Pepsinogen synthesis and secretion in isolated gastric glands

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SUMMARY De novo synthesis of pepsinogen was shown in isolated rabbit and human gastric glands after incubation of the glands in a ^{14}C labelled amino acid enriched minimum Eagles medium. At regular intervals, glands and medium were separated and analysed by polyacrylamide gel electrophoresis. Newly synthesised pepsinogen was shown by autoradiography. Incorporation of ^{14}C labelled amino acids was detected after only 30 min of culture and increased almost linearly in time for 4 h. By comparing the incorporation of label into total protein and into pepsinogen, it was concluded that pepsinogen formed 70-90% of the newly synthesised protein.

Cimetidine, at a concentration of 160 $\mu\text{g/ml}$, strongly inhibited the synthesis of pepsinogen. Spontaneous secretion of pepsinogen into the medium was very low and relatively constant. Dibutyryl cyclic AMP considerably stimulated the secretion of pepsinogen into the medium. Histamine and pentagastrin did not influence the release of pepsinogen.

These results show that isolated gastric glands are capable of synthesis and secretion of pepsinogen and that both can be selectively stimulated and inhibited.

Pepsinogen is synthesised in the gastric mucosa by the chief and neck cells of the glandular ducts and is secreted into the lumen of the gastric gland.¹⁻³ Human pepsinogen can be separated on polyacrylamide gel electrophoresis into seven isozymogens, consisting of two immunochemically distinct groups: pepsinogen A (formerly pepsinogen I, fractions 1-5) and pepsinogen C (formerly pepsinogen II, fractions 6 and 7). In contrast to pepsinogen C, pepsinogen A is secreted in the urine and shows considerable interindividual heterogeneity. Several genetic models have been proposed to explain the inheritance of urinary pepsinogen A.⁴⁻⁶

We have postulated a new multigene model for pepsinogen A, in which the main fractions, 3, 4, and 5 are each coded for by a specific gene, B, C, and D respectively.⁷ Furthermore, we recently described an association between distinct electrophoretic pepsinogen A patterns in biopsy samples of gastric mucosa and (pre)malignancy of the stomach.⁸ These findings suggest a predisposition of some genetic pepsinogen A types to malignancy of the stomach. There were, however, considerable intraindividual differences between the pepsinogen A isozymogen

patterns in stomach tissue and urine in about 10% of the patients studied. This discrepancy makes it questionable to use urinary pepsinogen analyses for the prediction of the gastric mucosa pepsinogen A patterns for clinical purposes.

There are several explanations for this discrepancy:

- 1 Differential secretion or reabsorption of the pepsinogen A isozymogens by the kidney.
- 2 Differential secretion by the zymogen cell.
- 3 Interconversion of the isozymogens.
- 4 Different synthesis regulation mechanisms for the separate isozymogens.

The secretion of pepsinogen has been studied extensively in vivo and in vitro.^{1-3 9 10} Koelz *et al*⁹ studied cholinergic and β -adrenergic stimulated pepsinogen release in isolated rabbit gastric glands and their results indicated that pepsinogen release is regulated in part by separate cholinergic and β -adrenergic mechanisms and that cyclic AMP plays a role in this regulation.

Few studies have been performed on pepsinogen synthesis either in vivo or in vitro. In 1976 Sutton and Donaldson reported the in vitro synthesis of pepsinogen in cultured rabbit gastric mucosa tissue.¹⁰ In these investigations, however, the secretion or synthesis of total pepsinogen and not that of the

separate isozymogens was studied. To investigate the inter-relation between the pepsinogen A fractions—that is, the effect of genetics, post-translational modifications, and differential secretion—a reproducible technique for studying de novo pepsinogen synthesis and secretion of this newly synthesised pepsinogen in vitro was needed. Using the tissue culture technique of Browning and Trier,¹¹ we have shown that whole biopsy samples of human gastric mucosa taken at routine gastroendoscopy are capable of pepsinogen synthesis (Pals and Défze, unpublished observations). This technique showed considerable intra- and interbiopsy variation in pepsinogen synthesis, which made it difficult to evaluate pulse and dose response studies. Furthermore, it was impossible to take multiple identical samples of cells and medium at different times. We report here the synthesis and secretion of pepsinogen by isolated rabbit and human gastric glands.

Material and methods

ISOLATION OF GASTRIC GLANDS

Isolation was done essentially according to the method of Berglindeh *et al.*¹² Briefly, the stomach of an anaesthetised rabbit was perfused with saline under high pressure through the aorta. The stomach was removed, and the mucosa stripped, minced into small pieces, and transferred to a collagenase solution (1 mg/ml, type I, Sigma) After 90 min incubation at 37°C the separated glands were repeatedly washed to remove free cells, and then resuspended in culture medium. Human biopsy samples, obtained by fibre endoscopy from patients, were treated with collagenase for 120 min. Viability of the glands was confirmed by the trypan blue exclusion test. As culture medium, amino acid free minimum essential medium was used, prepared from a minimum essential medium select amine kit (Gibco RPM1 1640). To this was added: penicillin (100 U/ml); streptomycin (100 µg/ml); insulin (porcine; 0.6 U/ml) and 10% fetal calf serum. Culture was performed in Eppendorf no 403 tubes in a rotating waterbath at 37°C. Labelling was done by adding 2.0 µCi ¹⁴C amino acid mixture (Amersham CFB 104, 250 µCi/ml) to a number of tubes containing 200 µl gland suspension. Reagents added were of the highest purity available: dibutyl cyclic AMP, carbachol, and histamine were obtained from Sigma, St Louis, USA; pentagastrin (peptavlon) from ICI, England; cimetidine was a gift from Smith, Kline and French, Hertfordshire, England.

In pulse chase experiments, glands were pulse labelled for 30 min, washed once in unlabelled culture medium, resuspended again in unlabelled cul-

ture medium, and cultured for additional different time intervals. Duplicate tubes were removed at regular intervals, and glands and medium were separated by centrifugation. Glands were resuspended in 100 µl 0.1M phosphate buffer, pH 8, and sonified; 50 µl aliquots of medium and gland homogenates were subjected to polyacrylamide gel electrophoresis, according to Pals and Défze.¹³ Gels were stained for pepsinogen activity or autoradiographed. Before autoradiography, gels were stained for protein, using a 0.1% Coomassie brilliant blue G250 solution (Biorad) in 12.5% trichloroacetic acid. Gels were then incubated in Enhance (New England Nuclear, Boston, USA) for 1 h and, after washing in water, dried and put on Kodak Safety film AR or RX-1 on an intensifying screen (SP 02810511) and stored in the dark at -70°C for one week. After autoradiography, the pepsinogen isozymogen pattern was cut out of the gel, put in vials, and ¹⁴C incorporation in pepsinogen was measured in a liquid scintillation counter. Total incorporation of label into protein was determined by trichloroacetic acid precipitation. Assessment of total protein content of the glands was done by the Biorad protein assay. Pepsinogen synthesis was expressed as ¹⁴C incorporation per milligram total protein content of the incubated glands. Secretion of pepsinogen was expressed as percentage of newly synthesised pepsinogen (intracellular and secreted) per incubation.

Fig. 1 Autoradiograph of cultured rabbit gastric glands after polyacrylamide gel electrophoresis. Glands were cultured in the presence of 2 µCi ¹⁴C amino acids for different time intervals.

Fig. 2 Time course of synthesis by isolated rabbit gastric glands. ●—● Incorporation into total protein/mg total protein content of the glands. ■—■ Incorporation into pepsinogen/mg total protein content of the glands. Results shown are derived from duplicate values of one experiment. This experiment is representative of three others. CPM = counts per minute.

Fig. 3 Synthesis of pepsinogen by rabbit glands in the presence of various compounds. Glands were cultured for 4 h. (A) control; (B) +1 mM dibutyryl cyclic AMP; (C) +1 mM sodium thiocyanide; (D) +40 µg cimetidine; (E) +80 µg cimetidine; (F) +160 µg cimetidine. Synthesis was expressed as CPM/mg total protein content of the glands. Results shown are derived from duplicate values of one experiment which is representative of three others. CPM = counts per minute.

Results

Isolated glands were cultured for a maximum of 4 h. At intervals, samples were taken in duplicate and analysed for pepsinogen synthesis. Fig. 1 shows an autoradiograph of cultured rabbit glands after electrophoresis. Incorporation of label was detectable already after only 30 minutes. Protein and pepsinogen activity staining of the same samples showed that incorporation of label was predominantly into pepsinogen. This was confirmed by comparing label incorporation in total protein and in pepsinogen as a function of time: 70–90% of the label was incorporated into pepsinogen (Fig. 2). When the glands were incubated in the presence of cimetidine (40–160 µg/ml; 0.15–0.6 mM) a strong dose dependent inhibition of the pepsinogen synthesis was observed, while pentagastrin or histamine (at a concentration of 1 mM) showed no effect (Fig. 3).

STIMULATION OF PEPSINOGEN RELEASE

Fig. 4 shows an autoradiograph of the secreted pepsinogen. After 2 h secretion of the newly synthesised pepsinogen was observed. Basal pepsinogen secretion was very low. An almost two fold increase of pepsinogen release compared with controls was

Fig. 4 Autoradiograph of secreted pepsinogen by rabbit gastric glands after polyacrylamide gel electrophoresis of the culture medium. Staining for protein of the same gel before autoradiography shows secretion of unlabelled pepsinogen at 0.5, 1 and 2 h. DBcAMP = dibutyryl cyclic AMP.

found when the glands were cultured in the presence of 1 mM dibutyl cyclic AMP (Fig. 5). As dibutyl cyclic AMP also stimulates acid secretion by the parietal cells, the pepsinogen secretion increase could be caused by increased acid output. Blocking the acid secretion by cimetidine or neutralising the secreted acid with 1 mM sodium thiocyanide, however, did not alter the stimulating effect of dibutyl cyclic AMP on the pepsinogen release. We therefore conclude that the effect of dibutyl cyclic AMP is a direct one and is not mediated by the increased acid output of the parietal cell (Fig. 6). Cholinergic stimulation by culturing the glands in the presence of 1 mM carbachol resulted in a less pronounced increase in pepsinogen release compared with dibutyl cyclic AMP.

The stimulation by both carbachol and dibutyl cyclic AMP was abolished by the simultaneous addition of 1 mM atropine. Glands cultured in the presence of cimetidine did not show an inhibition of pepsinogen release. Nor did we find a stimulating effect on the secretion by histamine or pentagastrin (Fig. 6).

ISOLATED HUMAN GASTRIC GLANDS

Isolation of human glands was more difficult than for rabbit glands. A longer collagenase digestion was needed (120 min), which also caused a considerable heterogeneity in length of the glands. Also, the gland suspension had to be more diluted because the

Fig. 5 Time course of pepsinogen secretion by rabbit gastric glands: ○—○ Basal; ●—● Stimulated by 1 mM dibutyl cyclic AMP. Secretion at different times was expressed as the amount of incorporated label (CPM) in secreted pepsinogen/mg total protein content of the incubated glands. Values are means of three experiments $\pm$ SD. CPM = counts per minute.

Fig. 6 Secretion of pepsinogen under influence of various compounds. Rabbit glands were cultured for 4 h and secretion was expressed as percentage of newly synthesised pepsinogen per incubation. (A) basal; (B) +1 mM dibutyl cyclic AMP; (C) +1 mM dibutyl cyclic AMP +1 mM NaSCN; (D) +1 mM dibutyl cyclic AMP +160 μ g/ml cimetidine; (E) +1 mM NaSCN; (F) +1 mM pentagastrin; (G) +1 mM histamine; (H) +160 μ g/ml cimetidine. Values are means of three experiments $\pm$ SD.

Fig. 7 Autoradiograph of cultured human glands after polyacrylamide gel electrophoresis showing synthesis of pepsinogen (PG) after different time intervals. Glands were cultured in the presence of 14 C labelled amino acids. Absence of other labelled proteins in the PG region of the gel was confirmed by electrophoresis of samples incubated with anti-pepsinogen-A and anti-pepsinogen-C.

glands aggregated very easily in concentrated suspensions. As in rabbit glands, incorporation of label was detectable after 30 min, but it did not increase significantly in time. The absence of other labelled proteins in the pepsinogen region of the gel was determined by polyacrylamide gel electrophoresis of samples preincubated with anti-pepsinogen A and anti-pepsinogen C. Label incorporation was again for 70–90% into pepsinogen and the action of several secretagogues was the same as described for the rabbit glands. Cimetidine again inhibited the synthesis of pepsinogen in a dose dependent manner.

No changes in time in the isozymogen pattern were noted; all the bands seemed to appear simultaneously, indicating that there is no common precursor for the isozymogens (Fig. 7). In pulse chase experiments we saw only an increase of pepsinogen A-2 at the expense of pepsinogen A-3, which confirmed the hypothesis that pepsinogen A-2 is a secondary product of pepsinogen A-3 (results not shown). No significant differences in the secreted and intracellular pepsinogen A pattern were found. In some cases the relative concentration of extracellular pepsinogen A-3 was slightly less than that of intracellular pepsinogen A-3.

Discussion

Berglinth *et al*¹² have shown that a preparation of isolated gastric glands is a good model system for studying gastric acid metabolism. Koelz *et al*¹⁰ explored the potential of isolated glands for the study of pepsinogen secretion and found that isolated glands met the criteria for a good model system—that is, a high zymogen content and stable low rate of spontaneous secretion. Our study shows that isolated glands also provide a good system for studying pepsinogen synthesis on a cellular basis. The viability of the glands is confirmed by the incorporation of labelled amino acids into proteins. From the experiments with cimetidine, we conclude that the ability of cimetidine to lower the pepsinogen content in the stomach *in vivo* is probably due to inhibition of synthesis rather than secretion.

Little is known about the influence of several drugs on the secretion of pepsinogen. There is evidence for a separate β -adrenergic (mediated by cyclic AMP) and cholinergic stimulation, the cholinergic stimulation possibly being dependent on external calcium.⁹ This is in agreement with zymogen release in the pancreas¹⁴ and salivary glands.¹⁵

In contrast to the secretion stimulation capacity of histamine and pentagastrin *in vivo*,^{16,17} we found no stimulation of pepsinogen secretion by these drugs

in isolated glands. This is in agreement with other studies using the organ culture technique.^{3,18} Studies on isolated parietal cells have shown that the stimulatory effect of histamine on acid secretion is mediated by the adenylate cyclase-cyclic AMP system.^{9,20} It has been postulated that the β -adrenergic stimulation of pepsinogen release is also mediated by cyclic AMP.¹⁰ This is supported by our findings those of others^{9,21} that dibutyl cyclic AMP stimulates pepsinogen secretion. We have established that this stimulation is a direct one and not mediated by an increased acid release of the parietal cell. The lack of stimulation by histamine (or its chemical derivative betazole hydrochloride) could be due to the rapid degradation of cyclic AMP by phosphodiesterase, as Ito *et al*²¹ found that simultaneous inhibition of phosphodiesterase by aminophylline considerably enhanced the stimulatory effect of betazole hydrochloride on pepsinogen release. In contrast, forskolin does stimulate the secretion of pepsinogen by isolated glands.²² This can be explained by the fact that forskolin is a much more potent stimulator of adenylate cyclase activity than histamine. Treatment with forskolin results in a 100 fold increase in tissue cyclic AMP concentration.²² In how far the effects of several secretagogues on pepsinogen secretion and synthesis are a direct result of their action on the zymogen cell or are mediated by the parietal cell remains to be elucidated. Increasing the cyclic AMP content of the parietal cell will stimulate the acid output, and Johnson²³ showed, for example, that topical acidification of gastric mucosa with diluted hydrochloric acid resulted in a two fold increase of pepsinogen secretion. It would therefore be of great interest to investigate the potential of isolated zymogen cells for secretion and synthesis of pepsinogen.

The experiments with human gastric glands show that there is probably no common precursor for the pepsinogen A isozymogens. The secreted pepsinogen A pattern, except for minor differences between pepsinogen A-2 and pepsinogen A-3, was the same as the intracellular, excluding differential secretion in these cases. This study also shows that preparations of isolated gastric glands are indeed an excellent tool for studying synthesis and secretion of pepsinogen.

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