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Prostaglandins inhibit secretion of histamine and pancreastatin from isolated rat stomach ECL cells

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1 The present study examines the effect of naturally occurring prostanoids and prostaglandin (PG) congeners on gastrin- and pituitary adenylate cyclase-activating peptide (PACAP)-evoked histamine and pancreastatin secretion from isolated rat stomach ECL cells.

2 ECL cells (75–85% purity) were isolated from rat stomach using pronase digestion followed by repeated counter-flow elutriation and cultured for 48 h before secretion experiments. The release of histamine and pancreastatin was determined by radioimmunoassay.

3 None of the PGs tested stimulated the release of either histamine or pancreastatin.

4 PGE_1 and PGE_2 inhibited both gastrin- and PACAP-evoked histamine and pancreastatin secretion ($IC_{50} = 1-2 \times 10^{-10}$ M). Most other naturally occuring prostanoids and PG congeners had no or little inhibitory effect. The PGE analogues misoprostol and sulprostone were more potent ($IC_{50} = 0.9 \times 10^{-11}$ M and 2×10^{-11} M respectively) than PGE_1 and PGE_2. The rank order of potency was misoprostol > sulprostone > $PGE_1 = PGE_2$, suggesting the involvement of the so-called EP_3 receptor.

5 The effects of PGs on the stomach ECL cells may be direct or indirect, for instance through the stimulated release of somatostatin from contaminating D cells (2–3%). However, the amount of somatostatin in the cell culture after 48 h was below the limit of detection, and somatostatin immunoneutralization did not prevent misoprostol from inhibiting secretion from the ECL cells.

6 The misoprostol-induced inhibition was reversed by pertussis toxin suggesting the involvement of G-protein subunits $G\alpha_0$ and/or $G\alpha_i$.

7 In view of the potency by which PGE_1, PGE_2, misoprostol and sulprostone inhibited the stimulated release of histamine and pancreastatin, we suggest that the ECL cells represent a primary target for prostaglandins acting via an EP_3 receptor in the oxyntic mucosa.

8 The results suggest that the clinically useful effect of misoprostol as an anti-ulcer drug reflects its ability to inhibit stomach ECL-cell histamine secretion.

Keywords: ECL cells; gastrin; misoprostol; histamine; pituitary adenylate cyclase activating peptide; pancreastatin; prostaglandins; rat; sulprostone

Introduction

Prostaglandins (PGs) stimulate the secretion of mucus and bicarbonate in the stomach (Allen & Garner, 1980; Konturek *et al*., 1983; Takeuchi *et al*., 1997) and inhibit the secretion of acid (Robert *et al*., 1967; Main & Whittle, 1973; Whittle & Vane, 1987; Sandvik & Waldum 1988a). The cytoprotective effect of the PGE_1 congener misoprostol explains its usefulness in the treatment of peptic ulcer (Bauer, 1985).

The parietal cell is one of several potential targets for PGs in the stomach (Soll 1980; Skoglund *et al*., 1982; Nylander *et al*., 1986; Tsai *et al*., 1987; Chen *et al*., 1988; Seidler *et al*., 1989; Choquet *et al*., 1990; Schepp *et al*., 1992). Another potential target may be the so-called ECL cell which plays a key role in the control of the parietal cell (Sandvik & Waldum, 1991; Håkanson *et al*., 1994; Andersson *et al*., 1996).

The ECL cells in the oxyntic mucosa produce, store and secrete histamine and pancreastatin (a chromogranin A-derived peptide) in response to gastrin (Håkanson *et al*., 1986; Prinz *et al*., 1993; Chen *et al*., 1994, 1996; Lindström *et al*., 1997) and there is much evidence to suggest that the acid-stimulating effect of gastrin is mediated by ECL-cell histamine (Waldum *et al*., 1991; Andersson *et al*., 1996). Also two neuropeptides, pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP), are powerful stimulators of the ECL cells, while two other peptides, the neuropeptide/hormone somatostatin and the neuropeptide galanin act as inhibitors (Lindström *et al*., 1997). Available evidence suggests that the ECL cells operate under the control of gastrin, somatostatin and the vagus (Håkanson *et al*., 1994). The vagal control is exercized by enteric neurons (conceivably with PACAP and/or VIP as stimulatory transmitters and with galanin as an inhibitory transmittor) (Ekblad *et al*., 1991; Sundler *et al*., 1992). PGs have been shown to inhibit histamine secretion from the isolated perfused rat stomach (Sandvik & Waldum, 1988a), from isolated canine oxyntic mucosal cells (Soll & Berglindh, 1994), and from isolated rat ECL cells (Prinz *et al*., 1997). In contrast, other reports suggest that PGs stimulate histamine release from rabbit gastric glands (Nylander *et al*., 1986), while potentiating the gastrin-evoked histamine release from isolated rabbit oxyntic mucosal cells (Hollande *et al*., 1993). Two studies using isolated rat ECL cells failed to observe either stimulatory or inhibitory effects of PGs on histamine release (Prinz *et al*., 1993; Song *et al*., 1996).

The aim of the present study was to examine the effect of various naturally occurring PGs and of the PGs congeners misoprostol, sulprostone and iloprost on gastrin- and PACAP-evoked histamine and pancreastatin secretion from isolated rat stomach ECL cells.

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Methods

Chemicals

The following prostanoids were purchased from ICN (Aurora, OH, U.S.A.): PGA_1, PGA_2, PGB_1, PGB_2, PGD_2, PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$ free acid, 11β-$PGF_{2\alpha}$, 9β,11α-PGF_2, PGG_2, PGH_2, PGI_2 sodium salt, carbocyclic TXA_2, TXB_2. PGG_2 and PGH_2 were delivered in acetone (0.1 mg/ml) and carbocyclic TXA_2 in ethanol (50 μg ml^{-1}). Stock solutions (10^{-3} M), made by dissolving the drugs in ethanol, were stored at -20°C except for PGG_2 and PGH_2 which were stored at -80°C. The ethanol concentration in the cell culture medium never exceeded 0.01% which does not affect secretion from the ECL cells. The PGE_1 analogue misoprostol was a kind gift from Searle (Skokie, IL, U.S.A.). The stock solution (10^{-2} M) was made by dissolving the drug in ethanol. The PGI_2 analogue iloprost and the PGE_2 analogue sulprostone were kind gifts from Schering (Berlin, Germany). Sulprostone was dissolved in ethanol (10^{-2} M stock solution). Iloprost (0.1 mg/ml) was delivered in 0.1 M Tris buffer enriched with ethanol. Rat gastrin-17 was from Research Plus (Bayonne, NJ, U.S.A.), rat PACAP-38 was from Peninsula (St. Helens, Merseyside, U.K.) and somatostatin was from Ferring (Malmö, Sweden). Anti-histidine decarboxylase antibody, raised in a guinea-pig, was a kind gift from Dr Lo Persson, Lund, Sweden (Dartsch *et al*., 1998). Anti-somatostatin antibody, raised in a rabbit, was from EuroDiagnostica (Malmö, Sweden) (Sundler *et al*., 1986). Pertussis toxin was from Calbiochem (LaJolla, CA, U.S.A.). TRITC-conjugated donkey anti-guinea-pig IgG and FITC-conjugated donkey anti-rabbit IgG were from Jackson ImmunoResearch (West Grove, PA, U.S.A.).

Isolation, fractionation and primary culture of ECL cells

The ECL cells were purified as described in detail by Lindström *et al*. (1997). Briefly, mucosal cells were dispersed using pronase digestion (0.9 mg ml^{-1} lot 83844924, Boehringer Mannheim) and calcium chelation (EDTA). The ECL cells were enriched by repeated counterflow elutriation using first a standard chamber and then a Sanderson chamber (Beckman, Palo Alto, CA, U.S.A.). The enriched cells from the standard chamber were collected at 25 ml min^{-1} and at a speed of 2000 r.p.m. (380–560 *g*). They were purified further in a Sanderson chamber and collected at 18 ml min^{-1} and 2000 r.p.m. The proportion of the ECL cells was assessed by immunocytochemistry using an anti-histidine decarboxylase antiserum (1 : 750). Immunoreaction was visualized by TRITC-conjugated donkey anti-guinea-pig IgG (1 : 80). At least 150–200 cells were examined on each slide and the proportions of the various cell types were calculated as described by Lindström *et al*. (1997). The isolated cell preparation consisted of 75–85% ECL cells. The number of D cells was determined using an anti-somatostatin antiserum (1 : 500). Immunoreaction was visualized by FITC-conjugated donkey anti-rabbit IgG (1 : 40). The contamination with D cells was 2–3%. The ECL cells were cultured in 96-well plates pre-coated with Matrigel® (1:10) (20,000 cells per well) in a humid atmosphere with 5% CO_2/95% air at 37°C for 48 h until the start of the experiments. The culture medium consisted of DMEM-Ham's F12 (1 : 1) supplemented with 2% fetal calf serum, 2 mM glutamine, 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 250 ng ml^{-1} amphotericin B, ITS (6.25 μg ml^{-1} insulin, 6.25 μg ml^{-1} transferrin, 6.25 μg ml^{-1} selenious acid), 1.25 mg ml^{-1} bovine serum albumin, 10 nM hydrocortisone, 15 mM HEPES, 10 μM pyridoxal-5-phosphate and 100 pM rat gastrin-17.

Secretion experiments

Secretion from the ECL cells was monitored by measuring histamine and pancreastatin in the medium. In preparation for the experiments, the medium was aspirated and replaced with fresh serum-free medium without gastrin. After equilibration for 2–3 h, the medium was again aspirated and replaced with fresh serum-free medium containing DMEM-Ham's F12 (1 : 1), 15 mM HEPES, 2 mM glutamine plus test substances. Thus, the cells were exposed to prostaglandins (10^{-12} M–10^{-7} M) alone or together with 10^{-9} M gastrin-17 or 10^{-9} M PACAP-38 (EC_{80} concentrations, Lindström *et al*., 1997) for 30 min. In one set of experiments, cells were pre-treated for 4 h with 250 ng ml^{-1} pertussis toxin (PTX) (a treatment known to block the inhibitory effects of PGE_2 on parietal cells, Schepp *et al*., 1992) in serum-free medium. After 4 h the PTX-medium was replaced with fresh serum-free PTX medium containing test substances. After incubation, the plates were centrifuged at 200 *g* for 1 min. The supernatants were collected and stored at -20°C until measurement of histamine and pancreastatin.

Determination of histamine

Histamine was measured using a commercial radioimmunoassay kit (Immunotech, Marseille, France). The amount of histamine released into the medium of each well (=20,000 cells) was calculated and expressed as % of stimulated secretion (10^{-9} M gastrin or PACAP-38).

Determination of pancreastatin-like peptides

The pancreastatin-like immunoreactivity was measured by radioimmunoassay using authentic rat pancreastatin as standard (Chen *et al*., 1994). The amount of pancreastatin released was expressed as % of stimulated secretion (10^{-9} M gastrin or PACAP-38) or as fmole equivalents of rat pancreastatin per well.

Determination of somatostatin

Somatostatin was measured by radioimmunoassay using a commercial kit (Euro-Diagnostica, Malmö, Sweden). The minimum detectable amount was 6 pmol/l.

Statistical analysis

All results are expressed as means ± s.e.m. Duplicate samples were analysed, n represents the number of independent cell preparations. The concentration of a secretagogue that releases 80% of maximum is referred to as EC_{80}. The concentration of an anti-secretagogue that suppresses gastrin- or PACAP (EC_{80})-evoked secretion by 50% is referred to as IC_{50}. EC_{80} and IC_{50} values were calculated from dose response curves by the use of a computer software-aided curve-fitter. Statistical significance of the difference between sets of data was assesed using the Scheffe *F*-test. $P<0.05$ was considered significant.

Results

PACAP and gastrin at 10^{-9} M induced a 4–5 fold increase in histamine (basal: 4.2 ± 0.5 pmoles/well versus 18.8 ± 1.9 (gastrin) and 22.2 ± 2.1 (PACAP)) and pancreastatin (basal: 1.2 ± 0.1 fmoles/well versus 5.5 ± 0.3 (gastrin) and 5.9 ± 0.4 (PACAP)) secretion. None of the prostanoids tested stimulated secretion of either histamine or pancreastatin. On the

contrary, both PGE_1 and PGE_2 were found to be effective and potent inhibitors of both gastrin- and PACAP-evoked secretion (Table 1, Figure 1a–d). They were quite effective already at 10^{-10} M concentration. Basal release of histamine and pancreastatin was unaffected. The stable PGE-analogues misoprostol and sulprostone were potent inhibitors of gastrin- and PACAP- stimulated histamine and pancreastatin secretion (Table 1, Figure 2a–d). The IC_{50} values for PGE_1-, PGE_2-, misoprostol- and sulprostone-mediated inhibition are given in Table 1. Other prostanoids were also able to inhibit gastrin-stimulated secretion but only at much higher concentrations ($>10^{-8}$ M) (Table 2). The PGI_2 analogue iloprost inhibited gastrin-stimulated secretion of pancreastatin, its potency ($IC_{50}=4.9\times10^{-9}$ M) being much lower than that of misoprostol, sulprostone, PGE_1 and PGE_2. The rank order of potency is misoprostol > sulprostone > $PGE_1=PGE_2>>$ iloprost.

Although radioimmunoassay failed to detect somatostatin in the cell cultures after 48 h (data not shown), it could not be excluded that the inhibitory effect of PGs was mediated by the release of small amounts of somatostatin from contaminating D cells. This was studied by immunoneutralization using a somatostatin antibody (Figure 3). Gastrin-17- and PACAP-38-stimulated secretion of histamine and pancreastatin is known to be inhibited by 10^{-8} M somatostatin (Lindström *et al.*, 1997). The effect of 10^{-8} M somatostatin was blocked by applying somatostatin antibody at a final dilution of 1 : 100. The same antibody concentration failed to affect the inhibition induced by misoprostol suggesting a direct effect of misoprostol on the ECL cells.

The possibility that PGs act on G-protein-coupled receptors was studied by treating cells with 250 ng ml^{-1} pertussis toxin (PTX) for 4 h before the secretion experiments (the medium was changed in control cells also, but without PTX). Treatment with PTX reversed the inhibitory effect of misoprostol (Figure 4), although a slight inhibitory effect could still be seen at high concentrations (10^{-7} M) of misoprostol.

Table 1 IC_{50} values for PGE-evoked inhibition of gastrin- and PACAP-evoked histamine and pancreastatin secretion

	Gastrin-evoked secretion		*PACAP-evoked secretion*	
Prostaglandin	*Histamine* (pM)	*Pancreastatin* (pM)	*Histamine* (pM)	*Pancreastatin* (pM)
PGE_1	98	169	101	122
PGE_2	108	159	79	78
Sulprostone	24	29	14	31
Misoprostol	9	8	7	9

Gastrin-17 and PACAP-38 were added to give a final concentration of 10^{-9} M. Mean values ($n=5-10$).

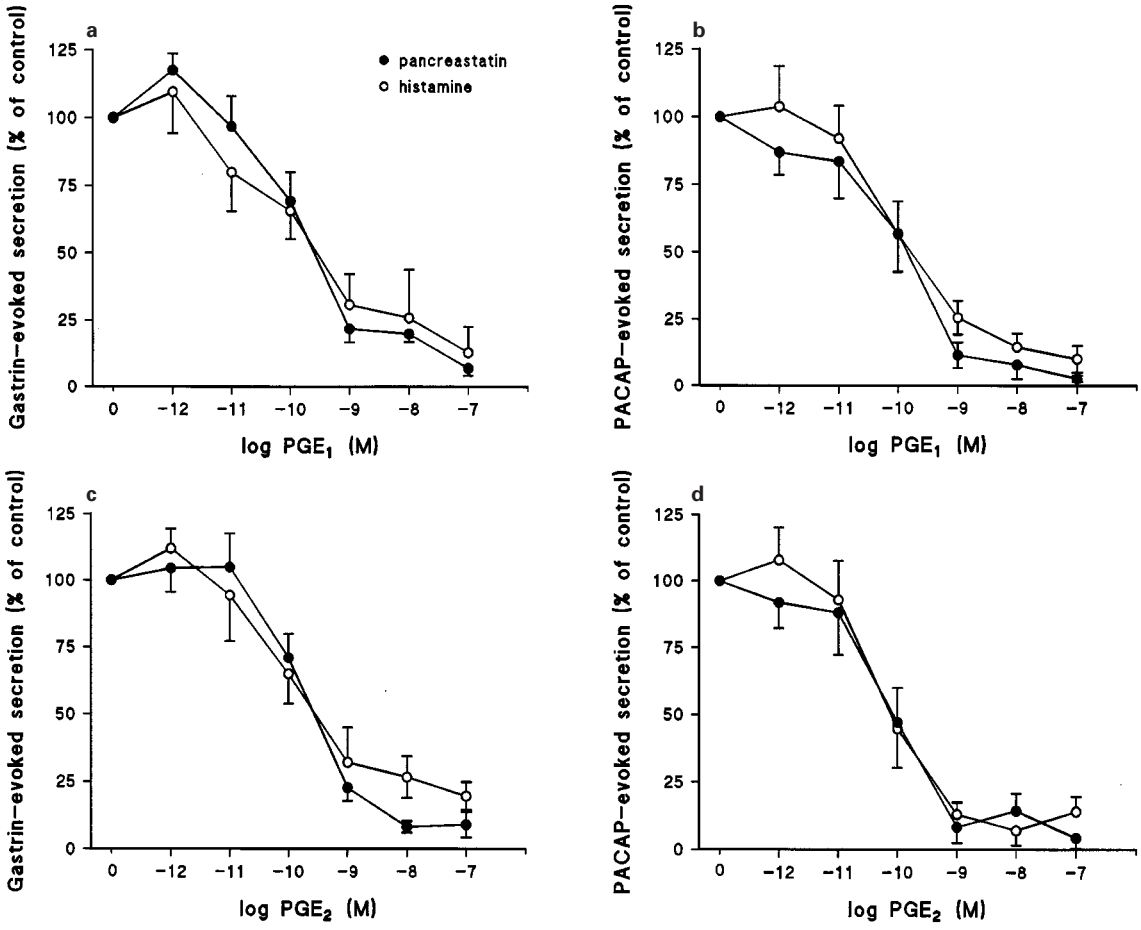

Figure 1 Prostaglandin-induced inhibition of histamine (○) and pancreastatin (●) secretion. Stimulatory effects of gastrin or PACAP (10^{-9} M) are set to 100%. Basal secretion is set to 0%. (a), effect of PGE_1 on gastrin-stimulated secretion. (b), effect of PGE_1 on PACAP-stimulated secretion. (c), effect of PGE_2 on gastrin-stimulated secretion. (d), effect of PGE_2 on PACAP-stimulated secretion. Mean values ± s.e.m. ($n=6-10$).

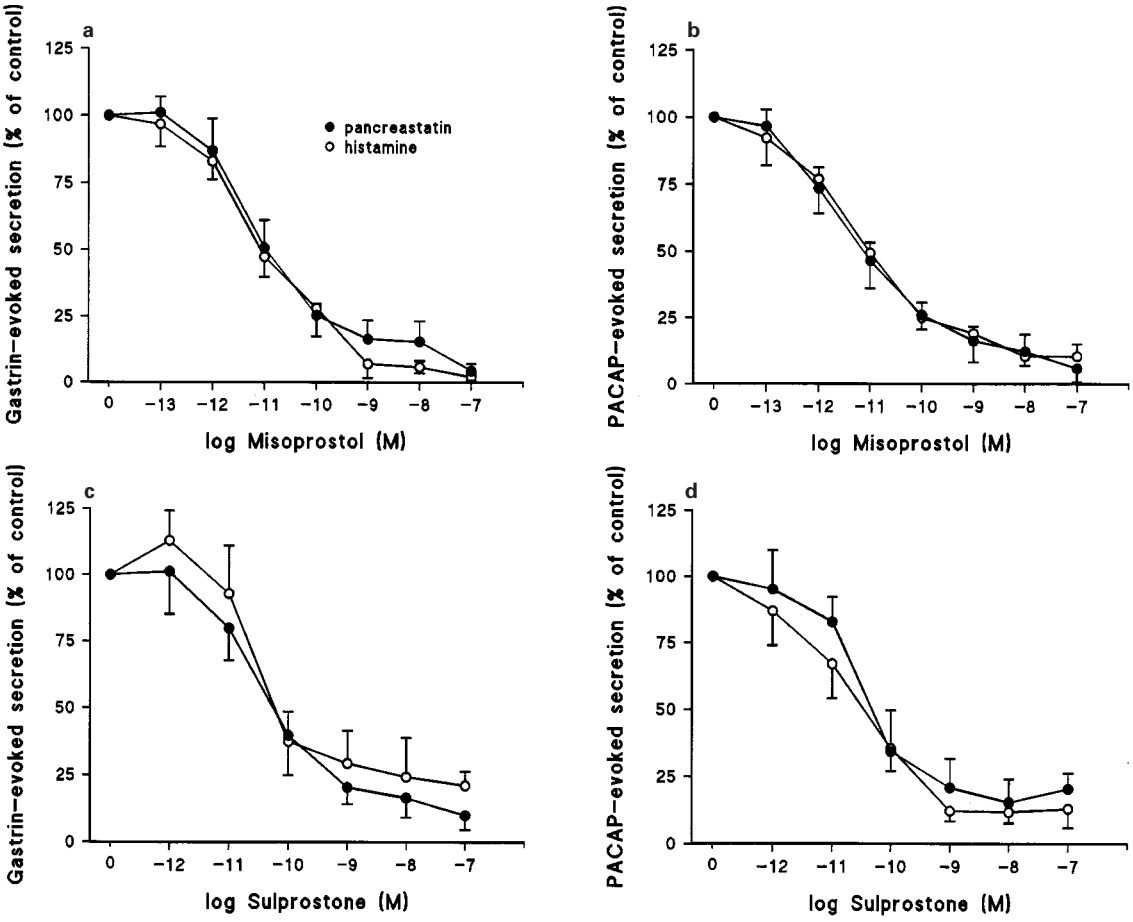

Figure 2 Misoprostol-and sulprostone-induced inhibition of histamine (○) and pancreastatin (●) secretion. Stimulatory effects of gastrin or PACAP (10^{-9} M) are set to 100%. Basal secretion is set to 0%. (a), effect of misoprostol on gastrin-stimulated secretion. (b), effect of misoprostol on PACAP-stimulated secretion. (c), effect of sulprostone on gastrin-stimulated secretion. (d), effect of sulprostone on PACAP-stimulated secretion. Mean values ± s.e.m. ($n = 6-10$).

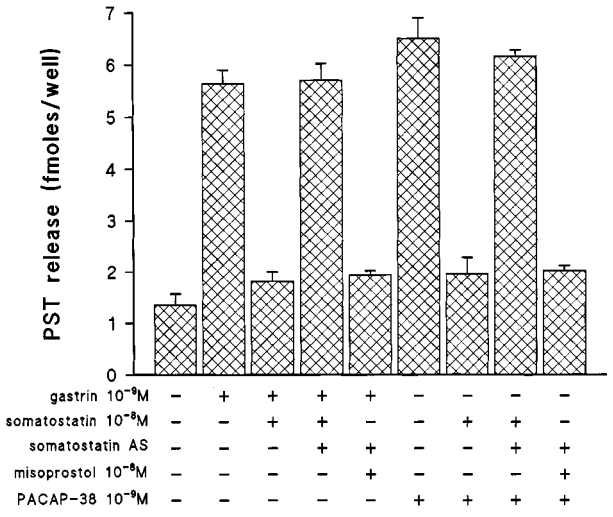

Figure 3 Release of pancreastatin from isolated ECL cells in response to 10^{-9} M gastrin or PACAP (30 min incubation) in the presence or absence of 10^{-8} M somatostatin, somatostatin antiserum (AS) or 10^{-8} M misoprostol. From the results it appears that the inhibitory effect of misoprostol on ECL cells is not mediated by somatostatin released from contaminating D cells, since misoprostol inhibited the release of ECL-cell pancreastatin also in the presence of somatostatin antibody added in amounts large enough to prevent the effect of somatostatin. Mean values ± s.e.m. ($n = 6$).

Discussion

The cytoprotective effect of PGs in the stomach reflects their ability to inhibit acid secretion (Robert *et al.*, 1967; Nezamis *et al.*, 1971; Konturek *et al.*, 1976). They may act directly on the parietal cells to inhibit acid production (Soll, 1980; Chen *et al.*, 1988) or on the stomach ECL cells to block histamine secretion (Sandvik & Waldum, 1991) or both. Data in the literature are conflicting, probably because of the wide range of experimental models that have been used to explore this issue. In the isolated, vascularly perfused rat stomach, misoprostol inhibited both basal and gastrin-stimulated histamine release (Sandvik & Waldum, 1988a). In the anesthetized dog, PGE_2 and PGI_2 inhibited the acid response to pentagastrin without affecting the stimulated histamine release, and it was concluded that PGs inhibit gastric acid output at the parietal cell level (Payne & Gerber, 1992). However, it may be argued that the effects of PGs on gastric functions are compromised by systemic effects and that the targets of PGs should be examined *in vitro* rather than *in vivo*. Unfortunately, also results of *in vitro* studies using either isolated glands or cells are in conflict. In rabbit gastric glands, PGs were found to stimulate histamine release (Nylander *et al.*, 1986), while isolated canine parietal cells responded to PGs with inhibition of histamine-stimulated acid production (Soll, 1980). Hollande *et al.* (1993) reported that PGs potentiated the gastrin-evoked histamine

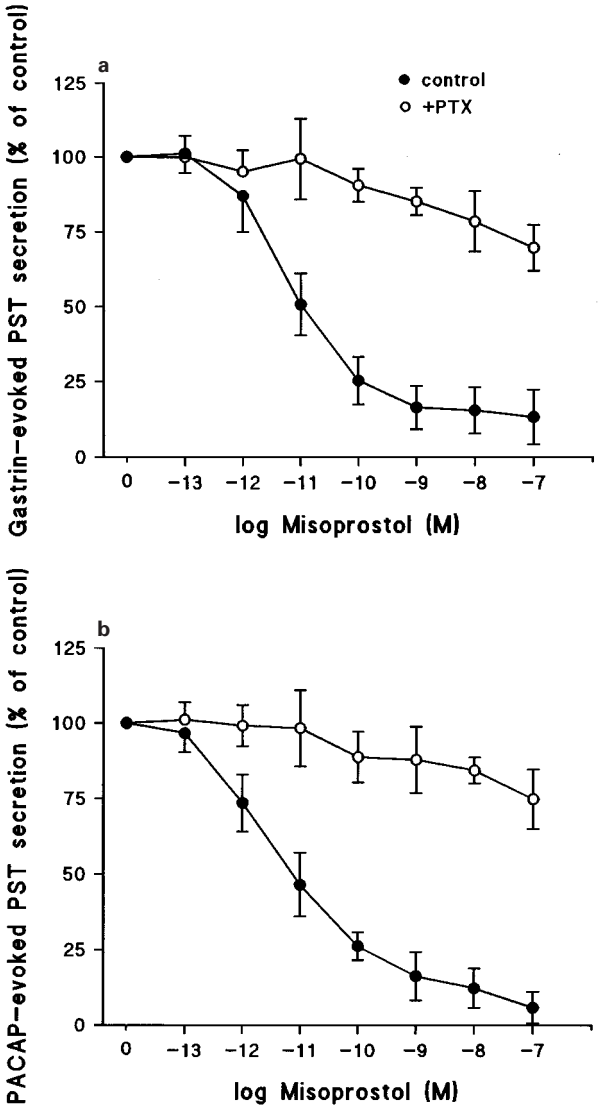

Figure 4 PTX-evoked reversal of the inhibitory effect of misoprostol on pancreastatin secretion from isolated ECL cells. Cells were treated with 250 ng/ml PTX for 4 h prior to secretion experiments. (○), PTX-treated cells, (●), control cells. (a), gastrin-stimulated (10^{-9} M) secretion, (b), PACAP-stimulated (10^{-9} M) secretion. Mean values ± s.e.m. ($n=6$).

release from a preparation of rabbit oxyntic mucosal cells. ECL cells isolated from the rat stomach failed to respond with either stimulated or inhibited histamine release (Prinz *et al.*, 1993; Song *et al.*, 1996) or responded with inhibition of histamine release (Soll & Berglindh, 1994; Prinz *et al.*, 1997).

The ECL cells have a pivotal role in the control of acid secretion (Håkanson *et al.*, 1994; Andersson *et al.*, 1996). They respond to gastrin with mobilization of histamine (Waldum *et al.*, 1991) which in turn stimulates the parietal cells by way of diffusion. The acid secretion-stimulating effect of gastrin is lost upon depletion of histamine from the ECL cells (Andersson *et al.*, 1996). Hence, any treatment that impairs the ability of the ECL cells to secrete histamine will impair also acid secretion. In the present study we show that some PGs, notably the PGE_1 congener misoprostol, are potent inhibitors of ECL-cell secretion. Interestingly, they turned out to inhibit gastrin- and PACAP-stimulated secretion with equal effectiveness.

Misoprostol is widely used as an anti-ulcer drug. Although misoprostol may have a direct inhibitory effect on gastrin secretion (Schepp *et al.*, 1994) and/or on acid production by the parietal cells (Tsai *et al.*, 1987), it cannot be excluded that its major action as an anti-secretagogue is to inhibit histamine secretion from the ECL cells (see also Sandvik & Waldum, 1988a). In the present study the various PGE derivatives tested were found to be potent and effective whereas most other prostanoids were inactive or poorly active. At this stage, we cannot exclude the possibility that the rate of metabolism of the various derivates in the cultures may differ, and that this could affect the measurement of potency. PGA and PGB type agents could be shown to induce a moderate inhibition of pancreastatin secretion (see Table 2), possibly reflecting their structural similarity to PGE (Middleditch 1975; Tepperman & Soper, 1981). Indeed, PGA has been shown to suppress acid secretion (Main & Whittle, 1973). Prostanoid receptors are classified into DP, EP, FP, IP, and TP in recognition of the existence of receptors that are specific for each of the five naturally occurring prostanoids (PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and Tromboxane A_2) (Coleman *et al.*, 1994). PGE receptors are of four different types, EP_1, EP_2, EP_3 and EP_4 (for reviews see Coleman *et al.*, 1994; Negishi *et al.*, 1995. Iloprost, which is generally considered to be an EP_1/IP receptor agonist (Sheldrick *et al.*, 1988), was recently found to bind to transfected mouse EP_3 receptors (Kiriyama *et al.*, 1997). This may explain its ability to inhibit ECL cell secretion at high concentrations.

Table 2 Inhibitory effects of various prostanoids and related compounds on gastrin stimulated secretion of pancreastatin

Prostanoid	*Secretion remaining* (%)		*Prostanoid*	*Secretion remaining* (%)	
Vehicle	94.4 ± 13.2		PGG_2	99.0 ± 7.5	n.s.
PGA_1	54.6 ± 12.7	n.s.	PGH_2	92.9 ± 8.9	n.s.
PGA_2	64.8 ± 6.1	n.s.	PGI_2	60.7 ± 10.3	n.s.
PGB_1	56.0 ± 12.2	n.s.	TXA_2	92.3 ± 8.1	n.s.
PGB_2	54.7 ± 4.7	n.s.	TXB_2	82.0 ± 10.1	n.s.
PGD_2	72.9 ± 11.7	n.s.	PGE_1	6.9 ± 2.8	***
$PGF_{1\alpha}$	99.1 ± 7.4	n.s.	PGE_2	8.9 ± 4.7	***
$PGF_{2\alpha}$	88.8 ± 12.0	n.s.	Misoprostol	4.4 ± 2.6	***
11β-$PGF_{2\alpha}$	83.0 ± 13.5	n.s.	Sulprostone	9.9 ± 5.4	***
$9\beta,11\alpha$-PGF_2	88.3 ± 9.9	n.s.	Iloprost	29.6 ± 5.6	***

Secretion of pancreastatin was stimulated by 10^{-9} M gastrin and prostanoids were added to give a final concentration of 10^{-7} M. 100% represents gastrin-stimulated secretion minus basal secretion, and all results are corrected for basal secretion. The effects of the various prostanoids are compared to that of the vehicle. Means ± s.e.m. ($n=5-10$). *$P<0.05$, **$P<0.01$, ***$P<0.001$. n.s. not significant.

The effectiveness and potency of PGE and the lack of effect of other prostanoids is in line with the results of previous studies of acid secretion (Skoglund *et al*., 1982; Payne & Gerber, 1987). Misoprostol is an EP_2/EP_3 receptor agonist and sulprostone is an EP_1/EP_3 receptor agonist. Sulprostone is suggested to be more potent than PGE_2 at EP_3 receptors and less potent at EP_1 receptors, while misoprostol is considered to be more potent than PGE_2 at EP_3 receptors and less potent at EP_2 receptors (Bunce *et al*., 1990; Coleman *et al*., 1994). The rank order of potency of the antisecretagogue effect of the various prostanoids tested in our study suggests the involvement of an EP_3 receptor. Indeed, such a receptor has been suggested to be present in ECL cell carcinoids of *Mastomys natalensis* (Naribayashi-Inomoto *et al*., 1995). However, unlike the receptor described by Coleman *et al*. (1994) the receptor of the ECL cells prefers misoprostol over sulprostone; perhaps an EP_3 receptor variant is involved. Our results agree with the findings of those who argue that PG-evoked inhibition of gastric acid secretion is mediated by EP_3 receptors (Reeves & Stables, 1985; Reeves *et al*., 1988; Hennies *et al*., 1992). Together with the fact that PGE_2 is the predominant prostanoid in the oxyntic mucosa of several species including the rat (Pace-Asciak & Wolfe, 1970; Creaghe *et al*., 1979; Skoglund *et al*., 1980; Ahlquist *et al*., 1982), this suggests that PGE_2 acts on ECL-cell EP_3 receptors to fulfill a physiologically important function.

Although it appears quite likely that PGs act directly on the ECL cells, it cannot be excluded that they affect the cells indirectly, e.g. through somatostatin released from contaminating D cells. Somatostatin is known to be a powerful inhibitor of ECL cell activity (Sandvik & Waldum, 1988b; Prinz *et al*., 1994; Lindström *et al*., 1997). However, we failed to detect somatostatin by radioimmunoassay and somatostatin immunoneutralization failed to prevent the action of misoprostol on ECL cell secretion, which makes it unlikely that somatostatin is responsible.

Available data suggest that PGE_2 and misoprostol inhibit both parietal cells and ECL cells. However, it appears that PGE-derivatives are more potent inhibitors of ECL cells (this study) than of parietal cells (Tsai *et al*., 1987, 1991; Choquet *et al*., 1990) (the apparent difference being at least one order of magnitude). Hence, it may be speculated that the ECL cells are the primary target for the acid-inhibiting effect of prostaglandins. We suggest that isolated ECL cells constitute a useful model system for the evaluation of EP_3 receptor agonists that might be exploited clinically as anti-secretory agents.

The EP-receptors contain seven-transmembrane segments characteristic of G-protein-coupled receptors. PTX inhibits $G\alpha_i$ and/or $G\alpha_0$ by causing ADP-ribosylation of these subunits (Ui, 1984; Hescheler *et al*., 1987). Pre-treatment with PTX for 4 h reversed the inhibitory effect of misoprostol, suggesting that the EP-receptor is coupled to one of these subunits. PTX has also been shown to reverse PG-evoked inhibition of isolated parietal cells (Choquet *et al*., 1990; Schepp *et al*., 1992).

PG-evoked effects are either paracrine or autocrine in nature. While the parietal cell has been suggested to be a source of PGs (Skoglund *et al*., 1980; Payne & Gerber, 1987), we cannot exclude the possibility that also the ECL cells are capable of producing PGs. However, hydrocortisone is routinely added to the culture medium because it improves the secretory response of the ECL cells to gastrin and PACAP. We assume therefore that little or no PGs are being produced during culture. Moreover, the ECL cells are washed prior to testing their responsiveness to gastrin or PACAP. Thus, any prostanoid that has accumulated during culture will be eliminated. Hence, it is unlikely that under the circumstances, endogenous PGs will be present in concentrations high enough to affect the ability of isolated ECL cells to respond to gastrin or PACAP. Nonetheless, it cannot be excluded that under normal circumstances ECL cells produce PGs that act as autocrine inhibitors of gastrin- or PACAP-stimulated secretion. This possibility is currently being investigated.

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