Biochemical Pharmacology, Vol. 46, No. 9, pp. 1551–1557, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

STIMULATION OF MUCUS GLYCOPROTEIN BIOSYNTHESIS IN RAT GASTRIC MUCOSA BY GASTRIN

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(*Received* 22 *March* 1993; *accepted* 20 *July* 1993)

Abstract—We examined the effects of the gastrin family of peptides on gastric mucus glycoprotein (mucin) biosynthesis in rat gastric mucosa using an organ culture technique. Radiolabeled mucin was obtained from the tissue and culture medium of the corpus and antrum of rat stomach incubated for 5 hr with [^{3}H]glucosamine (GlcN), [^{14}C]threonine (Thr), and [^{35}S]sulfate *in vitro*. With the addition of 10^{-8} and 10^{-7} M tetragastrin to the culture medium, [^{3}H]GlcN labeled mucin in the corpus tissue increased to 120–135% that of the control ($P < 0.01$). The biosynthetic responses to cholecystokinin (CCK)-8 and the 17-peptide gastrin were essentially the same as that to tetragastrin. Tetragastrin 10^{-8} M also increased the incorporation of [^{35}S]sulfate into the corpus mucin but failed to change [^{14}C]Thr incorporation. In the antrum, biosynthetic activity showed no significant change with $10^{-9} \sim 10^{-5}$ M tetragastrin. Ranitidine, diphenhydramine and omeprazole at 10^{-5} M did not suppress the tetragastrin-induced increase in [^{3}H]GlcN incorporation into mucin, but L-365,260 at a concentration of 10^{-6} M completely blocked this effect. These results suggest that gastrin stimulates mucin production via CCK-B/gastrin receptors in the oxyntic region of rat gastric mucosa.

Gastric mucus is known as one of the principal physiological defense mechanisms of the gastric mucosa and is mainly composed of gel-forming, high molecular weight mucus glycoprotein (mucin). The production of mucus is a major physiological function of the stomach [1–3]. Although little is understood about the regulatory mechanism of its biosynthesis, histamine and carbachol are claimed to be responsible for increasing mucin synthesis in isolated pig gastric gland and canine antral explants. These observations indicate that histamine and cholinergic stimulation may be factors controlling the biosynthesis of gastric mucin, as well as activating gastric acid production [4, 5].

Gastrin, initially considered to exist by Edkins in 1905 and isolated and characterized by Gregory and Tracy in 1964, has been extensively studied as an acid stimulatory hormone. This hormone also exerts several other important actions, such as trophic effects in the oxyntic region of gastric mucosa and contraction of antral smooth muscle [6]. The administration of gastrin has been shown to promote protection of the gastric mucosa in rat [7, 8]. The stimulatory effect of this hormone on mucin synthesis has been postulated by several laboratories [9, 10]. However, the physiological mechanism by which gastrin stimulates biosynthesis, particularly with regard to whether this occurs directly by gastrin or indirectly through other functions such as histamine release by gastrin [11], remains unclear.

The present study was conducted to examine the effects of the gastrin family of peptides on gastric mucin biosynthesis in rat gastric mucosa, using the organ culture method. For this purpose, the biosynthetic response of mucin to gastrin was compared with or without the addition of the following drugs: diphenhydramine and ranitidine, histamine H_1 and H_2 receptor antagonists, respectively; omeprazole, a proton pump inhibitor; and L-365,260, a specific gastrin receptor antagonist.

MATERIALS AND METHODS

Experimental animals. Seven-week-old male Wistar rats (SLC, Shizuoka, Japan) each weighing approximately 160 g were used. All were fasted for 24 hr before the experiments and had free access to water during this time.

Organ culture. The stomachs of the rats were excised immediately after they were euthanized by exsanguination from the carotid artery under light anesthetization with CO_2 and were washed in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline [PBS(−)§]. They were then cut along the greater curvature. The glandular part was selected, and separated into the corpus and antrum, both of which were then cut into small pieces of 2 × 2 mm. The tissue culture method of Eastwood and Trier [12] was used with modification [13]. Eight tissue fragments, with the mucosal surface facing up, were placed on a stainless steel grid in the central well of a plastic culture dish (60 × 15 mm, Falcon, U.S.A.) and then treated with 0.75 mL of medium and 0.05 mL of test substances. The following media were used: medium 1 consisted of

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§ Abbreviations: PBS(−), Ca^{2+}/Mg^{2+}-free phosphate-buffered saline; DMSO, dimethyl sulfoxide; CCK, cholecystokinin; GlcN, glucosamine.

90% Eagle's minimum essential medium and 10% dialysed fetal calf serum, with 370 kBq/mL [^{3}H]-glucosamine hydrochloride (GlcN) with or without 1.85 MBq/mL [^{35}S]sulfate, and medium 2 consisted of 90% modified Eagle's minimum essential medium, in which the L-threonine concentration was reduced to 40 mmol/L and 10% dialysed fetal calf serum, with 370 kBq/mL [^{3}H]GlcN and 37 kBq/mL [^{14}C]-threonine (Thr). Each of the gastrin family of peptide was diluted with dimethyl sulfoxide (DMSO) and added at concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M to the dishes, making the final concentration of DMSO 0.01%. The dishes were maintained at 37° mainly for 5 hr and occasionally for 7 or 9 hr in 5% CO_2 and 95% air.

Isolation of labeled mucin and radioactivity measurement. On completion of the culture period, the tissue fragments in a dish were harvested from the medium after being gently rinsed with PBS(−) and boiled at 100° for 3 min in 0.4 mL 0.05 M Tris–HCl buffer, pH 7.2. The extraction and isolation of gastric glycoproteins were performed as described previously [14]. The tissue fragments were homogenized with a Physcotron micro homogenizer (Niti-On, Chiba, Japan). Triton X-100 was added to a 2% (v/v) concentration and the fragments were shaken for 1 hr at 37°. The homogenate thus obtained was centrifuged at 8000 *g* for 30 min to obtain the supernatant. Of the supernatant 0.4 mL was applied onto a Bio-Gel A-1.5 m column (1 × 30 cm), previously equilibrated with the Tris buffer containing 2% Triton X-100, and the column was eluted with this buffer. Finally, fractions of 0.8 mL each were collected and the radioactivity was measured by a scintillation counter (Beckman, Model LS-2800, U.S.A.), using Aquasol-2 (New England Nuclear, Boston, MA, U.S.A.) as the scintillant. The incorporation of radioactive precursors into the gastric macromolecular fraction corresponding to mucin [15], which was eluted in the void volume of the column, was determined. To compare the synthetic activity of mucin, the total radioactivity of this fraction was divided by the tissue protein content of each homogenate and expressed as dpm/μg of tissue protein. To isolate the gastric mucin from the culture medium, it was treated with the ethanol precipitation method. To one volume of a combined solution of the culture medium and PBS(−) used for the tissue rinsing, three volumes of ethanol containing 1% potassium acetate were added to make a 75% concentration (v/v). The resultant precipitate was maintained at 4° overnight and collected by centrifugation. The pellet obtained was solubilized in the Tris–Triton solution. This precipitation–solubilization procedure was repeated five times and the final pellet was dissolved in the Tris–Triton buffer. The resultant solution was then analysed with the same method used for the intracellular material.

CsCl *equilibrium gradient centrifugation.* For further characterization and purification of the radioactive substances obtained as mucin, fractions corresponding to the void volume of the Bio-Gel column were collected and used for CsCl equilibrium density gradient centrifugation. The starting density was adjusted to 1.40 g/mL by the addition of CsCl dissolved in 3 M guanidine-HCl. After centrifugation at 1.5 × 10^5 *g* for 120 hr at 10° (Hitachi 65P, RPS-40T rotor), each gradient was separated into a 0.5 mL fraction, its radioactivity was measured and its density was determined by weighing a definite volume of the corresponding fraction.

Gel-filtration analysis of oligosaccharides obtained from mucin. For the preparation of the radiolabeled oligosaccharides derived from mucin, the purified [^{3}H]GlcN-labeled mucin obtained from the CsCl density gradient centrifugation was treated by alkaline borohydride reduction using the method of Carlson [16] with a slight modification [17]. The reaction mixture was cooled and neutralized by dropwise addition of acetic acid (final pH 5). The reduced oligosaccharides were applied onto a gel-filtration column (1.0 × 50 cm) of Toyopearl HW-50S previously equilibrated with 0.1 N acetic acid and eluted with the same solution at a flow rate of 12 mL/hr. Fractions (0.8 mL) were collected for the measurement of radioactivity. The column was calibrated by eluting Dextran T-500 (Pharmacia), Dextran T-10 (Pharmacia), maltose and NaCl.

Reagents. The following substances were used for this study: minimum essential medium deficient in L-threonine (Gibco, U.S.A.); tetragastrin (Mect, Tokyo, Japan); cholecystokinin (CCK)-8 (Sigma Chemical Co., MO, U.S.A.); 17-peptide gastrin (Sigma); diphenhydramine (Sigma); ranitidine hydrochloride (Sankyo, Japan); omeprazole (Fujisawa-Astra, Japan); L-365,260 (Merck Sharp & Dohme, Rahway, NJ, U.S.A.); D-[1,6-^{3}H(N)]GlcN (1950 GBq/mmol, New England Nuclear); [^{14}C]Thr (8.6 GBq/mmol, New England Nuclear); [^{35}S]sulfate (1.59 TBq/mg, New England Nuclear); bovine testicular hyaluronidase (Sigma); α-amylase from bacillus species (Sigma). L-365,260 was dissolved with DMSO and utilized at a final concentration of 0.01% DMSO.

Protein determination. Protein content in the tissue homogenate was determined by the bicinchoninic acid method [18] with a Pierce protein assay kit, using bovine serum albumin as the standard.

Statistical analysis. The results were expressed as means ± SD. The one-way analysis of variance (ANOVA) with Dunnett's test was used for statistical analysis, with $P < 0.05$ being considered as significant.

RESULTS

Effect of tetragastrin on [^{3}H]GlcN incorporation into gastric macromolecules

Figure 1 shows the elution profile of Bio-Gel A-1.5 m chromatography of the [^{3}H]GlcN-labeled extracts obtained from the control and 10^{-8} M tetragastrin-treated corpus specimen. In the control without the addition of tetragastrin, radioactivity incorporated into the gastric macromolecular fraction (Fr-1) was as much as 17.0 ± 2.6 dpm/μg tissue protein. The addition of 10^{-8} M tetragastrin significantly increased the radioactivity by about 35% beyond that of the control.

For further characterization of the radiolabeled gastric macromolecule, CsCl equilibrium density gradient centrifugation was carried out. Figure 2

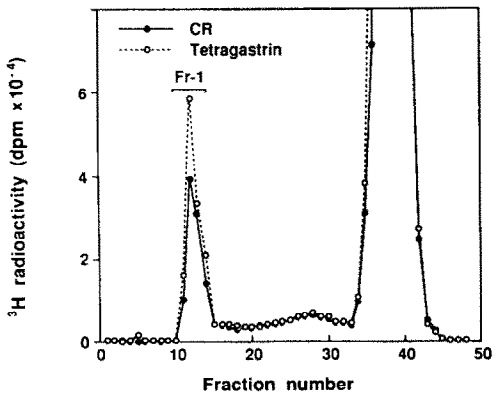

Fig. 1. Elution profiles of ^{3}H-labeled extracts from corpus tissue on Bio-Gel A-1.5 m column chromatography.

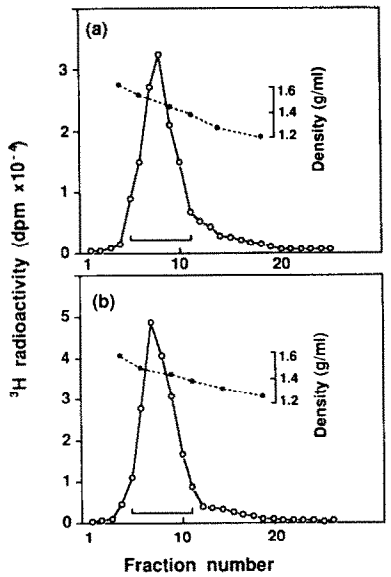

Fig. 2. CsCl equilibrium centrifugation of rat gastric macromolecules. ^{3}H-Labeled macromolecules from control (a), and 10^{-8} M tetragastrin-treated corpus tissue (b) were subjected to CsCl centrifugation as described in the text. The designated areas were pooled for further study.

shows the distribution of radioactivity of the high molecular glycoconjugates following the gradient centrifugation. The radioactive components displayed a sharp peak at approximately 1.44 g/mL density, and more than 90% of the activity was present at a density greater than 1.35 g/mL. These results indicate that most of the high molecular radioactive components coincide with the density of typical mucin-type glycoprotein [19]. The total radioactivity of the tetragastrin-treated group was greater than that of the control group by about 30% (Fig. 2b).

To determine the chain size distribution of the

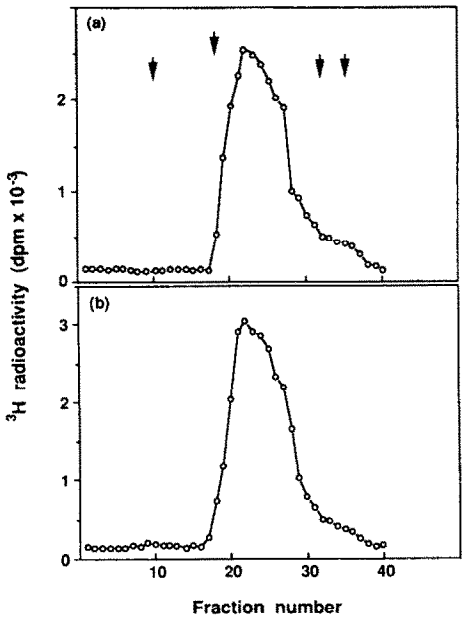

Fig. 3. Elution profiles of alkaline borohydride-treated ^{3}H-labeled mucin on Toyopearl HW-50S column chromatography. (a) Control; (b) tetragastrin, 10^{-8} M. Four arrows indicate, from left to right, void volume (T-500), peak positions of T-10 and of maltose, and total bed volume (NaCl), respectively. Molecular weight range corresponding to the peak of radioactivity was 2×10^3 to 3×10^3.

oligosaccharide of the mucin-like glycoconjugates, the radioactive materials obtained from the gradient centrifugation were subjected to alkaline borohydride treatment followed by Toyopearl HW-50S column chromatography (Fig. 3). The oligosaccharide mixture obtained from the control group formed a single broad peak. The calculated molecular weight of the radioactive oligosaccharide distributed from 1 to 5 kD, and the highest peak value was approximately 2.5 kD. The molecular weight distribution of the radioactive oligosaccharides was similar to that obtained from the purified rat gastric mucin [20]. The elution pattern of oligosaccharides of the tetragastrin group was essentially the same as that of the control. The profile of this chromatogram did not change after treatment of the oligosaccharide mixture with either testicular hyaluronidase of α-amylase.

Influence of the gastrin family of peptides on the biosynthesis of mucin in the corpus region

Figure 4 shows the biosynthetic activity of mucin in the corpus as measured by [^{3}H]GlcN incorporation. With the addition of 10^{-8} and 10^{-7} M tetragastrin, the biosynthetic activity significantly increased by about 20–35% beyond that of the control, but the addition of 10^{-9}, 10^{-6} and 10^{-5} M tetragastrin had no effect on the biosynthetic activity. The biosynthetic responses to CCK-8 and the 17-peptide gastrin were essentially the same as that to the tetragastrin group (Fig. 4).

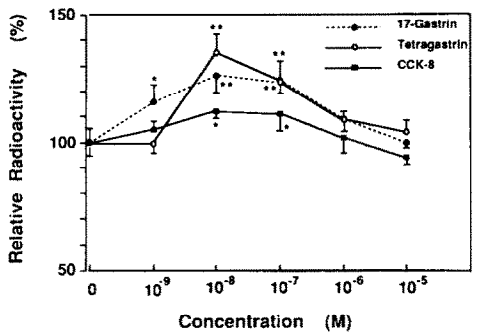

Fig. 4. Effects of the gastrin family of peptides on [^{3}H]-GlcN incorporation into gastric macromolecules in corpus tissue. Activities are expressed as percentages of the control, and represent means ± SD from eight different samples. *$P < 0.05$ and **$P < 0.01$ as compared with the control value (0 M).

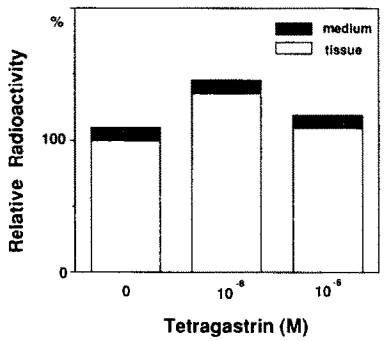

Fig. 5. Distribution of ^{3}H-labeled mucins in tissue and medium of the corpus specimens. Values are expressed as percentages of activity incorporated in control tissue.

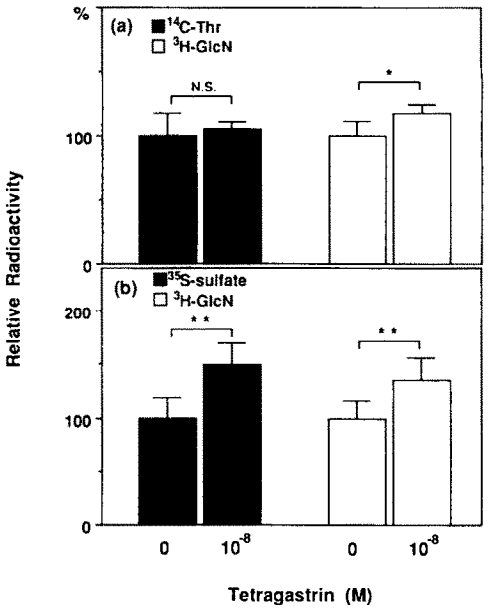

Fig. 6. Effects of tetragastrin on [^{14}C]threonine, [^{3}H]GlcN and [^{35}S]sulfate incorporation into mucin in corpus tissue. (a) [^{14}C]Thr and [^{3}H]GlcN double-labeled experiment was used for incubation in medium 2 described in Materials and Methods. (b) [^{35}S]Sulfate and [^{3}H]GlcN double-labeled experiment was used for incubation in medium 1. Values are expressed as percentages of the control and represent means ± SD from eight different samples. *$P < 0.05$ and **$P < 0.01$ as compared with the control value (0 M). NS, not significant.

Influence of the gastrin family of peptides on the biosynthesis of mucin in the antral region

No significant change could be detected in the mucin biosynthesis of the antrum on the addition of $10^{-9} \sim 10^{-5}$ M tetragastrin (sp. act.: 24.5 ~ 33.2 dpm/μg). The same results were obtained on the addition of CCK-8 and 17-peptide gastrin (data not shown).

Distribution of [^{3}H]GlcN-labeled mucin

Figure 5 shows the radioactivity distribution of [^{3}H]GlcN-labeled mucin in the corpus tissue and that excreted into the medium. Of the total ^{3}H-radioactivity incorporated into mucin, 10–15% was found in the medium and this rate was independent of tetragastrin addition. Although excretion of the radiolabeled mucin into the incubation medium of the antral mucosa was greater than that of the corpus, a distributional change was not obtained with the addition of tetragastrin. (Of the total mucin radioactivity 20–25% was recovered in the medium of the antrum.)

Influence of tetragastrin on the biosynthesis of apomucin in the corpus region

To confirm the effects of tetragastrin on apomucin biosynthesis, corpus tissue was cultured in the presence of [^{14}C]Thr and [^{3}H]GlcN. Figure 6a shows the biosynthetic activity of mucin in the corpus as measured by the simultaneous incorporation of [^{14}C]Thr and [^{3}H]GlcN with or without 10^{-8} M tetragastrin. Addition of tetragastrin enhanced [^{3}H]-GlcN incorporation into mucin by 17% ($P < 0.05$) but had no significant effect on [^{14}C]Thr incorporation (sp. act.: 2.84 ~ 2.98 dpm/μg tissue protein). Additionally, when the incubation time was further extended up to 9 hr (Fig. 7), the effects of tetragastrin on the incorporation of [^{14}C]Thr and [^{3}H]GlcN into the mucin molecule remained the same as those seen after the 5-hr incubation period.

In an alternate experiment, the effects of tetragastrin on the sulfation of mucin were studied. [^{3}H]GlcN and [^{35}S]sulfate incorporation into the corpus mucin was enhanced by 35% and 49% ($P < 0.01$) of the control, respectively, by the addition of 10^{-8} M tetragastrin (Fig. 6b).

Influences on the H_1 and H_2 receptor antagonist, proton pump inhibitor omeprazole, and specific gastrin receptor antagonist L-365,260 on the tetragastrin-induced increase in mucin synthesis

The 10^{-8} or 10^{-7} M tetragastrin-induced increase

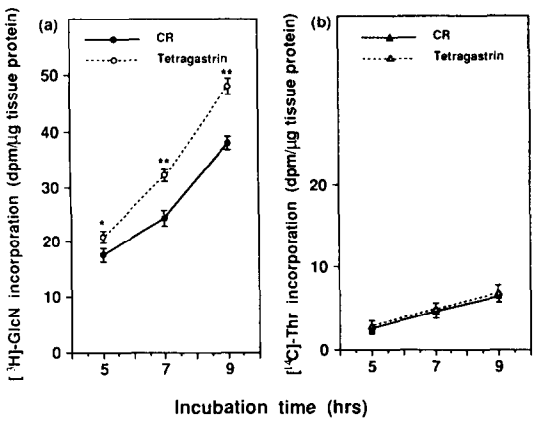

Fig. 7. Effects of tetragastrin on the incorporation of [^{3}H]-GlcN (a) and [^{14}C]threonine (b) into mucin in corpus tissue. Tissue fragments were incubated for the time indicated with the tracers in the absence (—●—, —▲—) or presence (···○···, ···△···) of 10^{-8} M tetragastrin. Values are expressed as dpm/μg of tissue protein and represent means ± SD from eight different samples. *$P < 0.05$ and **$P < 0.01$ as compared with the control value (0 M).

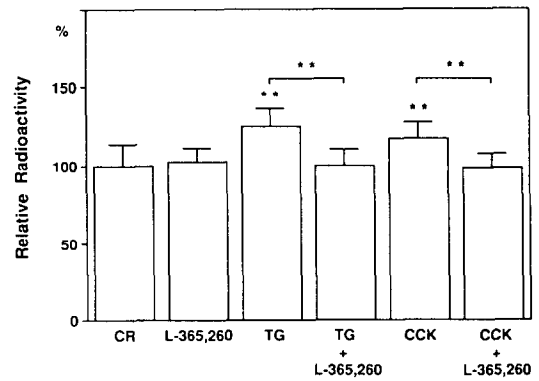

Fig. 8. Influence of L-365,260 (10^{-6} M) on the tetragastrin (TG) or CCK-8 (CCK) -stimulated incorporation of [^{3}H]-GlcN into mucin in corpus tissue. Values are expressed as percentages of the control and represent means ± SD from eight different samples. Asterisks indicate statistical significance (**$P < 0.01$), those just above the SD bar showing the significance versus the control value.

of [^{3}H]GlcN-labeled mucin in the corpus was not significantly suppressed by the addition of 10^{-5} M diphenhydramine, 10^{-5} M ranitidine or 10^{-5} M omeprazole (data not shown). In contrast, the addition of 10^{-6} M of a gastrin receptor antagonist, L-365,260, completely blocked the effect of tetragastrin on mucin biosynthesis (Fig. 8), as well as the CCK-8-induced activation of mucin synthesis.

DISCUSSION

In the present study, the high molecular [^{3}H]-GlcN-labeled substances isolated by chromatography on a Bio-Gel A-1.5 m column formed a single peak by equilibrium centrifugation in a CsCl density gradient at the typical density of mucin-type glycoproteins [19]. Furthermore, most of the radiolabeled oligosaccharides obtained from the high molecular glycoconjugates were distributed within the molecular weight corresponding to the oligosaccharides obtained from the purified rat gastric mucin [20]. The profile of the oligosaccharides in the HW-50S chromatogram did not change as a result of the treatment of the radioactive oligosaccharide mixture with either hyaluronidase or amylase, indicating that contamination with polysaccharides such as glycogen and hyaluronic acid was negligible. Goso and Hotta [17] reported that [^{35}S]sulfate-labeled mucin prepared by a procedure similar to that used in this study did not contain sulfated proteoglycans such as chondroitin sulfate, heparin or keratan sulfate. This indicates that most of the [^{3}H]GlcN-labeled substances obtained from the void volume peak of Bio-Gel A-1.5 m gel-filtration column chromatography are mucin-type glycoproteins [19], and the radioactivity measurement of this peak (Fr-1) is thought to be valid for the estimation of the biosynthetic activity of gastric mucin.

The stimulant effect of gastrin on mucin synthesis has been reported from several laboratories [9, 10]. Gerard *et al.* [10] showed that gastrin increased the apparent synthesis of gastric mucus of canine fundic crypt cells and the antral pyloric gland using *in vivo* incorporation of [^{35}S]sulfate followed by light microscopical autoradiography of the gastric epithelium. In the present study, for further clarification of the effects of gastrin on gastric mucin metabolism, rat gastric tissue was cultured in the presence of tetragastrin or its relevant peptides. With the addition of 10^{-8} and 10^{-7} M tetragastrin, [^{3}H]GlcN-labeled mucin in the corpus tissue increased significantly above the control level. [^{35}S]Sulfate incorporation into the corpus mucin was also significantly stimulated by the addition of 10^{-8} M tetragastrin, which was compatible with the histochemical findings of Gerard *et al.* [10]. The rate of radioactive mucin excreted into the medium from the tissue was essentially the same with or without tetragastrin addition. Accordingly, tetragastrin may accelerate mucin biosynthesis, rather than suppress the secretion, and consequently enhance the accumulation of this macromolecule in the tissue. The biosynthetic responses to CCK-8 and 17-peptide gastrin were essentially the same as that of tetragastrin, indicating that the activation of mucin synthesis is a feature common to the gastrin family of peptides.

Other than stimulation of gastric acid secretion, gastrin is known to exert many actions on epithelial and smooth muscle target cells in the gastrointestinal tract. Many of these actions, however, require pharmacological doses of this hormone [6]. In this study, the optimal condition for the stimulation of mucin synthesis was achieved at 10^{-8} and 10^{-7} M of the gastrin family of peptides. The gastrin concentration of rat serum was 10 and 100 ~ 150 pM, at fasting and after feeding, respectively, which was measured by radioimmunoassay using the antibodies

against the C-terminal active site of this peptide. Maximum acid secretion and histamine releases in the isolated vascularly perfused rat stomach were noted at 520 pM of the 17-peptide gastrin [11]. The concentration of the 17-peptide gastrin required for the stimulation of mucin synthesis in this study, $10 \sim 100$ nM, was rather higher than the normal serum level of gastrin in the rat. One possible explanation is the lack of cholinergic/histaminergic background stimulation in the model used in this study. The present results also showed that 10^{-6} and 10^{-5} M tetragastrin failed to stimulate mucin synthesis. A similar observation has been made by Scheiman *et al.* [9] in a cell culture system of highly enriched mucus cells of canine gastric mucosa. A higher dose of gastrin might have an inhibitory action competing with the stimulatory signal transduction pathways activated by a lower dose of the agonist. Although the differences between the tissue model and the isolated cell system should be kept in mind, it is possible that the stimulant effects of gastrin on the mucin synthesis would be negated at supraphysiologic doses of this peptide.

Heim *et al.* [4, 21] showed that histamine stimulated the production of high molecular glycoprotein and protein of isolated pig gastric non-parietal cells and concluded that at least the stimulation of the latter was mediated by H_2 receptors. Acetylcholine, another important mediator of gastric acid secretion, has also been shown by several laboratories to stimulate mucin synthesis and secretion in the stomach [5, 22]. Compared with these two substances, the physiological mechanism by which gastrin stimulates mucin biosynthesis remains unclear. Gastrin has been shown to stimulate gastric histamine release via the activation of histamine-storing cells and consequently to accelerate acid secretion of parietal cells [11, 23]. Thus, it is conceivable that the gastrin-induced stimulation of mucin synthesis occurs through histamine release induced by gastrin. Histochemical evidence showed that entero-chromaffin-like cells, the prominent histamine-storing cells of rat gastric mucosa are present exclusively in the oxyntic mucosa and are very rarely found in the antral region [24, 25]. In the present study, it was shown that mucin synthesis in the antral mucosa was not modulated by gastrin-related peptides. To clarify whether the gastrin-induced increase in mucin synthesis involves some intermediary activation mechanism for gastric mucosa, we examined the susceptibility of the tetragastrin effects to the H_1 receptor antagonist, diphenhydramine, the H_2 receptor antagonist, ranitidine, and the proton pump inhibitor, omeprazole. Ranitidine, at a concentration known to inhibit completely gastrin-stimulated acid secretion [26], did not suppress the increase in [^{3}H]GlcN incorporation induced by 10^{-8} and 10^{-7} M tetragastrin. Likewise, diphenhydramine and omeprazole did not affect the gastrin-induced increase in [^{3}H]GlcN incorporation. These results indicate that the histamine release and acid secretion which may occur concomitant with the addition of tetragastrin could not possibly be responsible for the gastrin-induced stimulation of mucin biosynthesis. The addition of L-365,260, a highly specific non-peptidic ligand for the gastrin receptor [27], at a concentration of 10^{-6} M, completely blocked the effects of both tetragastrin and CCK-8 on mucin biosynthesis. Thus, it may be concluded that the CCK-B/gastrin receptor is critically involved in the mechanism by which the activation of mucin biosynthesis occurs.

Our previous studies showed that the mucin obtained from the corpus of rat gastric mucosa differed in chemical composition of carbohydrate moieties and subunit structure from those present in the antrum [13, 17, 20]. In this study, gastrin-induced activation of mucin synthesis could not be observed in the antral mucosa of the rat stomach. This phenomenon may well reflect the fact that there are no receptors for gastrin on antral mucus cells. Different regulatory mechanisms might function in the mucin biosynthesis of the corpus and antrum.

Because threonine is one of the major amino acids of the backbone peptide of mucin [19], an increase in [^{14}C]Thr incorporation in the mucin would be expected following stimulation by tetragastrin. The addition of tetragastrin at a concentration which increased the incorporation of [^{3}H]GlcN and [^{35}S]-sulfate into mucin caused, however, no significant change in [^{14}C]Thr incorporation. This evidence suggests that tetragastrin may affect the process of post-translational modifications, including N- [28] and O-glycosylation and sulfation, rather than biosynthesis of the backbone peptide.

Histological observations show that the surface epithelial cells and gland mucus cells of gastric mucosa produce a distinct type of mucus with different staining characteristics [29]. Biochemical characterization of these two types of mucus was performed [30]. Komuro *et al.* [7] reported that subcutaneous administration of tetragastrin into rats increased the mucin content of the surface mucus cell layer but had no effect on that of the gland mucus cell-rich layer. These histochemical and biochemical findings suggest the possibility that tetragastrin stimulates the synthesis of a specific type of mucin. A comparison was thus made between the oligosaccharides obtained by gel-filtration of alkaline borohydride-treated mucins which had been synthesized in the absence or presence of tetragastrin. The elution profiles were similar with or without tetragastrin addition, indicating that the activation of mucin biosynthesis by tetragastrin was not accompanied by changes in the molecular size distribution of oligosaccharides in the corpus mucosa. Further studies are needed to clarify whether the gastrin-induced activation mechanisms are limited to the specific mucus cells or extend to mucus-producing cells in general in the corpus mucosa. To do this, new methods such as monoclonal antibodies have to be developed which are capable of detecting specific mucin species. Also a culture system of specific mucus cells isolated from the distinct site and layer of the gastric mucosa is needed.

In summary, the present observations demonstrate that [^{3}H]GlcN and [^{35}S]sulfate incorporation was stimulated by tetragastrin and related peptides in the corpus region of the rat stomach. This stimulation was not suppressed by either diphenhydramine, ranitidine or omeprazole but was completely blocked by L-365,260. The present data warrant the

conclusion that gastrin stimulates the process of mucin production via CCK-B/gastrin receptors distributed in the oxyntic region.

Acknowledgements—This work was supported in parts by Grants-in-Aid from the Japanese Ministry of Education, the Research Funds of Kitasato Gakuen and the Terumo Life Science Foundation. The authors express their sincere appreciation to Drs Susumu Ohara and Yukinobu Goso for their valuable comments and to Profs Haruya Okabe and Michihito Okubo for their encouragements throughout this study.

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