INFLUENCE OF HISTAMINE RELEASING AGENTS ON GASTRIC ACID SECRETION OF ISOLATED BULLFROG GASTRIC MUCOSA

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Abstract—The influence of histamine releasing agents on gastric acid secretion was studied in isolated bullfrog gastric mucosa preparations. Maximum acid secretory responses in our preparations were obtained by stimulation with tetragastrin (5×10^{-7} g/ml), histamine (1×10^{-8} g/ml) and bethanechol (1×10^{-6} g/ml). Compound 48/80 (1×10^{-4} g/ml) showed a transient stimulatory action which was followed by a gradual depression of basal acid secretion. The stimulatory phase of compound 48/80 was completely antagonized by burimamide (1×10^{-5} g/ml), a histamine H_2 -receptor antagonist. In gastric mucosa preincubated with compound 48/80, the secretagogue action of tetragastrin or bethanechol was not exerted, although this preparation continued to respond to histamine. The effects of Triton X-100, decylamine and polymixin B were quite similar to those of compound 48/80. After pretreatment with compound 48/80, the gastric mucosa preparation became refractory to the stimulatory action of compound 48/80 or Triton X-100. It is thus suggested that endogenous histamine may play an important role in the secretagogue action of tetragastrin and bethanechol.

The accumulation of knowledge relating to the role of endogenous histamine in gastric acid secretion (1-5) has been accelerated by the development of histochemical methods of detecting tissue histamine (6). The functional role of gastric histamine is, however, not fully known as it is difficult to study simultaneously the dynamic aspect of histamine movement in the tissue and the change in the gastric acid secretion response. Thus, study of the effect of histamine liberators on gastric acid secretion in isolated gastric mucosa is deemed worthy.

Although there are reports concerning the effect of histamine liberators on gastric acid secretion (7-8), most deal with the complex influence of these agents on gastric functions including systemic and peripheral effects. The effects of histamine liberators on isolated gastric mucosa have been reported (9), but these data appeared before development of the specific histamine H_2 -receptor antagonist.

The present communication describes the effects of some histamine liberators and a histamine H_2 -receptor antagonist on the isolated frog gastric mucosa preparation.

MATERIALS AND METHODS

The isolated bullfrog gastric mucosa preparation

The isolated frog stomach preparation was made following the procedure described by Davidson et al (10) with some modification (11-12). A bullfrog (*Rana catesbeiana*) was

decapitated and pithed. The gastric mucosa of the isolated stomach was immediately separated from the muscular layer and was mounted between the lucite chambers each with a volume of 5 ml, containing the following solution: (a) serosal side—NaCl 102.7 mM, CaCl_2 0.85 mM, KCl 1.0 mM, NaHCO_3 2.4 mM, glucose 11.1 mM, (b) mucosal side—NaCl 105.1 mM, CaCl_2 0.85 mM, KCl 1.0 mM, glucose 11.1 mM. The rate of acid output was measured by titration with N/500 NaOH to pH 6 using an automatic titrator with a recorder (Toa Electronics Ltd., HS-2A and EPR-3T). The transmucosal potential difference (P.D.) was measured by a pair of calomel electrodes electrically connected via agar-KCl bridges. The serosal side solution was gassed with the mixture of 95% O_2 -5% CO_2 , and the mucosal side solution was bubbled with 100% O_2 . The experiments were carried out at room temperature (15–25°C).

Histamine assay

Histamine content in gastric mucosa was determined by the method of Shore et al (13). The isolated stomach was incubated with histamine releasing agents for 30 min and homogenized for the histamine extraction. The histamine was fluorometrically assayed with a Farrand spectrofluorometer (Mark I). The released histamine in the serosal solution was also assayed according to Rangachari's method (14–15).

Materials

The test drugs were dissolved in Ringer's solution and added to the serosal side chamber. The concentrations of the test drugs in this experiment did not affect the pH of the Ringer solution on the serosal side. The tested concentrations of the drugs were expressed in g/ml in terms of the salt. The drugs used were as follows: compound 48/80 (Sigma, U.S.A.), Triton X-100 and histamine 2HCl (Wako Pure Chem., Japan), burimamide (Smith, Kline and French Laboratories, England, kindly provided by Dr. J.W. Black), tetragastrin (Nissui, Japan) and bethanechol chloride (Yoshitomi, Japan).

RESULTS

Effect of histamine liberators on spontaneous secretion

Isolated bullfrog gastric mucosa secreted acid spontaneously at a rate of about 380 $\text{m}\mu\text{eq.H}^+ / 4 \text{ cm}^2 / 10 \text{ min}$. The polymer amine, compound 48/80 showed a transient stimulatory action followed by a gradual depression of basal acid secretion in the concentration of $1 \times 10^{-4} \text{ g/ml}$ (Fig. 1A and 1C). The initial stimulatory action was observed during the first 10 min and thereafter the acid secretory rate declined gradually until reaching almost half of the basal secretory rate within 30 min. The nonionic detergent, Triton X-100 ($1 \times 10^{-3} \text{ g/ml}$) also stimulated gastric acid secretion to 468 $\text{m}\mu\text{eq.H}^+ / 4 \text{ cm}^2 / 10 \text{ min}$ at the first 10 min period, and then depressed acid secretory rate to 220 $\text{m}\mu\text{eq.H}^+ / 4 \text{ cm}^2 / 10 \text{ min}$ at the fourth 10 min period after the incubation. Fig. 1 also shows the effects of two agents on transmucosal P.D. In most of the preparations, compound 48/80 and Triton X-100 showed a transient increase in transmucosal P.D. immediately after they were added. However, the effects on P.D. were not uniform in each experiment. As shown in Table 1, polymixin B

FIG. 1. Effect of histamine releasing agents on gastric acid secretion in isolated bullfrog gastric mucosa. Ordinate: the rate of acid output ($\text{mEq. H}^+ / 4 \text{ cm}^2 / 10 \text{ min}$). Compound 48/80: $1 \times 10^{-4} \text{ g/ml}$. Triton X-100: $1 \times 10^{-3} \text{ g/ml}$. The upper trace in each column illustrates the change in transmucosal P.D. The base line indicates pH 6.

and decylamine also showed similar effects on basal acid secretion in isolated frog gastric mucosa preparation.

The cross-influence of compound 48/80 and Triton X-100 on acid secretion was also examined. The gastric mucosae used in this experiment were pretreated with either compound 48/80 ($1 \times 10^{-4} \text{ g/ml}$) or Triton X-100 ($1 \times 10^{-3} \text{ g/ml}$) for 40 min, and then each drug was added to the serosal solution with or without changing accommodation. As shown in Fig. 1, both compound 48/80 and Triton X-100 no longer showed the transient stimulatory action in the preparation preincubated with compound 48/80 or Triton X-100.

Interaction of histamine releasing agents with histamine H_2 -receptor antagonist

Burimamide was found to show a specific competitive antagonism to histamine H_2 -receptors without any significant interaction with histamine H_1 -, acetylcholine-, and catecholamine beta-receptors (16). Thus, it is expected that the action of histamine releasing agents would be blocked by a histamine H_2 -receptor antagonist, if the stimulation by these drugs of acid secretion is due to endogenous histamine released from the gastric mucosa. It was confirmed that this preparation responded well to the three representative secretagogues, histamine, tetragastrin and bethanechol, that their actions were concentration-dependent and that the maximum acid secretory responses to each stimulant were nearly equal (Fig. 2). In this figure also, it can be seen that the secretagogue effect of histamine ($1 \times 10^{-5} \text{ g/ml}$) was completely blocked by burimamide ($1 \times 10^{-4} \text{ g/ml}$). In addition, it was found that the actions of an equipotent concentration of tetragastrin ($5 \times 10^{-7} \text{ g/ml}$) and bethanechol ($1 \times 10^{-6} \text{ g/ml}$) could be fully inhibited by burimamide even in a lower concentration than that required to depress the action of histamine. Fig. 3 and Table 1 indicate that pretreatment with burimamide in the lower concentration completely blocked the transient stimulatory actions of compound 48/80 and Triton X-100. The effects of three secretagogues

FIG. 2. Effect of secretagogues on gastric acid secretion and inhibition by burimamide in isolated bullfrog gastric mucosa. T-Gas.: tetragastrin 5×10^{-7} g/ml. Bet.: bethanechol 1×10^{-6} g/ml. His.: histamine 1×10^{-5} g/ml. Bur.: burimamide 1×10^{-4} g/ml. Other abbreviations as in Fig. 1.

FIG. 3. Influence of burimamide on the stimulatory action of histamine releasing agents in isolated bullfrog gastric mucosa. Bur.: burimamide 1×10^{-5} g/ml. Compound 48/80.: 1×10^{-4} g/ml. Triton X-100.: 1×10^{-3} g/ml. Other abbreviations as in Fig. 1.

TABLE 1. Effect of histamine releasing agents on gastric acid secretion in isolated bullfrog gastric mucosa

Histamine releasing agents	g/ml	No.	Change in acid output first 10 min period	(mμeq.H ⁺ /4 cm ² /10 min) fourth 10 min period
i) compound 48/80	1×10^{-4}	10	$+82 \pm 10$	-78 ± 8
polymixin B	5×10^{-4}	5	$+78 \pm 15$	-118 ± 19
ii) Triton X-100	1×10^{-3}	10	$+84 \pm 13$	-164 ± 20
decylamine	1×10^{-3}	5	$+93 \pm 19$	-180 ± 24
iii) compound 48/80		10	-49 ± 8	-116 ± 23
pretreated with burimamide				
Triton X-100		10	-40 ± 6	-185 ± 18
pretreated with burimamide				

The mean basic acid output of 55 experiments was 384 ± 21 mμeq.H⁺/4 cm²/10 min. Histamine releasing agents were added 20 min after the pretreatment with burimamide (1×10^{-5} g/ml).

on transmucosal P.D. were not affected by the antagonist. From these results, it is suggested that stimulatory action by histamine liberators of acid secretion may be attributed to endogenous histamine.

Interaction of histamine releasing agents with histamine, tetragastrin or bethanechol

Histamine, tetragastrin and bethanechol stimulated acid secretion almost to the same degree in normal preparation *in vitro*. On the contrary, gastric mucosa pretreated with histamine releasing agents did not respond to tetragastrin and bethanechol. Fig. 4 shows

FIG. 4. Influence of compound 48/80 pretreatment on the stimulatory action of three secretagogues in isolated bullfrog gastric mucosa. Compound 48/80: 1×10^{-4} g/ml. Other abbreviations as in Figs. 1, 2.

FIG. 5. Influence of Triton X-100 pretreatment on the stimulatory action of three secretagogues in isolated bullfrog gastric mucosa. Triton X-100: 1×10^{-3} g/ml. Other abbreviations as in Figs. 1, 2.

FIG. 6. Influence of pretreatment by histamine releasing agents on the stimulatory action of three secretagogues in isolated bullfrog gastric mucosa. Each secretagogue was added 40 min after pretreatment with compound 48/80 (1×10^{-4} g/ml) or Triton X-100 (1×10^{-3} g/ml). Each column shows the mean of 6 experiments with S.E.M.

that three secretagogues stimulated acid production nearly to the maximum in normal tissue (upper trace), but the mucosal strips were not stimulated by tetragastrin and bethanechol after pretreatment with compound 48/80 (lower trace). It should be noticed that only histamine stimulated acid secretion in both normal and compound 48/80 pretreated preparations. A similar experiment with Triton X-100 is presented in Fig. 5. The results of repeated trials with histamine liberators as pretreatments are summarized in Fig. 6. It was found that after the pretreatment with compound 48/80 or Triton X-100, the gastric mucosa became refractory to the stimulatory effect of tetragastrin and bethanechol, but it continued to respond to histamine, even though the response was much less than in a normal preparation. Pretreatment with either histamine liberator produced little effect on P.D. responding to each secretagogue.

Effect of histamine releasing agents on gastric histamine content and histamine release in isolated gastric mucosa preparation

The isolated stomach was treated with compound 48/80 (1×10^{-4} g/ml) or Triton X-100 (1×10^{-3} g/ml). These concentrations were selected to obtain a clear increase in acid secretion. The gastric mucosa exposed to serosal Ringer solution served as the control. Thirty min later, the specimens (gastric mucosa and the serosal contents) were assayed to determine the amount of histamine. In eight experiments, the mean histamine content in gastric mucosa was 2.57 ± 0.15 $\mu\text{g/g}$ tissue (Table 2). It was found that the gastric histamine content was reduced by compound 48/80 and significantly decreased by Triton X-100. On the other hand, the amount of histamine liberated into the serosal side solution during 30 min was increased by compound 48/80 and Triton X-100 from 95.9 ng/30 min to 102.5 and 124.0 ng/30 min, respectively. From these results, it is suggested that the decrease of histamine content in gastric mucosa corresponded with the increase of histamine released into the serosal solution.

TABLE 2. Effect of histamine releasing agents on gastric histamine content and histamine release in isolated bullfrog gastric mucosa

Treatment	No.	Histamine content in gastric mucosa $\mu\text{g/g}$ tissue	Histamine released into serosal solution ng/4 cm ² /30 min
control	8	2.57 ± 0.15	95.9 ± 3.2
compound 48/80 1×10^{-4} g/ml	8	2.17 ± 0.16	102.5 ± 5.0
Triton X-100 1×10^{-3} g/ml	8	$1.71 \pm 0.08^{**}$	$124.0 \pm 7.9^{**}$

** $P < 0.01$: significantly different from control (Student's *t*-test)

DISCUSSION

In the first series of the present experiments, it was revealed that all of the tested histamine liberators, including selective (compound 48/80 and polymixin B) and non-selective liberators (Triton X-100 and decylamine), showed a transient stimulatory effect followed by a gradual depression of basal acid secretion by isolated bullfrog gastric mucosa *in vitro*. The concentration of compound 48/80 in our experiment was too high to be regarded as the dose which has selective action on mast cells. As to the stimulatory phase of the response, it was found that the gastric mucosa pretreated with a histamine liberator became refractory to a second application of the liberator. This observation was found to be applicable to cross interaction between compound 48/80 and Triton X-100. In addition, the stimulatory phase was completely inhibited by a histamine H₂-receptor antagonist, burimamide. These results indicate that the stimulatory phase and the refractory phase after histamine releasing agents may be due to endogenous histamine. A related observation was made by Limlomwongse et al (9) who found that compound 48/80 depressed acid output in isolated gastric mucosa of the frog (*Rana tigerina*), and Triton X-100 showed a transient stimulation followed by a depression. These authors investigated the role of mast cells in the mucosa, but the relationship found between the tissue histamine level and

secretory responses was not conclusive. Recently, Rangachari using isolated bullfrog gastric mucosa preparation (14), has directly demonstrated that gastric stimulants release histamine into the serosal solution. He assumed that the histamine released by stimulants in turn stimulated acid secretion. As shown in Table 2, compound 48/80 and Triton X-100 liberated histamine in this preparation. In addition, it was also observed that the histamine level in gastric mucosa was reduced by the application of compound 48/80 and Triton X-100. This observation was inconsistent with the result obtained by Limlomwongse et al (9) that the histamine content in *Rana tigerina* was not affected by compound 48/80. According to their report, *Rana tigerina* has a much higher gastric histamine content than *Rana catesbeiana*. Species differences may be the main cause of the discrepancy between the two observations. From our results, it seems likely that the secretagogue action of compound 48/80 and Triton X-100 is mediated by histamine in the bullfrog. Recent histochemical evidence has shown that histamine in gastric mucosa is concentrated in mast cells, enterochromaffin-like cells (6) and parietal cells (17), but knowledge of frog gastric mucosa is lacking. Thus, further histochemical investigations are necessary for discussion of the type of storage of the endogenous histamine in frog gastric mucosa.

Our second series of experiments was performed to elucidate the influence of the histamine liberators on secretagogue-stimulated acid secretion response. As shown in Figs. 4, 5 and 6, gastric mucosa pretreated with a histamine liberator was found to become non-responsive both to tetragastrin and bethanechol. On the other hand, the acid secretion induced by histamine was not affected by pretreatment with histamine liberators. Those results along with another series of our results, provide evidence that the action of the two secretagogues is closely related to endogenous histamine. Kahlson et al (18) found that the histamine forming capacity in rat stomach was significantly elevated by gastrin. Kasbekar et al (19) examined the relationship between pentagastrin and acetylcholine and endogenous histamine, and concluded that the secretagogue action of both pentagastrin and acetylcholine was mediated by histamine. Many other reports also support the idea that histamine is a chemomediator candidate for the stimulatory effect of all gastric acid secretagogues. The present observations on the effect of histamine releasing agents on isolated gastric mucosa are consistent with the ideas described above.

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