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INTRACELLULAR COUPLING OF PROSTAGLANDIN INHIBITION OF ACID SECRETION IN ISOLATED RABBIT GASTRIC PARIETAL CELLS

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Abstract—Acid secretion from isolated rabbit gastric parietal cells can be stimulated by gastric secretagogues, histamine (cyclic-AMP pathway) and carbachol (inositol phosphate pathway). Prostaglandins (PG) from E series are potent inhibitors of acid secretion. The intracellular mechanism of this inhibition was examined by using a stable PGE1-analogue, misoprostol. Aminopyrine (AP) accumulations due to histamine, IBMX and forskolin were dose-dependently inhibited by misoprostol, whereas a weak but significant biphasic effect on carbachol-induced AP accumulation was observed. The cyclic-AMP formation induced by histamine and IBMX were also inhibited by misoprostol in a non-competitive way. The potent effect of forskolin on cyclic-AMP levels was not modified by misoprostol in parietal cells, whereas it was potentiated in non-parietal cells. The inhibitory effect of misoprostol on AP accumulation was reduced by incubation of parietal cells with Bordetella pertussis toxin (IAP) but not with Cholera toxin (CT). Pretreatment of the cells with IAP did not alter cyclic-AMP levels of resting and histamine-stimulated parietal cells but abolished the inhibitory effect of misoprostol. Treatment with CT increased basal and histamine-stimulated cyclic-AMP levels and masked the inhibitory effect of misoprostol. The biphasic effect of misoprostol on carbachol-stimulated AP accumulation in parietal cells was confirmed on carbachol-stimulated phospholipase C activity and on $[Ca^{2+}]i$ stimulated by carbachol. These data confirm a direct and specific effect of the prostanoid on the Gi-subunit of the adenylate cyclase coupled to the histamine H_2-receptor, and a biphasic effect on the phospholipase C pathway of the parietal cells.

Histamine and acetylcholine are the most potent physiological secretagogues of acid secretion in isolated gastric parietal cells (for review, Refs 1 and 2). Many agents like somatostatin [3, 4], epidermal growth factor [5, 6] and prostaglandins (PGs) from the E series [7, 8] were shown to inhibit this secretory activity by a direct action on the parietal cell. The effect of these physiological inhibitors and particularly that of PGs of the E and I series [7] appeared to be selective of the histamine-induced gastric acid secretion. These PGs act on the adenylate cyclase system coupled to histamine-H2 receptor [9]. PGEs inhibited histamine-induced aminopyrine accumulation (AP accumulation was an index for *in vitro* acid secretion) and cyclic-AMP production but were inactive on AP accumulation due to db-cAMP. However, when parietal cells were stimulated by forskolin, which directly activated the catalytic subunit of the adenylate cyclase, some discrepancies appeared in the results according to the species studied: PGE_2 inhibited forskolin-stimulated acid production in the dog [10], whereas it was devoid of effect in the rat [11]. Bordetella pertussis toxin (IAP) and Cholera toxin (CT) are capable of discrimination between the two signal-transducing G-proteins (Gi and Gs): IAP caused ADP-ribosylation of Gi reducing its inhibitory effect on the catalytic subunit (C), whereas CT catalysed the ADP-ribosylation of Gs, leading to a permanent activation of the C subunit (for review, Refs 12–15). In isolated parietal cell from rabbit and dog, it was shown [16, 17] that IAP, which ADP-ribosylated a 41 kDa membrane protein in these cells, reduced the inhibitory effect of PGE_2 on histamine-stimulated acid secretion, presumably via ADP-ribosylation of a Gi subunit of the H_2 receptor-associated adenylate cyclase.

More recent studies showed that, in some tissues, PGs regulate the inositol phosphate pathway: PGE_2 stimulated PLC in fibroblasts [18], osteoblasts [19] and adrenal chromaffin cells [20], whereas PGI_2 inhibited PLC in platelets [21]. It was suggested that the IP_3-induced release of intracellularly bound Ca^{2+}, might mediate the inhibitory effect of PGs.

The purpose of this study will be to specify the intracellular effects of a stable PGE_1 analogue (misoprostol) following histamine (cyclic-AMP pathway) and carbachol (inositol phosphate pathway) stimulation of rabbit parietal cells.

MATERIALS AND METHODS

Materials. The sources of materials were the following: Prostaglandin E_1, carbachol, histamine, 3-isobutyl-1-methylxanthine (IBMX), forskolin, Pertussis toxin from Bordetella pertussis, Cholera toxin from Vibrio cholerae, N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (db-cAMP), *N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), FURA-2 AM and bovine serum albumin

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(fraction V) (BSA) were from the Sigma Chemical Co. (St Louis, MO). Misoprostol, [(±)-methyl-(11,13E)-11,16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oate] (SC-29233) 1:100 dispersion in hydroxypropylmethylcellulose) was from Searle (France). *myo*-[2-^{3}H]Inositol (10–20 Ci/mmol), dimethylamine-[^{14}C]aminopyrine (AP) (118 mCi/mmol), and [2,8-^{3}H]adenosine 3′,5′-cyclicmonophosphate (30–50 Ci/mmol) were from Amersham (Bucks, U.K.). Cyclic-AMP radioimmunoassay kit was from Dupont De Nemours-NEN Division (F.R.G.). Collagenase (0.8 units/mg, from *Clostridium histolyticum*) was from Serva Heidelberg, (F.R.G.). Earle's balanced salt solution was from Biomerieux (France). Medium A: 132 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM HEPES, 0.2% glucose, 0.2% BSA, 0.02% phenol red, pH 7.4. Medium B: Earle's balanced salt solution without bicarbonate containing 10 mM HEPES and 0.2% BSA, pH 7.4. PBS: phosphate buffer saline, pH 7.4.

Cell isolation. Cell isolation was carried out following the collagenase/EDTA procedure as already described [22]. Briefly, rabbit fundic mucosa was scraped, minced and extensively rinsed with PBS. Tissue fragments were dispersed in medium A (gassed with 95% O_2/5% CO_2) containing 0.25 mg/mL collagenase and 0.3 mg/mL pronase. After 15 min incubation at 37°, tissue fragments were allowed to settle and the medium was discarded. The fragments were rinsed twice in Ca^{2+}/Mg^{2+}-free medium A containing 2 mM EDTA and then incubated in this medium for 10 min. Mucosal fragments were transferred into medium A containing fresh collagenase (0.25 mg/mL) and incubated for 15 min at 37° under continuous gassing (95%O_2/5%CO_2). A new similar incubation was carried out for 20 min in medium A containing 0.25 mg/mL collagenase. The cell suspension was half-diluted with medium A, passed through a nylon mesh and the filtrate was centrifuged for 5 min at 200 *g*, then washed twice with medium B and resuspended. This procedure generated about 5×10^7 cells per g of wet mucosa with viability (trypan blue exclusion) always greater than 90%.

Cell sorting was performed using a BECKMAN elutriator rotor JE6-B. The cell suspension (2 to 4×10^8 cells in medium B) was loaded into the elutriation chamber at a flow rate of 17 mL/min and with a rotor speed of 2300 RPM. Under these conditions, erythrocytes, cell fragments and small cells (less than 9 microns) were eliminated. Three fractions were collected at a rotor speed of 2100 rpm by increasing the flow rate from 24 mL/min (fraction F1) to 43 mL/min (fraction F2) and 67 mL/min (fraction F3). The first fraction contained 80 ± 5% of mucus cells and the third fraction mainly contained parietal cells (70 ± 5%) and some clumps of mucus cells. The size distributions of the different fractions were tested by light scattering (FACS) and Coulter counting.

Aminopyrine accumulation. For aminopyrine accumulation experiments (a weak base which can accumulate into the acidic spaces of the parietal cell as a function of the pH gradient) [22], cells from fraction F_3 suspended in medium B (1.5×10^6 cells/mL) were incubated with or without stimulants and inhibitors at 37° for 20 min under continuous gassing (95% O_2/5% CO_2) in the presence of 0.05 μCi [^{14}C]aminopyrine (3 μM) in a final volume of 1.5 mL. Triplicate samples (0.4 mL of each tube) were layered over 0.9 mL ice-cold B medium and centrifuged for 1 min in an Eppendorf microfuge. The pellets were suspended in 0.1 mL 10% $HClO_4$ and the radioactivity was measured in a KONTRON liquid scintillation counter. AP accumulation was expressed as the per cent of total radioactivity associated with cells.

Cyclic-AMP determinations. Cyclic-AMP contents of isolated cells (fractions F_1 and F_3) from gastric fundic mucosa were determined by radioimmunoassay as follows: the cell suspension (1.25×10^6 cells/mL) was preincubated for 5 min at 30° in medium B. Then, 0.4 mL of the suspension was incubated in duplicate with various concentrations of histamine and/or misoprostol in the presence of 10 μM IBMX for 5 min at 30°.

The reaction was stopped by adding 0.1 mL 40% trichloracetic acid (TCA) and 4000 cpm per tube [^{3}H]cyclic-AMP and the tubes were centrifuged at 2500 *g* for 15 min at 4°. Supernatants were collected and TCA was extracted four times with 5 mL water-saturated ether. Ether phases were evaporated in a water bath at 60° for 30 min and residues were dissolved in 0.5 mL acetate buffer (0.06 M, pH 6.2). The radioactivity of 0.05 mL of each tube was measured in a liquid scintillation counter for evaluation of cyclic-AMP recoveries. Cyclic-AMP contents were then determined by radioimmunoassay in duplicate on 0.1 mL-aliquots. The amounts of cyclic-AMP were expressed after correction for recovery in picomoles cyclic-AMP generated per 10^6 cells and per 5 min incubation.

Inositol phosphates contents. Inositol phosphates analysis was performed as follows: cells (15×10^6 per mL) from fraction F_3 were incubated for 3 hr at 37° in oxygenated (95% O_2/5% CO_2) medium B with 40 μCi/mL *myo*-[^{3}H]inositol as previously described [23]. After two washings, cells were incubated for 15 min with 10 mM LiCl. Then, agents were added and incubation continued for 20 min at 37° under continuous gassing (O_2/CO_2). Radiolabelled inositol phosphates accumulated into cells were separated by ion-exchange chromatography (DOWEX AG-1X8) after extraction by perchloric acid and neutralization.

Intracellular calcium measurements. The fluorescent probe FURA-2 AM was used to determine intracellular free Ca^{2+} as described by Chew et al. [24]. Cells (2.5×10^6 per mL) were incubated for 20 min at 37° with 2 μM FURA-2 AM in medium B. Then, cells were washed by centrifugation (100 *g*) with BSA-free medium B and cell pellets were resuspended in 3 mL BSA-free medium B. Agents were added at the time of the fluorescence measurement. Emission fluorescence was measured at 540 nm under 340 nm excitation. Intracellular Ca^{2+} concentrations were expressed in nmoles per 10^6 cells after calibration of the emission fluorescence.

All statistical evaluations were carried out with the paired Student's *t*-test.

RESULTS

Aminopyrine accumulation

The PGE_1 analogue inhibited histamine and

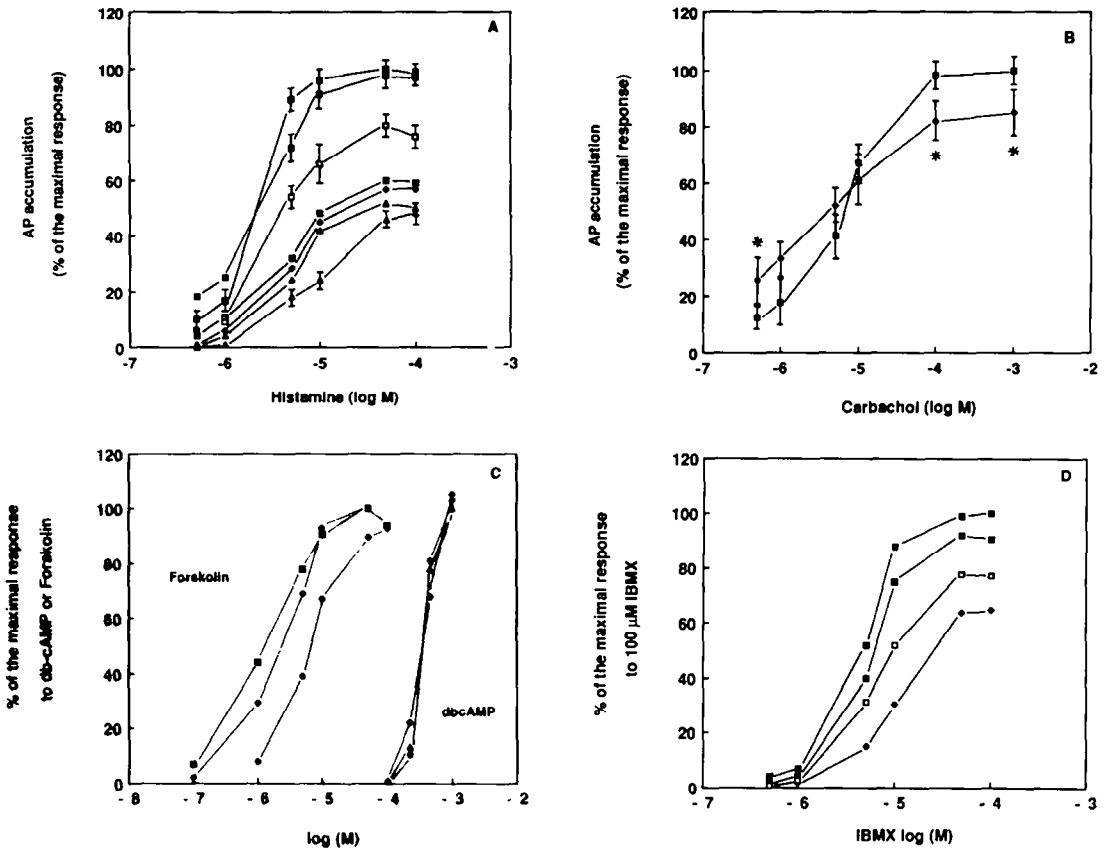

Fig. 1. Effect of misoprostol on secretagogue-stimulated AP accumulation. Cells (fraction F3, 1.5×10^6 per mL) were incubated in the presence of 3 µM [^{14}C]aminopyrine (AP) and indicated concentrations of stimulants and/or misoprostol under O_2/CO_2 for 20 min at 37°. AP accumulation was expressed as the per cent of total radioactivity associated with cells. (A) Histamine; (B) carbachol (± SD, * $P < 0.05$); (C) db-cAMP and forskolin; (D) IBMX. For all figures, misoprostol concentrations are indicated with the following symbols: (■) none; (▨) 1 nM; (□) 10 nM; (⊡) 100 nM; (◇)1 µM; (△) 2 µM; (▲) 10 µM. The data shown are representative of five experiments performed in triplicate.

IBMX stimulations of AP accumulation into parietal cells in a dose-dependent manner and this inhibition was non-competitive as regard to stimulants.

Misoprostol was slightly more effective in inhibiting histamine than IBMX stimulations (50% for maximal stimulation by histamine and 40% for maximal stimulation by IBMX with 1 µM misoprostol). For submaximal concentrations of histamine (5 µM) or IBMX (10 µM), misoprostol inhibited by 70 ± 5% AP accumulation induced by both secretagogues (Fig. 1A and D).

Stimulation of AP accumulation by forskolin was also inhibited by misoprostol in a dose-dependent manner, but the effect was reversed by high forskolin concentrations, in agreement with a competitive-like interaction. In contrast, the PGE_1 analogue did not modify AP accumulation induced by db-cAMP (Fig. 1C).

Misoprostol showed a weak but significant biphasic effect on carbachol-stimulated AP accumulation: it was stimulant at low concentrations of carbachol (0.5–5 µM) and inhibitor at higher carbachol concentrations (10 µM–0.5 mM) (Fig. 1B). This effect was maintained in the presence of cimetidine (histamine-H2 receptor antagonist).

Cyclic-AMP production

Cyclic-AMP generation was studied in fraction F_3 and in fraction F_1. Histamine dose-dependently increased cyclic-AMP contents of parietal cells, the maximal value being obtained at 0.1 mM histamine. When cells were incubated in the presence of histamine plus 10 µM IBMX, misoprostol inhibited this accumulation in a dose-dependent fashion (Fig. 2) and this inhibition was correlated with the inhibition of AP accumulation evaluated in the same conditions (Fig. 3). Although histamine (10 µM) caused a dramatic increase in cyclic-AMP content of fraction F_3 (3-fold), its effect was weak (1.8-fold) in fraction F_1 (Fig. 4). In contrast, the PGE_1 analogue (1 µM) produced a strong increase in fraction F_1 (7-fold) and a weak increase in fraction F_3 (1.3-fold). Moreover, histamine and misoprostol had additive effects on cyclic-AMP levels in fraction F_1, whereas, in fraction F_3, misoprostol inhibited histamine stimulation. The natural prostaglandin E_1 showed a similar

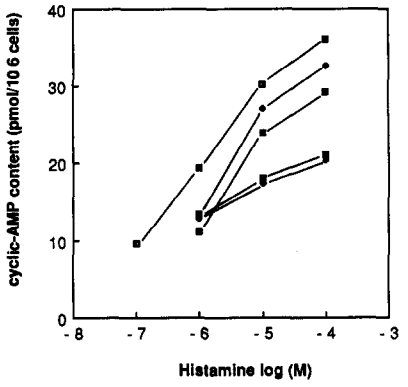

Fig. 2. Effects of misoprostol on histamine stimulation of cyclic-AMP contents. Same legend as in Fig. 2. Misoprostol concentrations are indicated with the following symbols: (⊡) none; (◆) 0.1 nM; (■) 10 nM; (■) 1 μM; (◇) 5 μM. The data shown are representative of four experiments performed in duplicate.

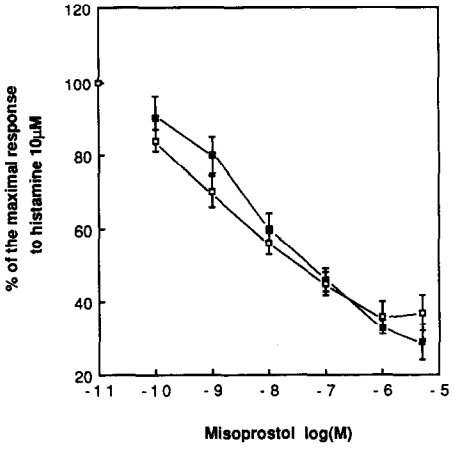

Fig. 3. Comparative dose–inhibition curves of misoprotol on histamine-stimulated AP accumulation and cyclic-AMP contents. AP accumulation and cyclic-AMP contents are expressed as per cent of the maximal response to histamine 10 μM. The data shown represent the mean ± SD of four experiments. (■) AP accumulation; (□) cyclic-AMP.

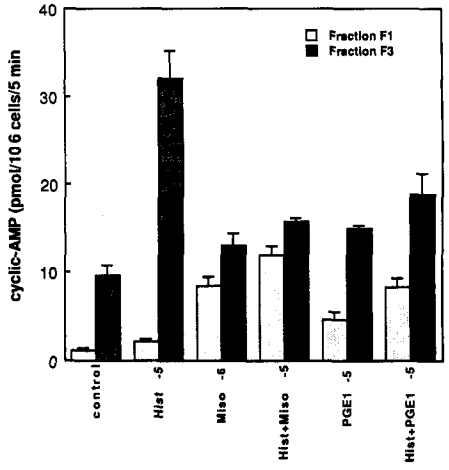

Fig. 4. Comparative effects of misoprostol and natural PGE_1 on basal and histamine-stimulated cyclic-AMP contents of cells from fractions F_1 and F_3. Cells (1.25×10^6 cells per mL) were incubated in the presence of histamine and/or misoprostol or PGE1 at indicated concentrations and with 10 μM IBMX for 5 min at 30°. Cyclic-AMP was then determined by radioimmunoassay as indicated in (Materials and Methods) and expressed as pmoles/10^6 cells/5 min. The data shown represent the mean ± SD of four experiments performed in duplicate.

Table 1. Effects of misoprostol on histamine and on forskolin-induced cyclic-AMP accumulations

	Control	+ Misoprostol (1 μM)
	(pmoles/10^6 cells/5 min)	
F3 cells		
Basal	9.8 ± 1.8	14.2 ± 3.2
Histamine (10 μM)	31.8 ± 7.1	16.0 ± 1.4
Forskolin (10 μM)	42.1 ± 3.0	71.1 ± 7.0
F1 cells		
Basal	1.6 ± 0.8	5.4 ± 1.1
Forskolin (10 μM)	8.0 ± 2.1	53.1 ± 11.0

F1: cells from fraction F1; F3: cells from fraction F3. Same legend as in Fig. 2. The data shown, expressed in pmoles/10^6 cells/5 min, represent the mean ± SD of four experiments performed in duplicate.

but less potent effect on the two cellular fractions (Fig. 4).

Forskolin dose-dependently increased cyclic-AMP contents in both fractions F_1 and F_3. Misoprostol potentiated forskolin stimulation in fraction F_1 (560%) but showed a weaker effect on fraction F_3. It is likely that the effect of misoprostol on this cellular fraction is due to the presence of about 20% mucus cells, in agreement with a specific effect of the prostanoids on parietal cells (Table 1).

Bordetella pertussis and Vibrio cholerae toxins treatments

When the enriched-parietal cell fraction was preincubated for 4 hr at 37° under O_2/CO_2 gassing, histamine-stimulated AP accumulation was still inhibited by 10 μM misoprostol (63 ± 3% of inhibition). But, when these cells were preincubated for the same period of time in the presence of 1.5 μg/mL IAP, misoprostol was less effective as inhibitor of histamine stimulation (29 ± 5% of inhibition). IAP alone was devoid of effect both on basal and on histamine-stimulated AP accumulations (Table 2A). In contrast, when the cells were preincubated for 2 hr with 0.12 μM CT, both basal and histamine-stimulated AP accumulations were increased. In the meantime, when cells were treated with CT, the inhibitory effect of misoprostol on histamine-stimulated AP accumulation was not affected (Table 2B).

Pretreatment of isolated parietal cells with IAP did not modify basal or histamine-induced cyclic-AMP production, but this pretreatment abolished the inhibitory effect of misoprostol (Table 2A). In

Table 2. Effects of IAP and CT on misoprostol-induced inhibition of AP accumulation and of cyclic-AMP contents

	AP accumulation (%)		Cyclic-AMP contents (pmoles/10^6 cells/5 min)	
	Control	+ Misoprostol (1 μM)	Control	+ Misoprostol (1 μM)
(A)				
Basal	4.2 ± 0.7	3.9 ± 0.5	3.2 ± 0.7	3.5 ± 0.8
Histamine (10 μM)	8.3 ± 1.2	5.4 ± 1.5	6.2 ± 2.2	4.7 ± 1.5
IAP (1.5 μg/mL)	4.7 ± 0.6	4.6 ± 0.5	3.4 ± 0.6	3.5 ± 0.6
+ Histamine (10 μM)	8.7 ± 0.8	7.1 ± 1.8	6.4 ± 2.2	6.6 ± 2.1
(B)				
Basal	6.2 ± 0.7	6.3 ± 0.5	3.2 ± 0.7	3.5 ± 0.8
Histamine (10 μM)	13.0 ± 1.2	8.5 ± 1.5	6.2 ± 2.2	4.7 ± 1.5
CT (0.12 μM)	9.5 ± 1.3	8.5 ± 1.3	30.1 ± 2.9	29.0 ± 3.1
+ Histamine (10 μM)	16.9 ± 0.9	10.4 ± 1.2	33.2 ± 3.5	31.7 ± 3.2

(A) Effect of IAP. Preincubation with IAP (1.5 μg/mL) was carried out for 4 hr before addition of stimulants. (B) Effect of CT. Preincubation with CT (0.12 μM) was performed for only 2 hr. AP accumulation was expressed as % of the total radioactivity associated with cells and cyclic-AMP contents as pmoles/10^6 cells/5 min. The data shown represent the mean ± SD of three separate experiments.

contrast, pretreatment of cells with CT induced a strong increase of basal (3.2 to 30.1 pmoles cyclic-AMP/10^6 cells/5 min) and of histamine-stimulated (6.2 to 33.2 pmoles cyclic-AMP/10^6 cells/5 min) cyclic-AMP accumulations and the inhibition by misoprostol was considerably reduced (60–8% inhibition) (Table 2B). This result is inconsistent with AP accumulation data where the treatment of parietal cells by CT did not modify the inhibitory effect of misoprostol on acid secretion. This discrepancy can be due to the stimulating effect of CT on adenylate cyclase systems which are not related to acid production and not regulated by PGs in an inhibitory way.

Inositol phosphates

Cholinergic stimulation of parietal cells is known to be mediated by an activation of a phospholipase C with a concomitant generation of inositol phosphates (IPs) and a subsequent rise of intracellular free Ca^{2+}. Carbachol dose-dependently increased IP formation with a maximum for 1 mM carbachol [22, 25]. The action of misoprostol was weak but permanent and biphasic, similar to that observed for AP accumulation. Histamine did not show any significant effect on the PLC activity of the parietal cell (Fig. 5A).

Intracellular free calcium

Carbachol dose-dependently stimulated intracellular Ca^{2+} levels with a maximal value for 100 μM carbachol (Fig. 6). Misoprostol by itself did not modify basal $[Ca^{2+}]i$ but enhanced $[Ca^{2+}]i$ released by low concentration of carbachol (1 μM), and inhibited $[Ca^{2+}]i$ released by high carbachol concentration (> 10 μM) (Fig. 5B). These results were similar to those obtained on carbachol-induced AP and IP accumulations.

DISCUSSION

The presence of different cell types in gastric mucosa is the major limitation to the study of the cellular regulation of gastric acid secretion. The development of an isolated cell preparation from fundic mucosa of the rabbit subsequently enriched in parietal (70 ± 5%) or mucus (80 ± 5%) cells by centrifugal elutriation, allowed us to clarify the hormonal regulation of the parietal cell itself. These cells were fully sensitive to physiological gastric secretagogues, histamine, carbachol and gastrin [26].

The effect of various types of prostaglandins on gastric acid secretion has been evaluated on isolated cells by the measurement of [^{14}C]aminopyrine accumulation in the canalicular system of the rabbit parietal cell. PGE_1, E_2, $F_{2\alpha}$, D_2 and TXB_2 were devoid of effect on the spontaneous AP accumulation (results not shown). Similar observation came from Nylander *et al.* [25] with PGE_1 on isolated rabbit gastric glands. This result confirms the absence of a direct effect of the PGs on the cellular mechanism of acid production (direct inhibition of the proton pump, for instance). We found a potent non-competitive inhibitory effect against histamine stimulation with prostaglandins from the E series [27]. Misoprostol, a stable PGE_1-analogue (SC 29233) used in this study, was described as a potent antisecretory and antiulcer agent in animals and humans [28, 29]. In our isolated cell system, this prostanoid was shown to be more potent than the natural PGE_1 and the inhibitory effect was mainly observed on histamine stimulation [27], whereas the effect on carbachol stimulation was weak and biphasic, as already found by Soll in dog parietal cells [30]. In this paper, we tried to clarify the intracellular biochemical pathway of this inhibitory effect.

The main observation came from AP accumulation experiments in parietal cells stimulated by agents which activated adenylate cyclase (forskolin) or increased intracellular cyclic-AMP levels (IBMX, db-cAMP): the specific inhibition by PGs of forskolin or IBMX stimulation, the absence of effect on db-cAMP stimulation and the non-competitive inhibition of histamine-stimulated cyclic-AMP levels

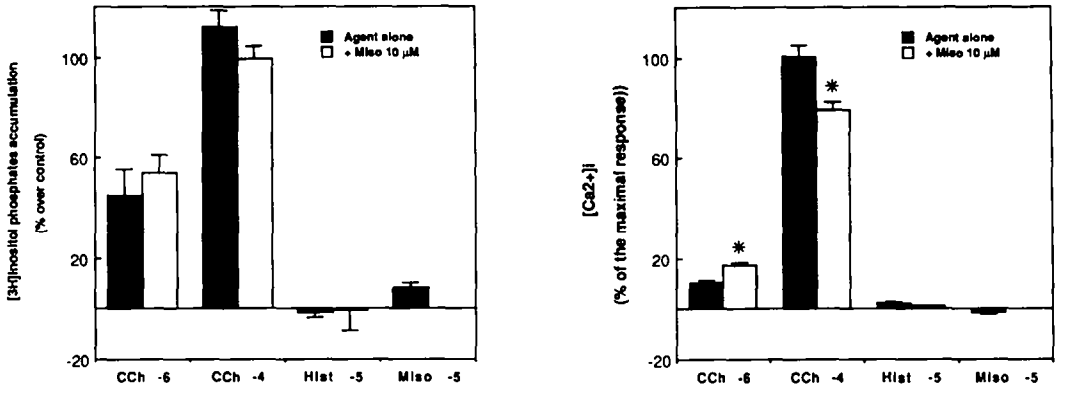

Fig. 5. (A) Effect of misoprostol on carbachol-induced inositol phosphates accumulation. Total inositol phosphates were determined from cells of fraction F_3 incubated for 3 hr at 37° with 40 µCi/mL *myo*-[^{3}H]inositol and after washing for additional 15 min with 10 mM LiCl. Agents (histamine 10 µM; carbachol 1 µM; carbachol 100 µM) ± misoprostol 10 µM were then added to the incubation medium for 20 min under gassing (O_2/CO_2). Radiolabeled inositol phosphates were separated by ion-exchange chromatography on DOWEX AG-1X8. Results are expressed as % of IPs accumulation over control value (resting cells). The data shown represent the mean ± SD of four experiments performed in duplicate. (B) Effect of misoprostol on carbachol-induced intracellular Ca^{2+} modification. Cells (2.5×10^6 per mL) were incubated for 20 min at 37° with 2 µM FURA-2 AM. After washing, cell fluorescence (340 nm excitation, 540 nm emission) was measured in the presence of agents and expressed as [Ca^{2+}]i after calibration with triton X-100 and 60 mM EGTA. Results are expressed as % of the 100 µM carbachol response. The data shown represent the mean ± SD of four experiments (* $P < 0.05$).

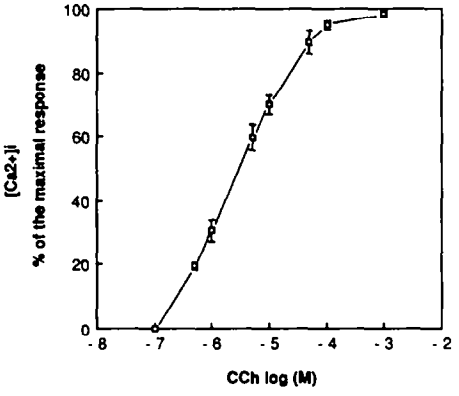

Fig. 6. Dose–response curve of carbachol on intracellular Ca^{2+} concentrations. Same legend as in Fig. 5B.

suggested that prostanoids may interact either with G-protein subunits or with the catalytic unit of the adenylate cyclase.

When acid secretion (AP accumulation) was stimulated by carbachol, misoprostol had a weak but significant biphasic effect with regard to carbachol concentrations. The same result was obtained on intracellular free Ca^{2+} release and on inositol phosphate production. This phenomenon involves a weak activation of the calcium pathway. It can be interpreted following two hypotheses: (i) misoprostol could exert a direct effect on PKC as it was shown with arachidonic acid [31]. Indeed, the activation of PKC by phorbol esters lead to either a stimulant or an inhibitory effect on acid secretion [16]. (ii) Misoprostol could potentiate PLC activity stimulated by carbachol. A more detailed study remains to be performed to define the type of inositol phosphate isomer involved in this stimulation.

On the non-parietal cell fraction (F_1), the results confirmed that histamine receptors are not coupled to adenylate cyclase, whereas forskolin and misoprostol had a stimulating effect on this system. Moreover, misoprostol strongly potentiated the effect of forskolin. This can explain the unexpected potentiating effect of PGs on forskolin-stimulated cyclic-AMP levels in the parietal cell fraction (F_3) which also contains about 30% of mucus, chief and endocrine cells.

In order to specify the type of regulatory protein involved in the inhibitory effect of PGs, the effects of Cholera toxin (CT) and of Bordetella pertussis toxin (IAP) were studied. CT, which caused ADP-ribosylation of the Gs subunit, increased basal and histamine stimulations of both AP accumulation and cyclic-AMP production. After treatment of parietal cells with CT, prostanoids still inhibited histamine-induced AP accumulation, whereas the rise of cyclic-AMP levels was not significantly affected. CT and forskolin are known potent stimulants of adenylate cyclase systems in many cell types, and, in turn, activate various cyclic-AMP-dependent protein-kinases (type I and II PKA) [24]. In parietal cells, only one type of PKA (cytosolic type I PKA) was shown to be related to acid production [24]. In addition, CT might also stimulate the adenylate cyclase of the contaminating mucus, chief and endocrine cells. This could explain the lack of efficacy of misoprostol on both forskolin- and CT-induced cyclic-AMP stimulations at concentrations where it still inhibited AP accumulation.

When parietal cells were incubated in the presence of IAP, the inhibitory effect of PGs was reduced on

both histamine-induced AP accumulation and cyclic-AMP production. As previously shown in isolated parietal cell from rabbit, rat and dog [8, 16, 17] with PGE_2, we can suppose that IAP reduced the inhibitory effect of misoprostol on histamine-stimulated acid secretion via ADP-ribosylation of a Gi subunit of the H_2 receptor-associated adenylate cyclase.

In conclusion, misoprostol shows a high specificity of action on the Gi-subunit of the adenylate cyclase coupled to histamine-H_2 receptor in rabbit parietal cells. This effect on Gi does not exclude an effect on the catalytic subunit which could explain the competitive-like inhibition of forskolin-induced AP accumulation. The potentiating parallel effect of PGs from E series on the inositol phosphate pathway remains to be clarified.

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REFERENCES

1. A. H. Soll and T. Berglindh, in *Physiology of the Gastrointestinal Tract* (Ed. Johnson L. R.), p. 883. Raven Press, New York, (1987).
2. M. J. Sanders and A. H. Soll, *Ann. Rev. Physiol.* **48**, 89 (1986).
3. S. Batzri and J. Dyer, *Biochim. biophys. Acta* **675**, 416 (1981).
4. C. Gespach, C. Dupont, D. Bataille and G. Rosselin, *FEBS Lett.* **114**, 247 (1980).
5. S. J. Konturek, M. Cieszkowski, J. Jamorek, J. Konturek, T. Brzozowski and H. Gregory, *Am. J. Physiol* **246**, G580 (1984).
6. B. J. Reichstein, C. Okamoto and J. G. Forte, *Fed. Proc.* **43**, 4607 (1984).
7. A. H. Soll, *J. clin. Invest.* **65**, 1222 (1978).
8. M. M. Atwell and P. J. Hanson, *Biochim. biophys. Acta* **971**, 282 (1988).
9. W. Schepp, H. K. Heim and H. J. Ruoff, *Agents Actions* **13**, 200 (1983).
10. A. H. Soll, MC. Y. Chen, D. A. Amirian, M. Toomey and M. J. Sanders, *Am. J. Physiol.* **81**, suppl. 2A, 5 (1986).
11. G. C. Rosenfeld, *J. Pharmac. exp. Ther.* **237**, 513 (1986).
12. A. G. Gilman, *J. clin. Invest.* **73**, 1 (1984).
13. T. Murayama and M. Ui, *J. biol. Chem.* **258**, 3319 (1983).
14. T. Katada, M. Oinuma and M. Ui, *J. biol. Chem.* **261**, 5215 (1986).
15. T. Katada, K. Kusakabe, M. Oinuma and M. Ui, *J. biol Chem.* **262**, 11897 (1987).
16. M. R. Brown and C. S. Chew, *Can. J. Physiol. Pharmacol.* **65**, 1840 (1987).
17. M. C. Y. Chen, D. A. Amirian, M. Toomey, M. J. Sanders and A. H. Soll, *Gastroenterology* **94**, 1121 (1988).
18. T. Yamashita and Y. Takai, *J. biol. Chem.* **262**, 5536 (1987).
19. R. W. Farndale, J. R. Sandy, S. J. Atkinson, S. R. Pennington, S. Meghji and M. C. Meikle, *Biochem. J.* **252**, 263 (1988).
20. H. Yokohama, M. Negishi, K. Sugama, H. Hayashi, S. Ito and O. Hayashi, *Biochem. J.* **255**, 957 (1988).
21. S. P. Watson, R. T. McConnell and E. G. Lapetina, *J. biol. Chem.* **259**, 13199 (1984).
22. R. Magous, J. C. Galleyrand, B. Baudière, A. Léonard, A. Choquet and J. P. Bali, in *Gastrin and Cholecystokinin. Chemistry, Physiology and Pharmacology* (Eds. J-P Bali and J. Martinez), p. 153 (1987).
23. B. Baudière, G. Guillon, J. P. Bali and S. Jard, *FEBS Lett.* **198**, 321 (1986).
24. C. S. Chew, *J. biol. Chem.* **260**, 7540 (1985).
25. O. Nylander, T. Berglindh and K. J. Obrink, *Am. J. Physiol.* **250**, G607 (1986).
26. R. Magous and J. P. Bali, *Regul. Peptides* **7**, 233 (1983).
27. A. Choquet, R. Magous, J. C. Galleyrand and J. P. Bali, *Diab. Mét.* **13**, 164 (1987).
28. E. R. Dajani, D. R. Driskill, R. G. Bianchi, P. W. Collins and R. Pappo, *Dig. Dis. Sci.* **21**, 1049 (1976).
29. D. E. Wilson, E. Quatros, T. Rajapaksa, A. Adams and M. Noar, *Dig. Dis. Sci.* **21**, (suppl). 126S (1986).
30. A. H. Soll, *J. clin. Invest.* **65**, 1222 (1980).
31. P. J. Blackshear, A. C. Nairn and J. F. Kuo, *Faseb J.* **2**, 2957 (1988).