S. TAKAHASHI, S. OKABE

A HISTAMINE H₂ RECEPTOR ANTAGONIST, ROXATIDINE, STIMULATES MUCUS SECRETION AND SYNTHESIS BY CULTURED RABBIT GASTRIC MUCOSAL CELLS

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto, Japan

We examined the effects of the known antisecretory and mucosal protective drug, roxatidine, on the secretion and synthesis of mucus by cultured rabbit gastric mucosal cells. The amounts of secreted and synthesized mucus were determined by the [³H] glucosamine labelling method. Exposure of the cells to roxatidine for 8 hr caused increases in the secretion and synthesis of mucus in a dose-related manner. The increase in mucus synthesis was maximally induced 4 hr after the addition of roxatidine, while mucus secretion was maximally enhanced a further 4 hr later. However, other H₂ antagonists such as cimetidine, ranitidine and famotidine failed to stimulate the secretion and synthesis of gastric mucus. In addition, neither indomethacin nor N⁵-nitro-L-arginine methyl ester affected the roxatidine-induced increases in mucus secretion and synthesis. We conclude that roxatidine directly acts on gastric mucosal cells, inducing increases in both the secretion and synthesis of mucus, and that an unknown regulatory pathway might be involved in these stimulatory actions of roxatidine.

Key words: roxatidine, gastric mucosal cells, mucus secretion, prostaglandin, NO.

INTRODUCTION

Roxatidine acetate hydrochloride (roxatidine, Fig. 1), a histamine H₂ receptor antagonist, is known to exert a mucosal protective effect as well as antisecretory effect in rats (1—5). The protective effect of roxatidine is considered to be due to enhancement of defensive factors, including gastric mucus, as well as to inhibition of aggressive factors such as acid and free radicals. Recently, roxatidine was reported to stimulate mucin synthesis by isolated rat stomachs (6). Mucus is known to play an important role in the gastric mucosal defensive mechanism (7). However, it remains unknown
whether or not mucus secretion and synthesis are stimulated through the direct action of roxatidine on gastric epithelial cells. Therefore, in the present study, we examined the effects of roxatidine on the secretion and synthesis of mucus by cultured mucosal cells prepared from rabbit stomachs.

![Chemical structure of roxatidine.](image)

**Fig. 1.** Chemical structure of roxatidine.

**MATERIALS AND METHODS**

**Preparation of Gastric Mucosal Cells**

Gastric mucosal cells were isolated from rabbit stomachs according to the method of Watanabe et al. (8). Briefly, male Japanese white rabbits (Nihon S.L.C., Shizuoka, Japan), weighing 2.5—3.5 kg, were anesthetized with pentobarbital (50 mg/kg, i.v.; Abbott, North Chicago, Ill.). After a stomach had been excised, the surface of the oxyntic mucosa was removed with a razor blade and minced immediately. The minced tissue was incubated in Hank’s balanced salt solution containing 0.07% collagenase (Wako Chemicals, Kyoto, Japan) for 15 min at 37°C, and then washed with Ca²⁺, Mg²⁺-free Hank’s solution containing 1 mM EDTA and 1% bovine serum albumin. These procedures were repeated twice. The mucosal cells were obtained by filtration through a metal mesh (diameter, 300 µm). The viability of the isolated cells was more than 85%, as determined by the trypan blue exclusion test (9).

**Cell Culture**

Coon’s modified Ham’s F12 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 U/ml streptomycin and 0.25 µg/ml amphotericin B was used. Gastric mucosal cells (2 × 10^5 cells/0.5 ml of medium) were inoculated into a 24-well culture plate coated with rat collagen type I (Sumitomo Bakelite, Tokyo, Japan). The culture was maintained at 37°C under 5% CO₂ in air, the medium being changed every day. The cells reached confluence 2 or 3 days later.

**Determination of Mucus Secretion and Synthesis**

The amounts of secreted and synthesized mucus were determined according to the methods of Terano et al. (10). Gastric mucosal cells grown to confluence were washed with Ca²⁺, Mg²⁺-free phosphate-buffered saline (PBS) and then incubated with 0.5 ml of the medium containing [³H] glucosamine (18.5 kBq, 1565.1 GBq/mmol; New England Nuclear, Boston, MA) in the presence of various drugs or the vehicle at 37°C. At appropriate times, the medium was recovered and then mixed with 0.1 ml of 50% trichloroacetic acid. The mixture was held on ice for 5 min and then
centrifuged at 10,000 × g for 5 min at 4°C. The resulting pellet was solubilized with 0.2 ml of 0.3 N NaOH, and then the radioactivity in an aliquot (0.1 ml) was measured as the amount of mucus secreted from the cells into the medium. For estimation of mucus synthesis in the cells, the amount of \(^{3}H\) glucosamine incorporated into the cells was determined. The remaining cells were washed twice with Ca\(^{2+}\), Mg\(^{2+}\)-free PBS and then solubilized with 0.25 ml of 0.3 N NaOH. The radioactivity in the cell lysate (0.1 ml) was measured.

**Determination of Prostaglandin E\(_2\) Production**

Gastric mucosal cells grown to confluence were incubated with 0.5 ml of medium in the presence of indomethacin (5 μM) or the vehicle 37°C for 8 hr. Thereafter, the medium was recovered, followed by 10-fold dilution. The amount of prostaglandin E\(_2\) (PGE\(_2\)) in the diluted medium was determined by enzyme-immunoassaying using a PGE\(_2\) EIA kit (Cayman Chemicals, Ann Arbor, MI).

**Drugs**

Roxatidine (Teikoku Hormone MFG. Co., Tokyo, Japan), cimetidine (Smith Kline Beecham, Tokyo, Japan), ranitidine (Sigma Chemicals, St. Louis, MO), famotidine (Yamanouchi Pharmaceutical Co., Tokyo, Japan), 16,16-dimethyl prostaglandin E\(_2\) (dmPGE\(_2\); Ono Pharmaceutical Co., Osaka, Japan), pentagastrin and indomethacin (Sigma Chemicals) were dissolved in dimethyl sulfoxide. For each assay, dimethyl sulfoxide was diluted to the final concentration of 0.5% in the medium. Histamine 2HCl (Nacalai Tesque, Kyoto, Japan), carbachol, isoproterenol, nitroprusside and N\(^{0}\)-nitro-L-arginine methyl ester (L-NAME; Sigma Chemicals) were dissolved in distilled water. All other chemicals were of reagent grade.

**Statistical Analysis**

Data are means ± S.E. for 7–9 cultures. Statistical significance was evaluated using Dunnett’s multiple comparison test or Student’s t-test, a P value of < 0.05 being regarded as significant.

**RESULTS**

**Characterization of Cultured Gastric Mucosal Cells**

Most of the cultured cells were morphologically epithelial cells, and 90% of the cells were confirmed to be mucus-producing ones by periodic acid-Schiff staining. As judged with the \(^{3}H\) glucosamine labeling method, the amounts of mucus secreted and synthesized by the cells increased in a time-dependent manner (see the inset in Fig. 4). The secretion and synthesis of mucus by the cells were dose-dependently enhanced when several agents, such as dmPGE\(_2\), pentagastrin, carbachol and isoproterenol, were added for 8 hr (Fig. 2). At 1 μM, these agents significantly stimulated both the secretion and synthesis of gastric mucus. However, histamine did not affect the secretion or synthesis at the indicated concentrations. Treatment with nitroprusside for 2 hr also
significantly stimulated mucus secretion and synthesis by the cells (control, 385.3 ± 21.4 and 387.6 ± 26.5; and nitroprusside, *488.0 ± 34.5 and *482.4 ± 18.7, for secretion and synthesis, respectively; *significantly different from the control, P < 0.05, n = 9). Furthermore, the cells constitutively produced PGE<sub>2</sub>, the production being potently inhibited by 5 μM indomethacin (control, 2.5 ± 0.16 ng/ml/hr, and indomethacin-treated, *0.25 ± 0.01 ng/ml/hr; *significantly different from the control, P < 0.05, n = 9).

Fig. 2. Effects of various agents on mucus secretion and synthesis by cultured rabbit gastric mucosal cells. Gastric mucosal cells were incubated with the indicated concentrations of each agent in the presence of [³H] glucosamine for 8 hr, and then the radioactivity of the acid-insoluble materials (secretion) and cells (synthesis) was measured. Data are means ± S.E. for 9 cultures.

*Significantly different from the control, P < 0.05.

**Effects of Roxatidine on Secretion and Synthesis of Gastric Mucus**

Exposure of gastric mucosal cells to roxatidine for 8 hr caused increases in the secretion and synthesis of mucus in a dose-related manner (Fig. 3). Roxatidine at 0.3 μM tended to increase mucus secretion by the cells, it significantly enhancing the secretion by 13.6% at 1 μM and 16.8% at 10 μM, as to the control, respectively. Similarly, the drug also significantly stimulated mucus synthesis by 12.7% at 1 μM and 14.6% at 10 μM, respectively.

The time courses of the effects of 1 μM roxatidine on the secretion and synthesis of gastric mucus are shown in Fig. 4. On 4 hr treatment with roxatidine, mucus secretion was significantly enhanced, the maximal increase in the secretion being observed 8 hr after the addition. Thereafter, mucus secretion tended to be slightly elevated up to 24 hr, as compared with that in
**Fig. 3.** Dose dependencies of the effects of roxatidine on the secretion and synthesis of mucus by gastric mucosal cells. Gastric mucosal cells were incubated with the indicated concentrations of roxatidine in the presence of $[^3]$H-glucosamine for 8 hr, and then the radioactivity of the acid-insoluble materials (secretion) and cells (synthesis) was measured. Data are means ± S.E. for 8 cultures. *Significantly different from the control, P < 0.05.

**Fig. 4.** Time courses of the effects of roxatidine on the secretion and synthesis of mucus by gastric mucosal cells. Gastric mucosal cells were incubated with 1 μM roxatidine or the vehicle (inset) in the presence of $[^3]$H-glucosamine for the indicated times, and then the acid-insoluble materials (secretion) and the cells (synthesis) was measured. Data are means ± S.E. for 8 cultures. *Significantly different from the control, P < 0.05.
the control. On the other hand, at 4 hr, the increase in mucus synthesis was maximally induced by roxatidine, and then the significant increase persisted for 8 hr. However, the increase in the synthesis had disappeared by more than 16 hr.

**Effects of Other H₂ Antagonists on Secretion and Synthesis of Gastric Mucus**

The effects of other H₂ antagonists such as cimetidine, ranitidine and famotidine were investigated (Fig. 5). When cells were exposed to the drugs at 0.1—10 μM for 8 hr, all antagonists failed to enhance mucus secretion and synthesis.

![Graphs showing mucus secretion and synthesis](image)

**Fig. 5.** Effects of cimetidine, ranitidine and famotidine on the secretion and synthesis of mucus by gastric mucosal cells. Gastric mucosal cells were incubated with the indicated concentrations of each drug in the presence of [³H] glucosamine for 8 hr, and then the radioactivity of the acid-insoluble materials (secretion) and cells (synthesis) was measured. Data are means ± S.E for 8 cultures.

**Effects of Indomethacin and L-NAME on the Roxatidine-induced Increases in Mucus Secretion and Synthesis**

To examine whether or not a mediator such as PGs and nitric oxide (NO) is involved in the action of roxatidine, the effects of indomethacin and L-NAME were studied (Fig. 6). The stimulatory effects of 1 μM roxatidine at 8 hr were unaffected by treatment with indomethacin at 5 μM and L-NAME at 0.1 mM, although the basal abilities of the cells to secrete and synthesize mucus were reduced by the agents.
Fig. 6. Effects of indomethacin and L-NAME on the roxatidine-induced increases in gastric mucus secretion and synthesis. Gastric mucosal cells were incubated with 1 μM roxatidine, with or without 5 μM indomethacin or 0.1 mM L-NAME, in the presence of [3H] glucosamine for 8 hr, and then the radioactivity of the acid-insoluble materials (secretion) and cells (synthesis) was measured. Data are means ± S.E. for 7 cultures. *Significantly different from the control, P < 0.05.

DISCUSSION

As judged on morphological observation and functional analysis of mucus secretion and synthesis, it is apparent that our cultured gastric cells were mucus-producing epithelial cells. These cells also released PGE₂, which was reported to be produced by gastric epithelial cells (11, 12). In addition, almost all known mucus secretors, such as dmPGE₂ (10, 13), pentagastrin (14), carbachol (14, 15), isoproterenol (15) and nitroprusside (16), significantly and dose-relatedly stimulate mucus secretion and synthesis by cells. Thus, it is evident that our mucosal cell preparations are quite sensitive to mucus-stimulating agents. Only histamine did not enhance mucus secretion, but this might be due to the cell strain and culture conditions. In fact, histamine significantly stimulates mucus secretion by canine and porcine gastric mucosal cells (14, 17), but it is not effective for cells prepared from rat stomachs (15).

Using these cells, we found that roxatidine directly acts on gastric mucosal cells, inducing increases in the secretion and synthesis of mucus. On treatment for 8 hr, the increases caused by 10 μM roxatidine were comparable with those by 1 μM dmPGE₂. Ichikawa et al. (6) reported that roxatidine promotes mucin biosynthesis in fragments of isolated rat stomachs, the stimulation at 10 μM being about 40%. Accordingly, it is possible that roxatidine may have indirect stimulatory effects on mucus secretion and synthesis by mucosal cells.
Based on the time courses of the effects of roxatidine, prior to elevation of mucus secretion, the increase in mucus synthesis was induced by roxatidine. As mucus was constitutively secreted and synthesized by the cultured cells, it can be easily considered that the accumulation of mucus in the cells, resulting from potentiation of synthesizing activity, causes an increase in mucus secretion. It is suggested that roxatidine might stimulate mucus synthesis, leading to acceleration of mucus secretion.

The possibility that roxatidine might stimulate the mucus secretion only through histamine H₂ receptors has been ruled out, since histamine itself and three other histamine H₂ receptor antagonists had no effect on mucus secretion and synthesis by the cells. Relating to the failure of cimetidine and ranitidine to stimulate mucus secretion and synthesis, similar results were obtained by Heim et al. (17), and Ichikawa et al. (6), who used pig and rat gastric mucosal cells, respectively.

It was shown that gastric mucosal cells exhibit NO synthase activity as well as PG synthesis activity (18). We confirmed that both dmPGE₂ and an NO generator enhance mucus secretion and synthesis by the cells, as described above. Furthermore, indomethacin and L-NAME reduced the basal secretion and synthesis of mucus by mucosal cells. Taken together, it is suggested that endogenous PGs and NO, produced by gastric mucosal cells, regulate the basal secretion and synthesis of mucus in the autocrine system. However, these agents could not inhibit roxatidine-induced increase in mucus secretion and synthesis, suggesting that neither PGs nor NO are involved in the stimulatory effects of roxatidine. Although the mechanism of the stimulation by roxatidine remains unclear, the drug might be a useful tool for elucidation of the regulatory mechanism for mucus secretion.

We conclude that roxatidine directly acts on gastric mucosal cells, inducing increases in both the secretion and synthesis of mucus, and that an unknown regulatory pathway might be involved in the stimulatory actions of roxatidine. Roxatidine has dual direct effects, inhibiting acid secretion by parietal cells and enhancing mucus secretion by gastric epithelial cells. These effects might contribute to the gastric mucosal protection by roxatidine in vivo.

Acknowledgements: We wish to thank Mr. N. J. Halewood for critical reading of the manuscript, and Ms. K. Hirose, Ms. M. Koba and Mr. T. Yamazaki for their technical assistance.

REFERENCES


Received: September 8, 1995. Accepted: September 27, 1995.

Author’s address: S. Takahashi, Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607, Japan.