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Stimulation of mucin biosynthesis in rat gastric mucosa by FRG-8813 and its structural analogs

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Abstract

Certain chemical properties, which may determine the stimulatory actions of the new histamine H_2 receptor antagonist, FRG-8813 (2-(furfurylsulfinyl)-*N*-(4-[4-(piperidinomethyl)-2-pyridyl]oxy-(*Z*)-2-butenyl)acetamide), on mucin biosynthesis, were identified by considering the derivation of this drug using an organ culture system of the rat stomach. [^{3}H]Glucosamine and [^{35}S]sulfate incorporation was stimulated in the corpus region by FRG-8813 and its structural analog, compound A (*N*-[4-[[4-(piperidinylmethyl)pyridyl]-2-oxy]-(*Z*)-2-butenyl]phthalimide). The chronotropic response to histamine in the guinea pig right atria was suppressed by FRG-8813 in a concentration-dependent fashion. In contrast, compound A did not suppress the histamine-induced response. Ranitidine at 10^{-4} M did not suppress the FRG-8813-induced increase in [^{3}H]glucosamine incorporation into mucin. These results suggest that the pyridine derivative and amide structure are chemically important in FRG-8813 as a stimulant on mucus metabolism. Also, this effect is not directly due to histamine H_2 receptor antagonism.

Keywords: FRG-8813; Histamine H_2 receptor antagonist; Gastric mucin biosynthesis; Organ culture; (Rat)

1. Introduction

The introduction of the histamine H_2 receptor antagonists (Black et al., 1972; Daly et al., 1981), cimetidine and ranitidine, has brought remarkable progress in the therapy for duodenal and gastric ulcers. However, ulcer relapse has become a clinical problem after cessation of these first-generation histamine H_2 receptor antagonists with fragility of the mucosal defensive factors (Martin et al., 1981; Goto et al., 1985). Since the concept of 'cytoprotection' was introduced by Robert et al. (1979) as an ability of prostaglandins, which is independent of the anti-acid secretory effect, attention has been paid to the mucosal defensive factors in the genesis and therapy of ulcer disease. Further improvements in peptic ulcer treatment, particularly with regard to the quality of healing, should be gained if, in addition to decreasing gastric acid secretion, therapeutic agents could enhance the ability of the mucosa to defend itself from aggressive factors.

Although little is understood about the regulation of the mucosal defensive mechanisms, gastrin is claimed to be responsible for the enhancement of the mucosal protective action (Takeuchi and Johnson, 1979; Nishizaki et al., 1994). We also reported that tetragastrin protected the gastric mucosa from necrotizing agents (Komuro et al., 1992) and significantly increased the biosynthesis of mucin (Ichikawa et al., 1993). Moreover, our recent studies showed that some acid inhibitory anti-ulcer drugs, which have gastro-protective actions, also stimulated the mucin biosynthesis in the corpus region of rat stomach, indicating that the stimulation of mucin synthesis by a drug was closely related to the presence of mucosal protective activity (Ichikawa et al., 1994a).

The newly synthesized anti-ulcer drug, FRG-8813, (±)-2-(furfurylsulfinyl)-*N*-(4-[4-(piperidinomethyl)-2-

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pyridyl]oxy-(*Z*)-2-butenyl)acetamide (Fig. 1), was developed to be a second-generation histamine H_2 receptor antagonist with an increased action on the integrity of the gastric mucosal defense (Ichikawa et al., 1994a; Yamaura et al., 1992). It has been suggested that the critical structural feature of the histamine H_2 receptor antagonist is the five-membered heterocyclic aromatic ring or ethylthiomethyl ring side chain of the various guanidine derivatives (Durant et al., 1978). FRG-8813, having neither of these structural components, significantly differs in chemical structure from cimetidine and ranitidine. It has a unique six-membered aromatic ring, pyridine derivative, instead of the five-membered heterocyclic ring (Fig. 1). Our recent studies indicated that FRG-8813 has a stimulant effect on corpus mucin biosynthesis in rat gastric mucosa, while the other two conventional histamine H_2 receptor antagonists, cimetidine and ranitidine, failed to provide the stimulation of the mucin biosynthesis (Ichikawa et al., 1994a). It might be assumed that the difference in the effects on the cytoprotective action is due to these structural differences.

The present study was conducted to provide some clarification of the critical structural features of FRG-8813 for the stimulant effect on mucin biosynthesis and its relation to histamine H_2 receptor antagonism. For this purpose, the biosynthetic response of mucin to the following five compounds and FRG-8813 was examined by using an organ culture system of the rat stomach, and the histamine H_2 receptor antagonistic properties of these compounds were investigated on histamine-induced positive chronotropic responses in isolated guinea pig right atria. The five compounds bearing a structural resemblance to FRG-8813 are; *N*-[4-[[4-(piperidinylmethyl)pyridyl]-2-oxy]-(*Z*)-2-butenyl]-phthalimide, compound A; 4-[4-(piperidinylmethyl)-pyridyl-2-oxy]-(*Z*)-2-buten-1-ol, compound B; 2-chloro-4-(piperidinylmethyl)pyridine, compound C; 2-(furfurylsulfinyl)acetamide, compound D; and *N*-acetyl-2-(furfurylthio)acetamide, compound E (Fig. 1).

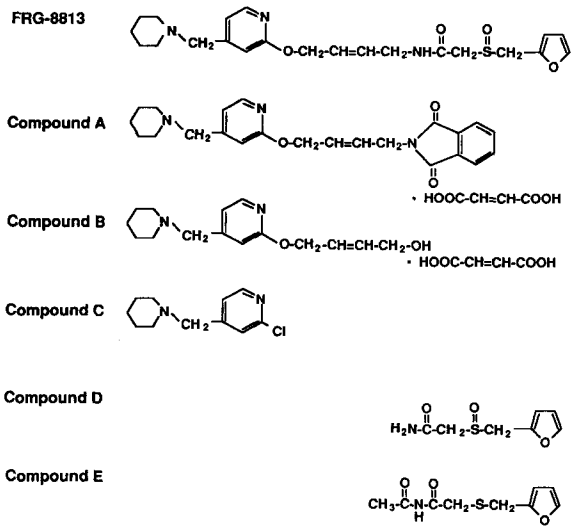

Fig. 1. Structures of the compounds used in this study.

2. Materials and methods

2.1. Experimental animals

7-week old male Wistar rats (SLC, Shizuoka, Japan) each weighing approximately 170 g were used. All were fasted for 24 h before the experiments and had free access to water during this time.

2.2. Organ culture

The stomachs of the rats were excised immediately after they were killed by light anesthetization followed by exsanguination from the carotid artery. They were then cut along the greater curvature, and the luminal surface was gently washed with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS(−)). The glandular part was selected, separated into the corpus and antrum and cut into small 2 × 2 mm sections. The tissue culture method of Eastwood and Trier (1973) was used with modification (Ishihara et al., 1988). Eight tissue fragments were randomly picked up from six to eight different stomachs, and were placed, with the mucosal surface facing up, on a stainless steel grid in the central well of a plastic culture dish (60 × 15 mm, Falcon, USA) containing 0.75 ml of the culture medium. The medium consisted of 90% Eagle's minimum essential medium and 10% dialyzed fetal calf serum, with 370 kBq/ml of D-[1,6-^{3}H(*N*)]-glucosamine hydrochloride (1565 GBq/mmol, New England Nuclear) with or without 1.85 MBq/ml [^{35}S]sulfate. For the addition of FRG-8813 or its structural analogs to the culture medium, the drug was dissolved in dimethyl sulfoxide (DMSO) and added at concentrations of 10^{-6} M to the dishes, making the final concentration of DMSO 0.01%. A DMSO solution without drug addition was added to the medium in the control well. All the dishes were maintained at 37°C for 5 h in 5% CO_2 and 95% air.

2.3. Isolation of labeled mucin and radioactivity measurements

Upon completion of the culture period, the tissue fragments on a grid were harvested from the medium, gently rinsed with PBS(−) and boiled at 100°C for 3 min in 0.4 ml of 0.05 M Tris-HCl buffer, pH 7.2. The extraction and isolation of mucin were performed as previously described (Azuumi et al., 1980). The tissue fragments were homogenized using a Physcotron micro homogenizer (Niti-On, Chiba, Japan). Triton X-100

was added to a 2% (v/v) concentration and the homogenate was shaken for 1 h at 37°C. The homogenate thus obtained was centrifuged at 8000 × *g* for 30 min to obtain the supernatant. A 0.4 ml portion of the supernatant was applied onto a Bio-Gel A-1.5m 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 using a scintillation counter (Beckman, Model LS-2800, USA) with Aquasol-2 (New England Nuclear, USA) as the scintillant. The radioactivity recovered into the void volume fractions of the column, which had been demonstrated to be the synthesized mucin (Ichikawa et al., 1993), was determined. To compare the synthetic activity of mucin, the total radioactivity of these fractions was divided by the tissue protein content of each homogenate to give the value relative to that of the control.

2.4. Histamine H_2 receptor antagonism of FRG-8813 and its structural analogs

Histamine H_2 receptor antagonistic properties of the compounds were investigated on the histamine-induced positive chronotropic responses in the isolated guinea pig right atria. Male Hartley guinea pigs (SLC, Shizuoka, Japan) were housed in a room at 20–24°C with a humidity of 45–65%. The hearts were rapidly removed and the atria were carefully dissected. Isolated right atria were mounted under 1 g of tension in a 30 ml organ bath containing Krebs-Henseleit solution at 37°C and continuously gassed with a mixture of 95% O_2 and 5% CO_2. Heart rate was picked up with an isometric force-displacement transducer (TB-651T, Nihon Kohden, Japan) and recorded on a pen recorder via a pulse-rate tachometer (AT-601G, Nihon Kohden, Japan). After administration of histamine (10^{-5} M), the test drug (3×10^{-7}–10^{-5} M) was added to the bath and the cumulative response curves to histamine were obtained. Inhibitory effects of each compound on histamine-induced chronotropic responses was reported as a percentage of the response without drug from the same preparation.

2.5. Drugs

The following drugs were obtained for use in this study: FRG-8813 and five compounds A–E, which bear a structural resemblance to FRG-8813 (Fujirebio, Japan); ranitidine hydrochloride (Sankyo, Japan).

2.6. Protein determination

Protein content in the tissue homogenate was determined using the Pierce BCA protein assay kit with bovine serum albumin as the standard.

2.7. Statistical analysis

The results were expressed as means ± S.D. The one-way analysis of variance (ANOVA) with Scheffé's test was used for statistical analysis with $P < 0.05$ taken as significant.

3. Results

3.1. Influence of FRG-8813 and its structural analogs on mucin biosynthesis in the corpus region

Fig. 2 shows the biosynthetic activity of mucin in the corpus as measured by the simultaneous incorporation of [^{3}H]glucosamine and [^{35}S]sulfate. In controls without addition of drugs, ^{3}H- and ^{35}S-radioactivities incorporated into the gastric mucin were 19.7 ± 2.3 and 11.4 ± 2.2 dpm/μg tissue protein, respectively. In the corpus, the addition of 10^{-6} M of FRG-8813 and compound A enhanced both [^{3}H]glucosamine and [^{35}S]sulfate incorporation into mucin, but the biosynthetic activity was not susceptible to the addition of the other compounds. The stimulation of [^{35}S]sulfate incorporation into mucin by compound A was significantly greater than that of FRG-8813 (Fig. 2). Compound A concentration dependently increased both [^{3}H]glucosamine and [^{35}S]sulfate incorporation into the mucin (Fig. 3).

3.2. Influences of FRG-8813 and its structural analogs on mucin biosynthesis in the antral region

In the antrum, no significant change could be detected during the mucin biosynthesis with the addition of each drug (^{3}H specific activity: 29.8–32.7 dpm/μg tissue protein, data not shown).

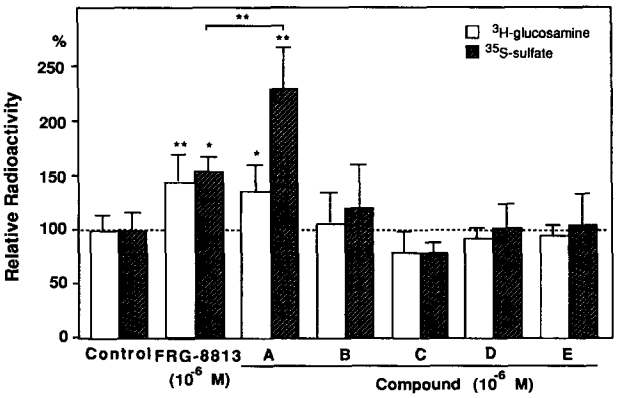

Fig. 2. Influence of FRG-8813 (10^{-6} M) and its structural analogs(10^{-6} M) on [^{3}H]glucosamine and [^{35}S]sulfate incorporation into mucins in the corpus region. Values are expressed as percentages of controls and represent means ± S.D. from six to ten different samples derived from six different animals. * $P < 0.05$ and ** $P < 0.01$ as compared with the control value.

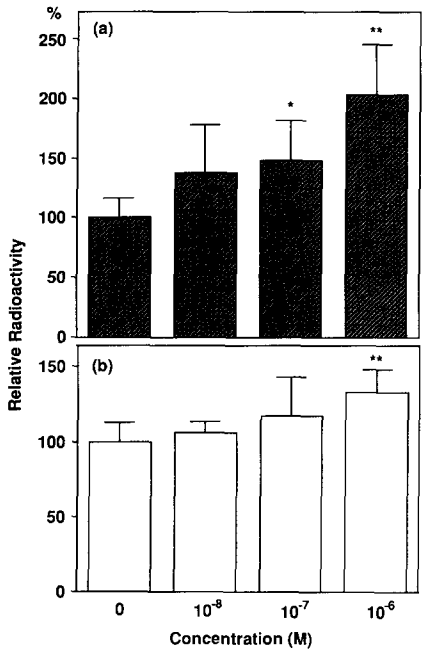

Fig. 3. Effect of compound A on the incorporation of [^{35}S]sulfate (a) and [^{3}H]glucosamine (b) into mucin in corpus tissue. Values are expressed as percentages of controls and represent means ± S.D. from six to twelve different samples derived from eight different animals. * $P < 0.05$ and ** $P < 0.01$ as compared with the control value (0 M).

3.3. Histamine H_2 receptor antagonistic effects by the compounds on chronotropic response of isolated guinea pig right atria to histamine

The effect of FRG-8813 and its structural analogs on the response of the guinea pig isolated right atria to histamine is shown in Fig. 4. FRG-8813 and compound B suppressed the 10^{-5} M histamine-induced response in a concentration-dependent fashion (Fig. 4). In contrast, the chronotropic response to histamine in the guinea pig right atria was not suppressed by the addition of other compounds (Fig. 4A,B,D,E).

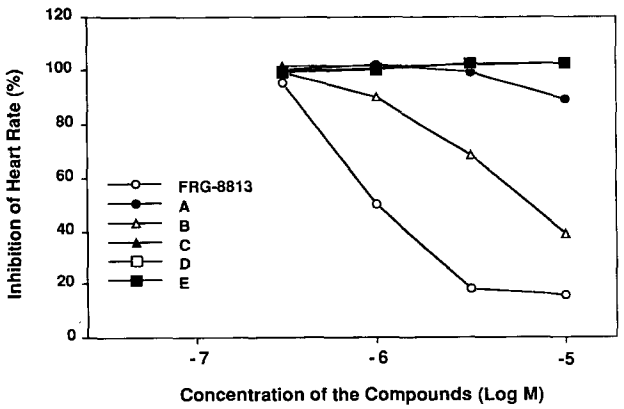

Fig. 4. Histamine H_2 receptor antagonistic effects by FRG-8813 and its structural analogs on the chronotropic response of isolated guinea pig right atria to histamine. Each point represents the mean inhibition percentage of the control response at 10^{-5} M histamine obtained from two to three preparations.

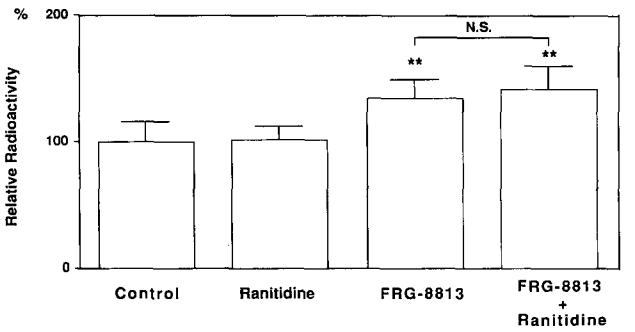

Fig. 5. Influence of ranitidine (10^{-4} M) on the FRG-8813 (10^{-6} M)-stimulated incorporation of [^{3}H]glucosamine into mucin in corpus tissue. Values are expressed as percentages of controls and represent means ± S.D. from six different samples. ** $P < 0.01$ as compared with the control value (0 M).

3.4. Influence of the histamine H_2 receptor antagonist, ranitidine, on the FRG-8813-induced increase in mucin synthesis

The 10^{-6} M FRG-8813-induced increase of [^{3}H]glucosamine-labeled mucin in the corpus was not significantly suppressed by the addition of 10^{-4} M ranitidine (Fig. 5).

4. Discussion

As noted above, FRG-8813 has a six-membered pyridine ring instead of a five-membered heterocyclic ring which has been considered the critical structural feature of the conventional histamine H_2 receptor antagonists, such as cimetidine and ranitidine (Fig. 1). Compared with the structural requirements for the histamine H_2 receptor antagonists, little is understood about the chemical aspects of drugs which exhibit cytoprotective/gastroprotective actions, because of the complicated mechanism of mucosal protection. FRG-8813 has been reported not only to inhibit acid secretion as a histamine H_2 receptor antagonist, but also to promote gastric mucosal protective action as well (Yamaura et al., 1992; Ichikawa et al., 1994b). On the other hand, many reports have indicated that cimetidine and ranitidine lack the mucosal protective activity, or so-called cytoprotection (Tarnawski et al., 1985). It may be assumed that the difference in the effects on the cytoprotective action is due to these structural differences. Recently, we reported that anti-ulcer drugs

bearing the mucosal protective activity, such as FRG-8813, roxatidine and Z-300, stimulated gastric mucin biosynthesis, while the drugs without cyto/gastroprotective properties, such as cimetidine and ranitidine, yielded no significant change in mucin biosynthesis (Ichikawa et al., 1994a, c). We, therefore, postulated that if a new histamine H_2 receptor antagonist has a six-membered aromatic ring, the drug might have gastric mucosal protective actions as well as accelerate mucus production (Ichikawa et al., 1994a). In this study, for clarification of the certain chemical properties of FRG-8813 as a stimulant of mucin production, which may be strongly correlated to the cytoprotective actions, rat gastric tissue was cultured in the presence of FRG-8813 and its structural analogs, and an assessment was made of mucin biosynthetic activity.

To judge whether differences in the chemical properties are significant requires a knowledge of structure-activity relationships, but the perception of such relationships depends on the manner in which the problem has been viewed and analyzed. Our notions of what is chemically important on the gastro-protective action have been conditioned by the results obtained in our search for new histamine H_2 receptor antagonists. Five derivatives were used in the present work as the FRG-8813 structural analogs (Fig. 1). Compounds A, B and C bear the pyridine ring and compounds D and E bear the furan ring which are commonly present in FRG-8813 structure. Radiolabeled mucin in the corpus tissue increased by about 35–125% above that of the control after the addition of two pyridine derivatives, FRG-8813 and compound A. The stimulation of [^{35}S]sulfate incorporation into the mucin by compound A was greater than that of FRG-8813, suggesting that compound A might strongly affect the process of sulfation in the mucin biosynthesis. In contrast, compounds D and E containing no pyridine ring failed to stimulate mucin biosynthesis. Similar results were obtained for compounds B and C, which have pyridine ring but lack an amide structure. These results indicate that the pyridine-based compounds including the amide structure may possibly be essential for activating the mucin biosynthesis in the gastric corpus mucosa.

All the drugs utilized in the present study failed to change the biosynthesis of antral mucin. Gastrin, known to be an acid stimulatory hormone, significantly accelerated mucin biosynthesis only in the oxyntic region, but yielded no significant change in the antral region of the rat stomach (Ichikawa et al., 1993). In previous studies, mucins obtained from the corpus and antrum of rat gastric mucosa were shown to differ in their subunit structures and the chemical composition of their carbohydrate moieties (Ohara et al., 1988; Goso and Hotta, 1989). These results and an immunohistochemical observation (Ishihara et al., 1993) strongly indicate that there are different types of mucus-producing cells in which a distinct regulatory mechanism on mucin biosynthesis might work in the corpus and antrum of the gastric mucosa.

For further clarification of the biological activities of FRG-8813 and its structural analogs, we compared the histamine H_2 receptor antagonistic characteristics of these compounds on the positive chronotropic responses to histamine in guinea pig atria. FRG-8813 dose dependently suppressed the histamine-induced positive chronotropic response and reflected the in vivo data of this drug which showed a potent gastric antisecretory activity in pylorus ligated rats and Heidenhain pouch dogs (Shibata et al., 1993). Of the five derivatives used in the present work, compound B was an antagonist of histamine at histamine H_2 receptor sites. In contrast, other drugs including compound A, which has a stimulatory effect on mucin biosynthesis, did not antagonize histamine-induced increases in contraction frequency of the guinea pig isolated right atrium. These results suggest that the histamine H_2 receptor antagonism was not directly correlated with the FRG-8813-induced stimulation of mucin biosynthesis.

In this study, the conventional histamine H_2 receptor antagonist, ranitidine, did not suppress the increase in [^{3}H]glucosamine incorporation induced by 10^{-6} M FRG-8813. Our data indicate that the histamine H_2 receptor, for which FRG-8813 competes with ranitidine, could not possibly be responsible for the FRG-8813-induced stimulation of mucin biosynthesis. Although little is understood about the regulatory mechanism of mucin production, acid secretagogues are claimed to participate in increasing mucin synthesis in isolated canine gastric mucosal cells. Scheiman et al. (1992) showed that histamine stimulated the production of glycoprotein of isolated canine mucous cells through the histamine H_2 receptor, analogous to histamine receptors present on the gastric parietal cells. Although the differences in the species and experimental systems between Scheiman's and ours should be kept in mind, the present data indicate that at least the FRG-8813 effect could not possibly be responsible for the histamine-induced stimulation of mucin biosynthesis. It is still uncertain whether FRG-8813 has a direct effect on mucus-producing cells or an indirect effect through the action of other cells. In any case, FRG-8813 might be a very useful tool for the detection and further clarification of the regulatory mechanism of mucin synthesis in the oxyntic region of the gastric mucosa.

In summary, the present finding demonstrates that certain chemical properties of the amide structure and ring structure including pyridine of FRG-8813 are responsible for the activation of mucin biosynthesis in the corpus region of the rat stomach. This effect of FRG-8813 is not directly due to histamine H_2 receptor antagonism.

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