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THE INFLUENCE OF OMEPRAZOLE ON THE SYNTHESIS AND SECRETION OF PEPSINOGEN IN ISOLATED RABBIT GASTRIC GLANDS

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Abstract—Regulation mechanisms of pepsinogen (EC 3.4.23.) synthesis and secretion were studied by following newly synthesized [^{14}C]-labeled pepsinogen during culture of isolated rabbit gastric glands.

Omeprazole, a substituted benzimidazole, while almost completely abolishing acid production at 10^{-4} M, strongly stimulated secretion of preformed and newly synthesized pepsinogen. Although the pepsinogen synthesis at this concentration of omeprazole was reduced to about 55% of the control rate, a two-fold absolute increase of total secreted pepsinogen was found. This increase was not due to a non specific leakage through disruption of chief cell membranes, as no increase of lactate dehydrogenase in the culture medium could be demonstrated.

The stimulated secretion was influenced neither by 10^{-3} M cimetidine, 10^{-3} M sodium thiocyanate nor 10^{-4} M atropine. No additivity was found between the carbachol (10^{-4} M) or dibutyryl cyclic AMP (10^{-3} M) and the omeprazole induced pepsinogen secretion.

Despite a vast amount of data on pepsinogen secretion obtained by *in vivo* [1–4] or *in vitro* studies [5–10], it remains unclear to what extent the regulation of pepsinogen secretion from the chief cell is affected by cholinergic stimulation, acid secretion or direct influence mediated by the parietal cell. Even less is known about the regulation of pepsinogen synthesis. *In vivo* stimulation of pepsinogen secretion by histamine or pentagastrin [4, 11, 12] has been suggested to be the result of a washout of preformed pepsinogen by the increased acid secretion [13] or stimulation by the increased acid production itself [14], as no stimulation of pepsinogen secretion by these compounds was found in isolated gastric mucosa [6, 10, 15]. Further evidence for the inability of histamine or pentagastrin to stimulate chief cell secretion was provided by Schepp *et al.* [16]. They showed that in the rat, intrinsic factor was synthesized and secreted by the chief cells and not stimulated by histamine or pentagastrin. Its release was mainly under cholinergic control.

Cholinergic agents such as carbachol are now generally recognized as potent stimulators of pepsinogen secretion [6]. Additivity of a beta-adrenergic stimulation by isoproterenol indicates a separate cholinergic and beta-adrenergic stimulation of pepsinogen secretion [15]. The beta-adrenergic stimulation is reported to be mediated by cAMP [15]. By blocking the simultaneously adrenergic stimulated acid secretion with cimetidine or sodium thiocyanate, we could confirm the observation of Hersey *et al.* [17], that the stimulation of pepsinogen secretion by adrenergic agonists was not mediated by the parietal cell. We also confirmed the inability of histamine and pentagastrin to stimulate pepsinogen secretion *in vitro* [10].

In 1975, Sutton and Donaldson [5] reported the synthesis of proteins including pepsinogen *in vitro*. They found an increase of [^{14}C]-leucine incorporation into total protein under influence of 10^{-9} M pentagastrin.

We have recently shown [10], that the actions of several secretagogues on pepsinogen synthesis and secretion could be studied in isolated human and rabbit gastric glands. Omeprazole, a substituted benzimidazole, reduces parietal cell acid output by selective inhibition of the H^+K^+ ATPase complex, which is responsible for the H^+ secretion [18]. In contrast to the inhibited acid secretion, Fimmel *et al.* [19] described an increase in the pepsinogen secretion by 10^{-4} M omeprazole in the *in vitro* perfused mouse stomach. We have recently observed a significant rise in serum pepsinogen levels after oral administration of omeprazole to healthy volunteers [20]. Serum pepsinogen is normally regarded as the result of leakage from disintegrating chief cells [4] and/or endocrine secretion [21].

In this study we have investigated the influence of omeprazole on the synthesis and secretion of pepsinogen in isolated rabbit gastric glands.

MATERIALS AND METHODS

Materials

[^{14}C]-labeled amino acid mixture (CFB 104; 50 μCi/ml) and [^{14}C]-aminopyrine were obtained from Amersham, England, collagenase (type II, 150 u/mg), hyaluronidase (380 u/mg), dibutyryl-cyclic adenosine monophosphate (dbcAMP)§, car-

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§ Abbreviations: dbcAMP, dibutyryl cyclic adenosine monophosphate; PAGE, polyacrylamide gel electrophoresis; TCA, trichloro acetic acid.

bachol and histamine from Sigma, St Louis, U.S.A. Cimetidine was a gift from Smith, Kline and French, Hertfordshire England, omeprazole from AB Hässle, Mölndal, Sweden.

Methods

Isolation of glands. Rabbit gastric glands were isolated according to Berglindh and Öbrink [22] with minor modifications. The perfusion step was omitted. Rabbit gastric mucosa was digested by a mixture of collagenase (1 mg/ml) and hyaluronidase (0.5 mg/ml), in 60 min at 37°.

Incubation of glands. Glands were incubated as previously described in 300 μl [^{14}C] amino acid supplemented RPMI 1640 medium [10]. Glands were incubated for a maximum of 5 hr in the presence of various compounds. Omeprazole was dissolved in 100% methanol, the final concentration of methanol in the culture medium was 1%. Incubations with 1% methanol only, were used as controls. After culture, glands and medium were separated and the glands were sonicated in 300 μl 0.1 M phosphate buffer pH 8. Aliquots of gland homogenates and medium (50 μl) were then subjected to polyacrylamide gel electrophoresis (PAGE) as described elsewhere [23, 24]. In short: electrophoresis was performed in a discontinuous system. The separating gel consisted of 10% acrylamide (2.5% bisacrylamide) in 35 mM Tris–HCl, pH 7.5. The stacking gel contained 3.5% acrylamide (20% bisacrylamide) in 10 mM Tris–H_3PO_4, pH 5.5. The electrode buffer consisted of 2.2 mM Tris–veronal, pH 7.0. Samples were diluted 2:1 with 50% sucrose in 20 mM Tris–H_3PO_4, pH 5.5 prior to application. Electrophoresis was performed for 70 min at +4° and 100 V/cm. Gels were stained either directly for protein, using a 0.2% Coomassie Brilliant Blue G250 solution in 12.5% trichloroacetic acid (TCA) or for acid proteolytic activity, performed by soaking the gel during 15 min in a solution of 2% bovine hemoglobin in 0.1 M HCl at 20°, followed by incubation in 0.1 M HCl for 40 min at 37°. After incubation, the gels were stained in a solution containing 0.2% Coomassie Brilliant Blue R250 in 10% (v/v) acetic acid/25% (v/v) isopropanol.

Gels, stained for protein, were processed in Amplify (Amersham) for autoradiography. After autoradiography, the pepsinogen isozymogen pattern, identified by activity staining after electrophoresis of identical samples as described above, was cut out of the dried gel and put into vials to which was added 4 ml LUMAGEL (Baker). [^{14}C] incorporation in intracellular and secreted pepsinogen was measured in a liquid scintillation counter (ISOCAP, Searle channel 8, 10 min). Incorporation of label into total protein was determined by TCA precipitation. Total protein content of the glands was determined for each sample by the Biorad protein assay. The total protein content of one gland suspension sample ranged from 1.0 to 3.0 mg.

Synthesis and secretion of pepsinogen. Pepsinogen synthesis was expressed as [^{14}C] incorporation in pepsinogen per mg total protein content of the incu-

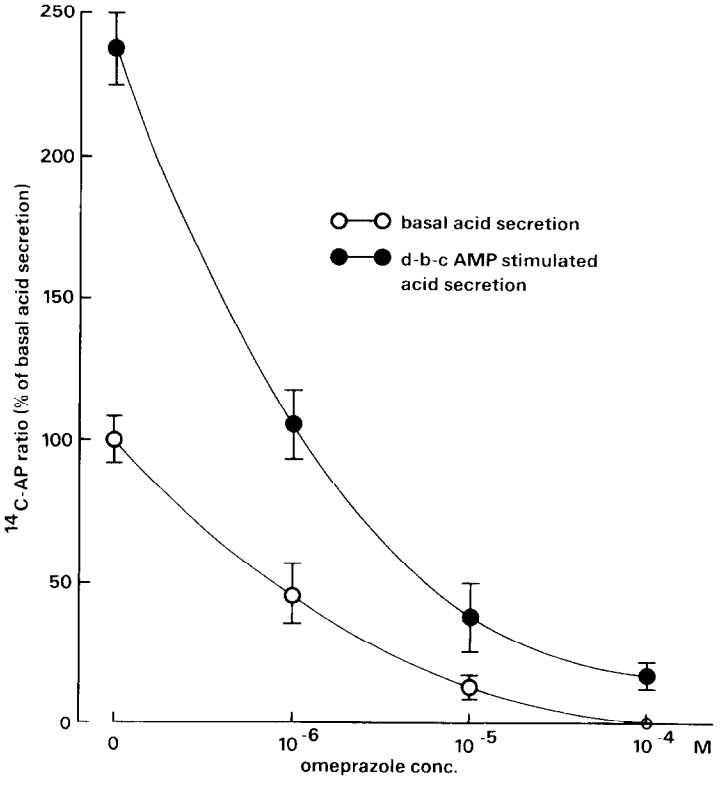

Fig. 1. Inhibition of basal and dbcAMP stimulated acid secretion by omeprazole: ○—○, basal acid production; ●—●, dbcAMP (10^{-3} M) stimulated acid production. Acid production was determined by aminopyrine accumulation. [^{14}C]-aminopyrine ratio is expressed as percentage of basal stimulation. Figures are means of three experiments ± S.D. The absolute AP ratio for the 100% value was 8.12 ± 2.1.

bated glands. Secretion of newly synthesized pepsinogen was calculated either absolutely as CPM in secreted pepsinogen per mg total protein content of the glands, or as percentage of total newly synthesized pepsinogen (intracellular and secreted) per incubation.

Determination of total pepsinogen secretion. Total secreted pepsinogen (labeled and unlabeled) in the medium (after culture) was determined according to Berstad [25] and was expressed as μg/mg total protein content of glands per incubation. Crystalline porcine pepsinogen (Sigma P7012, 2× crystallized) was used as a standard. Blanks contained all the agents used in the incubation.

Determination of acid secretion. Acid production of the parietal cell was assessed by the [^{14}C]-aminopyrine ([^{14}C]AP) uptake method as described by Berglindh *et al.* [26]. Stimulation or inhibition of acid formation was determined in aliquots of the gland suspension used for the synthesis studies, using 5 nmol (0.15 μCi/incubation). After 90 min incubation in a shaking water bath, glands and medium were separated, the glands were washed twice with phosphate buffered saline to minimize contamination with medium, dried with a cotton-wool stick and homogenized in 0.1 M phosphate buffer, pH 8. Aliquots of medium and gland suspension were counted for radioactivity in a liquid scintillation counter. Acid secretion was expressed as [^{14}C]AP ratio between medium and glands, stimulation and inhibition of secretion as [^{14}C]AP ratio percentage of basal secretion. Intraglandular water was determined as being equal to two times the dry weight of the glands [26]. Based on the fact, that the total protein content of the cell bears an almost constant relation to its dry weight (± 70%) [27], the total protein contents of the glands were used to calculate the [^{14}C]AP ratio.

Measurement of lactate dehydrogenase (LDH) release to the medium. LDH release was measured as a marker of chief cell membrane integrity [9, 15]. LDH activity was measured using a spectrophotometric assay with pyruvate and NADH as substrates [28]. 10 μl Aliquots of medium were added to the reaction mixture containing 50 mmol Tris, 5 mM EDTA, 0.15 mM NADH, 0.6 mM Na-pyruvate.

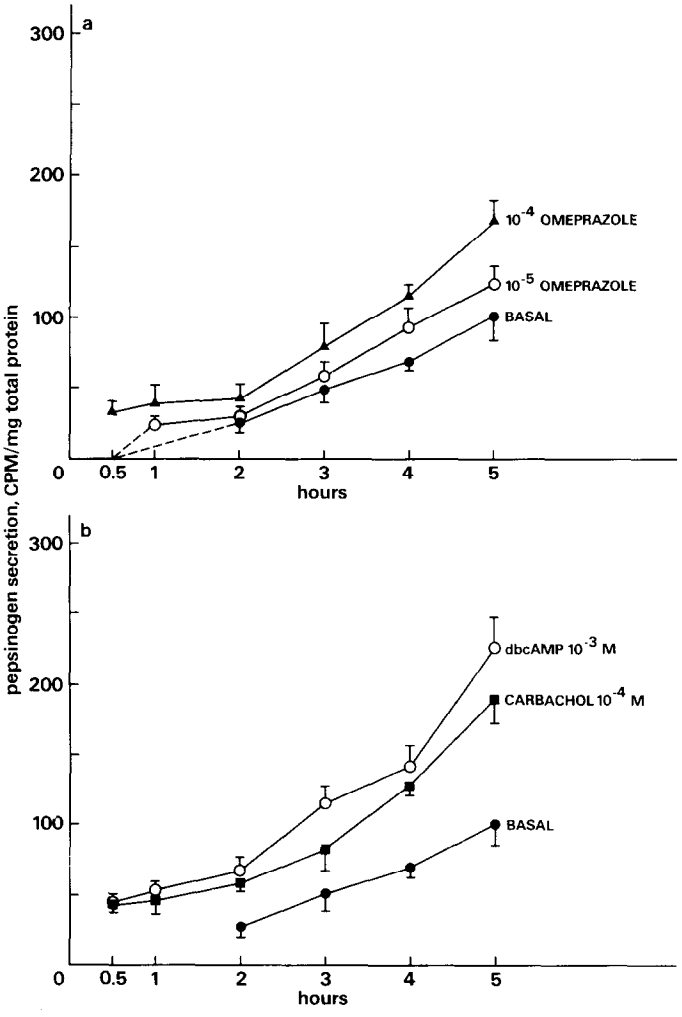

Fig. 2. Time course of labeled pepsinogen secretion. (a) During culture in the presence of omeprazole: ●—●, basal; ○—○, 10^{-5} M; ▲—▲, 10^{-4} M omeprazole. (b) During culture in the presence of 10^{-4} M carbachol (■—■) and 10^{-3} M dbcAMP (○—○). Pepsinogen secretion is expressed as CPM in secreted pepsinogen per total protein content of the glands. Experiment shown is representative for three others.

Table 1. Pepsinogen secretion by isolated rabbit gastric glands

	Omeprazole concentration			
	0	10^{-6} M	10^{-5} M	10^{-4} M
Basal	100 ± 23	123 ± 22*	120 ± 20*	179 ± 20*
Carbachol 10^{-4} M	191 ± 27*	176 ± 14*	181 ± 19*	191 ± 21*
dbcAMP 10^{-3} M	226 ± 19*	227 ± 25*	229 ± 17*	212 ± 19*

Pepsinogen secretion (CPM/mg total protein) is expressed as percentage of basal secretion after 5 hr. Values are means of three experiments ± S.D. The absolute value of the 100% basal secretion was 110 ± 21 CPM/mg total protein of glands.

* $P < 0.01$, compared to basal; + not significant vs no omeprazole (Student's *t*-test).

LDH activity was calculated from the decrease in extinction at 340 nm.

Statistical analysis

The statistical significance of differences was evaluated with Student's *t*-test, with n equal to the number of gland preparations.

RESULTS

Effect of omeprazole on pepsinogen synthesis and secretion

Omeprazole showed a dose dependent inhibition of both basal and dbcAMP (10^{-3} M) stimulated acid secretion, with a maximal effective dose of 10^{-4} M and an IC_{50} value of about 8.5. 10^{-7} M (Fig. 1).

In contrast to the inhibition of acid secretion, the secretion of newly synthesized pepsinogen was stimulated by 10^{-4} M omeprazole. Figure 2 shows the time course of labeled pepsinogen secretion, expressed as CPM in secreted pepsinogen per mg total protein, under influence of 10^{-5} and 10^{-4} M omeprazole (Fig. 2a), 10^{-4} M carbachol and 10^{-3} M dbcAMP (Fig. 2b). At a concentration of 10^{-4} M omeprazole, the amount of labeled pepsinogen in the medium after 5 hr was 1.75 times higher compared to basal, the dbcAMP and carbachol stimulated secretion was about twice as high as basal. At lower concentrations of omeprazole (10^{-6} and 10^{-5} M) no significantly higher secretion compared to basal was found (Table 1).

Figure 2 further shows, that without stimulation, labeled pepsinogen is detectable in the medium after 2 hr, reflecting the transit time between synthesis and secretion. Stimulation with carbachol, dbcAMP and omeprazole resulted in a transit time of 30 min.

In contrast to the stimulated secretion, synthesis of both total protein (determined by TCA precipitation, results not shown) and pepsinogen in the presence of 10^{-4} M omeprazole was inhibited, resulting in an 45% decrease in the rate of pepsinogen synthesis, compared to controls after 5 hr. (Fig. 3). Carbachol (10^{-4}) and dbcAMP (10^{-3} M) did not

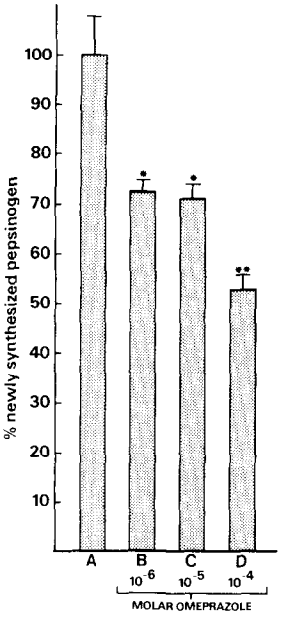

Fig. 3. Inhibition of pepsinogen synthesis by increasing doses of omeprazole. Glands were cultured for 5 hr and synthesis is expressed as percentage of basal pepsinogen synthesis. Figures are means of five experiments ± S.D. The 100% level was 3000 ± 240 CPM/mg total protein * $P < 0.01$; ** $P < 0.001$.

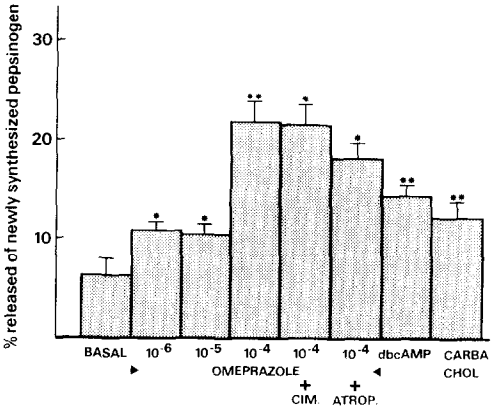

Fig. 4. Pepsinogen secretion under influence of various drugs. Glands were cultured for 5 hr in the presence of the following compounds: 10^{-6}; 10^{-5}; 10^{-4} M omeprazole; 10^{-4} M omeprazole + 10^{-3} M cimetidine; 10^{-4} M omeprazole + 10^{-4} M atropine; 10^{-3} M dbcAMP and 10^{-4} M carbachol. Pepsinogen secretion is expressed as percentage secreted of newly synthesized pepsinogen. The absolute value of the 100% level was 2.7 ± 0.9 μg/mg total protein. * $P < 0.001$ compared to control. Figures are means of five experiments ± S.D.

influence the pepsinogen synthesis. As a consequence of this diminished synthesis, the relative amount of secreted labeled pepsinogen, expressed as percentage of newly synthesized pepsinogen, was more than three times higher compared to basal (Fig. 4.) The lower concentrations of omeprazole (10^{-6}–10^{-5} M) also increased the relative pepsinogen secretion expressed as percentage of newly synthesized, but the effect seems to be non-linear.

The effect in time of omeprazole on the secretion of total pepsinogen is represented in Fig. 5(a), showing that omeprazole at 10^{-4} M also stimulated secretion of preformed, unlabeled pepsinogen. As a result of the initial secretion of preformed pepsinogen, the stimulation of total pepsinogen secretion under influence of omeprazole (10^{-4} M) was slightly higher than the stimulation of labeled pepsinogen secretion (twice versus 1.75 times basal) and did not differ significantly from the stimulation by carbachol and dbcAMP (Fig. 5b.). The lower concentrations of omeprazole did not significantly stimulate secretion of total pepsinogen.

Both carbachol (10^{-4} M) and dbcAMP (10^{-3} M) gave a ± two-fold stimulation of total and labeled pepsinogen secretion. However, no additivity was found between stimulation by omeprazole at all concentrations tested and the carbachol or dbcAMP stimulated secretion, either on total pepsinogen or labeled pepsinogen secretion (Table 1).

No effect on the pepsinogen secretion was observed when glands were cultured in the sole presence of 10^{-3} M NaSCN, thereby preventing acid formation by destroying the proton gradient over the parietal cell membrane.

As pepsinogen secretion is mainly under cholinergic control [15], we have investigated whether the omeprazole stimulated pepsinogen secretion could be influenced by atropine, an antimuscarinic inhibitor of cholinergic receptors. No influence on the omeprazole stimulated pepsinogen secretion was found by either atropine (10^{-4} M) or cimetidine (10^{-3} M) (Fig. 4), confirming the results of Fimmel *et al.* [19].

Short time secretion studies

The effect of omeprazole during short incubation times (0–120 min) is represented in Fig. 6. This figure shows, that the 10^{-4} M omeprazole stimulated pepsinogen secretion is almost linear in time during the first 60 min. After 60 min the stimulation declines, probably as a result of the simultaneously inhibited pepsinogen synthesis.

The combined lower synthesis and higher secretion of pepsinogen caused by omeprazole, were not the

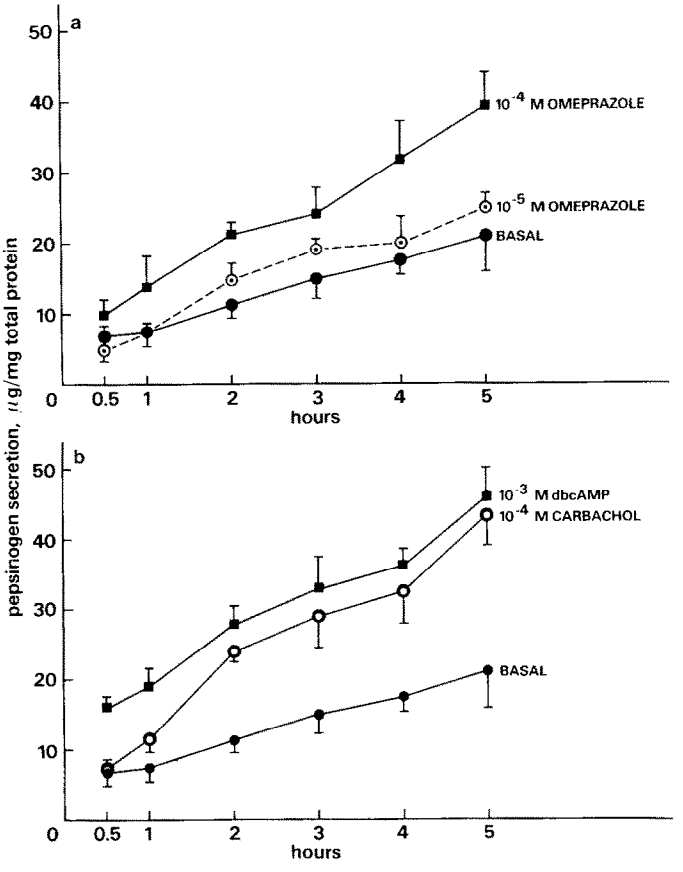

Fig. 5. Time course of total pepsinogen secretion. (a) During culture in the presence of omeprazole: ●—●, basal; ○—○, 10^{-5} M; ■—■, 10^{-4} M omeprazole. (b) During culture in the presence of 10^{-4} M carbachol (○—○) and 10^{-3} M dbcAMP (■—■). Pepsinogen secretion is expressed as µg/total protein content of the glands. Experiment shown is representative for three others.

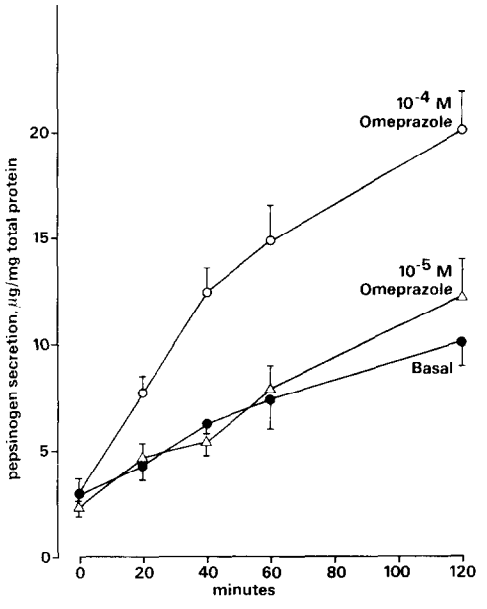

Fig. 6. Time course of total pepsinogen secretion, basal and stimulated by omeprazole, 10^{-5} M and 10^{-4} M. Pepsinogen secretion is expressed as µg/mg total protein content of the glands. Figures are means of three experiments ± S.D.

result of a non-specific disintegration of cells as no increase of LDH activity could be detected in the medium compared to controls after 5 hours of culture (Table 2).

Furthermore, measurement of total pepsinogen secretion by the glands at 4°, showed no increase of pepsinogen secretion over basal, indicating, that omeprazole does not cause a non-specific disruption of the zymogen granules (results not shown).

DISCUSSION

Omeprazole blocks acid formation by selectively inhibiting the H^+K^- ATPase complex in the membrane of the parietal cell [18]. Its action is reported to be far more effective than the action of H2 receptor antagonists [18], allowing almost complete inhibition of acid production. In spite of the inhibited pepsinogen synthesis at 10^{-4} M omeprazole, the simultaneous stimulation of pepsinogen secretion still resulted in an increase of labeled as well as total pepsinogen in the medium. The lack of effect of atropine on the stimulation by omeprazole indicates, that a cholinergic receptor mechanism is not involved in this omeprazole induced stimulation.

Table 2. Lactate dehydrogenase release by cultured isolated rabbit gastric glands

	LDH activity, % of total
Control	6.4 ± 0.8
10^{-6} M omeprazole	4.5 ± 0.9
10^{-5} M omeprazole	5.8 ± 0.9
10^{-4} M omeprazole	5.7 ± 0.5

LDH release after 5 hr of culture was expressed as percentage of total LDH activity in medium and glands.

Although omeprazole reduced the transit time between synthesis and secretion, the effect was not different from that of dbcAMP or carbachol. Furthermore, almost 80% of the newly synthesized pepsinogen has not been secreted after 5 hr. Therefore, omeprazole probably does not influence package of pepsinogen, but as a result of a stimulated secretion, the normal maturation of the zymogen granules is impaired, resulting in a faster secretion of newly synthesized pepsinogen [4]. This explains also the action of lower concentrations of omeprazole; by reducing the transit time, the effect of a diminished synthesis (±72% of control rate) is counteracted, resulting in an unchanged secretion of labeled pepsinogen. The experiments on total pepsinogen secretion indicate, that these lower concentrations of omeprazole do not stimulate secretion of preformed stored pepsinogen. The non-linearity of the effect of omeprazole may suggest a threshold concentration, necessary for stimulation of total pepsinogen secretion, although Fimmel *et al.* found a dose dependent effect of omeprazole on the pepsinogen secretion in the perfused mouse stomach [19]. The combined lower synthesis and higher secretion also indicate, that inhibition of synthesis does not necessarily impair secretion.

Comparison between Figs. 2 and 5 shows, that the increase of secreted total pepsinogen is parallelled by an increase of secreted labeled pepsinogen. This indicates, that during stimulation, in time, an increasing part of the secreted pepsinogen is labeled. Thus, measurement of labeled pepsinogen secretion gives an accurate estimation of the stimulatory effect of these agents on the secretion. Another advantage of combined studies on pepsinogen synthesis and secretion is shown by the short time secretion curves in Fig. 6. Here the non-linearity of the stimulation by omeprazole after 60 min can be explained as the result of the simultaneously inhibited pepsinogen synthesis becoming effective after 60 min. We did not observe a further increase of the omeprazole stimulated pepsinogen secretion by carbachol or dbcAMP. This suggests, that the pepsinogen secretion under influence of omeprazole has reached its maximal capacity or, in the case of dbcAMP, is using the same pathway. In contrast, an additivity was found in whole stomach preparations between the stimulation by 10^{-5} M omeprazole and submaximal concentrations of carbachol [19]. Therefore further testing should be done, using combinations of omeprazole with other pepsinogen secretagogues, such as the cholecystokinine like peptides.

Previously we have reported [10], that cimetidine has a dose dependent inhibitory effect on the pepsinogen synthesis and no effect on the pepsinogen secretion. In this study we have also found an inhibition of the pepsinogen synthesis by omeprazole. Both the inhibitory effect on the pepsinogen synthesis and the stimulatory effect on the pepsinogen secretion were, however, only significant at an omeprazole concentration where basal acid secretion was completely abolished. As preventing acid formation by NaSCN had no effect on either pepsinogen synthesis or pepsinogen secretion, we must conclude however, that the effect of omeprazole is not due to the absence of acid. It is likely, that the increased

pepsinogen output by isolated gastric glands, reflects both increased exocrine and endocrine pepsinogen secretion. This mechanism could then explain the rise in pepsinogen serum levels in volunteers, receiving oral omeprazole, as reported by Festen *et al.* [20].

The endocrine secretion of pepsinogen probably represents only a minor part of the total pepsinogen output by the chief cell. It seems therefore unlikely, that the increased total pepsinogen output caused by omeprazole *in vitro* can be attributed to endocrine secretion only, especially as the dbcAMP and carbachol induced pepsinogen secretion is regarded as exocrine secretion. In addition, the experiments on LDH release argue against a contribution from disintegrating cells. We suggest, that the effect of omeprazole is either directly on the chief cell, or that blocking of the H^+ K^+ ATPase complex, results in changed membrane potentials between parietal and chief cell [29], which could lead to a facilitation of the zymogen granule/chief cell membrane fusion. No effect of omeprazole on the pepsinogen secretion was found by Fryklund *et al.* [30] in preparations of isolated rabbit chief cells, further indicating, that the effect of omeprazole on the pepsinogen secretion is mediated by the parietal cell. For the inhibitory effect on pepsinogen synthesis, (reflecting inhibition of total protein synthesis), we have as yet no sufficient explanation. Electron microscopy studies have shown, that omeprazole not only decreases the canalicular expansion of the parietal cell during acid secretion but also causes an increase in condensed (at low energy state) mitochondria in the cell [31]. Furthermore, the compound benzimidazole itself is a microtubular inhibitor [31] and may therefore cause damage to the lumen of the endoplasmic reticulum, resulting in an inhibited protein synthesis.

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