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Investigation of the effects of platelet-activating factor (PAF) on ion transport and prostaglandin synthesis in human colonic mucosa *in vitro*

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1 We have investigated the effects of platelet-activating factor (PAF), an endogenous mediator of inflammation, on ion transport and prostaglandin synthesis in the human isolated colon.

2 Application of PAF to the serosal surface of human colonic mucosa induced a marked, concentration-dependent increase in ion transport. Mucosal application was without effect.

3 The secretory response to PAF was significantly inhibited by prior application of a specific PAF receptor antagonist WEB 2170, indicating that the response is dependent on PAF receptor activation.

4 The response to PAF was attenuated by prior application of indomethacin or piroxicam, implicating products of the cyclo-oxygenase pathway in the response.

5 The response to PAF was attenuated by the loop diuretic bumetanide, indicating an involvement of chloride ion secretion in the response.

6 Addition of PAF to the serosal surface induced a significant increase in serosal prostaglandin E_2 (PGE_2), but not 6-oxo-$PGF_{1\alpha}$ release. There was no effect on mucosal application of PAF.

7 In summary, we have shown that PAF is a potent secretagogue in isolated preparations of human colon and that the response is dependent on a specific PAF receptor, cyclo-oxygenase products and bumetanide-sensitive chloride ion transport.

Keywords: Platelet-activating factor (PAF); human colon; ion transport; prostaglandin synthesis

Introduction

Platelet-activating factor (PAF) is an acetylated phospholipid (1-*O*-alkyl-2-acetyl-*sn*-glycero-phosphocholine) which is synthesized in many cell types. It has a range of biological activities suggesting that it could be involved in pathological events in the gastrointestinal tract (Appleyard & Hillier, 1995), although its physiological role is uncertain. The expression of PAF is upregulated in inflammatory cells and the main route of enhanced synthesis is suggested to be via the remodelling pathway involving acetylation of the substrate 1-*O*-alkyl-2-lyso-*sn*-glycero-phosphocholine (lyso-PAF). Lyso-PAF can be generated by the action of phospholipase A_2 (PLA_2) on 1-*O*-alkyl-2-arachidonoyl-*sn*-glycero-phosphocholine (Snyder 1989), with the concomitantly-released arachidonate a potential substrate for eicosanoid synthesis. When PAF is catabolized, the obligatory initial product in human colonic mucosa is also lyso-PAF (Appleyard & Hillier, 1992). PAF is involved in hypersensitivity and inflammatory reactions, including platelet and neutrophil aggregation, vasodilatation, increased vascular permeability and leukocyte adhesion (see Camussi *et al*., 1987 for review).

Based on conclusions from laboratory animal studies, PAF has been implicated as a mediator of the pathogenesis associated with inflammatory bowel disease (IBD) and cholera (Guerrant *et al*., 1994; Nassif *et al*., 1996). By the use of selective PAF receptor antagonists it has been implicated as a mediator of the intestinal inflammatory and secretory effects and epithelial disruption caused by *Clostridium difficile* toxin A (Fonteles *et al*., 1995) and also of *Escherichia coli* endotoxic-shock in rats (Pons *et al*., 1991). It has also been shown that PAF plays a significant role in the transport changes observed during intestinal anaphylaxis (Wallace, 1987). Increased levels of PAF have been found in specimens of human colon taken from patients with IBD (Kald *et al*., 1990; Ferraris *et al*., 1993), and when culture medium from biopsies of patients with IBD is applied to sections of rat colon there is a significant increase in fluid secretion across the mucosa (Wardle *et al*., 1996). Exogenous application of PAF has been shown to increase chloride ion secretion in both the small and large intestine of the rat *in vitro* (Bern *et al*., 1989; Buckley & Hoult, 1989; Hanglow *et al*., 1989). The effect of PAF and its mechanisms of action in human intestinal secretion have not previously been examined. The aim of the present study was to investigate the effects of exogenously-applied PAF on fluid secretion and prostaglandin synthesis in human colonic mucosa. In addition, by using selective inhibitors the nature of the secretory response was investigated. A preliminary account of these findings has been made to the British Pharmacological Society (Borman *et al*., 1997).

Methods

Sections of macroscopically normal human sigmoid colon were obtained from specimens removed at operations for carcinoma and were placed in ice-cold Krebs solution for transportation. Specimens were placed on a cork mat and pinned mucosa downwards. By use of scissors and fine forceps, preparations of mucosa and submucosa were separated from the muscle layers by sharp dissection. Mucosal preparations were then mounted as a flat sheet in Ussing chambers (window area 1.43 cm^2) and bathed on either side by 5 ml of gassed (5% CO_2 in O_2) Krebs solution

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of the following composition (mM): NaCl 121, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.1. Krebs solution was maintained at 37°C by a water-jacket and circulated via a gas-lift system.

Ion transport

The tissue was clamped at zero potential by a high impedance voltage clamp (DVC-1000, World Precision Instruments) and transmucosal short-circuit current (I_{SC}) was continuously recorded on an Apple Macintosh computer via a MacLab interface, as described previously (Borman & Burleigh, 1993). Tissue conductance was calculated via Ohms Law, using the change in I_{SC} generated by clamping the tissue at 2 mV rather than zero. Tissue preparations were allowed to equilibrate for 60 min under short-circuit conditions, during which time Krebs fluid bathing both sides of the tissue was replaced at 15 min intervals. This was performed in order to prevent the rise in I_{SC} which is thought to be due to the accumulation of mediators in the fluid bathing the tissue.

After 60 min equilibration, inhibitors or control vehicle were applied to the preparations (to both sides unless otherwise stated) and left to equilibrate for a further 30 min. After this time, a single concentration of PAF (10^{-9} to 10^{-5} M) was applied to the serosal surface of each preparation and the resulting change in I_{SC} was monitored until a maximum response had been attained. The effect of mucosal application of PAF was also tested. To test tissue viability carbachol (100 μM) was applied to the serosal side of all preparations on completion of each experiment.

Prostaglandin synthesis

In a series of four experiments, three separate preparations of colonic mucosa were mounted in Ussing chambers under identical conditions to those used for the ion transport studies. After equilibrating the tissue for 15 min, the total volume of Krebs fluid (5 ml) was removed from both serosal and mucosal incubation chambers. In test tissues, 1 μM PAF in Krebs was applied either serosally or mucosally with Krebs alone being added to the opposite chamber. In control tissues Krebs was added to both serosal and mucosal chambers. Following 15 min incubation, 2 ml aliquots were removed from each chamber, rapidly frozen and stored at −20°C until assayed (within 24 h). Incubates were analysed for both prostaglandin E_2 (PGE_2) and 6-oxo-$PGF_{1\alpha}$ (the stable breakdown product of PGI_2) by radioimmunoassay.

Prostaglandin analysis

Prostaglandin analysis was based upon the radioimmunoassay methods of Hillier *et al*. (1991) and de la Hunt *et al*. (1988) with the following modifications. The PGE_2 antibody had the following cross reactivities: PGE_2 100%; PGE_1 30%; $PGF_{1\alpha}$ 0.9%; $PGF_{2\alpha}$ 0.4%; PGD_2 0.17%; 6-oxo-$PGF_{1\alpha}$ 0.04%; thromboxane B_2 (TXB_2) <0.01%; arachidonic acid <0.01%. Cross-reactivity data for the 6-oxo-$PGF_{1\alpha}$ antibody were: 6-oxo-$PGF_{1\alpha}$ 100%; $PGF_{1\alpha}$ 4.0%; PGD_2 1.4%; PGE_2 0.2%; TXB_2 0.05%; arachidonic acid <0.01%. The prostaglandin content of 0.1 and 0.2 ml of each sample was assayed directly and without dilution. The levels of PGE_2 and 6-oxo-$PGF_{1\alpha}$ were calculated from standard curves run in parallel with each assay and the values expressed as ng ml^{-1} (limits of detection of 0.2 ng ml^{-1} and 0.1 ng ml^{-1}, respectively). The total Ussing chamber volume was 5 ml. Before the analysis of Ussing chamber samples, experiments were performed to ascertain the level of interference on the radioimmunoassays from non-prostanoid substances within unextracted incubates. In seven experiments, colonic mucosa was incubated for 15 min, with 0.1 ml incubation fluid being assayed alone and with the addition of PGE_2 or 6-oxo-$PGF_{1\alpha}$. No significant difference was found between predicted and obtained values, when these samples were assayed with and without added exogenous standard PGE_2 (0.1 ng) or 6-oxo-$PGF_{1\alpha}$ (0.05 ng), indicating that samples could be assayed unextracted without interference.

Data are given as mean ± s.e.mean, with the exception of EC_{50} values which are given as geometric mean with 95% confidence limits (95% C.L.), where n indicates the number of patients. Data analysis used the Mann-Whitney U-test, with $P<0.05$ being taken to indicate statistical significance.

Drugs and reagents

PAF (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) and lyso-PAF (1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine) were obtained from Sigma and stored as a solution in chloroform:ethanol (1:1). Each day, an aliquot was evaporated with nitrogen and redissolved in Krebs solution with 0.5% bovine serum albumin added. Indomethacin (Sigma) was dissolved in 100 mM Tris buffer (pH 8.0) to give a 100 μM stock solution. WEB 2170 (4{-[6-(*o*-chlorophenyl)-8,9-dihydro-1-methyl-4*H*, 7*H*-cyclopenta[4,5]thieno-[3,2-*f*]-*s*-triazolo[4,3-*a*][1,4]diazepin-8-yl]carbonyl}morpholine; (a PAF receptor antagonist (MacNaughton & Gall, 1991) from Boehringer Ingelheim) was dissolved in 0.5% saline, piroxicam (ICN) and bumetanide (Sigma) were dissolved in a few drops of dimethylsulphoxide (DMSO) and diluted in Krebs solution. PGE_2 and PGI_2 (Cascade) were dissolved in Krebs fluid immediately before use, atropine sulphate (Sigma) and carbachol (Sigma) were dissolved in distilled water. Application of any of the vehicle controls had no effect on either basal electrical parameters or the response to agonists. The antibody to 6-oxo-$PGF_{1\alpha}$ was purchased from Advanced Magnetics Inc, the PGE_2 antibody was raised in our own laboratories. The radiochemicals [^{3}H]-PGE_2 and 6-oxo-[^{3}H]-$PGF_{1\alpha}$ were purchased from Amersham and NEN, respectively.

Results

Effect of PAF on I_{SC}

After 60 min equilibration, basal I_{SC} across the mucosa of human sigmoid colon was 70.3 ± 10.3 μA cm^{-2} and basal conductance was 12.4 ± 1.4 mS cm^{-2} ($n=15$). Serosal application of PAF caused an increase in I_{SC} (Figure 1a). The response was monophasic in nature, reached a maximum after approximately 10 min, but was not maintained. Initial experiments showed that it was not possible to obtain a second secretory response to PAF in a single preparation by use of either cumulative or non-cumulative dosing. Therefore, only a single concentration of PAF was applied to any one tissue, either in the absence or presence of inhibitors or antagonist. The secretory response to PAF was concentration-dependent within the range of 10^{-9} to 10^{-5} M PAF; concentrations in excess of 10^{-5} M were not available and were therefore not applied. The increase in I_{SC} at 10^{-5} M PAF was 74.3 ± 9.5 μA cm^{-2}, with the concentration producing 50% of the effect of 10^{-5} M (apparent EC_{50}) being 18.2 nM (95% C.L. 5.5–61.4 nM, $n=6$; Figure 2). We have used the term 'apparent EC_{50}', as it appears that a concentration of 10^{-5} M PAF may be insufficient to produce a maximum

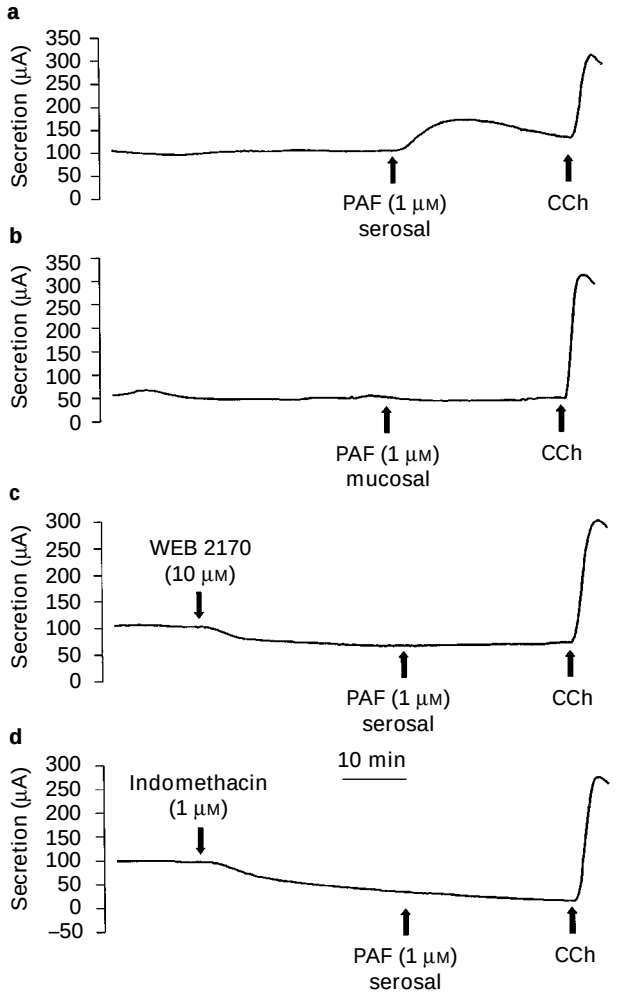

Figure 1 Typical recordings of I_{SC} from human sigmoid colonic mucosa. The traces represent responses to PAF (1 μM) applied (a) serosally, (b) mucosally, (c) serosally in the presence of WEB 2170 (10 μM), a PAF receptor antagonist, and (d) serosally in the presence of indomethacin (1 μM). In all panels secretion is expressed as raw data, i.e. in total microamps for an exposed window area of 1.43 cm^2, with positive currents indicating secretion and negative currents indicating absorption. The secretory response to carbachol (CCh; 100 μM) is indicated at the end of each experiment. Also shown is the pro-absorptive effect seen after administration of WEB 2170 or indomethacin.

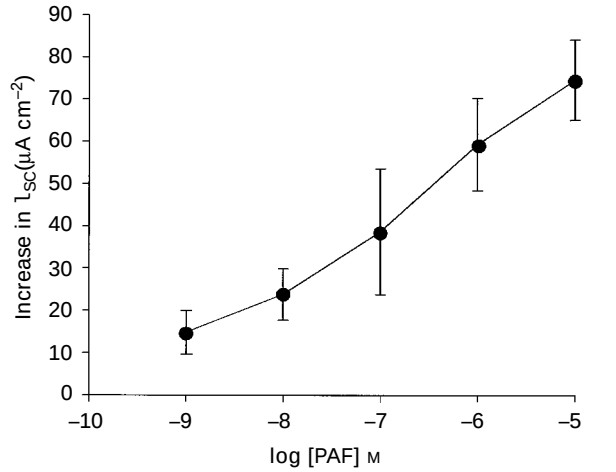

Figure 2 Concentration-response relationship for PAF. Figure shows the increase in I_{SC} in response to PAF, applied non-cumulatively, in the range 10^{-9} to 10^{-5} M. Data are given as mean for $n \geqslant 6$ specimens; vertical lines show s.e.mean.

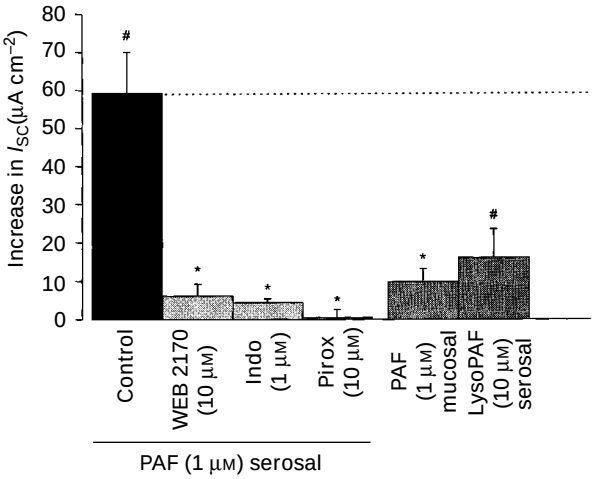

Figure 3 Effect of PAF and lyso-PAF on I_{SC}, and the effect of inhibitors on the I_{SC} response to PAF. Figure shows the response to PAF (1 μM) applied serosally and mucosally, and the response to lyso-PAF (10 μM) applied serosally. Also shown are the responses to serosally applied PAF (1 μM) in the presence of WEB 2170 (10 μM, a PAF receptor antagonist), indomethacin (1 μM) and piroxicam (10 μM). Data are given as mean $\pm$ s.e.mean for $n = 3$ to 15 specimens. #Indicates $P < 0.05$ compared to control vehicle. *Indicates $P < 0.05$ compared to 1 μM serosal PAF alone.

response. The effect of inhibitors was tested against PAF at a concentration of 1 μM, which was shown to produce a substantial and reproducible secretory response (Figure 2). Mucosal application of this concentration of PAF (10^{-6} M) had no significant effect on I_{SC} ($n = 6$, Figures 1b and 3). Application of lyso-PAF (10 μM), the biological precursor of PAF, caused a small but statistically significant rise in I_{SC} of 16.0 ± 7.5 μA cm^{-2} (Figure 3). At the end of each experiment, application of carbachol caused a significant increase in I_{SC} in all preparations (196 ± 11.0 μA cm^{-2}, $n = 15$, Figure 1), thereby proving tissue viability.

Effect of inhibitors on basal I_{SC}

In the 30 min following addition of WEB 2170 (10 μM), but before the application of PAF, the basal I_{SC} of human colonic mucosa was significantly reduced (reduction in I_{SC} of 23.2 ± 3.3 μA cm^{-2} compared to 12.0 ± 4.9 μA cm^{-2} in tissues not incubated with WEB 2170, $P < 0.05$, Figures 1c and 4). In addition, during the 30 min incubation period before the addition of PAF, both indomethacin and piroxicam significantly reduced basal I_{SC} of human colonic mucosa by 35.1 ± 6.7 μA cm^{-2} and 26.3 ± 9.5 μA cm^{-2}, respectively (compared to 12.0 ± 4.9 μA cm^{-2} in untreated (control) tissues, $P < 0.05$, Figures 1d and 4).

Effect of inhibitors on PAF-induced increase in I_{SC}

Pretreatment with the PAF receptor antagonist WEB 2170 (10 μM, $n = 4$, Figure 1c) significantly reduced the secretory response to 1 μM PAF (increase in I_{SC} of 6.1 ± 3.1 μA cm^{-2} compared to 59.1 ± 10.9 μA cm^{-2} in untreated (control) tissues, $n = 6$, $P < 0.05$; Figure 3). This concentration of WEB 2170 has previously been shown to inhibit significantly the I_{SC} response to PAF in rat jejunum (MacNaughton & Gall, 1991). Incubation with the cyclo-oxygenase inhibitors indomethacin (1 μM, $n = 4$) or piroxicam (10 μM, $n = 3$) caused a significant

inhibition of the response to 1 μM PAF. In the presence of indomethacin or piroxicam, application of 1 μM PAF evoked a rise in I_{SC} of 4.4 ± 1.0 μA cm^{-2} and 0.4 ± 2.2 μA cm^{-2}, respectively (Figure 3), compared to 59.1 ± 10.9 μA cm^{-2} in untreated (control) tissues. Subsequent challenge of the tissue with PGE_2 (3 μM) or PGI_2 (10 μM) could still evoke an increase in I_{SC} in the region of 100 μA cm^{-2} in tissues pretreated with indomethacin or piroxicam, indicating that the inhibition of the response to PAF was not due to a lack of ability of the tissues to respond to prostaglandins. Serosal application of the loop diuretic bumetanide (10^{-8} to 10^{-5} M), an inhibitor of chloride ion secretion, caused a concentration-dependent inhibition of the secretory response to 1 μM PAF ($n=4$; Figure 5). This compound had no significant effect on basal I_{SC} compared to untreated (control) tissues (Figure 4).

Effect of PAF on prostaglandin synthesis

In untreated (control) preparations, following 15 min incubation, levels of PGE_2 and 6-oxo-$PGF_{1\alpha}$ were approximately 2 and 3 fold higher, respectively, in the serosal incubate compared to the mucosal fluid. The effects of PAF on prostaglandin release from human colonic mucosa *in vitro* are shown in Table 1. Applied serosally, PAF (1 μM) significantly increased the release of PGE_2 into the serosal chamber, but had no effect on PGE_2 levels in the mucosal chamber. Serosal application of PAF had no effect on 6-oxo-$PGF_{1\alpha}$ release into either chamber. Mucosal application of PAF (1 μM) had no effect on the release of either PGE_2 or 6-oxo-$PGF_{1\alpha}$.

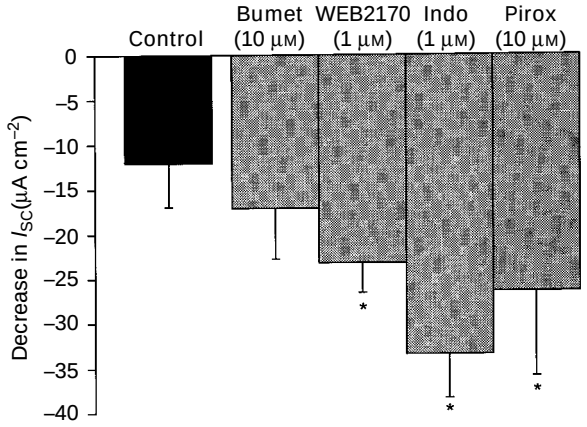

Figure 4 Effect of inhibitors on basal I_{SC}. Figure shows the change in I_{SC} during the 30 min incubation period in the absence (control) or presence of bumetanide (Bumet; 10 μM), WEB 2170 (10 μM), indomethacin (Indo; 1 μM) and piroxicam (Pirox; 10 μM). Data are given as mean$\pm$s.e.mean for $n \geqslant 3$ specimens, where $^*P<0.05$ compared to control tissues.

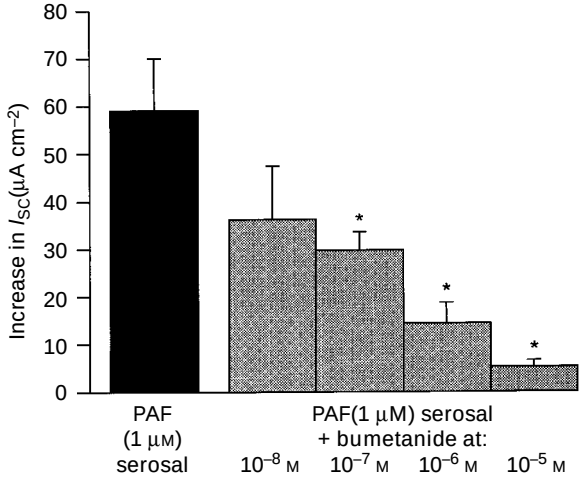

Figure 5 Effect of bumetanide on I_{SC} response to PAF. Figure shows the secretory response to PAF (1 μM applied serosally) in the absence (control) and presence of bumetanide at a concentration of 10^{-8} to 10^{-5} M. Data are given as mean$\pm$s.e.mean for $n \geqslant 4$ specimens, where $^*P<0.05$ compared to the response to 1 μM serosal PAF alone.

Discussion

In human sigmoid colon, we demonstrated that exogenously applied PAF causes an increase in short-circuit current across the mucosal wall. Short-circuit current has consistently been shown to be an indicator of electrogenic fluid secretion. The response to PAF was concentration-dependent and was only evoked when PAF was applied to the serosal surface; mucosally-applied PAF was without effect. This finding has implications for our understanding about the mechanisms by which some infections may induce secretory diarrhoea and also the mechanisms of secretory diarrhoea which can occur in diseases such as inflammatory bowel disease. It also provides preliminary evidence that basal I_{SC} may in part involve PAF generation.

The response to PAF of human colonic mucosa was substantially inhibited by pretreatment with the selective PAF receptor antagonist WEB 2170, the chemically dissimilar cyclo-oxygenase inhibitors indomethacin and piroxicam and by the addition of the loop diuretic, bumetanide. These data suggest that PAF is acting via a specific receptor to induce chloride ion secretion and that ongoing prostaglandin synthesis is necessary for the PAF-induced secretory response.

In rat jejunum the secretory response to PAF was blocked by pretreatment with WEB 2170 (MacNaughton & Gall, 1991). However, contradictory results have been obtained in rat colon, where Buckley & Hoult (1989) showed that the secretory response to PAF could not be blocked by a number of PAF receptor antagonists, including WEB 2086, a structural analogue of WEB 2170. This appears to indicate that the nature of the response to PAF may differ both between species and between different intestinal regions of the same species. The ability to inhibit the response to PAF by the use of a specific receptor antagonist is important as it has been shown that at high concentrations (100 μM), PAF may exhibit a general detergent effect and thus have a direct effect on the cell membrane (Sawyer & Anderson, 1989). In the present study

Table 1 Effect of PAF on prostanoid release from intact sheets of human colonic mucosa

	PGE_2		*6-oxo-$PGF_{1\alpha}$*	
	Serosal	*Mucosal*	*Serosal*	*Mucosal*
Control	0.27 ± 0.09	0.11 ± 0.03	0.33 ± 0.08	$0.11 \pm 0.08^{\#}$
PAF serosal	1.45 ± 0.56*	0.13 ± 0.03	0.39 ± 0.14	0.09 ± 0.02
PAF mucosal	0.18 ± 0.06	0.13 ± 0.01	0.29 ± 0.09	0.10 ± 0.02

Tissues were incubated for 15 min in the absence (control) or presence of PAF (1 μM), applied either serosally or mucosally, with the medium bathing both sides of the tissues being removed for subsequent assay. Data are given as mean$\pm$s.e.mean for $n=4$ preparations and are expressed as ng ml^{-1} in the medium bathing the tissues (total volume of 5ml). *Indicates $P<0.05$ compared to control. $^{\#}$Indicates $P<0.05$ serosal vs mucosal (for control only).

the effect was inhibited by a PAF receptor antagonist, so it is unlikely that the secretory response to PAF is due to such non-specific effects.

The I_{SC} response to PAF was inhibited by incubation with either indomethacin or piroxicam, implicating products of the cyclo-oxygenase pathway as mediators of the response. We cannot as yet unequivocally exclude the possibility that a small component of the effect of PAF may be due to a direct response, but inhibition of cyclo-oxygenase enzymes with either of the two inhibitors did reduce the effect of PAF by more than 90%. In this laboratory, incubation of human colon mucosal biopsies, obtained from patients without inflammatory disease, with 1 μM indomethacin inhibited PGE_2 synthesis by approximately 70% (data not shown). After treatment with either of these cyclo-oxygenase inhibitors, sheets of colonic mucosa were still able to respond in a secretory manner to application of either PGE_2 or PGI_2, indicating that the inhibitory effects were not due simply to non-specific effects on mucosal function. Indeed, the concentration of indomethacin used in this study was considerably less than that needed to cause non-specific effects (>200 mM, MacNaughton & Gall, 1991).

In our study, PGE_2 and 6-oxo-$PGF_{1\alpha}$ are released from both serosal and mucosal surfaces *in vitro*. In control tissues, serosal levels of 6-oxo-$PGF_{1\alpha}$ were significantly higher than mucosal levels. Serosal application of PAF significantly and selectively stimulated PGE_2 release into the serosal chamber, 6-oxo-$PGF_{1\alpha}$ synthesis was not stimulated. Both PGE_2 and PGI_2 have been shown to act as potent secretagogues in rabbit ileum (Musch *et al*., 1987) and from our own studies both are capable of inducing a rise in short-circuit current in human colon. In a previous study with human uninflamed colonic mucosa, Wardle *et al*. (1996) showed that with PAF at a concentration range of 10^{-9} to 10^{-4} M, only 10^{-5} M PAF increased PGE_2 synthesis. However, in inflamed tissue 10^{-6} M PAF was effective.

MacNaughton & Gall (1991) demonstrated a similar selectivity in rat jejunum, whereby application of PAF resulted in the increased release of PGE_2 but not PGI_2 into the bathing medium. However, in rat colon Bern *et al*. (1989) measured an increase in PGE_2 and 6-oxo-$PGF_{1\alpha}$ on PAF addition, whereas Buckley & Hoult (1989) were unable to stimulate prostaglandins with PAF. When the effect of indomethacin was studied, MacNaughton & Gall (1991) and Bern *et al*. (1989) were able to inhibit PAF-induced chloride secretion, whereas in rat colon Buckley & Hoult (1989) could not reduce PAF-induced secretion with indomethacin. Further investigations are needed to determine whether the nature of the effect of PAF on electrolyte transport differs with experimental protocols used and also between species and disparate intestinal regions of the same species.

The mechanism by which PAF increases PGE_2 levels in gastrointestinal mucosa is as yet not known, but PAF has been shown to upregulate phospholipases and cyclo-oxygenase enzymes (Bern *et al*., 1989; Ghandi *et al*., 1992; MacNaughton & Gall, 1991). Although we cannot at this stage rule out the involvement of other mediators, it is possible that the secretory effects of PAF may be partially accounted for by increased PGE_2 synthesis.

Inclusion of the loop-diuretic bumetanide in the medium bathing the serosal surface of the mucosa was found to attenuate the secretory response to PAF (1 μM), the effect being concentration-dependent. Bumetanide inhibits the basolateral Na^+-K^+-$2Cl^-$ cotransporter, the driving force for chloride ion secretion, and these results are consistent with the hypothesis that application of PAF induces active chloride ion secretion across the intestinal mucosa. This has also been shown to be the case in rat jejunum and colon, where the I_{SC} response to PAF was inhibited in chloride-free medium or by frusemide (Buckley & Hoult, 1989; MacNaughton & Gall, 1991).

An important observation is the ability of WEB 2170, indomethacin and piroxicam to decrease significantly the basal short circuit current in human colonic mucosa in the pre-incubation period before the addition of PAF. This appears to indicate that both PAF and cyclo-oxygenase products may play a role in the maintenance of the basal level of fluid transport in human colon. However, the possibility that cyclo-oxygenase inhibition may be influencing the levels of other eicosanoids has not been explored. Wardle *et al*. (1996), for example, were able to show that in human colonic mucosa, when PGE_2 production was suppressed by cyclo-oxygenase inhibitors a concomitant increase in leukotriene D_4 occurred. This lipoxygenase product has been shown to increase fluid secretion in rat colon (Hyun & Binder, 1993), although its effects in human colon are unknown.

Lyso-PAF is a precursor for PAF synthesis, particularly in inflamed cells. Human colonic mucosal cells also have the ability to catabolize PAF rapidly to a variety of products, allowing removal of this biologically active phospholipid with lyso-PAF being the obligatory initial metabolite (Appleyard & Hillier 1992; 1995). Lyso-PAF had only a small effect on electrolyte transport compared with PAF, although we were unable to determine if this is due to upregulation of prostanoid synthesis by lyso-PAF, the intermediate formation of PAF, or a direct effect of lyso-PAF. Because of the modest effect of lyso-PAF and the relatively rapid action of added PAF on secretion, it is unlikely that the effects of added PAF are substantially due to the presence of PAF degradation products.

In conclusion, application of PAF induces a significant increase in ion transport across human colonic mucosa. On the basis that the rise in I_{SC} can be inhibited by bumetanide, the increase in secretion can be accounted for by an increase in active chloride ion transport. The response is dependent on a specific PAF receptor and cyclo-oxygenase products. Application of PAF has been shown to induce the release of PGE_2 from human colonic mucosa, and it is possible that this increase in PGE_2 may be responsible for the secretory effect of PAF. If the role of PAF in the diarrhoea associated with bacterial endotoxins, such as cholera toxin and *Clostridium difficile* toxin A can be confirmed, these findings may have important implications in the treatment of such disorders.

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