Cytokine effects on pepsinogen secretion from human peptic cells

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Abstract
Background—Different cytokines, including epidermal growth factor (EGF) and interleukin 1β (IL 1β), participate in the pathogenesis of gastric mucosal damage and repair by means of different mechanisms that are either paracrine or autocrine in nature.
Aims—To study whether EGF and IL 1β affect pepsinogen secretion in vitro.
Methods—Dispersed human peptic cells were prepared from endoscopically obtained biopsy specimens after collagenase digestion, mechanical disruption, and density gradient centrifugation.
Results—EGF dose dependently increased basal pepsinogen secretion and mitogenic concentrations (0.1 nM) of EGF induced submaximal stimulation. Similar effects were observed with transforming growth factor α. EGF effects on pepsinogen secretion were in addition to that induced by CCK-OP and db-cAMP stimulated pepsinogen secretion. EGF stimulated pepsinogen secretion was completely inhibited by a human immunospecific EGF receptor antibody and reduced by both genistein and tyrphostin-25, two different tyrosine kinase inhibitors. IL 1β does not affect basal, CCK-OP or acetylcholine stimulated pepsinogen secretion. However, IL 1β dose dependently inhibited db-cAMP and histamine stimulated pepsinogen secretion.
Conclusions—These results show that both EGF and IL 1β modulate human pepsinogen secretion in vitro and suggest that the paracrine effects of these cytokines on pepsinogen secretion might be involved in some pathological conditions of damage and inflammation of the gastric mucosa.

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Keywords: epidermal growth factor, tyrosine-kinase, interleukin 1, pepsinogen.

Current evidence suggests that different cytokines including growth factors and interleukins participate in the pathogenesis of gastrointestinal repair and damage in different conditions such as peptic ulceration and inflammation.1 2 The involvement of cytokines in the peptic ulcer process has been reinforced by current data on Helicobacter pylori infection of the human gastric antrum mucosa.3 The complexity of the mechanisms involved in these conditions and the multiple biological actions of these peptides make further studies necessary to understand the role of cytokines in the regulation of gastric secretion and to establish potential pathophysiological bases for new therapeutic strategies. Furthermore, a potential role for the immune system in gastric acid secretion and peptic ulcer disease was suggested by Mezey and Palkovits.4 These authors found receptors for acetylcholine, histamine, gastrin, and dopamine in macrophages and IgA bearing plasma cells from the lamina propria of the gastric mucosa suggesting that these cells may be the targets of drugs that inhibit gastric acid secretion. The biology of these factors differs somewhat from classic hormones as neither the sites of synthesis nor the sites of actions are restricted to defined tissues.5 6 Many growth factors and interleukins operate in a paracrine fashion, and, in some instances, their actions are autocrine in nature. In this way, in vitro studies seem necessary to better understand the effects of these peptides in the control of gastric functions including acid and pepsinogen secretion.7 Furthermore, this approach has the advantage of being more accurate as the concentrations of the test substances can be controlled precisely and interactions with other organs and cell types can be eliminated.8 Unfortunately, current data on the effects of these factors in gastric secretion are mostly limited to experiments measuring acid secretion in different in vivo animal models, and, of all cytokines studied, both epidermal growth factor (EGF) and interleukin 1 (IL 1) have been shown to affect gastric secretion by inhibiting acid secretion in this type of studies.8 9 However, although available information provides sufficient evidence that the intramucosal activation of acid proteases, mainly pepsinogens, plays a major part in peptic diseases of the upper gastrointestinal tract, there are no data on the potential effects of cytokines on pepsinogen secretion by the stomach. Moreover, many of the actions described for different factors on gastric secretion are species specific, which indicates that data from one species cannot be fully extrapolated to others (for example, humans).10 In this study we have examined in vitro the effects of EGF and IL 1β on pepsinogen secretion by means of dispersed human peptic cells obtained from endoscopic biopsy specimens.

Methods

MATERIALS
Chemicals were obtained from the following sources: bovine serum albumin (fraction V),
acetylcholine (ACH), histamine, db-cAMP, cholecystokinin-8 (CCK-OP), HEPES, per-
coll, crystalline porcine pepsinogen, soybean
trypsin inhibitor, ethylene glycol tetraacetic acid
(EGTA) and glucose from Sigma Chemical
Company (St Louis, MO); human rEGF and TGF-α
from Calbiochem (La Jolla, CA); the antibody
against the hEGF receptor from Oncogene
Science (Uniondale, NY); collagenase (Type IV)
from Worthington Biochemical Corp (Freehold,
NJ); bovine haemoglobin from Gibco Diagnos-
tics, (Madison, WI). Human recombinant
interleukin 1β was obtained from two different
sources. Most experiments were performed
with that obtained from Calbiochem (La Jolla,
CA). However, in the experiments performed
in combination with db-cAMP the interleukin
1β used was from Boehringer Mannheim
(Mannheim, Germany).

Unless otherwise stated, the standard
Ringer solution contained 82.2 mM NaCl, 4.0 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgCl₂,
0.8 mM NaSO₄, 17.8 mM NaHCO₃, 0.8 mM
NaH₂PO₄, 11.5 mM glucose, and 0.2% bovine
serum albumin. The medium was equilibrated
with 95% O₂-5% CO₂ and the pH was
adjusted to 7.45. The Ca²⁺ low medium con-
tained 0.1 mM CaCl₂ and no MgCl₂, and the
Ca²⁺ free medium contained no CaCl₂ or
MgCl₂.

**Human material**

Gastric biopsy specimens were obtained from
83 patients undergoing upper gastrointestinal
endoscopy (60 men). The mean (SD) age of
this population was 42.8 (15-1) (18-68). In 21
of these patients the endoscopy was normal, 20
had an active duodenal ulcer, 16 a healed
duodenal ulcer, 13 oesophagitis, three a gastric
ulcer, and 10 duodenitis or antritis without
ulcer. Most of them were or had recently been
taking H₂ receptor antagonists, and the rest
were taking antacids or free from any treat-
ment. *Helicobacter pylori* status in the corpus
of the stomach was not determined, but it
was in the antrum (CLO-test, Delta West
LTD, Canning Vale, Bentley, Australia) in
49 patients (positive in 42). No biopsy spec-
imen's were obtained from patients who had
recently used non-steroidal anti-inflammatory
drugs (NSAIDs). The study was approved by
the Institutional Review Board and written
informed consent was obtained from all
patients.

**STUDY DESIGN**

**Cell isolation**

Dispersed peptic cells were obtained from
dissected biopsy specimens after
collagenase digestion, filtration, and density
gradient centrifugation according to a method
previously described. 11 This method yields a
cell population with approximately 90% of
peptic cells, 90% viable by trypan blue and
flow cytometry.

Eight to 10 regular size punch biopsy
specimens yielded approximately 10⁶ cells that
were suspended in Ringer's solution under
95% O₂-5% CO₂ atmosphere and refrigerated
overnight. The next morning, the cells were
allowed to warm at room temperature before
they were used in experiments. No experi-
ments were performed if cell viability was lower
than 89% and experiments with a low total
pepsinogen content were discarded.

**Experiments and calculations**

The warmed cells were re-suspended in fresh
Ringer's solution and counted (haemato-
cytometer). After appropriate dilutions, cells were
placed in 1.5 ml polypropylene test tubes con-
taining the appropriate agents and incubated in
a shaking water bath under an atmosphere of
95% O₂-5% CO₂ at 37°C for different
periods of time. In general, cells from a single
patient were sufficient for nine to 11 aliquots
(including duplicates). If the experiment was
high in the number of aliquots or samples, two
sets of cells from two different people were put
together. In this case n = 4 means eight people.
The experiments ended by centrifuging the
tubes for 17 seconds at 14,000 g and pipetting
off the supernatants. An aliquot of the super-
натant was assayed for pepsinogen activity
using a modified Anson-Mirsky method using
acid denatured haemoglobin as the substrate
as previously described. 12 Secretory responses
to stimuli were calculated in the output per
unit time with basal output subtracted. The
(mean) basal rate of pepsinogen secretion in
the experiments was 0.19 (0.008) of total
pepsinogen content per minute.

In some experiments carried out to study the
role of calcium in basal or stimulated
pepsinogen secretion, cells were washed once
in calcium free Ringer's solution resuspended
in calcium free Ringer's solution containing
0.5 mM EGTA for three minutes to remove
extracellular Ca²⁺, washed again, and finally
re-suspended in calcium free Ringer's solutions
plus the drugs to be tested. When the antibody
anti-EGF receptor (stock solution = 100 µg/ml)
was to be used, this was added one minute
before the other agents. According to the
manufacturer, the antibody is a mouse mono-
omonal IgG4, with applications on in vivo
studies and specificity for the cell surface
domain of the human EGF receptor. The
antibody inhibits the EGF binding to the EGF
receptor and tumour formation in nude mice. 13
Also, according to the manufacturer, one unit
of IL-1β was defined as the amount of IL-1β
that doubles the amount of [³H]-thymidine
incorporated by submitogenically activated
(PHA, 1 µg/ml) murine myeloma cells.

Unless otherwise specified, the pepsinogen
secretion obtained after different stimuli is
expressed as the percentage of total cell
pepsinogen content with basal secretion sub-
tracted. Data are reported as mean (SEM) and
the 't' in each experiment is equal to the
number of separate cell preparations. Analysis
of variance (ANOVA) and Student's 't' test for
paired or unpaired data and either equal or
unequal variances were used for statistical
analysis and applied when appropriate.
EGF dose dependently stimulated basal pepsinogen secretion from isolated human peptic cells (Fig 1). Maximal stimulation was achieved with 1 nM (Δ secretion=2.21 (0.25)%). Concentrations of EGF (0-1 nM) that have proved to be mitogenic in different cellular systems, also significantly increased basal pepsinogen secretion. Apparently, the presence of previous *H pylori* infection did not affect the EGF effect on pepsinogen secretion (Δ secretion=1.2 (0.08)% in *H pylori* positive vs 1.3 (0.15)% in *H pylori* negative after 0-1 nM EGF; n=16 and 6, respectively). When compared with other secretagogues, the EGF response was 46.3% of that induced by 0-1 μM CCK, 84% of 0.01 mM acetylcholine, and 52.5% of mM db-cAMP, similar to histamine, and 147% of μM gastrin (Table I). This effect was calcium dependent because removal of extracellular calcium inhibited the stimulating effect of EGF on pepsinogen secretion (0-1 nM M=1.3 (0-1)% with v 0-16 (0-2)% without calcium). A few experiments performed to evaluate the effects of TGFα, a factor structurally and functionally similar to EGF and which competes with EGF for binding to the same receptor on the surface of epithelial cells, also showed that mitogenic concentrations of this peptide stimulated basal pepsinogen secretion (Table I).

EGF also potentiated histamine and db-cAMP stimulated pepsinogen secretion. As previously shown, 0-1 mM concentrations of histamine significantly stimulated pepsinogen secretion from dispersed human peptic cells (Table I). When 0-1 nM EGF was combined with histamine and db-cAMP, pepsinogen secretion was stimulated in an additive manner (Fig 2). CCK-OP dose dependently increased pepsinogen secretion from dispersed human peptic cells. The addition of EGF further increased pepsinogen secretion in CCK stimulated cells and such an effect was in addition to that induced by CCK alone (Fig 3). However, EGF (100 nM–0-1 mM) did not increase pepsinogen secretion in acetylcholine (μM) stimulated cells (1-85 (0-3)% vs 2.31 (0-4)–2 (0-35)% n=6). The effect of 0-1 nM TGFα was also tested in combination with db-cAMP and CCK obtaining similar results to those presented with EGF (data not shown).

Table I: Pepsinogen secretion by isolated human peptic cells incubated for 30 minutes with various secretagogues

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Concentration</th>
<th>ΔPepsinogen secretion (% total-basal/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-OP</td>
<td>10-8 M</td>
<td>2.6 (0.58)</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>10-4 M</td>
<td>4.9 (0-9)</td>
</tr>
<tr>
<td>10-4 M</td>
<td>1.95 (0-3)</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>10-8 M</td>
<td>2.7 (0-5)</td>
</tr>
<tr>
<td>db-cAMP</td>
<td>10-8 M</td>
<td>2.2 (0-6)</td>
</tr>
<tr>
<td>10-9 M</td>
<td>4.32 (1-02)</td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>10-11 M</td>
<td>1.3 (0-15)</td>
</tr>
<tr>
<td>10-12 M</td>
<td>2.27 (0-25)</td>
<td></td>
</tr>
<tr>
<td>TGFα</td>
<td>10-13 M</td>
<td>2.1 (0-6)</td>
</tr>
<tr>
<td>10-9 M</td>
<td>0.98 (0-18)</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>10-13 M</td>
<td>1.8 (0-3)</td>
</tr>
<tr>
<td>10-12 M</td>
<td>1.66 (0-4)</td>
<td></td>
</tr>
<tr>
<td>Gastrin</td>
<td>10-12 M</td>
<td>1.5 (0-2)</td>
</tr>
<tr>
<td>10-9 M</td>
<td>1.8 (0-08)</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean (SEM) of four to 12 separate experiments. Basal secretion in experiments=3-34 (0-89)–5.34 (0-67)%. All values=Δp=0-05 v basal secretion.

CCK-OP=scholeystokinin-8; TGFα=transforming growth factor; EGF=epidermal growth factor.
To study whether the effects of EGF on pepsinogen secretion were non-specific or dependent on the activation of a cell surface receptor the cells were resuspended in the presence of two different concentrations (1:500 and 1:100 dilution) of an immunospecific antibody against human EGF receptor (100 μg/ml) and then stimulated with EGF. In these conditions the effect of EGF on basal pepsinogen secretion was partially or completely inhibited by both concentrations of the antibody respectively (Fig 4A). EGF effects on pepsinogen secretion were also inhibited (62%) by genistein, a membrane cytosolic tyrosine kinase inhibitor (Fig 4A). In a different set of experiments, tyrphostin-25, an inhibitor of EGF receptor tyrosine kinase activity, induced a small increase in pepsinogen secretion but also prevented the higher increase obtained with EGF (Fig 4B). However, genistein and tyrphostin at the same concentrations used in previous experiments did not significantly affect CCK stimulated pepsinogen secretion (2-03 ± 0-5) with 10 μM genistein+10 nM CCK-OP and 2-29 ± 0-29 with 10 μM tyrphostin+10 (nM) CCK-OP v 2-6 ± 0-58 with CCK-OP alone; n=4.

Effects of interleukin 1β on basal and stimulated pepsinogen secretion from isolated human peptic cells

Unlike EGF, neither high nor low concentrations of IL 1β induced any significant effect on basal pepsinogen secretion from isolated peptic cells. Neither did IL 1β significantly affect CCK or acetylcholine stimulated peptic cells. However, IL 1β completely inhibited maximally histamine stimulated pepsinogen secretion (Table II). In a different set of experiments, both submaximally (0-1 mM) and maximally (mM) db-cAMP stimulated pepsinogen secretions were dose dependently inhibited by IL 1β (Fig 5).

Discussion

In this study, it has been shown for the first time that EGF stimulates basal pepsinogen secretion from isolated human peptic cells
and that IL 1 inhibits stimulated pepsinogen secretion dependent on cAMP mobilisation in the same experimental model. Parenteral EGF reduces acid secretion stimulated by histamine, pentagastrin carbachol, and insulin induced hypoglycaemia in rats, dogs, and monkeys.\(^8\) \(^{15}\) \(^{16}\) In vitro studies in Ussing chambers, in isolated gastric glands or in isolated parietal cells from rats, guinea pig, frog, and dogs have also shown that EGF inhibits acid secretion stimulated by different agonists.\(^7\) \(^{12}\) \(^{22}\) In these studies, species differences have been reported, suggesting that data from one species cannot be fully extrapolated to others (for example, humans). Unlike gastric acid, we have found that EGF stimulates basal pepsinogen secretion from dispersed human peptic cells. This effect was still present when pepsinogen secretion was maximally or submaximally stimulated, or both, with CCK, histamine, and db-CAMP. These effects also seem to be induced by TGF\(\alpha\), a factor that is structurally and functionally similar to EGF and competes with EGF for binding to the same receptor on the surface of epithelial cells.\(^14\)

Although acid and pepsinogen responses to different stimuli are thought to be similar, different and opposite effects of prostaglandins on acid and pepsinogen secretion from isolated canine and human gastric cells had previously been reported that suggests that acid and pepsinogen secretions are not always regulated in parallel.\(^{11}\) \(^{22}\) As far as we know, the only previous report in the English literature dealing with the effects of EGF on pepsinogen secretion was reported by Miyamoto et al in abstract form.\(^23\) These authors found that EGF inhibited forskolin and db-cAMP but not carbachol or CCK stimulated pepsinogen secretion in organ culture of small fragments of rabbit gastric mucosa at 0.1 \(\mu M\) concentrations. As mentioned above, differences between this study and our results might depend on the experimental model used (fragments of the entire mucosa versus isolated cells) and that EGF effects on human peptic cells could be species specific.

EGF can stimulate the proliferation of epithelial cells at pico to nanomolar concentrations,\(^7\) but the inhibition of histamine stimulated acid secretion requires nano to micromolar concentrations in different in vitro animal models.\(^18\) \(^{20}\) \(^{24}\) An important finding of our research is that EGF (and also TGF\(\alpha\)) stimulates basal pepsinogen secretion at pico to nanomolar concentration, suggesting that EGF can induce this effect on pepsinogen secretion in physiological conditions.

Different intracellular mechanisms have been proposed to explain the effects of EGF on acid secretion and includes the inhibition of cAMP to explain the effects of EGF on histamine stimulated acid secretion in rabbit parietal cells.\(^{19}\) Ostrowski et al\(^{20}\) reported that ornithine decarboxylase inhibition blocks the inhibitory effect of EGF on acid production in rat parietal cells, suggesting that EGF acts by means of the induction of ODC activity and the synthesis of polyamines. More recently, Tsunoda et al\(^{25}\) have reported that TGF\(\alpha\) inhibited both histamine and carbachol stimulated acid secretion in rabbit parietal cells by a tyrosine kinase dependent pathway. Our results suggest that EGF effects on pepsinogen secretion by dispersed human peptic cells is dependent on binding to EGF receptors because the stimulation was completely inhibited by pre-incubation with a human immunospecific antibody against EGF receptors. The expression of EGF receptors was shown in different gastric cell populations including parietal and chief cells of different animal species and humans.\(^{25}\) \(^{26}\) TGF\(\alpha\)/EGF receptor mRNA expression was more intense in parietal cell fractions than in chief cell fractions obtained from the stomach of different animals,\(^{26}\) which may suggest the requirement of higher concentrations of EGF to obtain responses from peptic cells. However, this is not the case in this study. Possible explanatory factors include the existence of high affinity EGF binding sites, a higher potency of human recombinant EGF in human peptic cells when compared with the effect of this factor in animal cells,\(^{20}\) or an increase of EGF receptor expression as shown in certain pathological conditions.\(^{27}\)
In this system, two different kinds of tiroisine kinase inhibitors with different specificities including genistein, which is a cell permeant specific inhibitor of both membrane associated and other protein tyrosine kinase activities by means of competition with ATP binding,38 and tyrphostin-25, which is an inhibitor of the EGF receptor tyrosine kinase activity, reversed the stimulatory effect of EGF on pepsinogen secretion, suggesting that both receptor and soluble tyrosine kinase activities might be involved in the modulation of EGF on pepsinogen secretion. Furthermore, it seems that this effect is independent of cAMP used pathways because the EGF stimulation of pepsinogen secretion was additive to that obtained after histamine and db-cAMP stimulation. EGF effects on pepsinogen secretion seem dependent on extracellular calcium, because EGF failed to stimulate basal pepsinogen secretion when extracellular calcium was removed from the medium. However, EGF also potentiated CCK but not ACh stimulated pepsinogen secretion, two secretagogues that stimulate pepsinogen secretion through calcium mediated pathways. These results fit the apparently contradictory results and the enhancement of the EGF potentiation of CCK effect is not clear and requires further and probably extensive research because of the complexity and not fully understood mechanisms of action through which EGF stimulate cells, which suggests that different calcium independent mechanisms might be involved.39 40 In this way, we have shown that CCK and EGF have different mechanisms of action because CCK is still able to stimulate secretion in the presence of two different tyrosine kinase inhibitors. Furthermore, we have also seen that, in our system, the pepsinogen secretion obtained with the combination of both CCK and acetylcholine is even higher than the addition of the secretion obtained with each one of them alone (preliminary unpublished data). These interesting data would explain why it is possible that EGF increase the secretion obtained with CCK, and that CCK and acetylcholine may have at least, some independent ways of stimulating pepsinogen secretion in human pectic cells. In other systems, peptides that stimulate enzyme secretion by interacting with CCK receptors can cause maximal stimulation with minimal changes in calcium mobilisation and maximal changes in calcium mobilisation occur with minimal changes in inositol phosphates.31

IL 1 is one of the main products of activated macrophages/monocytes and other cell types in both normal and inflamed tissue, including *H pylori* induced gastritis. At present, interest in this cytokine is growing because of its potential involvement in pectic ulcer disease and other gastroduodenal disorders.42 43 Furthermore, receptors for acetylholine, histamine, gastrin, and dopamine in macrophages and plasma cells from the lamina propia of the gastric mucosa have been found, which suggests a potential role for the immune system in the paracrine control of gastric secretion and pectic ulcer disease.4 Different animal studies have shown that the administration of IL 1β, whether intraperitoneal, intravenous or intra-sternal, inhibits basal, pentagastrin, and vagally stimulated gastric acid secretion.2 9 34 Preliminary data in isolated murine gastric glands and canine gastric parietal cells suggest that IL 1β inhibits histamine and carbachol stimulated acid secretion by acting directly on the parietal cells.35 36 The effects of IL 1 on pepsinogen secretion are not known. A single report from Okumura and Uhera37 in the pyloric glands of female rats shows that both intravenous and, above all, intracisternal IL 1β inhibits basal pepsinogen secretion. In this study, we have found that IL 1β does not significantly affect either basal or calcium dependent (CCK, acetylcholine) stimulated pepsinogen secretion from dispersed human pectic cells, but it completely inhibits stimulated pepsinogen secretion dependent on cAMP mobilisation (histamine, db-cAMP). This and other in vivo37 and vitro reports on acid secretion35 36 suggest that IL 1β may not only act through the indirect release of substances that will eventually inhibit the gastric secretion but through specific receptors on gastric cells. The intrinsic mechanisms whereby IL 1β inhibits pepsinogen secretion are not known. This study suggests that IL 1β may interact with secretagogues distal to the site of the catalytic subunit of adenylate cyclase activation. In this way, as shown for other substances, it could induce the inhibition of protein kinase A, through a redistribution of other kinases (for example, PKC).36 IL 1 stimulates prostaglandin secretion from different cells, including epithelial cells from the digestive tract.39 In vivo studies have suggested that the inhibition of gastric acid secretion by IL 1β is a prostaglandin mediated mechanism, because the inhibition of endogenous prostaglandin secretion by indomethacin reverses the effects of IL 1 on gastric acid secretion.37 However, this does not seem to be the case for pepsinogen secretion because it has previously been shown12 22 40 that prostaglandins do not inhibit but stimulate pepsinogen secretion by either human or animal pectic cells. Esplugues et al41 showed that IL 1β induced inhibition of pentagastrin stimulated acid secretion in the rat involves nitric oxide. This study may suggest the possibility that IL 1β effects on pepsinogen secretion also could be a nitric oxide mediated process. However, more recent reports from Fiorucci et al42 43 have shown that nitric oxide increases rather inhibits pepsinogen secretion induced by leukotrienes and calcium dependent agonists in guinea pig gastric chief cells. Whether these results can be extrapolated to human pectic cells and whether nitric oxide is involved in the effects of IL 1β on pepsinogen secretion is uncertain but deserves further research. Finally, by using patient material it may be questioned whether long term drug use or the presence of *H pylori* infection may disturb the response of the cells to different agents. Basal pepsinogen secretion and the response to EGF is similar in samples obtained from either *H pylori* positive or negative patients in this
study, which suggests that the presence or absence of previous H. pylori infection does not significantly affect the response to EGF. In any case, as previously discussed, if the basal status of cells is changed by long term drug use or H. pylori infection, the potential differences between experiments and the responses to different secretagogues will largely be eliminated by expressing the results as a percentage of total pepsinogen content with basal secretion subtracted.

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