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Effect of gastric secretagogues on the formation of inositol phosphates in isolated gastric cells of the rat

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1 The effects of compounds affecting gastric acid secretion were studied on the formation of inositol phosphates after prelabelling with [^{3}H]-inositol in enriched gastric parietal cells of the rat, prepared by isopycnic centrifugation with Percoll.

2 In cell preparations with 60 to 70% parietal cells, carbachol (10^{-6}–10^{-2} M) enhanced the accumulation of [^{3}H]-inositol monophosphate ([^{3}H]-IP1), [^{3}H]-inositol bisphosphate ([^{3}H]-IP2) and [^{3}H]-inositol trisphosphate ([^{3}H]-IP3) in a concentration-dependent manner, an effect which was antagonized by 10^{-8} M atropine.

3 Li^+ (0.5–30 mM) enhanced the basal and carbachol-induced accumulation of all three [^{3}H]-inositol phosphates, the formation of [^{3}H]-IP1 being more sensitive to Li^+ than those of [^{3}H]-IP2 and [^{3}H]-IP3.

4 The concentration of Ca^{2+} in the incubation medium did not affect the relative stimulation of the accumulation of [^{3}H]-inositol phosphates by carbachol, although the basal formation was higher in the presence of Ca^{2+} in the medium. In the absence of added Ca^{2+}, the incorporation of [^{3}H]-inositol into phospholipids was increased – an effect which was further enhanced by the addition of EGTA to the medium.

5 Gastrin and pentagastrin (10^{-8}–10^{-5} M) enhanced the formation of [^{3}H]-inositol phosphates, although they were clearly less effective than carbachol. Histamine (10^{-6}–10^{-3} M) had no effect of its own, but slightly attenuated the effect of carbachol. Cholecystokinin octapeptide (10^{-9}–10^{-6} M) slightly increased the formation of [^{3}H]-inositol phosphates.

6 Indomethacin (10^{-4} M) had no consistent effect on the basal and carbachol-induced accumulation of [^{3}H]-inositol phosphates, nor did prostaglandin E_2 (10^{-5} M) modify it.

7 Adrenaline (10^{-3} M), 5-hydroxytryptamine (10^{-3} M), forskolin (10^{-5} M), vasopressin (10^{-5} M), angiotensin II (10^{-5} M) and bombesin (10^{-9}–10^{-6} M) were all without effect.

8 We suggest that the hydrolysis of inositol phospholipids may be involved in the signal transduction mechanism by which the activation of the muscarinic and gastrin receptors on the parietal cells leads to Ca^{2+} mobilization and the stimulation of hydrogen ion secretion.

Introduction

The function of the acid-secreting parietal cells in the gastric mucosa is regulated by neuronal and humoral factors. Acetylcholine released from the vagal nerve endings activates muscarinic M_1-receptors at the parietal cells resulting in the stimulation of acid secretion. Gastrin released by various factors from the antral G-cells and histamine originating from the gastric mucosal histaminocytes exert their secretagogue action via specific gastrin and histamine H_2-receptors, respectively. On the other hand, it has also been proposed that the effect of acetylcholine and gastrin, may be mediated, at least in part, by the release of histamine (for review, see Soll, 1981). In addition to these 'classical' physiological secretagogues, a number of gastrointestinal hormones affecting the parietal cell function have been identified more recently (Walsh, 1981).

The secondary effector mechanisms for the action of gastric secretagogues have been intensively investigated in recent years. As a consequence, there is now evidence that cyclic AMP mediates the secretory action of histamine on the parietal cells (Sachs & Berglindh, 1981; Schepp *et al.*, 1983; Schepp & Ruoff, 1984), although the recent report of Sewing *et al.*,

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(1985) does not support this view. In contrast to histamine, the secretory response to muscarinic receptor agonists or gastrin is not related to changes in the intracellular level of this nucleotide, so that their second messenger system(s) in the parietal cell must be other than cyclic AMP. In fact, the elevation of the cytosolic level of Ca^{2+} in the parietal cells seems to be involved in the transduction of the muscarinic or gastrin stimulation of hydrogen ion secretion, but not that induced by histamine (Sachs & Berglindh, 1981; Muallem & Sachs, 1984).

Recent findings indicate that the hydrolysis of inositol phospholipids is linked to Ca^{2+} mobilization by hormonal agents and neurotransmitters in many tissues (for review, see Berridge & Irvine, 1984; Hirasawa & Nishizuka, 1985). Of special interest with respect to the stimulus-secretion coupling in the parietal cells are the findings that acetylcholine which stimulates the exocrine secretion from the pancreas, parotid and avian salt gland also induces hydrolysis of inositol phospholipids in these tissues (Berridge & Irvine, 1984). Therefore, in the present work we have studied the possible involvement of the hydrolysis of inositol phospholipids in the stimulus-secretion coupling for the secretion of gastric acid by investigating the formation of [^{3}H]-inositol phosphates after prelabelling with [^{3}H]-inositol in isolated gastric cells.

Methods

Isolation of gastric cells

The gastric cells were isolated according to the method of Lewin *et al.* (1974) with the modifications described by Ruoff *et al.* (1982).Three different cell media were used: Medium A (mmol l^{-1}): NaH_2PO_4 0.5, Na_2HPO_4 1.0, $NaHCO_3$ 20, NaCl 70, KCl 5, glucose 11, EDTA 2, HEPES 50 and bovine serum albumin (BSA 20 mg ml^{-1}). Medium B was of the same composition as medium A, but was EDTA-free, supplemented with $CaCl_2$ 1.0 and $MgCl_2$ 1.5. and, contained BSA 10 mg ml^{-1}. Medium C differed from medium B in having a reduced BSA concentration (1 mg ml^{-1}). Briefly, 8–12 non-fasted male Wistar rats of weight 180–250 g were used per experiment. The animals were killed by decapitation after which stomachs were removed and transformed into everted sacs. The sacs were filled with 2 ml of pronase solution (1.5 mg ml^{-1}) and incubated at 37°C in medium A under continuous gassing with 95% O_2 plus 5% CO_2 for 90 min during which medium A was renewed twice. The sacs were then transferred to medium B and gently stirred for 45 min at 37°C by a magnetic stirrer. The cells released into the medium were collected by centrifugation after 5, 15, 30 and 45 min. The number of cells and the percentage of parietal cells and dead cells (trypan blue) were estimated by cell count. The parietal cells were identified under the light microscope by their size and granulation. The cell yield at this stage was on average 8×10^7 cells per stomach with 20–23% parietal cells. The isolated cells were stored at room temperature in medium C.

Enrichment of parietal cells

Enrichment of parietal cells was performed by using isopycnic centrifugation with Percoll as described by Sonnenberg *et al.* (1979): 6.5 ml cell-containing medium C was mixed with 2.7 ml Percoll and 0.8 ml HEPES (0.25 mol l^{-1}; density 1.03 g ml^{-1}) and centrifuged at 190 *g* for 40 min. Parietal cells were collected in the surface layer, which was carefully removed and washed three times with medium C to remove Percoll. The cell yield was on average 2×10^7 cells per stomach with 60 to 70% parietal cells, less than 5% of the cells being damaged.

Incubation of gastric cells

Isolated gastric cells were prelabelled by incubation with 15 μCi of [^{3}H]-inositol per ml cell suspension (4×10^7 cells per ml medium C) in a shaking water bath at 37°C for 1 h under an atmosphere of 95% O_2 plus 5% CO_2. The unincorporated label was removed by centrifuging and resuspending the cells twice in medium C. The incubation of the prelabelled cells was performed in a similar manner to that described by Berridge *et al.* (1982). One hundred μl of the cell suspension (4×10^6 cells) was pipetted into flat bottomed plastic vials containing 180 μl of medium C plus 20 mM LiCl if not indicated otherwise. The vials were then gassed with 95% O_2 plus 5% CO_2, capped and shaken at 37°C for 30 min after which test compounds (20 μl in medium C) were added. The incubation was terminated with 940 μl of chloroform/methanol/HCl (100 : 200 : 2) and the samples were allowed to stand for at least 5 min after which 310 μl of chloroform and 310 μl water was added to separate the phases. The samples were then centrifuged at 8,000 *g* for 5 min, and 900 μl of the upper aqueous phase was removed and diluted with 3.5 ml of water. The samples were stored at −20°C until assayed for ^{3}H-labelled inositol phosphates as described below. To estimate the incorporation of [^{3}H]-inositol into phospholipids in some experiments, 400 μl of the lower lipid phase was removed, dried and counted after addtion of 3 ml of Quickzint 212 (Zinsser Analytic, Frankfurt, F.R.G.).

Assay of ^{3}H-labelled inositol phosphates

For the assay ^{3}H-labelled inositol phosphates by anion-exchange technique the method of Berridge *et al.* (1983) with some modifications was used. The

samples were applied to columns containing 1 ml (0.5 × 2 cm) of Dowex anion-exchange resin (1 × 10; 100–200 mesh; formate form) and [^{3}H]-inositol phosphates were removed by a stepwise addition of formate solutions in increasing strength. [^{3}H]-inositol was eluted with 5 ml of water (buffer 1), [^{3}H]-glycerophosphoinositol with 10 ml of 5 mM disodium tetraborate plus 60 mM sodium formate (buffer 2), [^{3}H]-inositol monophosphate ([^{3}H]-IP1) with 15 ml 0.1 M formic acid plus 0.2 M ammonium formate (buffer 3), [^{3}H]-inositol bisphosphate ([^{3}H]-IP2) with 15 ml 0.1 M formic acid plus 0.4 M ammonium formate (buffer 4) and [^{3}H]-inositol trisphosphate ([^{3}H]-IP3) with 10 ml 0.1 M formic acid plus 1.0 M ammonium formate (buffer 5). As suggested by more recent reports, the fractions designated here [^{3}H]-IP1 and [^{3}H]-IP2 may not only contain [^{3}H]-inositol 1-monophosphate and [^{3}H]-inositol 4.5-bisphosphate respectively, but perhaps also their cyclic forms (Wilson *et al.*, 1985). Similarly, the [^{3}H]-IP3 fraction may consist of two isomers, viz. [^{3}H]-inositol 1, 4, 5- and 1, 3, 4-trisphosphate (Irvine *et al.*, 1984), and may also contain their cyclic forms (Wilson *et al.*, 1985) as well as the novel inositol polyphosphate, inositol 1, 3, 4, 5-tetrakisphosphate (Batty *et al.*, 1985).

Based on the elution profile shown in Figure 1 the following 'batch' technique was routinely used: [^{3}H]-inositol and [^{3}H]-glycerophosphoinositol were eluted with 3 × 5 ml of buffer 2 and discarded, [^{3}H]-IP1 with 3 × 5 ml of buffer 3, [^{3}H]-IP2 with 3 × 5 ml of buffer 4 and [^{3}H]-IP3 with 2 × 5 ml of buffer 5. The radioactivity in 5 ml of the eluate was determined by the scintillation counting in the gel phase using 15 ml Quickzint 212.

Drugs

The following drugs were purchased from the companies indicated: Pronase E, Na_2-EDTA, trypan blue (Merck, Darmstadt, F.R.G.); myo-[^{2}H(N)]-inositol, 16.5 Ci mmol^{-1} (New England Nuclear, Boston, Mass., U.S.A.); Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden); atropine sulphate, carbachol, histamine dihydrochloride, 5-hydroxytryptamine hydrochloride, adrenaline bitartrate, indomethacin, prostaglandin E_2, forskolin, porcine gastrin, pentagastrin, cholecystokinin octapeptide, bombesin, angiotensin II (human form) and [Arg^8] vasopressin (Sigma, Deisenhofen, F.R.G.). Dowex (1 × 10; 100–200 mesh) was purchased from Fluka (Neu-Ulm, F.R.G.) as the chloride form and converted to the formate form. All other reagents were of analytical grade.

Adrenaline, atropine, carbachol, histamine and 5-hydroxytryptamine were dissolved in medium C. Bombesin was dissolved in water and indomethacin in fresh 5% $NaHCO_3$ immediately before use. Gastrin and pentagastrin were dissolved in dimethyl sulphoxide, prostaglandin E_2 and forskolin in absolute ethanol, vasopressin, angiotensin II and cholecystokinin in water and all stored at −20°C.

Further dilutions were made with medium C and the corresponding vehicle was always added to the control samples.

Statistical analysis

The results are expressed as means ± s.e. Statistical analysis was performed by the one-way analysis of variance and the Newman-Keuls procedure as an *a posteriori* test. *P* values less than 0.05 were taken as significant.

Results

General conditions

Elution profile In the elution profile of the water-soluble extracts from the control samples the peaks corresponding to [^{3}H]-inositol, [^{3}H]-glycerophosphoinositol and [^{3}H]-IP1 were easily identified, whereas the peaks of [^{3}H]-IP2 and [^{3}H]-IP3 were hardly visible (Figure 1). When the extracts from the samples stimulated with 10^{-3} M carbachol were run through the Dowex columns the peaks corresponding to [^{3}H]-IP2 and [^{3}H]-IP3 were clearly detectable. Furthermore, carbachol caused an increase in the [^{3}H]-IP1 peak, but had no effect on the first two peaks in the elution profile.

Effect of Li^+ As shown in Figure 2, the pre-incubation of the prelabelled cells with Li^+ enhanced the basal and carbachol-induced accumulation of [^{3}H]-inositol phosphates. The formation of [^{3}H]-IP1 was strongly enhanced already by low concentrations of Li^+, the maximal effect on the carbachol-induced stimulation being found by 5 mM Li^+, whereas the basal level was further increased by higher concentrations of Li^+. The basal and carbachol-induced accumulation of [^{3}H]-IP2 and [^{3}H]-IP3 was increased by Li^+ to a lesser extent than that of [^{3}H]-IP1, and the maximal effect was found with 20 mM Li^+. Therefore, pre-incubation with 20 mM Li^+ was always used in the following experiments.

Effect of Ca^{2+} The effect of added Ca^{2+} and EGTA on the basal and carbachol-induced formation of [^{3}H]-inositol phosphates is shown in Table 1. When no external Ca^{2+} was added to the incubation medium both the basal and carbachol-induced accumulations of [^{3}H]-inositol phosphates were attenuated as compared with the values obtained in the presence of 1 mM Ca^{2+} (i.e. under standard conditions). This effect was more pronounced when 0.5 mM EGTA was added to the Ca^{2+}-free medium. The addition of 2 mM Ca^{2+} did

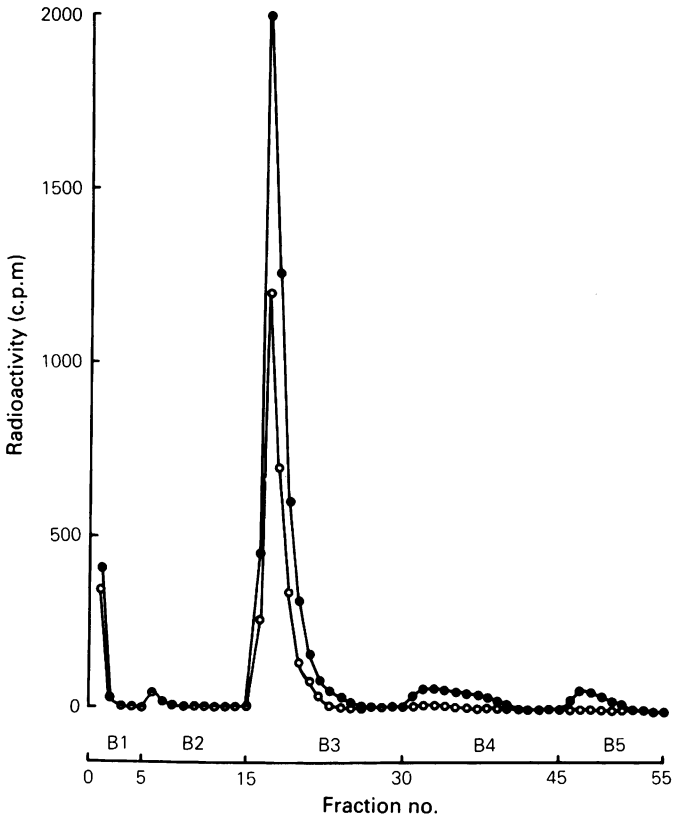

Figure 1 Elution profile of ^{3}H-labelled inositol phosphates extracted from isolated gastric cells on Dowex anion-exchange columns. The cells prelabelled with [^{3}H]-inositol were pre-incubated for 30 min with 20 mM LiCl and thereafter for additional 10 min in the presence (●) or absence (○) of 10^{-3} M carbachol. For further handling of the samples and the elution buffers used, see Methods. The data shown are representative of three separate experiments.

not increase the accumulation of [^{3}H]-inositol phosphates above the levels obtained with 1 mM Ca^{2+}. However, as compared with the corresponding basal value, the relative stimulation by carbachol always remained constant. As described under Methods, the labelling of the cells with [^{3}H]-inositol was usually performed in the presence of 1 mM Ca^{2+}. In a separate experiment, the effect of Ca^{2+} on the incorporation of [^{3}H]-inositol into phospholipids was studied (Table 2). It can be seen that the effects of Ca^{2+} on the incorporation of [^{3}H]-inositol and breakdown of [^{3}H]-inositol phospholipids were opposite. As compared to the standard conditions (1 mM Ca^{2+}) the incorporation in the buffer with no added Ca^{2+} was approximately two times higher, and when Ca^{2+} was totally removed from the medium by addition of 0.5 mM EGTA the incorporation was again doubled. Increase of the Ca^{2+} concentration to 2 mM did not affect the incorporation as compared with the values obtained in the presence of 1 mM Ca^{2+}.

Carbachol

Carbachol stimulated the formation of all three [^{3}H]-inositol phosphates. Increases in the accumulation of [^{3}H]-IP3 and [^{3}H]-IP2 were detected 30 s after the addition of 10^{-3} M carbachol and before any increase in the level of [^{3}H]-IP1 (Figure 3). The maximal effect on [^{3}H]-IP3 and [^{3}H]-IP2 was observed within 10 min, after which their levels gradually decreased. The increase in [^{3}H]-IP1 was observed after 5 min incubation with carbachol, and the carbachol-induced accumulation was approximately linear for at least 40 min. Figure 4 shows that the stimulation by carbachol was concentration-dependent and was antagonized by 10^{-8} M atropine, which had no effect of its own.

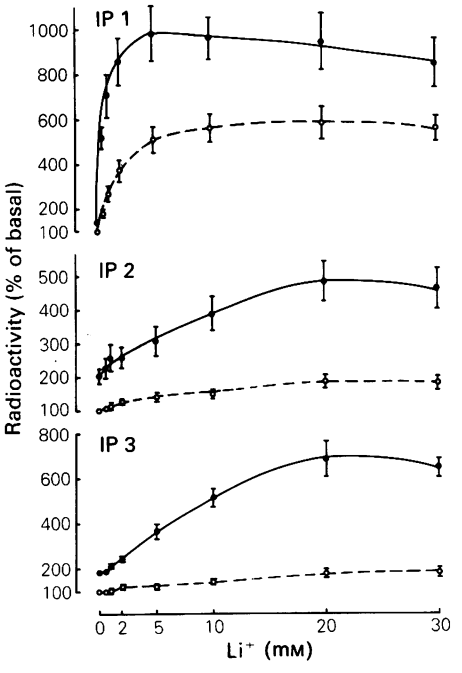

Figure 2 Effect of Li^+ on the formation of ^3H-labelled inositol phosphates in isolated gastric cells. After 30 min pre-incubation of the [^{3}H]-inositol prelabelled cells with various concentrations of LiCl they were incubated for 10 min in the presence (●—●) or absence (O---O) of 10^{-3} M carbachol and handled further as described under Methods. The 'batch' elution technique used here to separate [^{3}H]-IP1, [^{3}H]-IP2 and [^{3}H]-IP3 is described under Methods. Values are expressed as percentage of the basal formation without addition of LiCl. Each point is the mean of 4 samples from 4 cell preparations; s.e.mean indicated by vertical lines. IP1 = [^{3}H]-inositol monophosphate; IP2 = [^{3}H]-inositol bisphosphate; IP3 = [^{3}H]-inositol trisphosphate.

Gastrin and pentagastrin

Gastrin stimulated the formation of [^{3}H]-inositol phosphates, although it was clearly less effective than carbachol. The increase in the levels of [^{3}H]-IP3 and of [^{3}H]-IP2 was evident 30 s after addition of 10^{-7} M gastrin, wereas the increase in [^{3}H]-IP1 was observed after 5 min. The maximal stimulation of the formation of [^{3}H]-IP3 and [^{3}H]-IP2 by gastrin was observed after 2 min and already at 10 min its effect was largely attenuated (Figure 5). The time-course of the stimulation with 10^{-6} M pentagastrin was similar ($n = 6$, two preparations, data now shown). Table 3 shows that gastrin and pentagastrin stimulated the formation of [^{3}H]-inositol phosphates in a concentration-dependent manner in the same range of concentrations.

Table 1 Effect of Ca^{2+} on the accumulation of [^{3}H]-inositol phosphates in isolated gastric cells

Additions	*[^{3}H]-IP1*	*[^{3}H]-IP2*	*[^{3}H]-IP3*
	Radioactivity (c.p.m.)		
(A) Vehicle			
EGTA 0.5 mM	733 ± 25	42 ± 2	19 ± 1
No $CaCl_2$	993 ± 44	54 ± 2	26 ± 1
$CaCl_2$ 1 mM	1547 ± 144	93 ± 7	49 ± 5
$CaCl_2$ 2 mM	1601 ± 80	100 ± 3	65 ± 5
(B) Carbachol 10^{-3} M			
EGTA 0.5 mM	1568 ± 49	130 ± 5	55 ± 4
No $CaCl_2$	2180 ± 24	185 ± 8	87 ± 3
$CaCl_2$ 1 mM	2865 ± 120	269 ± 8	149 ± 8
$CaCl_2$ 2 mM	2969 ± 81	285 ± 14	169 ± 7

The cells prelabelled with [^{3}H]-inositol were preincubated for 30 min in the presence of 20 mM Li^+ the medium also containing 0.5 mM EGTA without $CaCl_2$, no added $CaCl_2$, 1 mM $CaCl_2$ or 2 mM $CaCl_2$. Ten min after addition of 10^{-3} M carbachol, dissolved in the corresponding medium, or 20 µl of the vehicle, the reaction was stopped and the samples analysed for [^{3}H]-inositol phosphates. [^{3}H]-inositol monophosphate = [^{3}H]-IP1; [^{3}H]-inositol bisphosphate = [^{3}H]-IP2; [^{3}H]-inositol trisphosphate = [^{3}H]-IP3. The results are means ± s.e.mean of 5 samples from one cell preparation.

Histamine

No changes in the formation of [^{3}H]-inositol phosphates were observed when the cells were incubated for 1, 5, 10 or 30 min with 10^{-3} M histamine in the

Table 2 Effect of Ca^{2+} on the incorporation of [^{3}H]-inositol into phospholipids in isolated gastric cells

Additions	*Radioactivity* (c.p.m.)
EGTA 0.5 mM	123216 ± 7166[a, b]
No $CaCl_2$	59645 ± 4100[a]
$CaCl_2$ 1 mM	34677 ± 2217
$CaCl_2$ 2 mM	36587 ± 1395

Cells (4×10^6) were incubated with 3 µCi of [^{3}H]-inositol in 300 µl of the medium containing 0.5 mM EGTA without $CaCl_2$, no added $CaCl_2$, 1 mM $CaCl_2$ or 2 mM $CaCl_2$ for 60 min at 37°C. For further information, see Methods. The results are means ± s.e.mean of 6 samples from one cell preparation. [a] $P < 0.01$ vs. $CaCl_2$ 1 mM; [b] $P < 0.01$ vs. no $CaCl_2$.

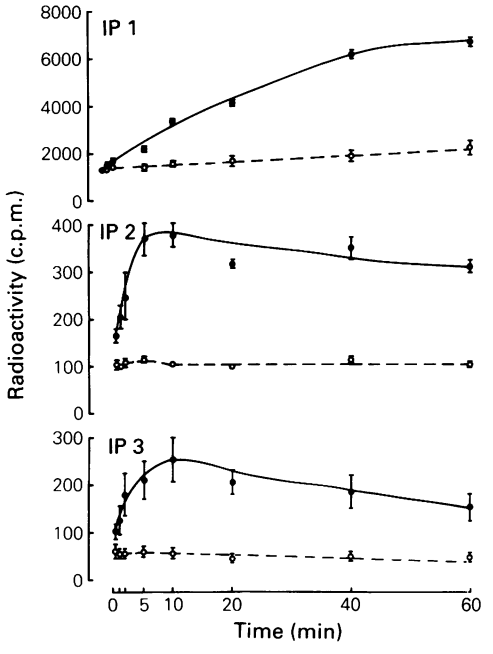

Figure 3 Time-course of the carbachol-induced formation of ^{3}H-labelled inositol phosphates in isolated gastric cells. After 30 min pre-incubation with 20 mM LiCl the cells were incubated for various times in the presence (●—●) or absence (O---O) of 10^{-3} M carbachol. Each point is the mean of 4 samples from 2 cell preparations; s.e.mean indicated by vertical lines. Abbreviations as for Figure 2.

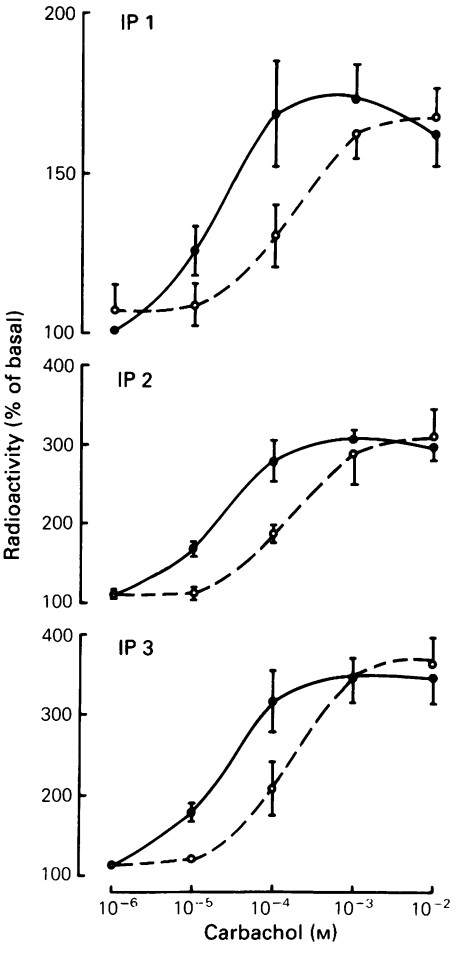

Figure 4 Concentration-effect curve for carbachol-induced stimulation of the formation of [^{3}H]-inositol phosphates and its antagonism by atropine in isolated gastric cells. The cells prelabelled with [^{3}H]-inositol were preincubated for 30 min with 20 mM LiCl in the presence (O---O) or absence (●—●) of 10^{-8} M atropine. The cells were then incubated for further 10 min with various concentrations of carbachol. Each point is the mean of 5 samples from 5 cell preparations; s.e.mean indicated by vertical lines. Abbreviations as for Figure 2.

presence or absence of 20 mM Li^+ (Table 4). Similarly, lower concentrations of histamine (10^{-6}–10^{-4} M) had no effect in the presence of 20 mM LiCl as determined after 10 min incubation (one preparation, $n = 4$, data not shown).

Interactions between carbachol, gastrin and histamine

Figure 6 shows the results of an experiment in which the effects of one concentration of carbachol, gastrin and histamine were assayed alone or in combination. It was found that the stimulant effects of carbachol and gastrin on the formation of [^{3}H]-inositol phosphates ($P < 0.01$) were less than additive. Histamine did not modify the effect of gastrin, but significantly ($P < 0.01$) inhibited the stimulation by carbachol.

Cholecystokinin

Cholecystokinin octapeptide (CCK-8) slightly stimulated the formation of [^{3}H]-inositol phosphates. The time-course of the stimulation of 10^{-6} M CCK-8 was similar to that produced by gastrin (two preparations; $n = 6$, data not shown). Table 5 shows that the effect of CCK-8 was concentration-dependent.

Other compounds

Pre-incubation of the cells with indomethacin margin-

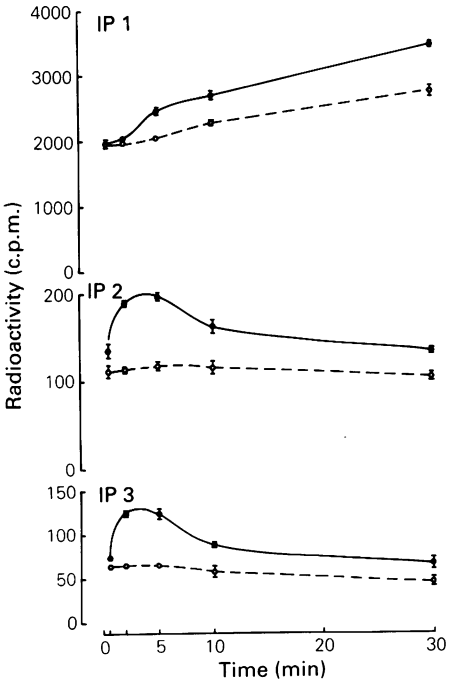

Figure 5 Time-course of the gastrin-induced accumulation of [^{3}H]-inositol phosphates in isolated gastric cells. After 30 min preincubation with 20 mM LiCl, prelabelled cells were incubated for various times with (●—●) or without (O---O) 10^{-7} M gastrin. Each point is the mean of 4 samples from one cell preparation; s.e.mean indicated by vertical lines. A similar time-course was found in another cell preparation. Abbreviations as for Figure 2.

ally increased the basal accumulation of [^{3}H]-IP1 and the carbachol-induced accumulation of [^{3}H]-IP1 and [^{3}H]-IP2 without affecting the ^{3}H-labelling of other fractions (Table 6). Prostaglandin E_2 10^{-5} M, had no effect on the basal formation of [^{3}H]-inositol phosphates, nor did it modify the response to carbachol (Table 7). Table 8 shows that adrenaline, 5-hydroxytryptamine, forskolin, vasopressin, angiotensin II and bombesin did not affect the formation of [^{3}H]-inositol phosphates after 10 min incubation. The effect of bombesin (10^{-6} M) was also tested after shorter (0.5, 2 and 5 min) or longer (30 min) incubation periods. In this experiment bombesin had no effect on the accumulation of [^{3}H]-inositol phosphates (one preparation, $n = 3$, data not shown).

Discussion

The signalling system which utilized the hydrolysis of

Table 3 Effect of various concentrations of gastrin and pentagastrin on the accumulation of [^{3}H]-inositol phosphates in isolated gastric cells.

Additions	*[^{3}H]-IP1*	*[^{3}H]-IP2*	*[^{3}H]-IP3*
	Radioactivity (c.p.m.)		
Vehicle	2696 ± 65	141 ± 2	68 ± 3
Gastrin			
10^{-10} M	2716 ± 77	138 ± 5	73 ± 7
10^{-9} M	2777 ± 39	142 ± 7	76 ± 6
10^{-8} M	2834 ± 65	164 ± 2[a]	88 ± 3[a]
10^{-7} M	3198 ± 71[b]	210 ± 6[b]	115 ± 9[b]
Pentagastrin			
10^{-9} M	2791 ± 45	157 ± 5	75 ± 5
10^{-8} M	2852 ± 62	178 ± 7[b]	86 ± 3
10^{-7} M	2934 ± 65	187 ± 7[b]	97 ± 5[b]
10^{-6} M	2907 ± 45	190 ± 3[b]	106 ± 5[b]
10^{-5} M	3020 ± 33[b]	187 ± 7[b]	100 ± 8[b]

The cells prelabelled with [^{3}H]-inositol were preincubated for 30 min with 20 mM LiCl and after addition of the agonists for a further 5 min. The results are means ± s.e.mean of 4 samples from one cell preparation. Abbreviations as in Table 1. [a]$P < 0.05$ and [b]$P < 0.01$ vs. vehicle.

inositol phospholipids as the second messenger system for mobilizing Ca^{2+}, first proposed by Michell (Michell, 1975; Michell *et al.*, 1981) has been intensively investigated in recent years. There is now evidence that inositol 1, 4, 5-trisphosphate formed from phosphatidylinositol 4, 5-bisphosphate upon receptor activation acts as the second messenger for the release of Ca^{2+}, although some other inositol polyphosphates may also be involved (Berridge & Irvine, 1984; Irvine *et al.*, 1984; Batty *et al.*, 1985; Wilson *et al.*, 1985). As mentioned in the introduction, the secretagogue action of acetylcholine and gastrin, both of physiological significance in the regulation of gastric acid secretion, is related to an increase in intracellular Ca^{2+} of the acid-secreting parietal cells (Sachs & Berglindh, 1981; Muallem & Sachs, 1984). Therefore, our present finding that the activation of muscarinic and gastrin receptors in isolated gastric cells with 60–70% parietal cells stimulates the formation of inositol phosphates is generally in agreement with this novel second messenger theory.

Berridge *et al.* (1982) have found that Li^+ enhances the agonist-induced accumulation of [^{3}H]-inositol phosphates in rat brain, rat parotid and the insect salivary gland, the effect being most pronounced in the [^{3}H]-inositol monophosphate fraction. This action of Li^+ is probaby due to an inhibition of inositol monophosphatase resulting in a decreased hydrolysis of inositol 1-monophosphatase (Sherman *et al.*, 1981). In the present work, Li^+ caused a concentration-depen-

Table 4 Effect of histamine on the formation of [^{3}H]-inositol phosphates in isolated gastric cells

	No LiCl		*20 mM LiCl*	
Time min	*Vehicle*	*Histamine*	*Vehicle*	*Histamine*
		[^{3}H]-IP1 (c.p.m.)		
1	234 ± 11	225 ± 12	1077 ± 40	1092 ± 52
5	263 ± 20	258 ± 14	1249 ± 42	1216 ± 44
10	249 ± 11	235 ± 9	1293 ± 51	1284 ± 55
30	285 ± 12	275 ± 12	1688 ± 46	1631 ± 34
		[^{3}H]-IP2 (c.p.m.)		
1	62 ± 8	56 ± 7	80 ± 9	87 ± 9
5	71 ± 9	65 ± 7	82 ± 5	93 ± 4
10	57 ± 5	52 ± 4	82 ± 3	86 ± 7
30	52 ± 4	49 ± 5	89 ± 7	80 ± 4
		[^{3}H]-IP3 (c.p.m.)		
1	20 ± 1	22 ± 2	39 ± 2	39 ± 2
5	25 ± 2	21 ± 1	42 ± 2	40 ± 3
10	18 ± 1	18 ± 1	34 ± 2	35 ± 2
30	17 ± 2	18 ± 1	43 ± 2	39 ± 3

After 30 min preincubation of the prelabelled cells in the presence or absence of 20 mM LiCl they were further incubated for various times with or without 10^{-3} M histamine. The results are means ± s.e.mean of 9 samples from 3 cell preparations.

dent enhancement of the basal and carbachol-induced accumulation of all three [^{3}H]-inositol phosphates in isolated gastric cells, although the formation of [^{3}H]-IP1 was affected by lower concentrations of Li^+ than that of [^{3}H]-IP3 or [^{3}H]-IP2. This implies that the phosphatases which degrade inositol tris- and bisphosphate are also inhibited by higher concentrations of Li^+ in gastric cells. In rabbit platelets, Li^+ has little effect on the thrombin-stimulated accumulation of [^{3}H]-IP1, while ^{3}H-labelling of IP3 was enhanced in a concentration-dependent manner. The effect on [^{3}H]-IP2 was biphasic: lower concentrations of Li^+ enhan-

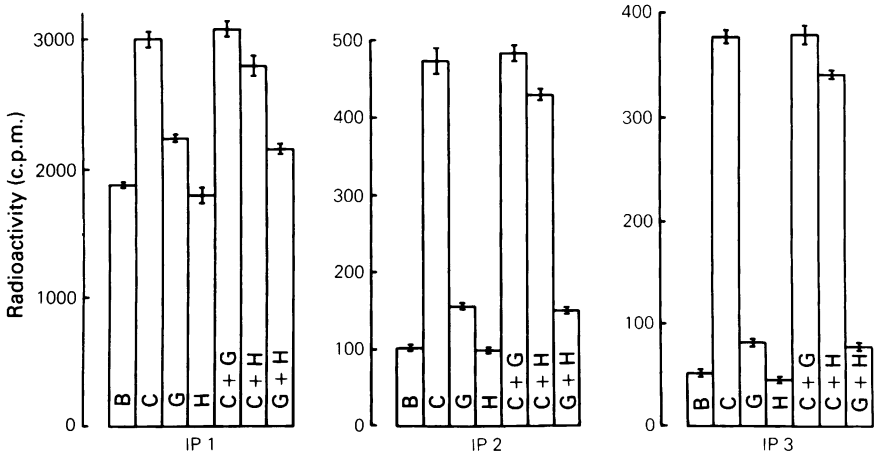

Figure 6 Combined effects of carbachol (C, 3×10^{-5} M), gastrin (G, 10^{-7} M) and histamine (H, 10^{-3} M) on the accumulation of [^{3}H]-inositol phosphates in isolated gastric cells; B = basal. The cells prelabelled with [^{3}H]-inositol were preincubated for 30 min with 20 mM LiCl after which the test compounds were added. The reaction was stopped after 5 min. Each column represents the mean of 6 samples from one cell preparation; s.e. mean indicated by vertical lines.

Table 5 Effect of cholecystokinin octapeptide (CCK-8) on the formation of [^{3}H]-inositol phosphates isolated gastric cells

Additions	*[^{3}H]-IP1*	*[^{3}H]-IP2*	*[^{3}H]-IP3*
	Radioactivity (c.p.m.)		
Vehicle	3451 ± 103	168 ± 7	82 ± 2
CCK-8			
10^{-9} M	3674 ± 77	195 ± 5[a]	94 ± 3[a]
10^{-8} M	4035 ± 166[a]	211 ± 7[b]	104 ± 1[b]
10^{-7} M	3866 ± 87[a]	209 ± 8[b]	107 ± 2[b]
10^{-6} M	3860 ± 77[a]	208 ± 9[b]	107 ± 5[b]

Prelabelled cells were preincubated for 30 min with 20 mM LiCl and then incubated for 5 min in the presence of various concentrations of CCK-8. The results are means ± s.e.mean of 4 samples from one cell preparation. Abbreviations as in Table 1. [a]$P < 0.05$ and [b]$P < 0.01$ vs. vehicle.

Table 7 Effect of prostaglandin E_2 (PGE_2) on the basal and carbachol-induced formation of [^{3}H]-inositol phosphates in isolated gastric cells

Additions	*[^{3}H]-IP1*	*[^{3}H]-IP2*	*[^{3}H]-IP3*
	Radioactivity (c.p.m.)		
Vehicles	1251 ± 32	77 ± 4	39 ± 3
PGE_2 10^{-5} M	1220 ± 54	74 ± 6	45 ± 2
Carbachol 10^{-3} M	2577 ± 77	355 ± 20	261 ± 19
Carbachol 10^{-3} M + PGE_2 10^{-5} M	2524 ± 107	344 ± 19	258 ± 18

After 30 min preincubation of the prelabelled cells with 20 mM LiCl, carbachol and/or prostaglandin E_2 or the corresponding vehicles were added and the reaction terminated after 10 min. The results are means ± s.e.mean of 5 samples from one cell preparation. Abbreviations as in Table 1.

ced its formation, whereas concentrations higher than 5 mM had an inhibitory effect (Vickers *et al.*, 1984). In the rat liver, the phosphatases degrading inositol mono- and bisphosphate are inhibited by Li^+, whereas inositol trisphosphatase is insensitive to Li^+ concentrations up to 10 mM (Storey *et al.*, 1984). Furthermore, it has been reported that in rat brain, Li^+ potentiates the carbachol-induced accumulation of [^{3}H]-IP1 and [^{3}H]-IP2 (Batty & Nahorski, 1985; Jacobson *et al.*, 1985) whereas that of [^{3}H]-IP3 was not affected (Jacobson *et al.*, 1985) or was inhibited by Li^+ in a concentration-dependent manner (Batty & Nahorski, 1985). There seems thus to be clear differences in the action of Li^+ on the formation of the inositol phosphates in various tissues.

Table 6 Effect of indomethacin on the basal and carbachol-induced accumulation of [^{3}H]-inositol phosphates in isolated gastric cells

Additions	*[^{3}H]-IP1*	*[^{3}H]-IP2*	*[^{3}H]-IP3*
	Radioactivity (c.p.m.)		
Vehicles	1178 ± 57	73 ± 4	45 ± 3
Indomethacin 10^{-4} M	1353 ± 46[a]	88 ± 5	52 ± 3
Carbachol 10^{-3} M	2289 ± 68	303 ± 13	236 ± 9
Indomethacin 10^{-4} M + carbachol 10^{-3} M	2508 ± 55[b]	339 ± 16[b]	249 ± 21

The cells prelabelled with [^{3}H]-inositol were preincubated for 30 min with 20 mM LiCl in the presence or absence of indomethacin. Ten min after addition of carbachol or its vehicle the reaction was stopped and the samples analysed for [^{3}H]-inositol phosphates. The results are means ± s.e.mean of 5 samples from one cell preparation. Abbreviations as in Table 1. [a]$P < 0.05$ vs. vehicles; [b]$P < 0.05$ vs. carbachol 10^{-3} M.

As mentioned above, the recent theory suggests that inositol 1, 4, 5-trisphosphate and perhaps also some other inositol polyphosphates may be the second messenger system for Ca^{2+} mobilization. However, the interactions between Ca^{2+} and inositol phospholipids seem to be much more complicated. Ca^{2+} may not only modify the receptor sensitivity to agonists inducing the hydrolysis of inositol phospholipids, but also affects the metabolism of inositol phospholipids in a complex manner, this action varying from tissue to tissue (Kendall & Nahorski, 1984 and the refs. therein). The present finding that the percentage stimulation of the formation of [^{3}H]-inositol phosphates by carbachol was independent of Ca^{2+} in the medium, supports in principle the hypothesis that the agonist-induced hydrolysis of inositol phospholipids is a prerequisite for, rather than a consequence of the Ca^{2+} mobilization. The finding that the basal accumulation of [^{3}H]-inositol phosphates was, however, clearly attenuated by the ommission of external Ca^{2+} and further inhibited in the presence of EGTA are in agreement with reports that the phosphodiesterase hydrolyzing phosphotidylinositol 4, 5-bisphosphate is Ca^{2+}-dependent (see Hirasawa & Nishizuka, 1985). As described by Kendall & Nahorski (1984) Ca^{2+} inhibits the incorporation of [^{3}H]-inositol into phospholipids in rat cerebral cortical slices, and we confirmed this observation in rat isolated gastric cells.

In agreement with the previous findings in various tissues (see Berridge & Irvine, 1984) carbachol, an agonist at muscarinic receptors, was a potent stimulator of the formation of [^{3}H]-inositol phos-

Table 8 Effect of several receptor agonists on the accumulation of [^{3}H]-inositol phosphates in isolated gastric cells

Additions	*[^{3}H]-IP1*	*[^{3}H]-IP2*	*[^{3}H]-IP3*
	Radioactivity (c.p.m.)		
(A)			
Vehicle	3207 ± 46	191 ± 5	90 ± 4
Adrenaline 10^{-3} M	3237 ± 50	197 ± 5	94 ± 4
5-Hydroxytryptamine 10^{-3} M	3251 ± 47	195 ± 5	91 ± 2
Forskolin 10^{-5} M	3182 ± 31	183 ± 4	81 ± 4
Vasopressin 10^{-5} M	3247 ± 18	194 ± 2	93 ± 1
Angiotensin II 10^{-5} M	3295 ± 56	199 ± 4	92 ± 2
(B)			
Vehicle	1453 ± 45	78 ± 4	53 ± 9
Bombesin 10^{-9} M	1547 ± 74	89 ± 7	48 ± 7
10^{-8} M	1535 ± 82	90 ± 8	50 ± 9
10^{-6} M	1526 ± 27	87 ± 4	53 ± 9

The cells prelabelled with [^{3}H]-inositol were preincubated for 30 min with 20 mM LiCl after which the test compounds were added. The reaction was terminated after 10 min. The results are means ± s.e.mean of 6 (A) or 3 samples (B) from one cell preparation. Abbreviations as in Table 1.

phates in isolated gastric cells. This effect was due to a specific action at muscarinic receptors as demonstrated by the ability of a low concentration of atropine to antagonize it. As compared with carbachol, gastrin is *in vitro* not only much less effective than carbachol, but also its duration of action on the intracellular Ca^{2+} concentration in dog isolated gastric cells (Muallem & Sachs, 1984) and on the electrical membrane response in rat parietal cells (Okada & Ueda, 1984) is shorter than that of carbachol. In the present work, gastrin and pentagastrin enhanced the formation of [^{3}H]-inositol phosphates in all cell preparations tested in the range of the concentrations which stimulate the parietal cell function *in vitro*. The maximal effect of gastrin or pentagastrin was much less than that of carbachol and the increases in the formation of [^{3}H]-IP3 and [^{3}H]-IP2 were of shorter duration than in the case of carbachol. Interestingly, the differences between the action of gastrin and carbachol on inositol phospholipids are in accord with those between their *in vitro* activities on the function of the parietal cell mentioned above.

Histamine stimulates the formation of inositol phosphates in rat brain through an effect on histamine H_1-receptors (Daum *et al.*, 1984), while the secretory action of histamine on the parietal cells is mediated via histamine H_2-receptors, cyclic AMP probably acting as the second messenger (for review, see Sachs & Berglindh, 1981). On the other hand, there is no evidence that the parietal cells contain histamine H_1-receptors. The lack of any effect of histamine on the basal accumulation of [^{3}H]-inositol phosphates in isolated gastric cells therefore agrees well with the above-mentioned findings. Histamine did not modify the gastrin-induced accumulation of [^{3}H]-inositol phosphates, whereas that induced by carbachol was slightly diminished. Our recent findings indicate that the increase in the intracellular level of cyclic AMP through the addition of dibutyryl cyclic AMP or the stimulation of adenylate cyclase and/or inhibition of cyclic AMP phosphodiesterase attenuates the carbachol-induced accumulation of inositol phosphates in isolated gastric cells (Puurunen, Lohse & Schwabe, unpublished observations). Therefore, the histamine-induced inhibition of the accumulation of inositol phosphates observed here may be due to an increased formation of cyclic AMP.

In addition to the 'classical' gastric secretagogues the effects of some other endogenous compounds affecting gastric acid secretion and/or stimulating the hydrolysis of inositol phospholipids in other tissues were studied. The main biological action of cholecystokinin in the gastrointestinal tract is the stimulation of the pancreatic enzyme secretion (Walsh, 1981). It is also a weak stimulant of gastric acid secretion *in vivo* (Walsh, 1981) and *in vitro* (Hersey, 1983) acting probably via specific receptors at the parietal cells. The profile of cholecystokinin in stimulating the formation of [^{3}H]-inositol phosphates in isolated gastric cells proved to be very similar to that of gastrin. As compared with the approximate two fold increase in ^{3}H-labelling of total inositol phosphates in isolated gastric glands of the guinea-pig containing approximately 20% parietal cells (Chang *et al.*, 1985), the effect of cholecystokinin on our gastric cell preparations consisting of 60–70% parietal cells was less pronounced. This may be due not only to species differences, but is also consistent with the fact that, at

least *in vitro*, cholecystokinin is a more potent stimulant of pepsinogen secretion than acid secretion (Hersey *et al.*, 1983). Bombesin is a potent stimulant of gastric acid secretion in many species (Walsh, 1981), and it has also been reported to enhance the degradation of inositol phospholipids in Swiss 3T3 cells (Brown *et al.*, 1984). The inability of bombesin to increase the formation of [^{3}H]-inositol phosphates in isolated gastric cells is in agreement with the suggestion that its gastric secretory effect may be mediated by the release of gastrin (Walsh, 1981). Forskolin, a potent secretagogue *in vivo* and *in vitro*, which probably operates via cyclic AMP, had no effect on the levels of [^{3}H]-inositol phosphates in gastric cells. Adrenaline, 5-hydroxytryptamine, vasopressin and angiotensin II, which all have been reported to enhance the hydrolysis of inositol phospholipids in other tissues (Berridge & Irvine, 1984), were similarly ineffective in gastric cells.

Prostaglandin E_1 has been reported to inhibit the hydrolysis of inositol phospholipids induced by thrombin or collagen in platelets (Ieyasu *et al.*, 1982; Sano *et al.* 1983). On the other hand, prostaglandins of A, E and I types are potent inhibitors of gastric acid secretion in various species. Studies with isolated gastric cells have demonstrated that this effect is, at least in part, due to a direct action on the parietal cells (Soll, 1981), although a central component of action seems also be of importance in the antisecretory effect of prostaglandins (Puurunen, 1983; 1985). *In vitro*, the inhibitory action of prostaglandin E_2 shows a clear selectivity in inhibiting hydrogen ion secretion induced by histamine, without having any effect on those induced by gastrin or carbachol (Soll, 1981). The interaction between histamine and prostaglandin E_2 seems to take place at the level of adenylate cyclase (Schepp *et al.*, 1983). In the present work, the pretreatment of the cells with indomethacin, which has been reported to attenuate the formation of prostaglandin E_2 in an identical cell preparation (Postius *et al.*, 1985) had only a marginal and inconsistent effect on the basal or carbachol-induced accumulation of [^{3}H]-inositol phosphates. Similarly, prostaglandin E_2 modifed neither the basal nor carbachol-induced hydrolysis of inositol phospholipids in isolated gastric cells.

In summary, the major findings of the present work are that carbachol and gastrin, the secretagogue action of which is reportedly related to an increase in intracellular Ca^{2+}, stimulated the formation of [^{3}H]-inositol phosphates in gastric cell preparations consisting of 60–70% of the acid-secreting parietal cells, whereas histamine, an agonist at histamine H_2-receptors, acting probably via cyclic AMP was ineffective. The hydrolysis of inositol phospholipids may therefore be involved in the signal transduction mechanism, by which the activation of the muscarinic and gastrin receptors of the parietal cells leads to Ca^{2+} mobilization and stimulation of hydrogen ion secretion.

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