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# Secretion of Somatostatin and Gastrin by Human Antral Mucosa — An *in vitro* study in duodenal ulcer patients and control subjects under stimulation of bombesin

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## ABSTRACT

In order to ascertain possible abnormalities in somatostatin and gastrin secretion in patients with duodenal ulcer, the author compared bombesin-stimulated somatostatin and gastrin secretion by antral mucosal explants in patients with duodenal ulcer, those with atrophic gastritis and normal controls. An organ culture technique was employed. This excluded neurogenic, hormonal and circulatory influences. Bombesin in concentrations of $10^{-7}$ M to $10^{-5}$ M stimulated gastrin and somatostatin secretion at a dose-dependent manner. In all subjects, bombesin ($10^{-7}$M) stimulated antral gastrin release and increased explant gastrin content significantly ($p<0.05$). Bombesin significantly increased somatostatin release and explant somatostatin content in normal subjects ($p<0.05$) but not in patients with duodenal ulcer ($p>0.05$). In the presence of bombesin, the total net increase of gastrin in medium and explants was greater in duodenal ulcer patients (31.57 ± 5.20 ng/mg wet w.) compared with normal subjects (19.63 ± 4.50 ng/mg wet w.) ($p<0.01$). The total net increase of somatostatin in the presence of bombesin was significantly less in duodenal ulcer patients (0.10 ± 0.02 ng/mg wet w.) than in normal subjects (1.45 ± 0.24 ng/mg wet w.) ($p<0.01$). The results suggest that abnormalities in somatostatin and gastrin secretion of the antrum contribute to the pathogenesis of increased gastric acid secretion in duodenal ulcer.

***Key words***: *Somatostatin and gastrin secretion, Bombesin, Organ culture, Duodenal ulcer*

Hypersecretion of gastric acid is generally accepted as a characteristic feature of duodenal ulcer[17,28]. Somatostatin and gastrin are two of the principal hormonal factors that inhibit and stimulate gastric acid secretion, respectively. Somatostatin, a peptide found in abundance in the stomach, is present in D cells, which are in close contact with gastrin-containing G cells in the antral mucosa[19]. It has been reported that, in patients with duodenal ulcer, the content of mucosal somatostatin in antrum is decreased and gastrin in the blood is elevated[6,18,32]. These abnormalities contribute to an increase in gastric acid secretion and are believed to be one of the factors involved in the pathogenesis of duodenal ulcer[17,25,35]. However, most studies on somatostatin and gastrin secretion have been conducted *in vivo*, and have paid little attention to the functional aspect of D cells and G cells[5,6,8,10-12,30,31,33]. Few reports have been published on the secretion of somatostatin and gastrin in experimental studies excluding neurohormonal and circulatory factors. In this experiment, the author attempted to exclude these factors in the secretion of somatostatin and gastrin by utilizing an organ culture technique of the antral mucosa. In a bombesin-stimulated state, the capability of secretion of somatostatin and gastrin in human antral mucosa was compared between four groups : patients with duodenal ulcer, moderate atrophic gastritis, severe atrophic gastritis and normal subjects.

## MATERIALS AND METHODS

### Subjects

Nine patients with duodenal ulcer (8 men and 1 woman, mean age ± SEM : 49.70 ± 3.49 years) were studied. The control group consisted of 15 subjects with normal gastric mucosa (9 men and 6 women, mean age ± SEM : 43.12 ± 2.46 years), 11 subjects with moderate atrophic gastritis (4 men and 7 women, mean age ± SEM : 59.25 ± 3.16 years), and 7 subjects with severe atrophic gastritis (5 men and 2 women, mean age ± SEM : 58.03 ± 3.87 years ). All subjects received gastrofiberscopic examination; diagnosis was made endoscopically and histologically. Subjects with atrophic gastritis were divided into moderate and severe subgroups according to the

severity of structural damage to the pyloric glands and infiltration of the inflammatory cells. Informed consent was obtained from each subject. Patients with both duodenal ulcer and severe atrophic gastritis were excluded. None of the subjects had any other serious illness.

**Organ culture**

After overnight fasting, patients received endoscopic examination under local anesthesia of the pharynx. Antral mucosae were obtained with a standard Olympus endoscopic biopsy forceps (FB-25K, Olympus Optical Co., Tokyo, Japan) without a central spike. Six pieces of the antral mucosae for organ culture and two pieces for histological examination were obtained from each subject. The tissues for culture were transferred immediately to the organ culture system. Three were cultured in the basal medium with a vehicle (normal saline) and the rest in a experimental medium containing bombesin.

The organ culture technique previously described by Harty et al[15] was employed. Antral mucosal explants were cultured at 37°C in 75% humidity in a $CO_2$ incubator (CP Series, Hirasawa Works, Japan) and gassed continuously with a 5% $CO_2$-95% $O_2$ mixture (Fig.1). The Minimum Essential Medium 410-1100 (Gibco Laboratories, N.Y.,) containing penicillin (100 U/ml) and streptomycin (100 μg/ml) was used as the culture medium. Antral mucosal explants were first stabilized in the basal culture medium for 1 hr. The culture medium was then removed and renewed.

To examine the dose-dependent effects on gastrin and somatostatin release, explants were cultured with 0, $10^{-7}$ M, $10^{-6}$ M or $10^{-5}$ M bombesin (final concentration, Peptide Institute Co., Osaka. Japan). Bombesin was added to the culture dishes for 1 hr consecutively with a recovery interval of 30 min between addition of different doses of bombesin. $10^{-7}$ M bombesin was selected as the lowest effective concentration according to dose response study to stimulate gastrin and somatostatin release (data shown in the Results).

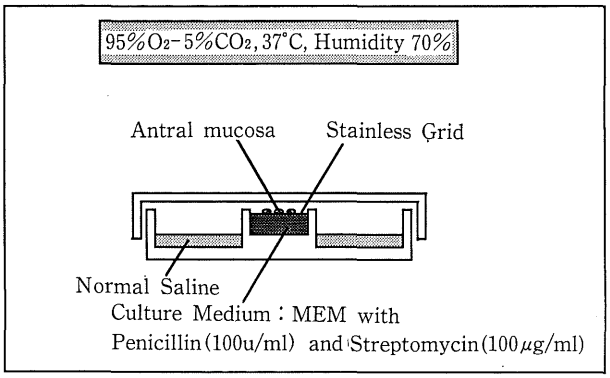


**Fig. 1**. Schematic representation of organ culture system

Gastrin and somatostatin release in response to $10^{-7}$M bombesin or the vehicle for an additional 6 hrs (basal secretion for the initial 1 hr and bombesin-stimulated secretion for the following 5 hrs) was determined in the patients with duodenal ulcer and the controls. The culture medium was changed hourly and kept at -20 °C until the concentrations of gastrin and somatostatin were determined. After 6 hrs of culture, the explants were weighed and promptly frozen, maintained at -20 °C until gastrin and somatostatin were extracted. The concentrations of gastrin and somatostatin in the medium and antral tissue were determined by means of the radioimmunoassay (RIA) method[32] described previously.

**Viability of antral mucosal explants**

The viability of antral mucosal explants during 6 hrs of organ culture in the basal medium was assessed by histological examination of the explants and immunohistochemical staining with bromodeoxyuridine (BrDU) of the proliferating cells in the antral mucosal explants.

On histological examination, the tissue structures of the explants collected at the initiation and completion of the organ culture were compared.

In order to examine the DNA replication of the antral mucosal explants during organ culture, they were exposed to BrDU (Takeda Chemical Industries Ltd., Osaka, Japan) at a final concentration of 400 μM for 30 min at the initiation and completion of the culture period. Specimens fixed in 70% ethanol were embedded in paraffin, and 3-μm sections were cut and placed on slides. The ABC (avidin-biotin-peroxidase complex) technique was used for the immunohistochemical detection of BrDU. Sections were covered first with normal horse serum and then with a 1:80 dilution of an anti-BrDU monoclonal antibody (Becton Dickinson Immunocytometry Systems, California, USA) for 60 min. A biotinylated anti-mouse IgG serum (Vectastain ABC kit, Vector Laboratories Inc., CA. USA.) was applied and avidin-biotin complex (10 μg/ml avidin and 2.5 μg/ml biotin-horse-radish peroxidase, Vectastain ABC kit, Vector Laboratories Inc., CA. USA.) was added. The presence of the antibody was visualized by a 5-min treatment with a freshly prepared solution of 0.02% 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, LTD., Japan) and 0.01% $H_2O_2$ in 0.05 M Tris buffer (pH 7.2). The slides were lightly counterstained with hematoxylin. All procedures were carried out at room temperature. Cells containing nuclei with an overlying brown pigment were considered to be positively stained.

### Statistical analysis

The data are presented as mean ± SEM (standard error of mean). A p value of <0.05 was considered statistically significant. Data were analyzed by paired and unpaired Mann-Whitney U tests.

## RESULTS

### Viability of antral mucosal explants

On histological examination, the tissue structure of the antral mucosal explants was kept intact at the end of the organ culture, though the stroma of the lamina propria was mildly edematous. The structure of the epithelium and glands of the explants did not change markedly during 6-hr organ culture.

The number of cells positively stained with BrDU and identified in the antral mucosal explants, was nearly identical at the initiation and completion of the organ culture (Fig.2).

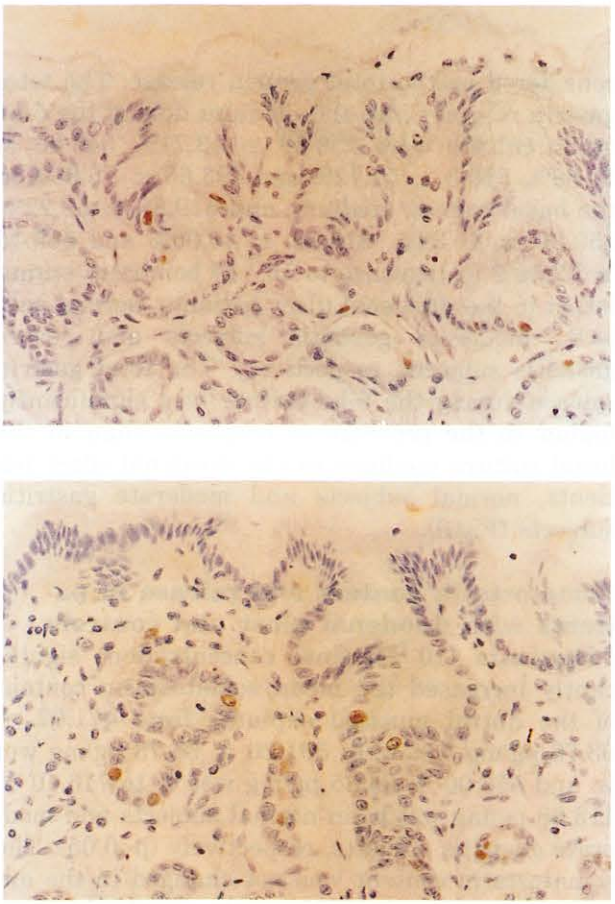

**Fig. 2**. Light minographs of antral mucosal explants at the beginning (A) and completion (B) of organ culture. Cell and tissue structures of the antral mucosa explants were kept intact during 6-hr organ culture, though the stroma was mildly edematous at the completion of culture. The BrDU-uptake was consistent, and the number of positive nuclei was nearly identical at both time points.

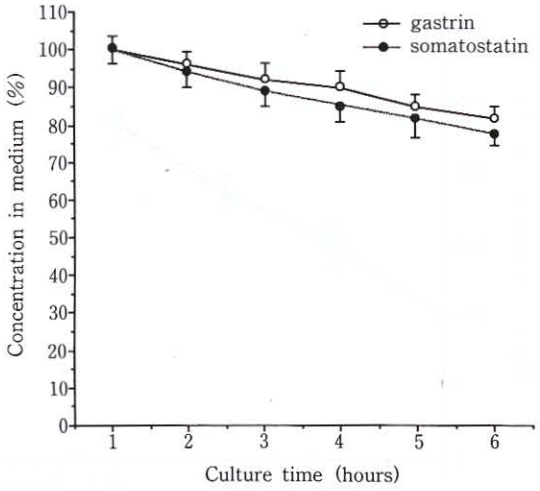


**Fig. 3.** Basal secretion of gastrin and somatostatin by antral mucosal explants obtained from normal subjects during 6-hr culture period. Gastrin or somatostatin concentration in the medium in each hr of the 6-hr organ culture was expressed in a percentage, with 100% concentration in the initial 1 hr after stabilization. Antral mucosal explants released gastrin and somatostatin steadily throughout 6 hrs of organ culture.

### Basal release and dose-dependent release of gastrin and somatostatin in normal subjects

Secretion of somatostatin and gastrin in various conditions is expressed in percentage, referring to the release of somatostatin and gastrin during the first 1 hr of culture. Basal gastrin release into the basal culture medium decreased from 100% to 81.67%, and basal somatostatin release decreased from 100% to 77.47% over the 6-hr culture period (Fig.3).

Bombesin at the concentrations of $10^{-7}$ M, $10^{-6}$ M or $10^{-5}$ M significantly stimulated the release of gastrin and somatostatin from antral mucosal explants ($p<0.05$) in a dose-dependent manner. Release of gastrin and somatostatin in the initial 1 hr, without bombesin, was 250.35 ± 20.21 pg/ml and 81.26 ± 20.36 pg/ml, respectively. Maximal secretion of gastrin (241.62% compared with 100% in the initial 1 hr) and somatostatin (234.38%) was induced by $10^{-5}$ M bombesin (Fig.4).

### Gastrin content and release in patients with duodenal ulcer and controls

After 6 hrs of organ culture in the basal culture medium, the mean gastrin contents in the antral mucosa from duodenal ulcer patients, normal subjects, moderate and severe gastritis subjects were 2039.32 ± 462.14 pg/mg wet weight (w.), 2307.15 ± 552.64 pg/mg wet w., 2092.90 ± 546.85 pg/mg wet w. and 1249.67 ± 445.67 pg/mg

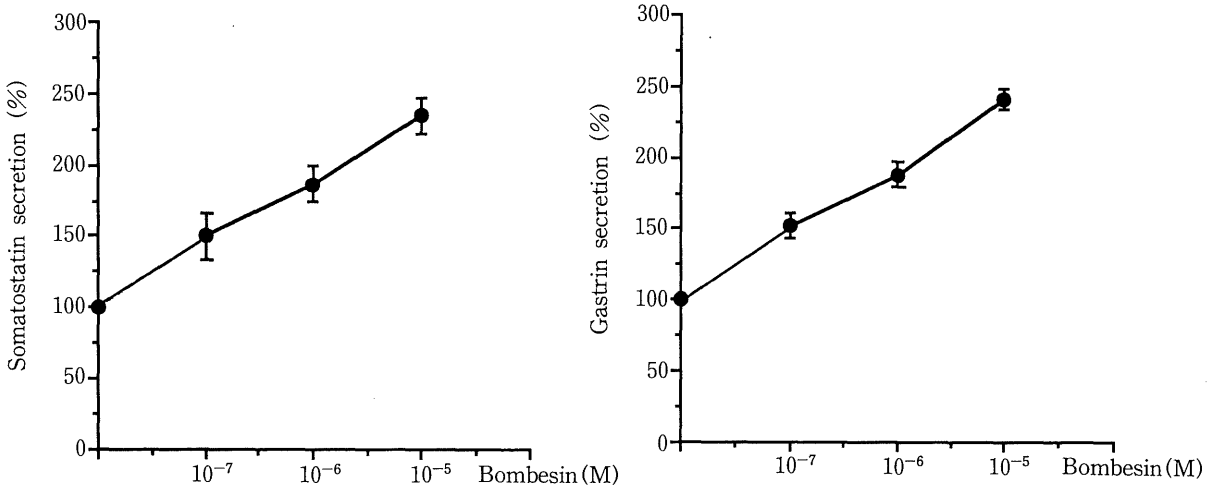


**Fig. 4**. Effect of bombesin on somatostatin (left) and gastrin (right) release by antral mucosal explants obtained from normal subjects. Concentration of gastrin or somatostatin in the medium in the initial 1 hr culture in the absence of bombesin was expressed as 100%. Bombesin stimulated gastrin and somatostatin release in a dose-dependent manner. The maximum stimulatory effect was observed at a concentration of $10^{-5}$ M.

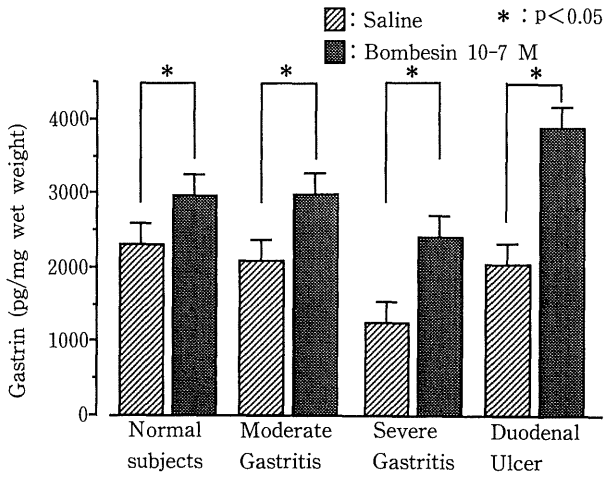


**Fig. 5**. Explant gastrin content in duodenal ulcer patients, normal subjects, and moderate and severe atrophic gastritis subjects at the completion of 6-hr culture. Gastrin content was significantly higher in the presence of bombesin than in the absence of bombesin ($p<0.05$).

wet w., respectively. In the presence of bombesin ($10^{-7}$M, final concentration), the mean gastrin contents were 3895.11 ± 1439.94 pg/mg wet w., 2970.73 ± 570.13 pg/mg wet w., 2983.70 ± 981.85 pg/mg wet w. and 2409.83 ± 1215.22 pg/mg wet w., respectively. The gastrin content was significantly higher in the presence of bombesin than in the basal culture medium in duodenal ulcer patients, normal subjects and moderate gastritis subjects, respectively ($p<0.05$) (Fig.5).

The sum of the percentage of gastrin released into the medium after each hr of the culture was considered as the total gastrin release. The total gastrin releases into the medium during the 6-hr organ culture were 556.49 ± 43.71%, 540.26 ± 30.89%, 510.3 ± 51.72% and 493.66 ± 75.65% in the basal culture medium, and 619.88 ± 59.22%, 650.20 ± 40.32%, 613.60 ± 45.90% and 565.16 ± 72.58% in response to $10^{-7}$ M bombesin stimulation in the duodenal ulcer patients, normal subjects, moderate gastritis subjects and severe gastritis subjects, respectively. The total gastrin release during the 6-hr culture was significantly higher in the presence of bombesin than in the basal culture medium in the duodenal ulcer patients, normal subjects and moderate gastritis subjects (Fig.6).

## Somatostatin content and release in patients with duodenal ulcer and controls

Bombesin ($10^{-7}$M, final concentration) significantly increased the mean somatostatin content of the antral mucosal explants from 471.07 ± 93.76 pg/mg wet w. to 591.26 ± 95.73 pg/mg wet w. and 555.00 ± 95.85 pg/mg wet w. to 716.10 ± 113.88 pg/mg wet w. in normal subjects and moderate gastritis subjects, respectively ($p<0.05$). The somatostatin content was not changed in the antral mucosal explants from duodenal ulcer patients and severe gastritis subjects (Fig.7).

The addition of bombesin ($10^{-7}$M, final concentration) to the culture medium significantly increased the total somatostatin release from 440.40 ± 35.49% to 544.61 ± 35.42% ($p<0.01$) and 465.60 ± 55.05% to 599.50 ± 47.54% ($p<0.05$), in normal and moderate gastritis subjects, respectively. However, significant change in somatostatin secretion was not noted in the duo-

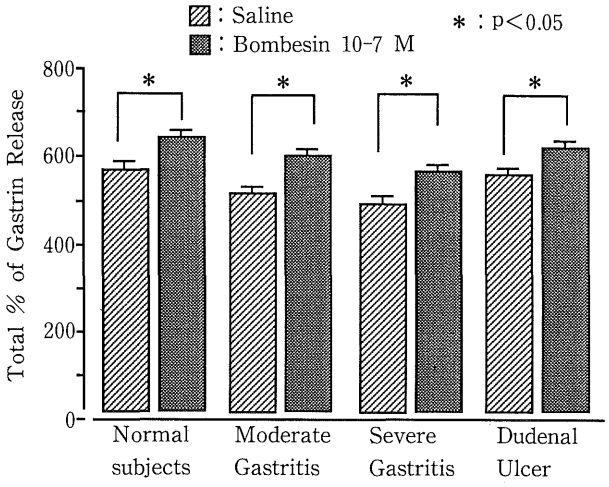


**Fig. 6.** The sum of the percentage of gastrin concentration in the medium at the end of each hr of the 6 hrs of culture. The gastrin concentration in the medium after the initial 1 hr of the basal culture was considered as 100%. Bombesin significantly increased gastrin release in duodenal ulcer patients, normal subjects, and moderate gastritis subjects ($p<0.05$).

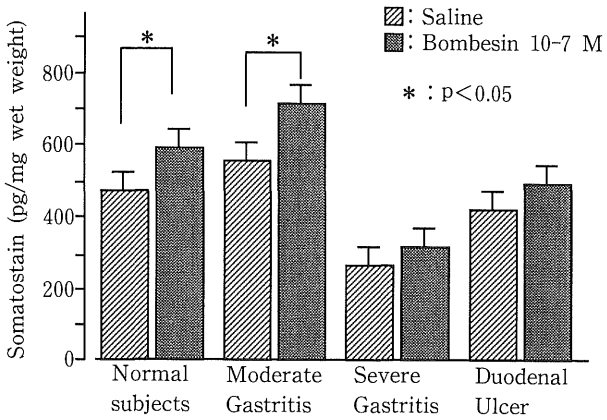


**Fig. 7.** Somatostatin content in explants at the completion of 6-hr culture. Bombesin increased somatostatin content of explants significantly in normal and moderate gastritis subjects ($p<0.05$), while it did not alter the contents in duodenal ulcer patients and severe gastritis subjects.

denal ulcer patients and severe gastritis subjects ($p>0.05$) (Fig.8).

### Total net increase in gastrin and somatostatin in patients with duodenal ulcer and controls

The total net increase of gastrin was defined as the sum of net increases in gastrin released into the medium during 6 hrs of bombesin-stimulated culture and stored in antral mucosal explants at the completion of a 6-hr bombesin-stimulated culture. It was calculated as the difference in the

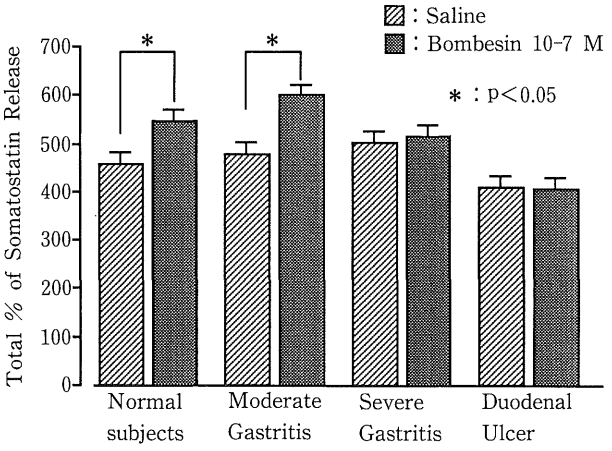


**Fig. 8.** The sum of the percentage of somatostatin concentration in the medium at the end of each hr of 6 hrs of culture. Somatostatin concentration in the medium after the initial 1 hr of the basal culture was considered as 100%. Significant increases in antral somatostatin release in response to bombesin were observed in normal subjects and moderate gastritis subjects ($p<0.05$) in contrast to the lack of obvious changes in release in duodenal ulcer patients and severe gastritis subjects.

total amount of gastrin released into the medium and stored in the explants between basal culture and bombesin-stimulated culture. The mean total net increases of gastrin were 31.57 ± 5.20 ng/mg, 19.63 ± 4.50 ng/mg, 17.25 ± 3.80 ng/mg and 0.77 ± 0.12 ng/mg for the duodenal ulcer patients, normal subjects, moderate and severe gastritis subjects, respectively. Bombesin stimulation resulted in a total net increase of gastrin significantly higher in the duodenal ulcer patients compared with the normal subjects ($p<0.01$) (Fig.9).

The total net increase of somatostatin by bombesin-stimulation was calculated in the same way as the total net increase of gastrin. The total net increase of somatostatin was significantly less in duodenal ulcer patients (0.10 ± 0.02 ng/mg) than in the normal subjects (1.45 ± 0.24 ng/mg) ($p<0.01$) (Fig.9).

## DISCUSSION

A number of methods have been used to study the synthesis and release of gut hormones in physiological condition and in the presence of stimulators or inhibitors. These methods include : *in vivo* test meal stimulation [30], vascular perfusion of the stomach[26], electrical stimulation of the vagus nerve [29], perfusion of oriented mucosal sheet[8,33], organ or explant culture[15], perfusion of mucosal gland[23,24] and primary culture of isolated cells[4]. Organ culture allows gastric epithelial and glandular cells to be maintained *in vitro* for a prolonged period, and has been used to

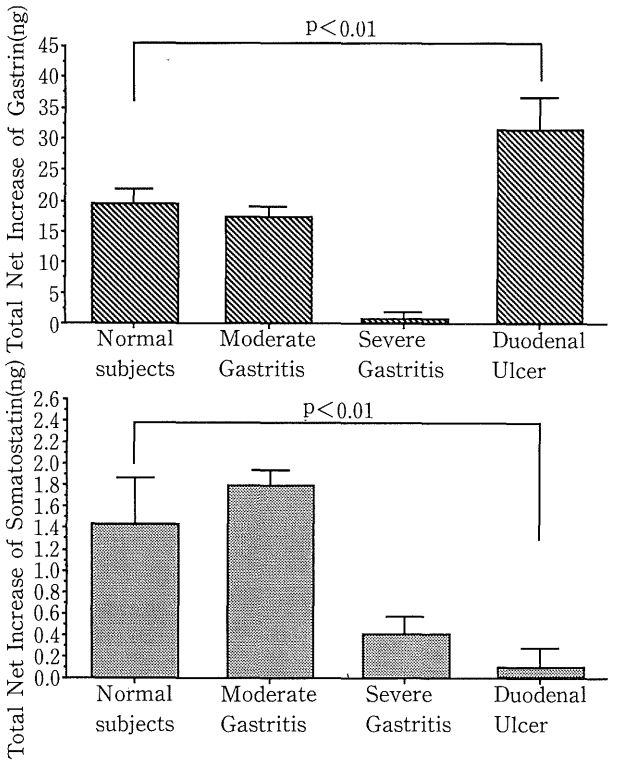


**Fig. 9.** Total net increase in gastrin and somatostatin. Total net increase in gastrin in the presence of bombesin was significantly higher in duodenal ulcer patients than in normal subjects ($p<0.01$). Total net increase in somatostatin in the presence of bombesin was substantially less in duodenal ulcer patients than in normal subjects ($p<0.01$). A reciprocal relationship existed between the total net increases in gastrin and somatostatin in duodenal ulcer patients.

study cell proliferation in normal and malignant tissues. Because the structure of explants in organ culture is kept relatively intact and since explants are not affected by neurohumoral factors as they are *in vivo*, this technique can be used to study the secretion and interaction of gut hormones. Moreover, the organ culture provides a convenient model for the study of gut hormones in the human gastrointestinal tract since human biopsies can be used as explants. Harty et al[15] and Lichtenberger et al[20] showed that, over 24 hrs, murine antral explants retained intact structure and stable synthesis of proteins including gastrin. In the present study, cell and tissue structure were kept intact and showed little change during the culture. Immunohistochemical identification of proliferating cells by BrDU provided further evidence of the good viability of explants. Moreover, the release of gastrin and somatostatin remained stable and the explants responded well to bombesin during the 6-hr organ culture.

Bombesin, as a stimulant of gastrin and somatostatin release, was selected because GRP (gastrin-releasing peptide, the counterpart of bombesin in humans) exists in the antrum as a neurotransmitter regulating the physiological secretion of gastrin from G cells and somatostatin from D cells. Bombesin is known to stimulate gastrin secretion, but its effects on somatostatin secretion are controversial. Chiba et al[7] and Buchan et al[4] reported that bombesin did not stimulate somatostatin release from D cells in a primary cell culture, but Martindale et al[22] and Guo et al[14] found that bombesin stimulated somatostatin release in the isolated vascularly perfused stomach. Alino and Unas-Moberg[1] demonstrated that bombesin stimulated the intravascular release of gastrin and somatostatin in the rat. DuVal et al[9] found that bombesin stimulated both gastrin and somatostatin release in the rat stomach, and suggested that bombesin and somatostatin acted in concert to regulate antral gastrin secretion. In the present study, bombesin significantly increased the mucosal content and release of gastrin and somatostatin in the antrum. This is consistent with the findings reported by DuVal et al[9]. Bombesin stimulated somatostatin release in a dose-dependent manner. The results from the present study, in which an extrinsic neurohumoral influence has been ruled out, suggest a stimulatory action of bombesin on D cells.

Hypersecretion of gastric acid has been generally accepted as a characteristic feature of duodenal ulcer[28]. Gastric acid secretion is regulated by complicated neurohumoral factors, and the mechanism of gastric acid hypersecretion is not yet completely understood. Gastrin is one of the principal humoral factors that regulate gastric acid secretion, and a feedback regulation is considered to exist between gastrin and gastric acid secretion[11]. Patients with duodenal ulcer have been found to release more gastrin into the circulation than control subjects in response to a standard meal[12,35], suggesting a disturbance of the feedback inhibition of gastrin secretion by gastric acid[34]. Mucosal gastrin content has been reported to increase[30,31] in patients with duodenal ulcer.

Morphologically, the cytoplasmic processes of somatostatin-producing D cells are in close contact with gastrin-producing G cells in the antrum[19]. In addition, a functional linkage between gastrin and somatostatin secretion may be reflected at the mRNA level. Omeprazole increases gastrin mRNA, and decreases somatostatin mRNA[36] in the antrum. Thus, the increased gastrin secretion in patients with duodenal ulcer may be caused, at least in part, by an enhanced functional activity of G cells and a reduced functional activity of D cells[8]. In the present study, bombesin significantly increased gastrin content and release in

antral mucosal explants in patients with duodenal ulcer. The total net increase of gastrin in the presence of bombesin was higher in patients with duodenal ulcer compared with normal subjects, suggesting that the synthesis, intracellular storage and release of gastrin by G cells were enhanced.

In a previous study, we found that the antral mucosal content of somatostatin in duodenal ulcer patients was low[32], which is consistent with the reports of other investigators[6,18]. Somatostatin, as a paracrine regulator of antral mucosal gastrin release, has thus been considered to play a role in the pathogenesis of duodenal ulcer. In the present study, bombesin did not significantly change the concentration of somatostatin released from antral mucosal explants and of somatostatin stored in the explants in duodenal ulcer patients, while bombesin stimulation increased both of them in normal subjects. In addition, the total net increase of somatostatin in the culture medium and antral mucosal explants in the presence of bombesin was substantially less in duodenal ulcer patients than in normal subjects. These findings suggest a reduced responsiveness of D cells to bombesin stimulation or a reduced number of D cells in duodenal ulcer patients. Whichever the mechanism, a reduced level of somatostatin probably contributes to the enhanced responsiveness of G cells in duodenal ulcer, leading to the hypersecretion of gastric acid.

Results in the control group showed that the secretory status of G cells and D cells was impaired according to the severity of atrophic gastritis. The total net increases of gastrin and somatostatin were lower in subjects with severe atrophic gastritis than in normal subjects, possibly because the numbers of G cells and D cells were reduced by the destruction of the antral glands. No patients with duodenal ulcer in this study had severe atrophic gastritis, although some had mild or moderate atrophic gastritis. Therefore, the significantly low somatostatin in the presence of bombesin, in patients with duodenal ulcer, was considered abnormal and unusual.

Our results, based on *in vitro* organ culture, indicated that storage and release of somatostatin were reduced in the antral mucosa in patients with duodenal ulcer. Abnormalities of synthesis and release of somatostatin in the antral mucosa appeared to weaken the paracrine inhibition of somatostatin on G cells, leading to an enhanced responsiveness of G cells and an increased secretion of gastrin. These disturbances of somatostatin and gastrin secretion in the antral mucosa itself may play an important role in the pathogenesis of duodenal ulcer.

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