Pepsinogen Secretion from Cultured Rat Gastric Mucosal Cells

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Rat gastric mucosal cells were isolated with the aid of 0.1% collagenase and Dispase®. Pepsinogen secretion from these cells was stimulated by carbachol, cholecystokinin octapeptide (CCK(S)-8) and pentagastrin, but not by histamine. Attempts to obtain a sufficient number of cells using a higher concentration of Dispase resulted in disappearance of the responses to secretagogues. However, when gastric mucosal cells thus prepared were cultured for 24 h in a CO_2 incubator, they were found to respond not only to carbachol, CCK(S)-8 and pentagastrin, but also to histamine, resulting in an increase in pepsinogen secretion. The secretagogue-induced pepsinogen secretion was inhibited by its antagonist in a dose-dependent manner. These results suggest that the receptor present in chief cells for pepsinogen secretion was destroyed during the isolation procedure and regenerated during culture.

Keywords rat; pepsinogen secretion; isolated gastric mucosal cell; chief cell; culture; carbachol; cholecystokinin; histamine; pentagastrin

Pepsinogen is secreted from gastric chief cells and converted by acid to pepsin, an acid proteinase, functioning as a digestive enzyme of ingested food. Effects of secretagogues on pepsinogen secretion had previously been studied by measuring the peptic activity of the gastric juices, whose secretion had been induced *in vivo*. However, *in vivo* pepsinogen secretion is influenced by many factors including hormones, autacoids, and neurotransmitters; in addition, a considerable amount of pepsinogen is secreted basally. From the time cells were isolated from canine gastric mucosa,[1] mechanisms of pepsinogen secretion from chief cells have been primarily studied in either dogs[2] or guinea pigs.[3] Although many experimental gastric-ulcer models, in addition to studies of *in vivo* pepsinogen secretion, have been developed using rats, there has been no report on pepsinogen secretion using rat chief cells. Thus in this study, we attempted to isolate rat chief cells.

Materials and Methods

Chemicals Carbamylcholine (carbachol), collagenase (type I) and bovine serum albumin (BSA) (fraction V) were purchased from Sigma Chem. Co. (St. Louis, U.S.A.). Cholecystokinin octapeptide, sulfated form, (CCK(S)-8) was from the Peptide Institute Inc. (Osaka), pentagastrin from Sumitomo Seiyaku Co. (Osaka), histamine and atropine sulfate from Wako Pure Chemical Industries (Osaka) and Dispase®, which is a collagenase-type proteinase from *B. polymyxa*, was from Godo Shusei Co. (Tokyo). Cimetidine and proglumide were gifts from Smith, Kline and Fujisawa Co. (Tokyo) and Kaken Seiyaku Co. (Tokyo), respectively.

Isolation of Gastric Mucosal Cells The stomach was removed from a 24-h-starved Wistar rat weighing about 200 g under urethane anesthesia. Gastric mucosal cells were isolated by a modification of the method of Lewin *et al.*[4] The stomach, which had been everted and filled with about 7 ml of medium A, was incubated with 0.1% collagenase and 0.1% Dispase in medium A gassed with 95% O_2 and 5% CO_2 for 1 h at 37 °C. The stomach was transferred to medium B and incubated for 30 min at room temperature under continued gassing with 95% O_2 and 5% CO_2. The cell suspension in medium B was filtered through a nylon filter (150 mesh), and centrifuged at $50 \times g$ for 2 min. The mucosal cells were resuspended in medium C. When the relationship between numbers of cells and their response to carbachol, and concentrations of Dispase was investigated, only concentrations of Dispase were changed in this procedure. For the measurement of pepsinogen release, gastric mucosal cells (3.5×10^4 cells/ml) in medium C were incubated with a secretagogue for 15 min at 30 °C. The pepsinogen content in the gastric mucosal cells used was about 25 ng/1×10^4 cells.

Medium A contained NaH_2PO_4 0.5, Na_2HPO_4 1.0, $NaHCO_3$ 20, NaCl 70, KCl 5.0, glucose 11, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) 25 (mM) and BSA 20 (mg/ml) (pH 7.4). Medium B contained EDTA (ethylenediamine tetraacetic acid, disodium salt) 2(mM) in medium A (pH 7.4). Medium C contained $CaCl_2$ 0.1 and $MgCl_2$ 1.0 (mM) in medium A (pH 7.4).

Culture of Gastric Mucosal Cells Gastric mucosal cells were cultured according to the method of Sanders *et al.*[2] as follows. The gastric mucosal cell suspension obtained by the above procedure with 0.75% Dispase and 0.1% collagenase was added to a mixture of Dulbecco's modified MEM and Ham's nutrient mixture F-12 (1 : 1) containing 10% fetal calf serum in collagen-treated dishes. The cells were cultured for 24 h at 37 °C in a CO_2 incubator. For the measurement of pepsinogen release, after replacement of the medium, gastric mucosal cells were incubated in medium C with a secretagogue for 30 min at 37 °C in a CO_2 incubator.

Measurement of Pepsinogen Concentration After centrifugation, pepsinogen release in an aliquot of the supernatant was measured by radioimmunoassay, as described previously,[5] and was expressed as a percentage of the total cellular pepsinogen which was estimated after freezing and thawing of residual cells.

Student's *t* test was used for the statistical analyses.

Results

Pepsinogen Secretion from Isolated Gastric Mucosal Cells Freshly isolated gastric mucosal cells were suspended in medium C and incubated with increasing doses of carbachol, CCK(S)-8, pentagastrin and histamine. The results are shown in Fig. 1. Carbachol stimulated pepsinogen secretion in a dose-dependent manner at doses above 1×10^{-5} M. CCK(S)-8 stimulated secretion at doses above 1×10^{-10} M; the maximal response was obtained at a dose of 1×10^{-8} M. Pentagastrin stimulated secretion at a dose of

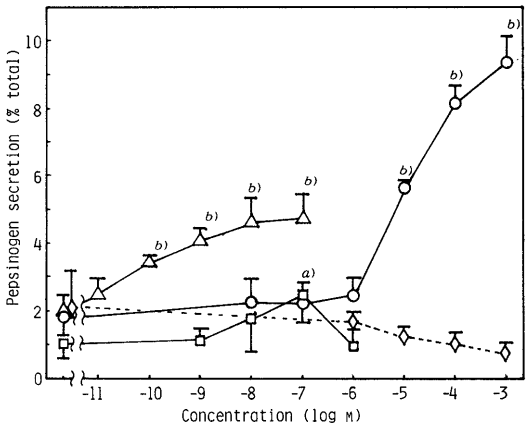

Fig. 1. Dose-Response Curves of Pepsinogen Secretion from Isolated Gastric Mucosal Cells Induced by Carbachol, CCK(S)-8, Pentagastrin and Histamine

○, carbachol; △, CCK(S)-8; □, pentagastrin; ◇, histamine. Each value is the mean ± S.E. from 7 separate experiments. *a*) Significant difference between the value induced by a secretagogue and the basal one. $p < 0.05$, *b*) $p < 0.01$.

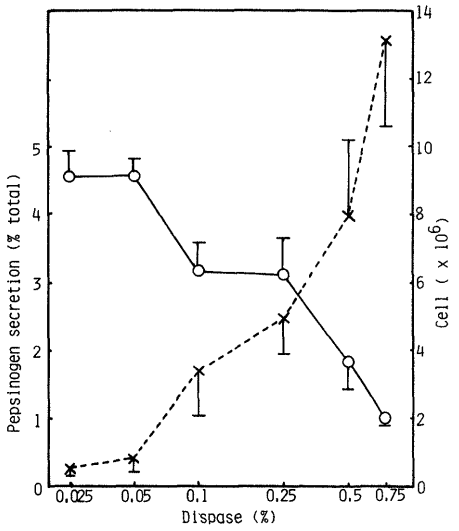

Fig. 2. Changes of the Number of Isolated Cells and Their Response on Digestion with Various Concentrations of Dispase

○, carbachol (1×10^{-5} M) induced pepsinogen secretion; ×, number of isolated cells. Basal pepsinogen release was about 1% of total cellular pepsinogen at any point. Each value is the mean ± S.E. from 5 separate experiments.

1×10^{-7} M, but the response was very weak. Histamine did not stimulate secretion at all. The percent of secreted pepsinogen induced by carbachol was about twice as much as that by CCK(S)-8.

The Relationship between the Number of Isolated Cells and Their Response to Carbachol as a Function of the Dispase Concentration There was some doubt as to whether the receptor was damaged during the isolation of gastric mucosal cells with Dispase, because unresponsive cells were occasionally obtained. Gastric mucosal cells were obtained after incubation of the everted rat stomach with various concentration of Dispase. The number of isolated cells and their response to carbachol (1×10^{-5} M) were plotted as a function of the Dispase concentration (Fig. 2). Cell viability, estimated by a trypan blue exclusion test, was over 90% at any point tested. An inverse relationship between the number of isolated cells and their response was observed: at a low concentration of Dispase, about 5×10^5 cells were obtained and the amount of secreted pepsinogen induced by carbachol (1×10^{-5} M) was about 4.5% of the total pepsinogen content. At a high concentration of Dispase, more than 8×10^6 cells were obtained, but no response to carbachol was observed.

Effects of Secretagogues on Pepsinogen Secretion from Cultured Gastric Mucosal Cells We studied whether the receptors for the secretagogues in the chief cells isolated by the use of a high concentration of Dispase were regenerated during cell culture or not. Freshly isolated gastric mucosal cells were cultured for 24 h. After washing the dishes with fresh medium C, pepsinogen release from the cells in medium C by incubation with various concentrations of secretagogues was measured (Fig. 3). Carbachol significantly stimulated pepsinogen secretion at doses above 1×10^{-7} M. CCK(S)-8 stimulated secretion at doses above 1×10^{-11} M. Pentagastin stimulated secretion at a dose of 1×10^{-8} M, but the response was low. Histamine stimulated pepsinogen secretion significantly at doses above 1×10^{-7} M. Thus, cultured chief cells were stimulated not only by carbachol, CCK(S)-8 and pentagastrin but also by

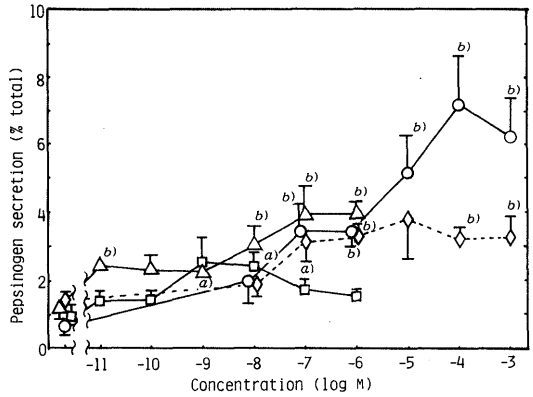

Fig. 3. Dose-Response Curves of Pepsinogen Secretion from Cultured Gastric Mucosal Cells

○, carbachol; △, CCK(S)-8; □, pentagastrin; ◇, histamine. Each value is the mean ± S.E. from 5 separate experiments. *a*) $p < 0.05$, *b*) $p < 0.01$.

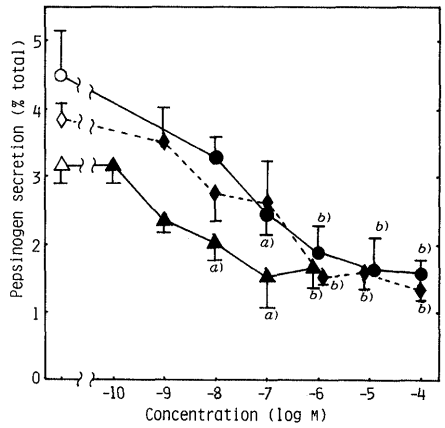

Fig. 4. Inhibition of Pepsinogen Secretion from Cultured Gastric Mucosal Cells by Secretagogue-Specific Antagonists

●, carbachol (1×10^{-5} M) with atropine; ▲, CCK(S)-8 (1×10^{-7} M) with proglumide; ◆, histamine (1×10^{-6} M) with cimetidine. Each value is the mean ± S.E. from 5 separate experiments. *a*) $p < 0.05$, $p < 0.01$.

histamine, although they did not respond to the secretagogues after isolation with 0.75% Dispase.

Inhibition of Pepsinogen Secretion from Cultured Gastric Mucosal Cells by Antagonists As specific antagonists, we used atropine for carbachol, proglumide for CCK(S)-8 and cimetidine for histamine. Pepsinogen secretions induced by submaximal doses of secretagogues were inhibited by their antagonists in a dose-dependent manner, as shown in Fig. 4. Atropine inhibited carbachol (1×10^{-5} M)-induced pepsinogen secretion at doses above 1×10^{-7} M in a dose-dependent manner. Proglumide significantly inhibited the CCK(S)-8 (1×10^{-7} M)-induced secretion at doses above 1×10^{-8} M. Cimetidine inhibited the histamine (1×10^{-6} M)-induced secretion at doses above 1×10^{-7} M. Inhibition by proglumide of pentagastrin-induced secretion could not be tested, because the response to pentagastrin was too low.

Discussion

Since Soll studied gastric acid secretion using isolated gastric mucosal cells,[1] many reports about the mechanisms of gastric acid and pepsinogen secretion have been published.[6-8] In the first report about pepsinogen secretion from isolated chief cells, cells were prepared from canine gastric mucosa.[2] Raufman *et al.* carried out studies on

pepsinogen secretion using the gastric gland of the rat[9] and then using chief cells of the guinea pig.[3] They used a 0.1% collagenase (type I) solution to isolate the chief cells. However, we were unable to obtain a sufficient number of cells from the rat gastric mucosa with 0.1% collagenase. By applying the method of Lewin *et al.*,[4] combining 0.1% collagenase and Dispase, we finally succeeded in isolating enough cells. However, we occasionally obtained cells which did not respond to the secretagogues, even though the cells were considered to be viable, as estimated by the dye exclusion test. We considered that the receptor for the secretagogues might have been digested by a proteolytic enzyme during cell isolation. We demonstrated that the use of a high concentration of Dispase to obtain a larger number of cells resulted in a decreased response of the cells. These results indicated that the receptor was degraded during cell isolation. However, we also showed that the receptors for secretagogues were regenerated during the culture. The rat chief cell response to secretagogues described in this paper is similar to that obtained from guinea pig cells.[3] Yet, disappearance of the response to histamine in the freshly isolated rat cells suggested that the receptor for histamine was more sensitive to Dispase digestion than other receptors.

The existence of histamine receptors on chief cells has been previously discussed. It is well known that histamine can stimulate pepsinogen secretion *in vivo*. Recently, Sanders *et al.*[2] and Sutliff *et al.*[10] showed that histamine stimulated pepsinogen secretion from isolated chief cells *in vitro*. However, Kasbekar *et al.*,[11] reported that histamine did not stimulate pepsinogen secretion from rabbit gastric glands. In the present report, we clarified that histamine stimulated pepsinogen secretion in a dose-dependent manner, and that the stimulation was inhibited by cimetidine, a histamine H_2 receptor antagonist. This evidence indicates that the histamine receptor for pepsinogen secretion is the same histamine H_2 receptor as that for acid secretion.

Recently, Cherner *et al.*[12] demonstrated that in terms of pepsinogen secretion in guinea pigs, there are two kinds of receptors for the gastrin family: the G-receptor for gastrin, and the C-receptor for CCK. In this paper, pentagastrin was about 50% as efficacious as CCK(S)-8. Therefore, the present results are consistent with the hypothesis that the receptors for pentagastrin and CCK(S)-8 are independent of each other.

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