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Isolated rat stomach ECL cells generate prostaglandin E_2 in response to interleukin-1β, tumor necrosis factor-α and bradykinin

Erik Lindström [a,*], Ulf H. Lerner [b], Rolf Håkanson [a]

[a] *Institute of Physiological Sciences, Department of Pharmacology, University of Lund, Sölvegatan 10 S-223 62, Lund, Sweden*
[b] *Department of Odontology, Oral Cell Biology, Umeå University, Umeå, Sweden*

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Abstract

The ECL cells control parietal cells by releasing histamine in their immediate vicinity. Gastrin and pituitary adenylate cyclase-activating peptide (PACAP) stimulate histamine secretion from isolated ECL cells, while somatostatin and galanin inhibit stimulated secretion. Prostaglandin E_2 and related prostaglandins likewise suppress ECL-cell histamine secretion. Conceivably, that is how they inhibit acid secretion. In the present study, we examined if prostaglandin E_2 can be generated by isolated ECL cells. Rat stomach ECL cells were purified (> 90% purity) by counterflow elutriation and gradient centrifugation and cultured for 48 h. ECL cell stimulants (gastrin and PACAP) and inflammatory agents (interleukin-1β, tumor necrosis factor-α and bradykinin) were tested for their ability to induce prostaglandin E_2 accumulation (24-h incubation), measured by radioimmunoassay. Gastrin and PACAP did not affect prostaglandin E_2 accumulation but interleukin-1β (300 pg/ml), tumor necrosis factor-α (10 ng/ml) and bradykinin (1 μM) induced a 2- to 3-fold increase in the amount of prostaglandin E_2 accumulated. While the combination of interleukin-1β and bradykinin induced a 9-fold increase, the combination interleukin-1β + tumor necrosis factor-α and bradykinin + tumor necrosis factor-α induced additive effects only. The combination of interleukin-1β + tumor necrosis factor-α + bradykinin did not induce a greater effect than interleukin-1β + bradykinin. The effect of interleukin-1β + bradykinin was abolished by adding 10 nM hydrocortisone (suppressing phospholipase A_2 and cyclooxygenase) or 1 μM indomethacin (inhibiting cyclooxygenase). Incubating ECL cells in the presence of interleukin-1β + bradykinin for 24 h reduced their ability to secrete histamine in response to gastrin. The inhibitory effect was reversed by 1 μM indomethacin. Also, increasing the concentrations of hydrocortisone in the medium resulted in an enhanced gastrin-stimulated histamine secretion. Hence, the previously described acid-inhibiting effect of inflammatory agents may be explained by inhibition of ECL-cell histamine mobilization, consequent to enhanced formation of prostaglandin E_2 by cells in the oxyntic mucosa, including the ECL cells themselves.

Keywords: Bradykinin; ECL cell; Gastrin; Histamine; Interleukin-1β; PACAP (pituitary adenylate cyclase-activating peptide); Prostaglandin E_2; TNF-α (tumor necrosis factor-α)

1. Introduction

ECL cells are the predominant endocrine/paracrine cell type in the rat stomach (for a review, see Håkanson et al., 1994). They play a pivotal role in the control of acid secretion in that they respond to gastrin by mobilizing histamine (Sandvik et al., 1987; Kitano et al., 2000), which in turn stimulates parietal cells to secrete acid (Waldum et al., 1991). The stimulatory effects of gastrin on acid secretion can be blocked by histamine H_2 receptor antagonists (Black and Shankley, 1987; Black, 1993) or by inhibitors of histamine synthesis (Andersson et al., 1996).

The ECL cells secrete not only histamine but also chromogranin A-derived peptides, such as pancreastatin, in response to gastrin. This has been shown in conscious rats (Chen et al., 1994; Kitano et al., 2000) as well as in experiments using the isolated vascularly perfused stomach (Chen et al., 1996) and isolated ECL cells (Lindström et al., 1997). Recently, pituitary adenylate cyclase-activating peptide (PACAP) was identified as a stimulant of ECL-cell secretion (Lindström et al., 1997; Zeng et al., 1999), while somatostatin and galanin were found to exert inhibitory effects (Lindström et al., 1997; Zeng et al., 1998). In a recent study, it was also shown that prostaglandins such as

* Corresponding author. Tel.: +46-46-222-75-85; fax: +46-46-222-44-29.
E-mail address: erik.lindstrom@farm.lu.se (E. Lindström).

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prostaglandin E_1 and prostaglandin E_2 are powerful inhibitors of ECL-cell histamine and pancreastatin secretion (Lindström and Håkanson, 1998).

Prostaglandins protect the oxyntic mucosa by stimulating secretion of mucus and bicarbonate, by stimulating mucosal blood flow and by inhibiting acid secretion (for a review, see Whittle and Vane, 1987). At least part of the acid-inhibiting effect is likely to reflect inhibition of histamine mobilization from ECL cells (Sandvik and Waldum, 1988; Lindström and Håkanson, 1998). Oxyntic mucosa produces prostaglandins, especially prostaglandin E_2 (Bennett et al., 1967; Pace-Asciak and Wolfe, 1970; Creaghe et al., 1979; Singh, 1980; Skoglund et al., 1980; Boughton-Smith and Whittle, 1983), but how much the various mucosal cell types contribute is not known. Parietal cells are thought to be one source of oxyntic mucosal prostaglandin E_2, but if they are responsible for the bulk of local prostaglandin production is not clear (Skoglund et al., 1980; Schepp et al., 1986; Ota et al., 1988; Hatt and Hanson, 1988). Also non-parietal gastric mucosal cells have been shown to generate prostaglandins (Postius et al., 1985; Chen et al., 1989; Olson et al., 1989) but it is not yet known if and how much the ECL cells contribute to local prostaglandin formation.

Cyclooxygenase, the rate-limiting step in the biosynthesis of prostaglandins, exists in two isoforms cyclooxygenase-1 and cyclooxygenase-2. Cyclooxygenase-1 is constitutively expressed while the expression of cyclooxygenase-2 is induced in relation to inflammation and ulcer-healing (Schmassmann et al., 1998; Takahashi et al., 1998). The expression of these enzymes in ECL cells has not been studied.

The aim of the present study was to examine if isolated ECL cells generate prostaglandin E_2 and if they respond to well-known stimuli of ECL-cell secretion, such as gastrin and PACAP, with accelerated formation of prostaglandin E_2. In addition, inflammatory mediators, known to stimulate the formation of prostaglandin E_2, such as interleukin-1β, tumor necrosis factor-α and bradykinin (Farmer, 1997), were investigated for their ability to induce formation of prostaglandin E_2 in isolated ECL cells.

2. Materials and methods

2.1. Chemicals

Recombinant human interleukin-1β and tumor necrosis factor-α were purchased from R&D Systems (Abingdon, Oxon, UK). Rat gastrin-17 was obtained from Research Plus (Bayonne, NJ, USA). PACAP-38 and bradykinin were from Peninsula Europe (St. Helens, Merseyside, UK). Indomethacin, hydrocortisone, Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F-12 and all culture medium supplements were from Sigma (St. Louis, MO, USA). Matrigel® was from Collaborative Biomedical Products (Bedford, MA, USA).

2.2. Isolation and primary culture of ECL cells

The ECL cells were purified as described earlier (Lindström et al., 1997) with modifications. Briefly, mucosal cells from four male Sprague–Dawley rats were dispersed using pronase digestion (0.9 mg/ml, Boehringer Mannheim, Mannheim, Germany) and calcium chelation (EDTA). After dispersion, the cell preparation (M cells for mucosal cells) contained 2–3% ECL cells, identified by immunocytochemistry (see below). The ECL cells were enriched by repeated counterflow elutriation using first a standard chamber and then a Sanderson chamber (Beckman, Palo Alto, CA). The enriched cells from the standard chamber were collected at 25 ml/min and at a speed of 2000 rpm ($380–560 \times g$). They were purified further in a Sanderson chamber and collected at 18 ml/min and 2000 rpm. This cell preparation consisted of about 80% ECL cells.

The ECL cell preparation was then subjected to density gradient centrifugation. A stock solution of 60% iodixanol (Optiprep®, Nycomed Pharma, Oslo, Norway) was enriched with (final concentration) 1.2 mM $MgCl_2$, 15 mM HEPES at pH 7.4 and 10 mg/ml bovine serum albumin. The stock solution was diluted to 15% and 10.8%, respectively, with medium consisting of (mM): 140 NaCl, 1.2 $MgSO_4$, 1 $CaCl_2$, 15 HEPES at pH 7.4, 11 glucose, 0.5 dithiothreitol and 10 mg/ml bovine serum albumin. In a 15-ml centrifuge tube, 5 ml of the 15% iodixanol solution was overlaid with 5 ml 10.8% iodixanol. Enriched ECL cells (2×10^6 in one ml) were layered on the two layers of iodixanol and centrifuged (Beckman, Spinchron R centrifuge). A slow acceleration period (400 rpm/min) was followed by 5-min centrifugation when the speed reached 1000 rpm. Deacceleration lasted 5 min. The cells in the light density fraction (above the 10.8% cushion) were collected (estimated density: 1.058 g/ml). The yield of cells in this fraction was $0.8 \pm 0.1 \times 10^6$ cells (mean ± S.E.M., $n = 17$).

The purity of each ECL cell preparation was assessed by immunocytochemistry using an anti-histamine antiserum (1:1000) (Håkanson et al., 1986). A drop of the cell suspension was applied to a glass slide and allowed to dry. They were fixed in 4% 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma) (Panula et al., 1988). The primary antiserum was applied for 12 h at 4°C. Immunoreaction was visualized by the use of fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit immunoglobulin G (IgG) (1:40) (Dako, Glostrup, Denmark). At least 150–200 cells were examined on each slide. The final cell preparation consisted of 94.0% ± 1.2 ECL cells (Fig. 1).

The ECL cells were cultured in 96-well plates pre-coated with Matrigel® (diluted 1:10 in DMEM–Ham's F12).

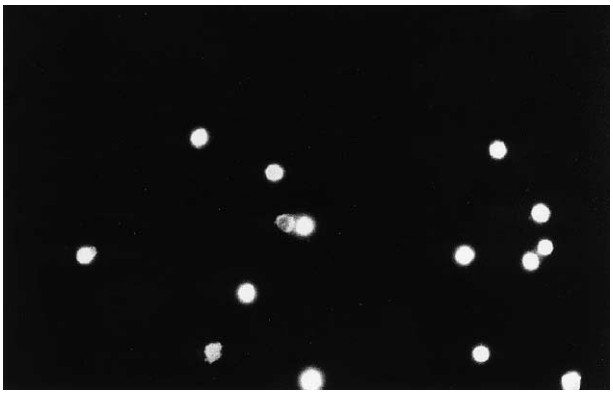

Fig. 1. Immunofluorescence of isolated ECL cells (> 90% purity) stained with anti-histamine antiserum (see Materials and methods for details).

20,000 cells were plated per well in 100-μl volume of culture medium 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 penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B, 10 μg/ml insulin, 5.5 μg/ml transferrin, 5 ng/ml selenious acid, 0.5 μg/ml bovine serum albumin, 15 mM HEPES, 10 μM pyridoxal-5-phosphate and 100 pM gastrin-17. In one series of studies, the effect of hydrocortisone on prostaglandin production and histamine secretion was studied by adding various concentrations of hydrocortisone to the culture medium. In previous studies of histamine and pancreastatin secretion 10 nM hydrocortisone was routinely added to the culture medium (Lindström and Håkanson, 1998).

2.3. Formation of prostaglandin E_2

Following culture for 48 h, the medium was aspirated and replaced with fresh serum-free medium containing DMEM–Ham's F12 (1:1), 15 mM HEPES, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B plus test substances (e.g., the inflammatory mediators interleukin-1β, tumor necrosis factor-α and bradykinin, the peptides gastrin and PACAP and the cyclooxygenase-1 and-2 inhibitor indomethacin). Twenty-four hours later, the plates were centrifuged and the supernatants collected. Supernatants were immediately acidified by addition of 4 μl 0.65 M HCl/100 μl and stored at −20°C until prostaglandin E_2 radioimmunoassay. Prostaglandin E_2 was determined using a commercially available radioimmunoassay kit (E.I. Du Pont de Nemours, Boston, MA, USA).

2.4. Histamine secretion

In one experiment, 5000 purified ECL cells were cultured as mentioned previously without hydrocortisone. Following culture for 48 h, the medium was aspirated and replaced with fresh serum-free medium containing DMEM–Ham's F12 (1:1), 15 mM HEPES, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphoteracin B plus test substances. Incubation lasted for 24 h. The experimental groups were: (1) controls, (2) 300 pg/ml interleukin-1β + 1 μM bradykinin, (3) 300 pg/ml interleukin-1β + 1 μM bradykinin + 1 μM indomethacin. Five microliters of gastrin (final concentration 10 nM) or saline were added to the medium 30 min before concluding the incubation. The plates were centrifuged and the supernatants collected and stored at −20°C until histamine analysis.

In another experiment, 5000 purified ECL cells were cultured as mentioned previously with different concentrations of hydrocortisone (0.1 nM–100 μM). Following culture for 48 h, the medium was aspirated and replaced with fresh serum-free medium (see above). After 1–2 h this medium was again aspirated and replaced with secretion medium (containing (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 10 HEPES at pH 7.0) with or without 10 nM gastrin for 30 min. The plates were centrifuged and the supernatants collected and stored at −20°C until histamine analysis. Histamine was determined using a commercially available radioimmunoassay kit (Immunotech, Marseilles, France).

2.5. Statistical analysis

All values are expressed as means ± S.E.M. n is the number of cell preparations. EC_{50} values (the concentration eliciting half-maximal effect) were calculated using Graph Pad Prism software (San Diego, CA, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA) with post-hoc Dunnett's or Bonferroni's tests. P values of < 0.05 were considered to be statistically significant.

3. Results

3.1. Induction of prostaglandin E_2 accumulation by ECL cells

ECL cells generated prostaglandin E_2 in response to interleukin-1β, tumor necrosis factor-α and bradykinin during the 24-h incubation (Fig. 2A–C). An incubation time of 24 h was chosen because incubation for 1 or 4 h caused only marginal accumulation of prostaglandin E_2 over basal (data not shown). Interleukin-1β (300 pg/ml) increased the amount of prostaglandin E_2 3-fold, with an EC_{50} concentration of 30 pg/ml. Tumor necrosis factor-α (10 ng/ml) increased the amount of prostaglandin E_2 2-fold (EC_{50} 1 ng/ml). Also bradykinin (1 μM) enhanced the amount of prostaglandin E_2 2-fold (EC_{50} 0.2 μM). Using maximally effective concentrations, potentiating effects were seen when combining interleukin-1β with

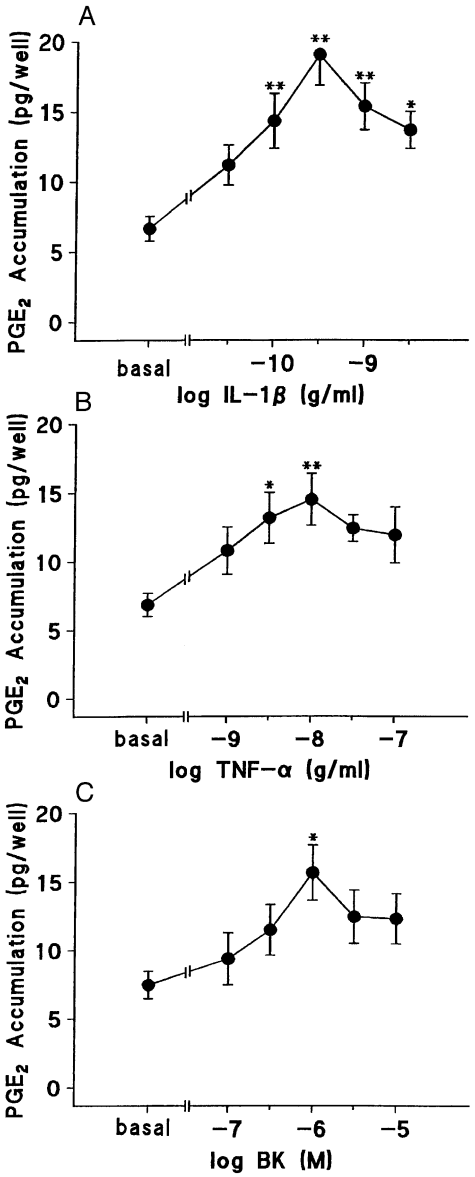

Fig. 2. Concentration–reponse curves demonstrating prostaglandin E_2 accumulation (24 h) in the medium surrounding the ECL cells in response to (A) interleukin-1β (IL-1β), (B) tumor necrosis factor-α (TNF-α) and (C) bradykinin (BK). All values were compared statistically to controls (Dunnett's test). Mean ± S.E.M., $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$.

bradykinin (9-fold increase) (Fig. 3). Combinations of interleukin-1β + tumor necrosis factor-α or bradykinin + tumor necrosis factor-α did not induce an effect greater than merely adding the effects of the individual agents (Fig. 3). The effect of interleukin-1β + tumor necrosis factor-α + bradykinin did not differ from that of interleukin-1β + bradykinin (data not shown). Gastrin-17 and PACAP-38 (at 10 nM, EC_{100} concentrations for histamine and pancreastatin release, Lindström et al., 1997) had no effect on the amount of prostaglandin E_2 accumulated (Fig. 3). The amounts of prostaglandin E_2 accumulated after exposing cultured, unfractionated M cells (2–3% ECL cells) to interleukin-1β + bradykinin did not differ

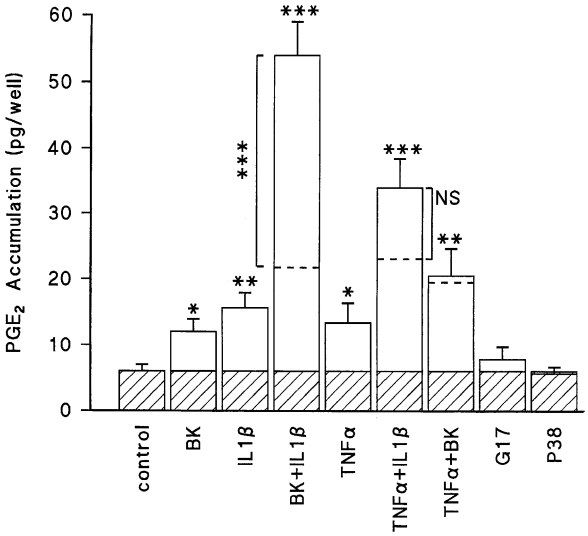

Fig. 3. The effects of various agents on the accumulation of prostaglandin E_2 in the medium surrounding the ECL cells (24 h) in response to **BK**: 1 μM bradykinin, **IL-1β**: 300 pg/ml interleukin-1β, **TNF-α**: 10 ng/ml tumor necrosis factor-α, **G17**: 10 nM gastrin-17, **P38**: 10 nM PACAP-38. Basal (control) prostaglandin E_2 level is indicated by the shaded area. The broken line through the bars BK + IL-1β, TNF-α + IL-1β and TNF-α + BK is the calculated additive effect of the two agents. A strong synergistic effect was seen with BK + IL-1β ($P < 0.001$), while the effects of TNF-α + IL-1β and TNF-α + BK appeared to be purely additive in nature. Statistical analysis was performed by comparing the different groups to controls (Dunnett's test) unless otherwise indicated by brackets. Mean ± S.E.M., $n = 10–12$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

from the amounts produced by purified ECL cells (Fig. 4). Also the basal release of prostaglandin E_2 from M cells did not differ from that of ECL cells.

3.2. Effects of hydrocortisone and indomethacin on prostaglandin E_2 accumulation

Hydrocortisone (10 nM; present throughout the 48-h culture period and during the subsequent 24-h incubation

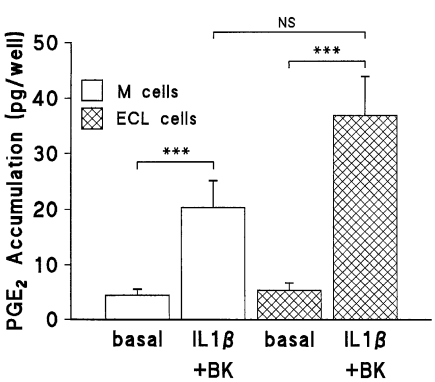

Fig. 4. The effects of 300 pg/ml interleukin-1β + 1 μM bradykinin on prostaglandin E_2 accumulation, comparing unfractionated mucosal (M) cells (containing 2–3% ECL cells, open bars) and purified ECL cells (> 90% ECL cells, hatched bars). Mean ± S.E.M., $n = 7–9$. $^{***}P < 0.001$ (Bonferroni's test).

period) prevented the accumulation of prostaglandin E_2 observed after challenging the ECL cells with interleukin-1β + bradykinin (Fig. 5A); basal levels were not affected. Hydrocortisone, at 10 nM, also prevented the accumulation of prostaglandin E_2 upon incubation with the various individual inflammatory mediators alone (data not shown). Indomethacin (1 μM; included during the 24-h incubation period) prevented the accumulation of prostaglandin E_2 in response to interleukin-1β + bradykinin (Fig. 5B), while basal levels of prostaglandin E_2 were unaffected.

3.3. Effects of hydrocortisone and indomethacin on ECL-cell histamine release

In the absence of hydrocortisone, basal histamine release was low and the response to gastrin-17 was greatly impaired (Fig. 6A). Also the response to PACAP-38 was reduced (data not shown). Increasing concentrations of hydrocortisone in the culture medium resulted in enhanced basal and gastrin-evoked histamine secretion.

Interleukin-1β + bradykinin suppressed basal ($P < 0.05$) and gastrin-stimulated ($P < 0.001$) histamine release from ECL cells during the 24-h incubation period compared to controls (Fig. 6B). Indeed, ECL cells incubated with interleukin-1β + bradykinin did not respond to gastrin at all. The inhibitory effect on basal and gastrin-stimulated secretion disappeared when including 1 μM indomethacin together with interleukin-1β and bradykinin, suggesting the involvement of prostaglandins.

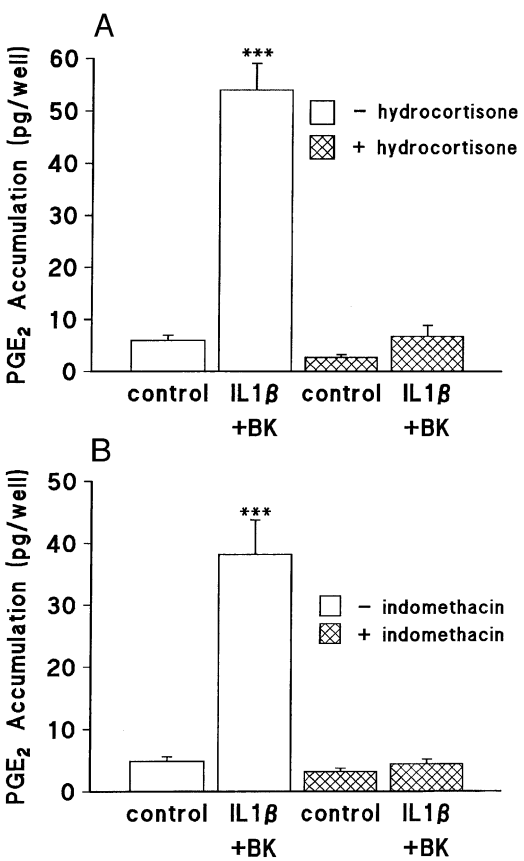

Fig. 5. The effects of hydrocortisone (10 nM) (A) and indomethacin (1 μM) (B) on basal and stimulated (300 pg/ml interleukin-1β + 1 μM bradykinin) ECL-cell prostaglandin E_2 accumulation. Mean ± S.E.M., $n = 8–12$. $^{***}P < 0.001$ (Bonferroni's test).

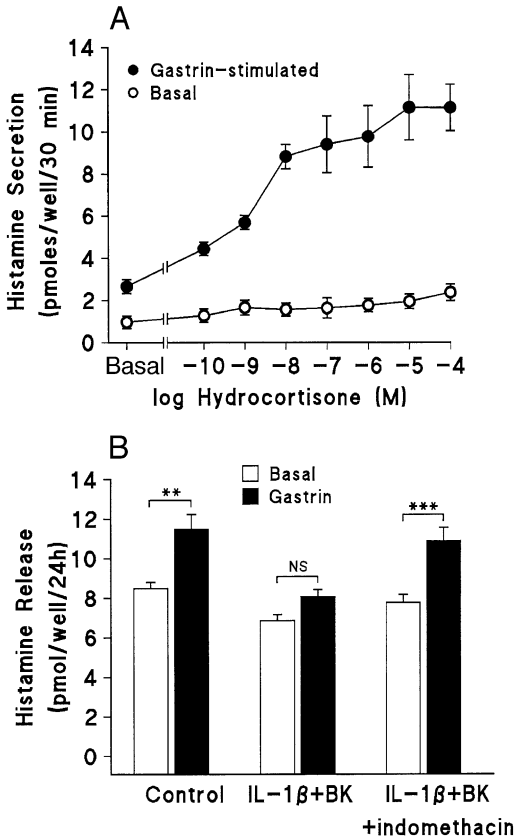

Fig. 6. (A) Effect of different concentrations of hydrocortisone in the culture medium (48 h) on the ability of ECL cells to release histamine. Open circles: basal release, closed circles: secretion evoked by 10 nM gastrin. (B) The effect of 300 pg/ml interleukin-1β + 1 μM bradykinin on basal and gastrin-evoked histamine release during 24 h. 10 nM gastrin was added during the last 30 min. The inhibitory effect of interleukin-1β + bradykinin on basal and gastrin-evoked secretion was reversed by adding 1 μM indomethacin to the medium. Mean ± S.E.M., $n = 8$. NS: not significant, $^{**}P < 0.01$, $^{***}P < 0.001$ (Bonferroni's test).

4. Discussion

Prostaglandin E_2 (both exogenously administered and endogenously produced) modulates gastric blood flow, stimulates bicarbonate and mucus secretion and blocks acid secretion (Whittle and Vane, 1987) either by direct inhibition of parietal cell activity or by inhibiting ECL-cell histamine secretion. We have previously shown that prostaglandin E_2 and its congeners are powerful inhibitors of ECL-cell histamine secretion (Lindström and Håkanson, 1998). It has also been suggested that prostaglandin E_2 impairs growth of ECL cells in situ (Kapraali et al., 1999). Based on the results of the present study we suggest that

not only are ECL cells inhibited by prostaglandin E_2, they also have the ability to generate the prostanoid.

Neither gastrin nor PACAP, known stimulators of ECL-cell secretion (Prinz et al., 1993; Lindström et al., 1997), seemed to accelerate prostaglandin E_2 formation. This seems to rule out the idea that prostaglandin E_2 is generated by the ECL cells in response to gastrin or PACAP to restrain the stimulated secretion of histamine by auto-feedback inhibition. Instead, inflammatory mediators, such as interleukin-1β, bradykinin and tumor necrosis factor-α, induced prostaglandin E_2 formation in isolated ECL cells. Interleukin-1β and tumor necrosis factor-α are produced in inflamed gastric mucosa and their expression is upregulated during healing of gastric ulceration (Noach et al., 1994). In fact, ECL cells are thought to express both interleukin-1 receptors (Prinz et al., 1997) and tumor necrosis factor-α receptors (Prinz et al., 1999). Interleukin-1β and tumor necrosis factor-α share many biological properties and at times they act synergistically (Okusawa et al., 1988; Pfeilschifter et al., 1989). A tendency for a potentiating interaction between interleukin-1β and tumor necrosis factor-α was noted with the ECL cells (not statistically significant). However, the combination of interleukin-1β and bradykinin induced a quite powerful synergistic effect (9-fold activation).

Interleukin-1β is a potent inhibitor of gastric acid secretion and protects against gastric injury produced by a wide variety of noxious stimuli (Wallace et al., 1990, 1992; Robert et al., 1991; Shibasaki et al., 1991; Perretti et al., 1992; Mugridge et al., 1995). Indeed, interleukin-1β has been shown to inhibit acid secretion from isolated parietal cells (Nompleggi et al., 1994; Beales and Calam, 1998; Schepp et al., 1998). Wallace et al. (1991), however, showed that interleukin-1β was an effective inhibitor of gastrin-evoked acid secretion but not of the secretion evoked by bethanechol or histamine, suggesting that interleukin-1β interferes specifically with the gastrin–ECL-cell axis. Pretreatment with interleukin-1β was also able to inhibit gastrin-induced histamine secretion from isolated ECL cells (Prinz et al., 1997). These earlier findings, together with our results, suggest that ECL cells are a target for interleukin-1β. Interestingly, interleukin-1β-stimulation has been demonstrated to induce the expression of inducible nitric oxide synthase (iNOS) and the subsequent increase in NO production in ECL cells (Prinz et al., 1997; Mahr et al., 2000). NO is a known stimulator of cyclooxygenase-enzymes (Salvemini et al., 1993). Hence, the stimulatory effect of interleukin-1β on prostaglandin E_2 production may be mediated by NO.

The acid-inhibitory and ulcer-protective effects of interleukin-1β are suggested to be prostaglandin-dependent (Uehara et al., 1989; Wallace et al., 1990; Shibasaki et al., 1991; Saperas et al., 1992). Indeed, two studies showed that interleukin-1β could accelerate the formation of prostaglandin E_2 in rat oxyntic mucosa in vivo (Robert et al., 1991) and in vitro (Mugridge et al., 1989). Since prostaglandin E_2 impairs both synthesis and release of ECL-cell histamine (Kondo et al., 1994; Lindström and Håkanson, 1998), it is conceivable that ECL cells respond to interleukin-1β with production of prostaglandin E_2 and with the consequent inhibition of histamine secretion (Fig. 7).

An acute challenge with bradykinin per se had no effect on the secretion of histamine and pancreastatin from ECL cells (Lindström et al., 1997). However, in the present study we found bradykinin (24-h incubation) to induce prostaglandin E_2 accumulation; the effect of interleukin-1β + bradykinin was even more pronounced. Previously, bradykinin has been reported to induce prostaglandin E_2 formation/release from isolated gastric cells (Chen et al., 1989; Brown et al., 1992).

Purified ECL cells produced at least as large amounts of prostaglandin E_2 (on a cell to cell basis) as a crude preparation of mucosal cells (M cells) (see Fig. 4). Prostaglandin E_2 generated by ECL cells (as well as by other cells in the mucosa) can inhibit parietal cells directly or indirectly via autocrine, inhibitory effects on ECL-cell histamine secretion. The demonstration of receptors to interleukin-1β (Prinz et al., 1997) and tumor necrosis factor-α (Prinz et al., 1999) on ECL cells would seem to support the view that these agents act directly on the ECL cells. However, an acute challenge with these agents does not seem to affect either basal or stimulated histamine/pancreastatin secretion (Lindström and Håkanson, 2001). Conceivably, their effects are delayed rather than immediate, causing prostaglandin E_2 to accumulate with time, eventually suppressing the activity of the ECL cells. Assuming that ECL cell-histamine mediates the acid-stimulating effect of gastrin, it appears conceivable that the acid-inhibiting effects of interleukin-1β (Wallace et al., 1991; Schepp et al., 1998) and tumor necrosis factor-α (Nakatsuji et al., 1990) reflect their ability to induce oxyntic mucosal

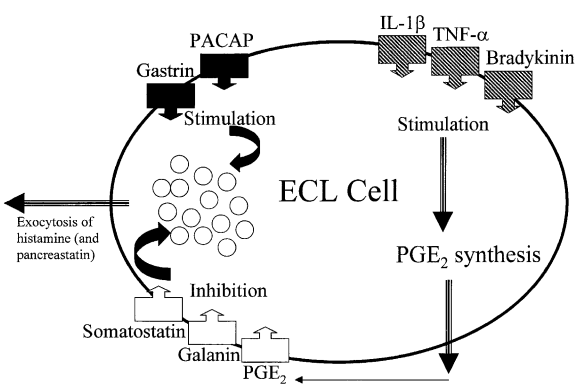

Fig. 7. Cartoon illustrating the stimulatory effects on the ECL cell of gastrin and PACAP and the inhibitory effects of somatostatin, galanin and prostaglandin E_2 on the process of exocytosis, and the stimulatory effects of inflammatory mediators such as interleukin-1β, tumor necrosis factor-α and bradykinin on the accumulation of prostaglandin E_2. The possibility of an autoinhibitory autocrine effect of prostaglandin E_2 on ECL-cell activity is suggested but not proven.

(ECL cell) prostaglandin E_2 accumulation with consequent suppression of ECL-cell histamine mobilization and acid secretion.

The amounts of prostaglandin E_2 generated by isolated ECL cells cultured without hydrocortisone are enough to inhibit gastrin-induced histamine secretion, based on the concentration–response curves reported previously (Lindström and Håkanson, 1998). Certainly, prostaglandin E_2 generated by ECL cells in response to interleukin-1β + bradykinin can be suspected to inhibit gastrin- or PACAP-stimulated ECL-cell histamine secretion. Indeed, in previous studies of histamine secretion, hydrocortisone was routinely added to the culture medium in concentrations that block prostaglandin E_2 formation. If hydrocortisone was not added, basal histamine secretion was suppressed and the response to gastrin (and to PACAP) was much impaired. Since gastrin- and PACAP-stimulated histamine secretion occurs within minutes, while prostaglandin E_2 synthesis requires longer periods of time, it was difficult to examine the role of endogenously produced prostaglandin E_2 in the present experimental setting. However, when measuring the amount of histamine released into the medium during 24 h of incubation, ECL cells maintained in the presence of interleukin-1β + bradykinin had a reduced basal histamine release compared to controls and failed to respond to gastrin. The effect of interleukin-1β + bradykinin was reversed by indomethacin suggesting that endogenously formed prostaglandin E_2 can indeed inhibit ECL-cell histamine secretion (Fig. 7).

An inhibitory action of glucocorticoids on cytoplasmic phospholipase A_2 ($cPLA_2$), the enzyme mediating arachidonic acid formation, could contribute to their inhibitory effect on prostaglandin E_2 formation (Dolan-O'Keefe and Nick, 1999). Indeed, several studies have shown that glucocorticoids are able to suppress interleukin-1β and tumor necrosis factor-α-induced $cPLA_2$ activity at both the mRNA level and the enzyme level in a variety of cell types (Lin et al., 1992; Newton et al., 1997; Dolan-O'Keefe and Nick, 1999). In addition, during the 48 h culture period, hydrocortisone may be expected to stimulate the expression of lipocortins which in turn can inhibit $cPLA_2$ activity (Almawi et al., 1996).

Recently, inflammation in the oxyntic mucosa was found to be associated with down-regulation of ECL-cell histidine decarboxylase (Mei and Sundler, 1999). Our observations seem to offer an explanation of this finding in that inflammatory mediators by inducing prostaglandin E_2 formation can be expected to impair the ability of the ECL cells to respond to gastrin and other stimulatory agents.

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