Gastroenterologia Japonica
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Vol. 21, No. 5
Printed in Japan

—Original Article—

CYCLIC AMP IN GASTRIC JUICE DOES NOT REFLECT HISTAMINE H_2 RECEPTOR ACTIVITY IN HEIDENHAIN POUCH DOG

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Summary

It is strongly believed that cAMP mediates histamine H_2 receptor activity, but does not mediate gastrin and acetylcholine stimulation of gastric acid secretion. Therefore, cAMP production could be a marker of H_2 receptor activity. Whether endogenous histamine mediates gastrin and/or acetylcholine stimulation, at least partialy, remains to be elucidated. If cAMP in the gastric juice reflects H_2 receptor activity, we can investigate whether endogenous histamine mediates gastrin and/or acetylcholine stimulation in vivo. In this study, we investigated whether cAMP in the gastric juice reflected histamine H_2 receptor activity in the Heidenhain pouch dog in vivo using different kinds of inhibitors of gastric secretion. Our hypothesis was as follows: Upon betazole stimulation, cimetidine, an H_2 receptor antagonist, should decrease cAMP output into the gastric juice, but omeprazole, an H^+, K^+-ATPase blocker, should not, because it blocks at a site more peripheral than the H_2 receptor and the production of cAMP. Sixty minutes after betazole administration, 4.0 μmol/kg of cimetidine and 0.18 μmol/kg omeprazole were administered intravenously and they inhibited gastric juice volume to a similar degree, that is, 49.6% and 52.1%, respectively. However, omeprazole caused a greater decrease in cAMP output than cimetidine. Inhibition with 4 μmol/kg/h of cimetidine or 0.2 μmol/kg of omeprazole from the beginning of betazole stimulation also caused similar decreases in gastric juice volume, 66.6% and 60.6%, respectively. Both inhibitors decreased cAMP output into the gastric juice in a similar fashion in the first two 30 minute periods. These results do not agree with our hypothesis. It seems that cAMP in the gastric juice does not reflect histamine H_2 receptor activity following betazole stimulation, at least as a major component.

Key Words: *Cyclic AMP, Gastric juice, Histamine H_2 receptor.*

Introduction

Histamine must have a very important role in gastric secretion, because H_2 receptor antagonists inhibit gastric secretion induced by histamine, gastrin and acetylcholine. However the mechanism of endogenous histamine participation is not clear. One possibility is that

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This study was presented in part at the 71st meeting of the Japanese Society of Gastroenterology (may 22, 1985).

This work was suported by a Grant-in Aid for Encouragement of Young Scientists (No. 59770483) of the Ministry of Education, Science and Culture.

endogenous histamine may enhance the effect of other stimulants, such as gastrin and acetylcholine[1,2]. Another possibility is that endogenous histamine may function as a mediator of gastrin and/or acetylcholine stimulation, at least in part[3-6].

Histamine increases cAMP production in parietal cells, but gastrin and acetylcholine do not[7-10]. An increase in cAMP production could be a marker of H_2 receptor activity. Since some of the cAMP produced in the cells leaks into extracellular fluid such as urine[11], plasma[12,13], bile[14], saliva[15,16] and cerebrospinal fluid[17,18], it is theoretically plausible that the stimulation of H_2 receptors on parietal cells results in the increase of cAMP output into the gastric juice. Therefore, if cAMP in the gastric juice reflects H_2 receptor activity, we can investigate the role of endogenous histamine in gastrin and acetylcholine stimulation in vivo by measuring cAMP in gastric juice. In this study, we used two inhibitors which block gastric secretion at different loci following betazole, an analogue of histamine, stimulation. The first inhibitor was cimetidine, an H_2 receptor antagonist, which decreases cAMP production in parietal cells. The second inhibitor was omeprazole, an inhibitor of H^+, K^+-ATPase, which inhibits a site more peripheral than H_2 receptor-adenylate cyclase coupling. Omeprazole was expected not to inhibit cAMP production in the parietal cells[19-21]. Our hypothesis was that, if cAMP in the gastric juice reflects H_2 receptor activity, then, following betazole stimulation, cimetidine should decrease cAMP output into gastric juice but omeprazole should not. If such were the case, the measurement of cAMP in gastric juice could be a useful instrument for studying the role of endogenous histamine in gastric acid secretion stimulated by gastrin, or acetylcholine. We also compared tetragastrin stimulation and betazole stimulation with regard to the change in cAMP level in gastric juice and plasma.

Materials and Method

Heidenhain pouch dogs weighing 8-10 kg were made to fast but allowed free access to water for 18 hours. Gastric secretion was stimulated by intravenous administration of 2 mg/kg/h of betazole for two hours and gastric juice was collected every 30 minutes through the cannula of the Heidenhain pouch. The gastric juice was filtered with gauze to remove mucus and its volume measured and titrated with 0.2 N NaOH to pH 7.0 with pH stat. Blood samples were collected from a vein before and at 15, 30, 60, 90 and 120 minutes after the infusion of stimulants with a syringe that contained EDTA, at a final concentration of 5 nMol. The blood samples were placed in ice water and centrifuged within 30 minutes. Cyclic cAMP in the gastric juice and plasma were measured by radioimmunoassay kits (Yamasa Company, Choshi, Japan).

Inhibition of gastric secretion at 60 minutes: Four μmol/kg of cimetidine or 0.18 μmol/kg of omeprazole was administered intravenously at 60 minutes after the beginning of betazole infusion.

Inhibition of gastric secretion from the beginning: Four μmol/kg/h of cimetidine was infused from the beginning, or 0.2 μmol/kg of omeprazole was injected intravenously 1 hour prior to the betazole infusion.

Study of gastrin stimulation: Tetragastrin at a dose of 4 μg/kg/h was infused intravenously for two hours and gastric juice and venous blood were collected in the same manner as in betazole stimulation.

Statistical analysis: All results were expressed as mean $\pm$ S.E. Student's unpaired t-test was used for statistical analysis. P-values of less than 0.05 were considered to be significant.

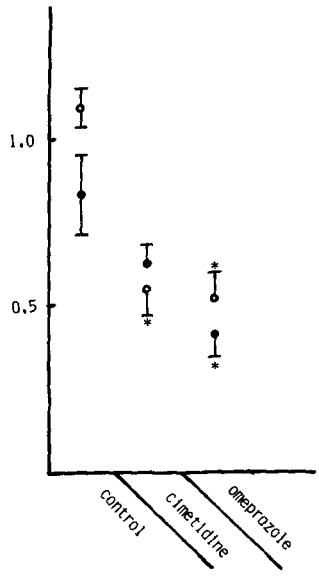

Fig. 1. The effect of the inhibitors cimetidine (4 μmol/kg i.v.) and omeprazole (0.18 μmol/kg i.v.) administered at 60 minutes, on gastric juice volume and cAMP output. Gastric secretion was stimulated by betazole (2 mg/kg/h). The degree of inhibition is expressed as the ratio of values of gastric juice volume and cAMP output during the period following inhibition to the same value during a 30-minute period prior to inhibition (mean ± SEM). Circles depict the ratio of gastric juice volume. Solid dots depict the ratio of cAMP output. Asterisks indicate significant differences from control.

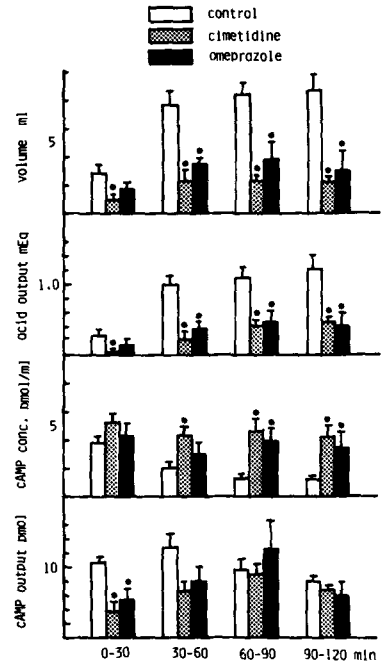

Fig. 2. The effect of inhibitors, cimetidine (4 μmol/kg/h) and omeprazole (0.2 μmol/kg) from the begining of betazole (2 mg/kg/h) stimulation on gastric juice volume, and acid output, cAMP concentration and cAMP output. Columns depict values for each thirty-minute period (mean ± SEM). Asterisks indicate a significant difference from control.

Results

Inhibition at 60 minutes: The ratio of the volumes of gastric juice and cAMP output during a 30-minute period following inhibition to the same values during a 30 minute period prior to inhibition are shown in (**Fig. 1**). We intended to use a dose that would inhibit gastric secretion at least one-half. Four μmol/kg of cimetidine and 0.18 μmol/kg of omeprazole decreased gastric juice volume by 49.6% and 52.1%, respectively. However, compared to controls, a significant decrease of cAMP output was found with omeprazole but not cimetidine.

Inhibition from the beginning of betazole stimulation: Four μmol/kg/h of cimetidine and 0.2 μmol/kg of omeprazole inhibited gastric juice volume 71.7% and 55.2% and gastric acid output, 66.6% and 60.6%, respectively. The cimetidine group was suppressed more than the omeprazole group in gastric juice volume, but the difference was not significant. The cyclic AMP concentration in the gastric juice was higher in both inhibitor groups than in the control group, but there was no significant difference in cAMP concentration between the two inhibitor groups. Cyclic AMP output decreased significantly in the first 30 minute period with both inhibitors but after that there were no significant differences between the control group and the inhibited groups or between the inhibitor groups (**Fig. 2**).

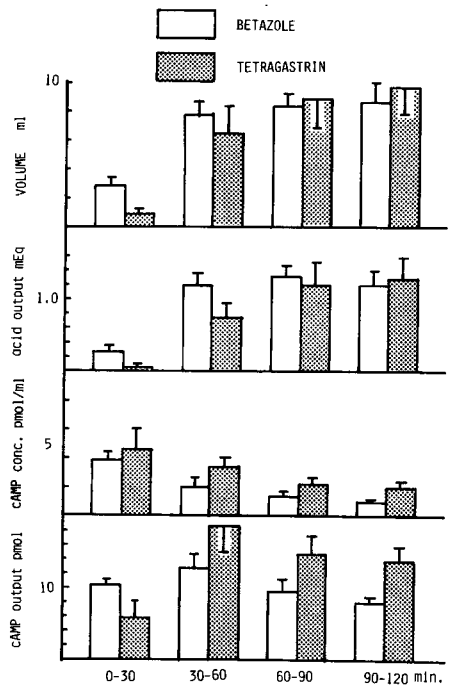

Fig. 3. The comparison of tetragastrin (4 μg/kg/h) stimulation and betazole (2 mg/kg/h) stimulation on gastric juice volume, acid output, cAMP concentration and cAMP output. Columns depict the value for each thirty minute period (mean ± SEM).

Two mg/kg/h of betazole stimulation and 4 μg/kg/h of tetragastrin stimulation caused similar increases of gastric juice volume and acid output, with the betazole stimulation occurring somewhat earlier. Both stimulants caused a similar change in cAMP concentration in the gastric juice and cAMP output. That is, cyclic AMP concentration in the gastric juice was highest in the first 30 minute period and decreased with time, but its output into the gastric juice was maximal during the second 30 minute period and then decreased with both stimulants (**Fig. 3**).

Plasma cAMP concentration did not change significantly with either betazole or tetragastrin stimulation (**Fig. 4**).

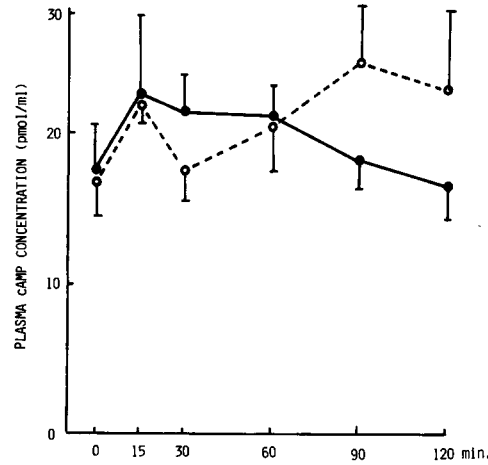

Fig. 4. Plasma cAMP level. Circles depict the value for tetragastrin (4 μg/kg/h) stimulation. Solid dots depict that for betazole (2 mg/kg/h) stimulation (mean ± SEM).

Discussion

There are a few reports on cAMP in the gastric juice. For example, Bieck[22] reported that, in Heidenhain pouch dogs, cAMP output into gastric juice was increased by histamine and pentagastrin stimulation and decreased by prostaglandin E_2 and insulin. Levine[23] reported that betazole stimulation alone caused a slight increased of cAMP concentration in the gastric juice in a group of peptic ulcer patients, but not in normal human subjects. Bower[24] reported that only betazole but not pentagastrin stimulation increased cAMP output into the gastric juice but not pentagastrin in humans. There is, however, no direct evidence that cAMP in the gastric juice reflects H_2 receptor activity. In this study, we investigated in this question using two different types of inhibitors of gastric acid secretion. The first, cimetidine, is an H_2 receptor antagonist that inhibits at a point more proximal than cAMP production. The second is omeprazole, an H^+, K^+-ATPase inhibitor that blocks gastric acid secretion at a site more peripheral from cAMP production.

We intended to use doses that inhibit gastric secretion by at least one-half. Inhibition by 4 μmol/kg of cimetidine or 0.18 μmol/kg of omeprazole at 60 minutes caused a similar inhibition of gastric juice volume, 52.1% and 49.6%, respectively. Yet cAMP output decreased significantly with omeprazole but not with cimetidine. No significant difference in cAMP output was found between the two inhibitor groups.

On the assumption that desensitization of the H_2 receptor occurred after 60 minutes of continuous stimulation with betazole, these inhibitors were administered before or at the time of stimulation. Cimetidine at a dose of 4 μmol/kg/h inhibited gastric secretion more than omeprazole at that dose but there was no significant difference between the two groups. The two inhibitors both caused a similar decrease of cAMP output in the first 30 minute period only. After that period no significant difference was found compared with control group. Both inhibitors increased cAMP concentration after the second 30 minute period. These data do not support the hypothesis that cAMP in gastric juice can be a marker of H_2 receptor activity.

Cyclic AMP in gastric juice probably reflects some factor other than H_2 receptor activity as a major component. We cannot identify the source of cAMP in the gastric juice in this study but the possibility of its origin from blood circulation is unlikely, because Schwartzel[25] reported that during infusion of cAMP, plasma cAMP concentration increased 500 fold but gastric cAMP levels did not increase. The concentration of cAMP in the plasma did not change after either betazole or tetragastrin stimulation, and there was no significant difference between the two stimulants. We need not consider the plasma cAMP level because it did not change significantly with either betazole or tetragastrin stimulation. Cyclic AMP concentration in the gastric juice was highest in the first 30 minute period and then decreased. Inhibition with both inhibitors increased cAMP concentration. These findings suggest that a washout phenomenon or diluting effect are likely. The main source of cAMP in the gastric juice is mediated by a mechanism other than the H_2 receptor. The component of H_2 receptor-mediated cAMP may be too small to be differentiated in gastric juice.

Because we cannot discern a reflection of H_2 receptor activity by studying cAMP in the gastric juice in Heidenhain pouch dogs, we cannot determine whether endogenous histamine plays a role in gastrin stimulation.

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