Regulation of Gastric Mucosal Pepsinogen and Intrinsic Factor Contents, and Their mRNA Levels during Starvation and Refeeding in Rats

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Gastric mucosal pepsinogen and intrinsic factor (IF) contents, and their mRNA levels during starvation and refeeding were studied. During starvation for 4 d, gastric mucosal pepsinogen and IF contents significantly decreased, whereas pepsinogen and IF mRNA levels increased by 30—50%. These results suggested that the mRNAs of pepsinogen and IF could be preserved for a long time so as to prepare for refeeding. After ceasing the starvation for 72 h, gastric mucosal pepsinogen and IF contents were significantly decreased at 1 h after refeeding, and their mRNA levels were increased by 20—30% at 30 min after refeeding. We examined whether the refeeding-induced changes in gastric mucosal pepsinogen and IF contents and their mRNA levels could be reproduced by the exogenous administration of secretagogues. They were not found to be affected by the administration of each secretagogue during starvation for 72 h at 30 min. However, by the simultaneous administration of 2 or 3 secretagogues (carbachol, cholecystokinin octapeptide (CCK-8) or secretin), the contents of pepsinogen and IF decreased to 70—80% and 50—80% of the control, respectively. However, their mRNA levels increased to 140—160% and 120—135% of the control, respectively. Therefore, refeeding-induced changes in pepsinogen and IF contents and their mRNA levels were partially reproduced by exogenously administered secretagogues. This showed that food intake influences huge changes in neural, hormonal and physical conditions on the stomach. It was indicated that the secretagogues stimulated not only pepsinogen and IF secretion, but also had a tendency to increase their mRNA.

Key words pepsinogen; intrinsic factor; mRNA; starvation; refeeding; rat

Although the IF of many mammalians, including human, is secreted from parietal cells, we previously confirmed that pepsinogen and IF are synthesized in gastric chief cells in rat, and both are secreted by the same mechanism.[1] Many observations have been obtained from studies on isolated gastric chief cells.[2] However, the regulation of pepsinogen and IF *de novo* synthesis is not yet well clarified. It is supposed that the expression of pepsinogen and IF mRNA is implicated at least in glucocorticoids from experiments using sucking and adrenalectomized rats.[3] On the other hand, starving and feeding cause various changes in the digestive tract. Majumdar observed that gastric mucosal DNA synthesis was decreased by starvation and increased by refeeding in rats.[4] According to Wu *et al.*, starvation caused a decrease in antral gastrin mRNA level and an increase in somatostatin mRNA level concomitantly, whereas inverse phenomena were observed with refeeding.[5] In relation to digestive enzymes, it was reported that starvation caused a decrease in amylase, chymotrypsinogen B and trypsinogen I mRNA in the pancreas.[6] However, no plausible explanation for the decrease in mRNA level was discussed. Generally speaking, after the ingestion of food, gastric acid and pepsinogen must be immediately secreted from the stomach, followed by pepsinogen mRNA biosynthesis for replenishment of pepsinogen content. In this report, we examined changes in pepsinogen and IF contents and their mRNA levels in the rat gastric mucosa after starvation, refeeding and the administration of secretagogues.

MATERIALS AND METHODS

Chemicals Carbamylcholine chloride (carbachol) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cholecystokinin octapeptide (CCK-8) and secretin were from Peptide Institute, Inc. (Osaka). Histamine and tetragastrin were from Wako Pure Chemical Industries (Osaka) and MECT Co. (Tokyo), respectively. All other reagents were of the best commercial quality available.

Animals and Treatment with Secretagogues Male Wistar rats weighing about 250 g were used in all experiments. Rats were starved for 72 h and were injected with secretagogues through the femoral vein under urethane anesthesia. The secretagogue doses were determined as previously described.[7,8] At the end of the treatment, rats were sacrificed after 30 min.

Extraction of RNA and Northern Blot Analysis Total RNA was extracted from the glandular portion of the stomach by the acid guanidium thiocyanate–phenol–chloroform (AGPC) method.[9] Northern blot hybridization was carried out according to the standard method.[10] Briefly, the electrophoresis of 5—20 μg of total RNA was carried out in 0.9% agarose formalin gel. RNA in gel was transferred onto a sheet of nylon membrane (Gene Screen; Du Pont NEN Res. Pro., Boston, MA, U.S.A.) by capillary action. The membrane was prehybridized for 4 h at 58 °C in a prehybridization buffer. Probes used for northern blot analysis were 27-base oligodeoxynucleotide of rat pepsinogen (877-903: 5′-TGTGTCTACAATGCCTTGGCAGCCCTG-3′)[11] and a 35-base oligodeoxynucleotide of rat IF (808-842: 5′-GGGAGA-ATCTGGGCAATGGACATGGGGTTCTGGAA-3′),[12] which were synthesized by Greiner Japan (Tokyo). Oligodeoxynucleotides of rat pepsinogen and IF were labeled with [γ-^{32}P]-ATP by a 5′-end labeling kit (Oligonucleotide 5′-End Labeling System; Du Pont NEN Res. Pro.). After hybridization, the membrane was washed three times with 0.1% SDS in 2× SSC buffer at room temperature for 15 min, and twice with 0.1% SDS in 0.1×SSC buffer at 50 °C for 15 min. The cDNA fragment of human β-actin was used as the control

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probe for the housekeeping gene and was labeled with [α-^{32}P] dCTP by a random priming kit (Random Primer *Plus* Extension Labeling System; Du Pont NEN Res. Pro.). After exposing the membrane to a sheet of X-ray film, the obtained autradiogram was analyzed by NIH Image software.

Measurement of Pepsinogen and IF The homogenate of the rat glandular stomach (10%) with 50 mM phosphate buffer (pH 7.5) was centrifuged at 10000×***g*** for 10 min after freezing and thawing. The supernatant was used to measure pepsinogen and IF concentration; peptic activity was measured by the method of Anson and Mirsky,[13] and the IF concentration was measured by the avidin-biotin complex enzyme-linked immunosorbent assay (abcELISA).[1] Protein concentrations were estimated by the method of Bradford (Bio-Rad Protein Assay; Bio-Rad Lab. Co. Richmond, CA, U.S.A.).[14]

Statistical Analysis The results were expressed as mean±S.E. Differences between the 2 groups were analyzed by Student's *t*-test.

RESULTS

Pepsinogen and IF Contents and Their mRNA Levels in Rat Gastric Mucosa during Starvation and Refeeding Changes in pepsinogen and IF contents and their mRNA levels in rat gastric mucosa during starvation are shown in Figs. 1A and B. Control values were from the stomachs of rats at 5 h after their pelleted chow was removed. Pepsinogen and IF contents were significantly decreased to 65% and 70% of their control values at 24 h after the starvation, respectively, and the decrease continued during the course of starvation. In contrast, pepsinogen mRNA levels were significantly increased by the starvation. IF mRNA levels were gradually increased, and reached about 130% of the control level at 72 h after the starvation.

Changes in the above values by refeeding after 72 h of starvation were measured. Pepsinogen and IF contents were significantly decreased to 55% and 65% at 60 min after refeeding (Fig. 2A), respectively, and gradually recovered by 3—6 h. However, mRNA levels were transiently increased at 30 min after the refeeding (Fig. 2B).

Effects of Secretagogues on Pepsinogen and IF Contents, and Their mRNA Levels in Rat Gastric Mucosa Effects of secretin, carbachol, CCK-8, tetragastrin and histamine on gastric mucosal pepsinogen and IF contents, and their mRNA levels in 72 h starved rats were examined. These mucosal concentrations were measured at 30 min after a single injection of each secretagogue through the femoral vein under urethane anesthesia (Fig. 3). The profiles of these contents and mRNA levels induced by the refeeding, and those induced by the single injection of each secretagogue, were not similar. Next, the effects of the combined administration of each secretagogue were examined. Pepsinogen and IF contents in gastric mucosa were decreased to 70—80% and 50—80%, respectively, in a 30 min period by the combined administration of secretin, carbachol and CCK-8. In contrast, pepsinogen and IF mRNA levels were increased to 140—160% and 120—135%, respectively (Fig. 4). However, pepsinogen and IF contents and their mRNA levels were not significantly different from the controls.

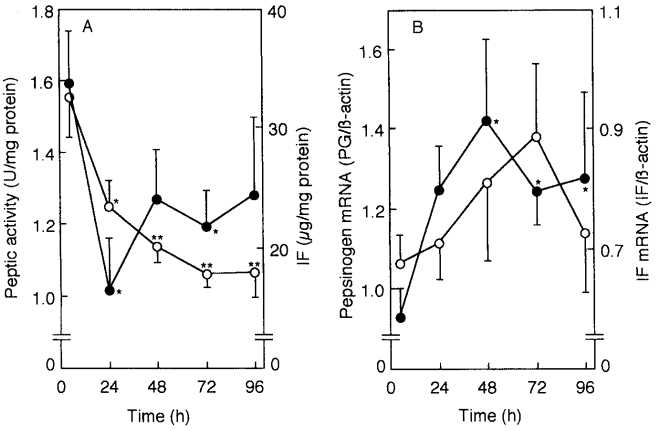

Fig. 1. Pepsinogen and IF Contents and Their mRNA Levels in Rat Gastric Mucosa during Starvation

Pepsinogen and IF contents are indicated by ● and ○, respectively (A), in which the pepsinogen content was represented by peptic activity. Pepsinogen (●) and IF (○) mRNAs are shown according to the ratios of their mRNAs to β-actin mRNA (B). Each value was the mean±S.E. of 5—7 experiments. * $p<0.05$, ** $p<0.01$.

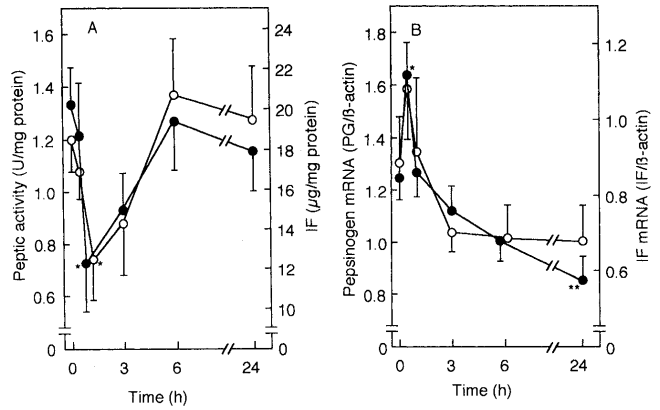

Fig. 2. Pepsinogen and IF Contents and Their mRNA Levels in Rat Gastric Mucosa by Refeeding

Pepsinogen and IF contents were indicated by ● and ○, respectively (A). Pepsinogen (●) and IF (○) mRNAs are shown according to the ratios of their mRNAs to β-actin mRNA (B). Each value is the mean±S.E. of 5—7 experiments. * $p<0.05$, ** $p<0.01$.

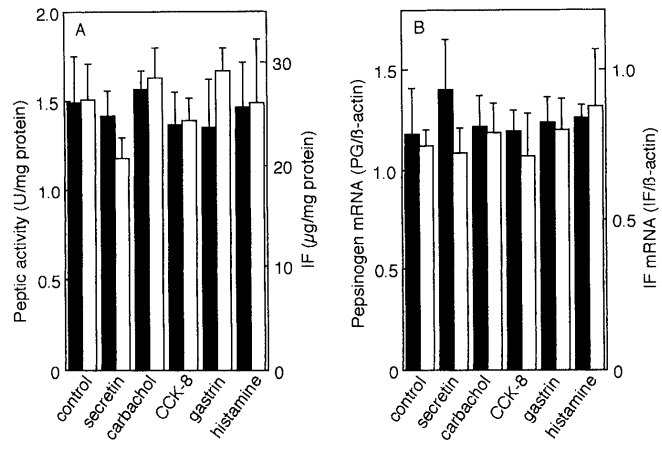

Fig. 3. Effects of Secretagogues on Pepsinogen and IF Contents and Their mRNA Levels in Starved Rat Gastric Mucosa

Each secretagogue was singly injected through the femoral vein of 72 h starved rat under urethane anesthesia. At 30 min after injection, samples were prepared from excised stomachs, according to the method in the text. Pepsinogen and IF contents are indicated by ■ and □, respectively (A). Pepsinogen (■) and IF (□) mRNAs are shown according to the ratios of their mRNAs to β-actin mRNA (B). The doses of chemicals used were as follows: secretin (20 μg/kg), carbachol (5 μg/kg), CCK (5 μg/kg), tetragastrin (10 μg/kg) and histamine (500 μg/kg). Each value is the mean±S.E. of 4—6 experiments.

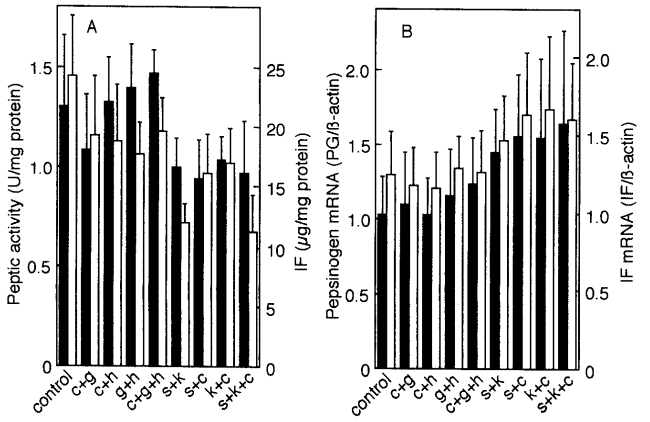

Fig. 4. Effects of Combined Administration of Secretagogues on Pepsinogen and IF Contents and Their mRNA Levels in Rat Gastric Mucosa

Mixtures of 2 or 3 secretagogues were singly injected through the femoral vein of 72 h starved rat under urethane anesthesia. At 30 min after injection, samples were prepared from excised stomachs. Pepsinogen and IF contents are indicated by ■ and □, respectively (A). Pepsinogen (■) and IF (□) mRNAs are shown according to the ratios of their mRNAs to β-actin mRNA (B). The doses of chemicals used were as follows: S, secretin (20 μg/kg); C, carbachol (5 μg/kg); K, CCK-8 (5 μg/kg); G, tetragastrin (10 μg/kg); H, histamine (500 μg/kg). Each value is the mean±S.E. of 5 experiments.

DISCUSSION

Gastric chief cells produce not only pepsinogen but also IF in the rat.[1,15] In this study, the effects of starvation and feeding on changes in pepsinogen and IF contents and their mRNA levels in rat gastric mucosa were examined. Since the weights of the body and gastrointestinal tract and gastric mucosal DNA synthesis are all decreased by starvation,[4] it is inevitable that the levels of amylase, cymotrypsinogen B and trypsinogen I mRNA in the pancreas are also decreased by starvation.[6] Therefore, it might be natural that gastric mucosal pepsinogen and IF mRNA levels are decreased by starvation. Certainly, the data from these studies demonstrates that gastric mucosal pepsinogen and IF contents are decreased by starvation. However, pepsinogen and IF mRNA levels were increased in terms of the ratios of pepsinogen and IF mRNAs to β-actin mRNA. These increases in pepsinogen and IF mRNA levels during starvation could mean that the mRNAs are prepared for a sudden feeding. Pepsinogen and IF stores dropped rapidly, along with their discharge, after refeeding. The presence of their mRNAs may work to restore gastric mucosal pepsinogen and IF. Pepsinogen and IF mRNA levels increased rapidly, then decreased gradually after the refeeding. Their mRNA levels might participate in the digestion.

We examined whether changes in gastric mucosal pepsinogen and IF contents and their mRNA levels could be reproduced by the administration of secretagogues instead of the refeeding. Generally, it is thought that feeding induces many changes in the neural, hormonal and physical conditions in the digestive tract. It is also thought that in the cephalic and gastric phases of gastric secretion, the parasympathetic nervous system works mainly in the stomach. Moreover, it is known that secretin and CCK concentrations in serum increase during the 30 min period after the feeding.[16] Since many factors would be closely connected with gastric secretion, we selected some secretagogues: carbachol as a substitute for the parasympathetic nervous system, gastrin and histamine for the gastric phase, and CCK and secretin for the intestinal phase. When each secretagogue was intravenously injected in 72 h starved rats, pepsinogen and IF contents and their mRNA levels were not significantly affected. Then, we examined the effects of the combined administration of the secretagogues. Previously, we described that carbachol, CCK-8 and secretin stimulated pepsinogen secretion, and their combination induced potentiated secretion from gastric chief cells. Furthermore, high pepsin secretion was obtained with the combined administration of these secretagogues *in vivo*.[8] Pepsinogen and IF contents in gastric mucosa were decreased to 70—80% and 50—80% with the combined administration of carbachol, CCK-8 and secretin which stimulate pepsinogen secretion from the chief cells; however, pepsinogen and IF mRNA levels were increased to 140—160% and 120—135% of the control, respectively. Thus, similar changes induced by both the refeeding and by the administration of the above secretagogues. In this paper, we showed that the administration of secretagogues increases the biosynthesis of pepsinogen and IF mRNA. The refeeding would stimulate not only the function of the gastrointestinal tract but also the metabolic pathway induced by the absorbed nutrient. These factors would modify the results in this paper. However, when secretions from chief cells are stimulated, it is anticipated that gastric mucosal pepsinogen and IF contents will decrease, and their mRNA levels will increase.

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