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RESEARCH PAPER

Cyclic AMP-mediated chloride secretion is induced by prostaglandin $F_{2\alpha}$ in human isolated colon

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Background and purpose: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is implicated in the pathogenesis of inflammatory bowel disease and colorectal cancer. This study investigates the effects of $PGF_{2\alpha}$ on electrophysiological parameters in isolated human colonic mucosa.
Experimental approach: Ion transport was measured as changes in short-circuit current across human colonic epithelia mounted in Ussing chambers. Colonic crypts were isolated by calcium chelation and cyclic adenosine monophosphate (cAMP) was measured by ELISA.
Key Results: $PGF_{2\alpha}$ stimulated chloride secretion in a concentration-dependent manner with an EC_{50} of 130 nM. The $PGF_{2\alpha}$ induced increase in chloride secretion was inhibited by AL8810 (10 μM), a specific $PGF_{2\alpha}$ receptor antagonist. In addition, $PGF_{2\alpha}$ (1 μM) significantly increased levels of cAMP in isolated colonic crypts.
Conclusions and implications: $PGF_{2\alpha}$ stimulated chloride secretion in samples of human colon *in vitro* through a previously unrecognizd cAMP-mediated mechanism. These findings have implications for inflammatory states.
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Keywords: Prostaglandin $F_{2\alpha}$; gut motility/secretion; gastrointestinal pharmacology; eicosanoids; Ussing chamber

Abbreviations: CCh, carbachol; Cl-, chloride; EP receptor, prostaglandin E receptor; FP receptor, prostaglandin F receptor; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PI3K, phosphoinositide-3-kinase; PKC, protein kinase C; TXA_2, thromboxane A_2

Introduction

Prostaglandins (PGs) are products of arachidonic acid (AA) metabolism. The primary prostanoids PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 are formed by the actions of specific synthases on AA metabolites and are often cell type specific (Breyer *et al.*, 2001). $PGF_{2\alpha}$, one of the primary prostanoids, is a locally acting, paracrine and autocrine, signalling molecule associated with inflammation, cell growth and motility. It is a potent constrictor of smooth muscle including myometrium (Senior *et al.*, 1992; Sugimoto *et al.*, 1997; Chen *et al.*, 1998), vascular smooth muscle (Griffin *et al.*, 1998) and iris sphincter muscle (Linden and Alm, 1999) but has been most extensively studied for its role in luteolysis during pregnancy (Diaz *et al.*, 2002).

$PGF_{2\alpha}$ activates a specific FP receptor, a 7 transmembrane spanning G-protein coupled receptor, to stimulate intracellular events (Lake *et al.*, 1994). It also binds to EP_1 and EP_3 receptors with significant affinity (Breyer *et al.*, 2001). Signalling following FP receptor activation occurs primarily via a G_q-mediated mechanism leading to activation of protein kinase C (PKC; Ito *et al.* 1994; Fujino *et al.*, 2000) and increases in intracellular calcium (Coleman *et al.*, 1994; Pierce *et al.*, 1997). However, modulation of other second messenger cascades has been described including activation of cyclic adenosine monophosphate (cAMP) (Heindel *et al.*, 1978; Yurko-Mauro and Reenstra, 1998) and phosphatidylinositol 3-kinase (PI3K)

PGs are known to play a role in ion transport across gastrointestinal epithelia although effects are complex and differ between species. PGD_2, for example, increases chloride secretion in guinea pig colon (Frieling *et al.*, 1994) yet inhibits chloride secretion in rat models (Goerg *et al.*, 1992). PGE_2 and TXA_2 both increase chloride secretion in murine and human colon (Sakai *et al.*, 1997; Halm and Halm, 2001). Despite the distribution of FP receptors throughout the gastrointestinal (GI) tract (Sugimoto *et al.*, 1994; Hasumoto *et al.*, 1997; Qualtrough *et al.*, 2007), the effects of $PGF_{2\alpha}$ on the functions of human colon remain relatively unexplored. Previous studies have been limited to animal models and cultured epithelial cell lines. $PGF_{2\alpha}$ increases chloride secretion in porcine small

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intestine (Unmack *et al*., 2001) and also increases smooth muscle contractility in rat, guinea pig and human colon *in vitro* (Kamikawa *et al*., 2002; Gallagher *et al*., 2009). Interestingly, raised levels of $PGF_{2\alpha}$ are a particular feature in patients with Crohn's disease (Cracowski *et al*., 2002) and up-regulation of the FP receptor is found in colorectal carcinoma tumour tissue (Gustafsson *et al*., 2007).

Given the clinical implications of $PGF_2\alpha$ production in GI disorders, we have, here, investigated the actions of $PGF_{2\alpha}$ on human colonic mucosa *in vitro*.

Methods

Colon samples

Institutional review board approval, including informed patient consent, was granted for this study. Human colon was obtained at surgical resection for colonic carcinoma. The normal histological appearance of tissues was confirmed by routine pathological examination of samples obtained during dissection. Tissues from the resection margins were immediately transferred to the laboratory in pre-oxygenated Krebs–Henseleit solution (in mM; NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and D-glucose 11. 1, pH 7.4).

Ussing chamber experiments

Tissues were stripped of overlying muscle layers by blunt dissection, mounted in modified Ussing chambers (0.63 cm^2 aperture), bathed in physiological buffer at 37°C and gassed with 95% O_2/5% CO_2. Short circuit current and voltage were continuously measured by means of attached Ag-AgCl agar-salt bridge electrodes (3% agar in 3M KCl). Up to eight sheets of mucosa were obtained from a single patient. The transepithelial potential difference (PD) generated by the epithelium was continuously short circuited by passing current across the tissue and was adjusted by a feedback amplifier (World Precision Instruments, Stevenage, UK) to keep the clamp voltage at 0 mV. The amount of current required was recorded using a MacLab data acquisition system (AD Instruments, Hastings, UK). The change in short-circuit current (I_{sc}) measured under voltage-clamp conditions was used as an indicator of induced active ion transport and is expressed as microamperes per square centimetre (μA cm^{-2}). Transepithelial electrical resistance (TER) was calculated by applying Ohm's law (R = V/I).

Drugs were added to solutions bathing either the basolateral side or the apical side of the preparation. DMSO and ethanol concentration in the final solution never exceeded 0.1%.

cAMP assay

cAMP was measured by ELISA (R&D systems, Bristol, UK) in isolated colonic crypts. Colonic crypts were isolated as previously described (Winter *et al*., 2000). Briefly, mucosal segments were exposed to a calcium chelation solution (composition in mM : NaCl 96, KCl 1.5, HEPES/Tris 10, NaEDTA 27, sorbitol 45, sucrose 28) for 30 min at room temperature. A pellet of isolated crypts was formed by gentle centrifugation at 10× *g* for 1 min and was resuspended in Krebs solution. Crypts were treated with vehicle, 1 μM $PGF_{2\alpha}$ or 1 μM forskolin for 10 min. cAMP in crypt lysates was measured according to the manufacturer's instructions.

Statistical analysis

All results are expressed as mean ± SEM for a series of *n* independent experiments. Statistical analysis was performed using GraphPad Prism (San Diego, CA, USA). Statistically significant differences between mean values were calculated using the Student's paired and unpaired *t*-test or by ANOVA. Concentration–response curves were analysed by non-linear fitting of parameters in the Hill equation to provide values of EC_{50} or IC_{50}. Differences between means were considered statistically significant if $P < 0.05$. Drug/molecular target nomenclature follows Alexander *et al*. (2008).

Materials

$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, PGE_2, PGD_2, carbachol, bumetanide, forskolin and amiloride were acquired from Sigma-Aldrich Ireland Ltd. (Dublin, Ireland). The $PGF_{2\alpha}$ antagonist AL 8810, [(9S,11S,15R)-9,15-dihydroxy-11-fluoro-15-(2-indanyl)-16,17,18,19,20-pentanor-5,13-prostadienoic acid] was purchased from Cayman Chemicals (Cayman Europe, Estonia). Stock solutions were made up in dimethyl sulphoxide (AL8810, forskolin), ethanol (PGE_2, PGD_2, bumetanide and amiloride) or de-ionized water (carbachol, $PGF_{2\alpha}$) and diluted to working concentration in Krebs solution. Tissue were pre-treated with inhibitors for 15 min or until a stable baseline was obtained. For chloride-free Krebs, sodium gluconate and potassium gluconate were added instead of sodium chloride and potassium chloride. Carbachol (10 μM) was used to confirm functional viability of tissue preparations at the end of experiments.

Results

Baseline readings for human colonic mucosa were as follows; I_{sc} = 107.5 ± 6.5 μA cm^{-2} PD = 16.7 ± 2.7 mV, TER = 100.8 ± 13.2 Ω cm^2 (*n* = 15). There was no significant difference between males and females. None of the patients were taking non-steroidal anti-inflammatory drugs.

Effects of prostaglandins on bioelectrical parameters

Serosal addition of $PGF_{2\alpha}$ produced a sustained increase in I_{sc} that lasted up to 30 min followed by a steady decline to baseline values (Figure 1). No effect was seen with 8-iso-$PGF_{2\alpha}$ or with apical addition of $PGF_{2\alpha}$.(data not shown)

Determination of the ionic component

Tissues were pre-treated with bumetanide (100 μM), amiloride (100 μM) or bathed in chloride-free Krebs solution (chloride substituted with gluconate). The ΔI_{sc} in response to 1 μM

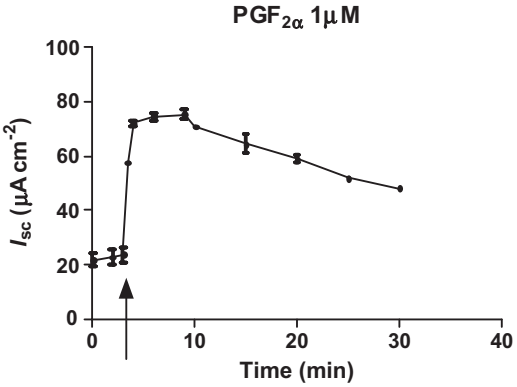

Figure 1 Representative time-course following addition of 1 μM $PGF_{2\alpha}$. There was a sustained increase in short circuit current (I_{SC}) lasting approximately 30 min, followed by a steady decline to baseline values.

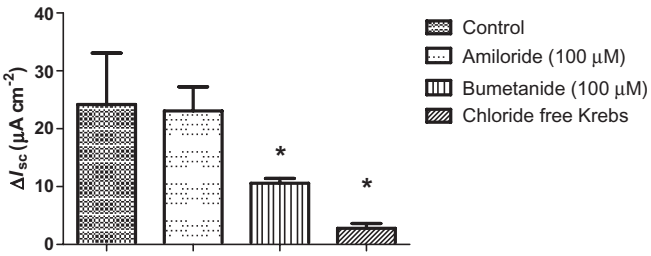

Figure 2 Responses to 1 μM $PGF_{2\alpha}$ (shown as control) were inhibited by pre-treatment with bumetanide (NKCC1 inhibitor), $P = 0.023$, and chloride-free Krebs ($P = 0.01$, $n = 3$), but amiloride (inhibitor of ENaC) did not change the response to $PGF_{2\alpha}$. *$P < 0.05$.

$PGF_{2\alpha}$ was decreased following pre-treatment with bumetanide (ΔI_{sc} = 10.4 ± 0.9 μA cm^{-2}) an inhibitor of the NKCC1 chloride transporter and was virtually abolished in chloride free Krebs (ΔI_{sc} = 2.7 ± 0.7 μA cm^{-2}). In contrast, pre-treatment with amiloride (ENaC inhibition) had no inhibitory effect on Isc compared with control (ΔI_{sc} = 23.2 ± 4 μA cm^{-2}) confirming that $PGF_2\alpha$ increased chloride secretion with little effect on sodium absorption (Figure 2).

Concentration-response experiments

Serosal addition of $PGF_{2\alpha}$ (1 nM–10 μM) caused a concentration dependent increase in I_{sc} in human colon with an EC_{50} of 130 nM (Figure 3). Successive applications of $PGF_{2\alpha}$ resulted in decreased responses, therefore concentration-response experiments and pharmacological studies were performed on different preparations.

Effect of the $PGF_{2\alpha}$ receptor antagonist AL8810

AL8810 is an antagonist at the prostaglandin FP receptor, inhibiting $PGF_{2\alpha}$ in A7r5 rat aortic smooth muscle cells and Swiss mouse 3T3 fibroblasts (Griffin *et al.*, 1999). Samples of human colonic mucosa were pre-treated with the antagonist for 15 min or until a stable baseline was achieved. AL8810 at

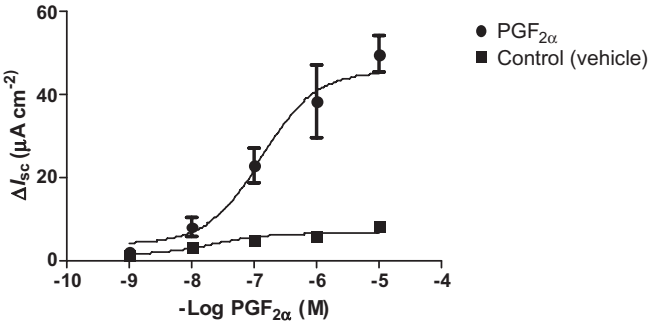

Figure 3 Concentration response curve for $PGF_{2\alpha}$ (1 nM–10 μM). The EC_{50} for $PGF_{2\alpha}$ was 1.31 × 10^{-7} M, (95% CI = 2.1 × 10^{-8}–8.3 × 10^{-7}), $n = 8$.

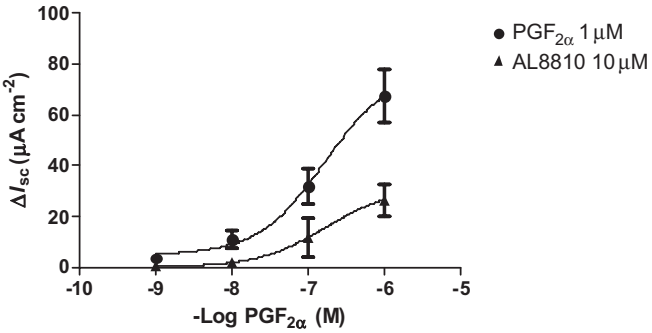

Figure 4 AL8810 (10 μM) inhibited the ΔI_{sc} of 1 μM $PGF_{2\alpha}$, (P = 0.017). The IC_{50} of AL8810 was 1.9 × 10^{-7} M (95% CI = 1.3 × 10^{-9}–2.1 × 10^{-5}), $n = 4$.

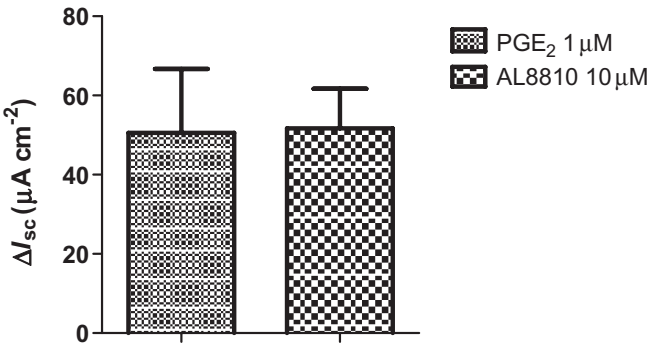

Figure 5 Effect of pre-treatment with AL8810 on ΔI_{sc} generated by PGE_2 (1 μM). There was no significant inhibition of PGE_2-mediated secretion following pre-treatment with the FP receptor antagonist AL8810.

a concentration of 10 μM inhibited the $PGF_{2\alpha}$ rise in I_{sc}. ($n = 4$, $P < 0.01$) (Figure 4). Furthermore, AL8810 (10 μM) did not inhibit ΔI_{sc} associated with PGE_2 (1 μM; $n = 3$, Figure 5).

cAMP

Levels of intracellular cAMP in isolated colonic crypts were significantly increased following exposure to $PGF_2\alpha$ (1 μM) and forskolin compared with control ($n = 8$; $P < 0.01$). Figure 6

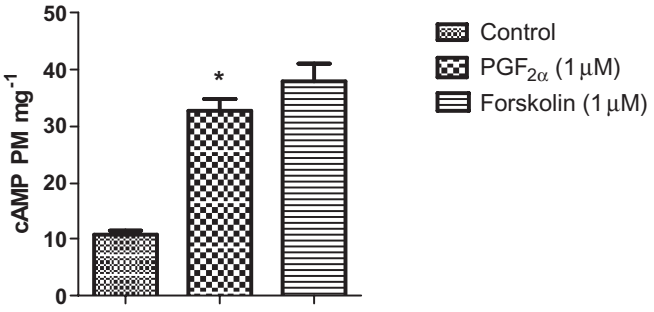

Figure 6 $PGF_{2\alpha}$ significantly increased levels of cAMP in isolated colonic crypts compared with control, $*P < 0.01$, $n = 8$.

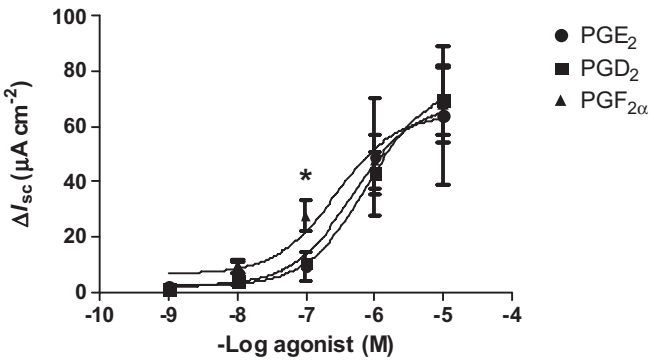

Figure 7 Comparisons between ΔI_{sc} induced by PGE_2, PGD_2 and $PGF_{2\alpha}$. $PGF_{2\alpha}$ was as potent as PGE_2 in eliciting a chloride secretory response in human colon: $n = 4$.

Effects of PGE_2 and PGD_2 on ion secretion

PGE_2 and PGD_2, two other major prostanoids, also elicited increases in chloride secretion in a concentration-dependent manner. A comparison of the effects of PGE_2, PGD_2 and $PGF_{2\alpha}$ (Figure 7) showed that $PGF_{2\alpha}$was equipotent to PGE_2 in paired (i.e., from the same patient) tissues ($n = 4$). The mean (with 95% confidence interval) EC_{50} value for PGE_2 was 5.7×10^{-7} M (4.6×10^{-8}–6.8×10^{-6}), for PGD_2 was 7.7×10^{-7} M (2.3×10^{-7} M–2.5×10^{-6} M) and for $PGF_{2\alpha}$ was 1.3×10^{-7} M (2.1×10^{-8} – 8.3×10^{-7}).

Discussion

The present study demonstrates that $PGF_{2\alpha}$ causes a concentration-dependent increase in chloride secretion in human colonic epithelium with an EC_{50} of 130 nM. The NKCC1 co-transporter located in the basolateral membrane is the principal mechanism for intracellular accumulation of chloride to a level above its electrochemical equilibrium (Matthews *et al*., 1994). Regulated opening of apical channels leads to chloride secretion and subsequent passive diffusion of sodium and water into the colonic lumen. Inhibition of the NKCC1 transporter with the loop diuretic bumetanide or substitution of chloride with gluconate in the bathing solution significantly inhibited the effects of $PGF_{2\alpha}$, confirming that the ΔI_{sc} observed in our preparations was due to activation of chloride secretion.

cAMP-mediated secretion

The sustained increase in chloride secretion found in this study is consistent with a cAMP-mediated response (McNamara *et al*., 1999). Levels of cAMP in isolated colonic crypts were increased following treatment with $PGF_{2\alpha}$. Previous classification of the FP receptor suggests it is a G_q-coupled receptor with activation leading to increases in intracellular calcium or PKC. Although it displays homology to other mammalian (mouse and rat) receptors, the human FP receptor is not well characterized and second messenger events are not fully elucidated (Bos *et al*., 2004). It is worthwhile to note that $PGF_{2\alpha}$ has significant affinity for EP_1 receptors, a G_s-coupled receptor which acts through adenylate cyclase (Narumiya *et al*., 1999). Our results demonstrate that approximately 75% of the current induced by $PGF_{2\alpha}$ was inhibited by AL8810. It may be possible that the remainder of the ΔI_{sc} was due to activation of an EP receptor subtype, coupled to cAMP. However, in NIH/3T3 cells, $PGF_{2\alpha}$ causes an increase in cAMP and subsequent activation of the CFTR leading to ion secretion (Gusovsky, 1991). Studies performed by Salesa *et al*. (2008) in endometrial adenocarcinoma cells also found that $PGF_{2\alpha}$ increased cAMP. Although there may be crosstalk between EP and FP receptors, activation of cAMP may be an alternative mechanism of action of $PGF_{2\alpha}$ similar to the recently discovered cAMP pathway for TXA_2 (Horikawa *et al*., 2005).

Comparison with other prostanoids

In addition to investigating the effects of $PGF_{2\alpha}$, we also examined its potency in relation to the other main prostanoids, PGE_2 and PGD_2. In this study, PGE_2, PGD_2 and $PGF_{2\alpha}$ all increased chloride secretion. Interestingly, $PGF_{2\alpha}$ proved to be as potent as PGE_2 and more potent than PGD_2 in eliciting secretory responses.

Conclusion

Infective and inflammatory insults are characterized by increased fluid secretion into the colonic lumen causing diarrhoea. Active ion secretion (induced by endogenous mediators or exogenous toxins) in the form of electrogenic chloride (Cl^-) secretion drives this process (Barrett and Keely 2006). Prostaglandins are well established as modulators of intestinal ion transport, and local eicosanoid production maintains basal secretory tone in colonic epithelia. However, little has been published on the effects of $PGF_{2\alpha}$ in human colon. This study demonstrates that $PGF_{2\alpha}$ increases chloride secretion in a cAMP-dependent manner. This mechanism may have implications for a variety of inflammatory disorders including infectious colitis (*Clostridium difficile*) as well as inflammatory bowel diseases (Lauritsen *et al*., 1988). Pharmacological modulation of $PGF_{2\alpha}$ production may therefore represent a potential therapeutic target for diarrhoeal disease.

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Conflict of interest

None

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