin has been previously shown to be synthesized by chopped and vortexed strips of gastric mucosa of several species and from the rat ileum (Whittle, 1980; Whittle, 1981b). The higher levels of prostacyclin generated by rat forestomach homogenate may reflect differences in the levels or activation of phospholipase A_2 or other lipases, cyclo-oxygenase or prostacyclin synthetase. Interestingly, this tissue has been reported to have only low levels of phospholipase A_2 (Pace-Asciak, 1972). Using RIA, we have found the levels of 6-oxo-$PGF_{1\alpha}$ to be greater than those of PGE_2, in homogenates of both rat mucosa and forestomach, as found previously with strips of rat and rabbit gastric mucosa (Whit-

tle, 1980). Likewise, using mass spectrometry, other workers have shown 6-oxo-$PGF_{1\alpha}$ to be the predominant product following arachidonic acid incubation with both muscular forestomach and whole stomach homogenates of the rat (Pace-Asciak & Rangaraj, 1977) as also found using radiolabelled precursors (Pace-Asciak & Nashat, 1977). The formation of considerably higher levels of 6-oxo-$PGF_{1\alpha}$ than PGE_2 from radiolabelled PGH_2 in microsomal preparations of gastric mucosa, jejunum and ileum from dog has also been reported (Le Duc & Needleman, 1979). Recent studies on a whole-cell preparation of rat jejunum indicated comparable levels of 6-oxo-$PGF_{1\alpha}$ and PGE_2 formed from endogenous substrate during a 5 min incubation (Peskar, Weiler, Kroner & Peskar, 1981). The formation of PGE_2 and its metabolite as well as PGD_2 and $PGF_{2\alpha}$ from washed whole-cell preparations of rat stomach and jejunum following 30–90 min incubation has also been detected by gas chromatography-mass spectrometry techniques (Knapp, Oelz, Sweetman & Oates, 1978). In the present study, similar levels of prostacyclin were generated from the crude homogenates as from the whole-cell preparations or chopped strips of rat gastric mucosa. Although this preparation gives limited information about the overall level and activity of cyclo-oxygenase in the gastric mucosa *in vivo*, the degree of reproducibility within and between batches of this preparation does suggest that it may provide a rapid method for the *in vitro* screening of potential cyclo-oxygenase inhibitors in gastrointestinal tissue.

Indomethacin, flurbiprofen, naproxen, ketoprofen and meclofenamate were potent inhibitors of prostacyclin formation from endogenous precursor in rat gastric mucosa, forestomach and ileum. The concentration of anti-inflammatory agents that inhibited prostacyclin production (determined by bioassay) or 6-oxo-$PGF_{1\alpha}$ and PGE_2 production (determined by RIA) from endogenous precursor in the rat gastric mucosa and ileum were similar to those inhibiting cyclo-oxygenase activity in other tissue preparations (Vane, 1971; Flower, 1974). Indomethacin, in these concentrations has also been shown to inhibit prostaglandin biosynthesis *in vitro* in homogenates and microsomal preparations of human gastric mucosa (Bennett, Stamford & Unger, 1973; Peskar, 1977). However, BW755C inhibited prostacyclin production *in vitro* from gastric mucosa and ileum only at high concentrations. This contrasts with the comparable potency of BW755C to indomethacin as an inhibitor of cyclo-oxygenase *in vitro* in horse platelets (Higgs *et al.*, 1979) and rat leukocytes (Randall, Eakins, Higgs, Salmon & Tateson, 1980) and its potency in inhibiting prostacyclin generation from the forestomach. Thus, as in the previous studies *in vivo* (Whittle *et al.*, 1980), such effects may suggest a selectivity of action of BW755C against different cyclo-oxygenases *in vitro* although the mechanisms underlying such effects are unclear. The current *in vitro* studies on tissue homogenates should have minimized differences in uptake and penetration of the drugs under investigation. In a recent study using 'whole-cell' preparations of rat forestomach and gastric mucosa, BW755C was likewise demonstrated to inhibit preferentially in the forestomach, with comparable ID_{50} values for the two tissues as reported in the present work (Peskar, Weiler & Peskar, 1982).

In the absence of inhibition by low doses of BW755C, a substantial enhancement of prostacyclin formation from endogenous substrate in the rat gastric mucosa was observed. Elevated prostacyclin production was also found in rat forestomach and ileal homogenates, but to a lesser extent. Paracetamol also stimulated prostacyclin formation but unlike BW755C, was equally effective in both mucosal and forestomach homogenates, perhaps suggesting a different mechanism of action. In other studies, stimulation of prostaglandin synthetase by paracetamol in bovine and ram seminal vesicle preparations only occurred in the absence of cofactors, thus suggesting that paracetamol itself was acting as a cofactor (Robak, Wieckowski & Gryglewski, 1978) or perhaps as a free-radical scavenger.

The mechanism by which BW755C enhances prostacyclin formation *in vitro* has not yet been characterized. Anti-oxidants such as ascorbic acid and propylgallate enhance 6-oxo-$PGF_{1\alpha}$ formation from radiolabelled arachidonic acid and PGH_2 in ram seminal vesicle microsomes (Beetens, Claeys & Herman, 1981). Likewise, the phenolic compound MK 447 and its analogues enhance PGE_2 formation by bovine seminal vesicle microsomes (Kuehl, Humes, Egan, Ham, Beveridge & Van Arman, 1977; Payne, Dewald, Siegl, Gubler, Ott & Baggiolini, 1982) probably by scavenging the enzyme-inactivating oxidative radicals produced in prostaglandin biosynthesis. Whether such mechanisms could contribute to the actions of BW755C awaits further study. The enhancement of prostacyclin formation by BW755C could also be related to inhibition of the lipoxygenase pathway since the hydroperoxy fatty acids formed by this pathway potently inhibit the enzyme, prostacyclin synthetase (Salmon, Smith, Flower, Moncada & Vane, 1978) and can reduce cyclo-oxygenase activity (Siegel, McConnel, Abrahams, Porter & Cuatrecasas, 1979). Thus, removal of this inhibitory influence of the lipoxygenase products could be expected to enhance prostacyclin production. Such modulator actions would necessarily depend on the relative activity of these lipoxygenase enzyme systems which have yet to be clearly demonstrated in the gastric mucosa and it is therefore not yet known whether BW755C can inhibit these pro-

ducts in gastric tissue. The presence of 12-HETE (the hydroxy derivative of 12-hydroperoxy-eicosatetraenoic acid) has, however, been recently detected in human gastric tissue, although this may be platelet-derived (Bennett, Hensby, Sanger & Stamford, 1981). A modulator role for the lipoxygenase product 12-HPETE on arachidonate metabolism in platelets has previously been proposed by Siegel and co-workers (Siegel *et al.*, 1979). A further consequence of inhibition of lipoxygenase pathway could be the diversion of substrate to the cyclo-oxygenase pathway thus increasing prostaglandin formation. Interestingly, the anti-oxidant NDGA which is known to inhibit the lipoxygenase pathway (Hamberg, 1976), also was found to enhance prostacyclin synthesis in the rat gastric mucosa in our *in vitro* studies.

Studies on the metabolism of arachidonic acid by gastro-intestinal tissues are important in elucidating the potential physiological and pathological roles of both cyclo-oxygenase and lipoxygenase products. For *in vitro* studies, some microsomal preparations have the disadvantage in requiring exogenous cofactors for efficient enzymatic conversion of exogenous arachidonic acid and these co-factors may alter drug effect (Robak *et al.*, 1978) and distort the profile of products (Cottee, Flower, Moncada, Salmon & Vane, 1977). Tissue homogenates, which can generate prostacyclin and other prostaglandins from endogenous substrate, thus provide a simple, rapid and reproducible method for measuirng the effect of anti-inflammatory drugs on prostaglandin formation *in vitro* from gastro-intestinal and perhaps other tissues. The relative insensitivity of gastric mucosal and ileal prostacyclin formation *in vitro* to inhibition by paracetamol as well as by BW755C, which supports the previous studies *in vivo* (Whittle *et al.*, 1980; Whittle, 1981a; Van Kolfschoten, Dembinska-Kiec & Basista, 1981), is of interest when considering the lack of gastro-intestinal damage induced by these compounds.

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