STUDIES ON THE EFFECT OF HEMICHOLINIUM-3 ON GASTRIC ACID SECRETION IN THE ISOLATED BULLFROG GASTRIC MUCOSA WITH SPECIAL REFERENCE TO THE MODE OF ACTION OF GASTRIN-LIKE TETRAPEPTIDE

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Gastrin and gastrin-like peptides are the powerful stimulants of gastric acid secretion both in vivo and in vitro (1-5). Although some possible mechanisms of their action have been postulated by several authors, the conclusive evidence has not been obtained. Some authors presumed the presence of certain mediators in the mode of action of gastrin (2). Among the mediator candidates, histamine and acetylcholine seem most important. Bennett (6) found that the contractions by gastrin of the isolated ileum of guinea-pig were mediated by acetylcholine. Kasbekar et al. (2) examined the relation of pentagastrin

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and acetylcholine to histamine, and concluded that the secretagogue action of both pentagastrin and acetylcholine was mediated by histamine.

Hemicholinium-3, which inhibits the synthesis of acetylcholine in the nerve terminal, is widely used in the field of pharmacology to examine the participation of acetylcholine in various physiological functions. The effect of hemicholinium-3 on gastric acid secretion has not been reported. Therefore, it seems worthy of studying the influence of hemicholinium-3 on secretagogue action of gastrin-like tetrapeptide, histamine and bethanechol.

This communication describes the selective inhibitory effect of hemicholinium-3 on gastrin-like tetrapeptide-stimulated acid secretion of the isolated bullfrog gastric mucosa.

Materials and methods: The experiments were performed on two kinds of preparations, “fresh” and “resting” isolated gastric mucosa of bullfrog.

i) “Fresh” gastric mucosa preparation

The isolated bullfrog gastric mucosa preparation was made following the procedure of Davidson et al. (1965) (3) with some alterations. Bullfrogs (Rana catesbiana) were killed by decapitation and pithing. The gastric mucosa was immediately separated from the muscular layer of the stomach and mounted between two chambers containing the following solutions: (a) nutrient side—NaCl 102.7 mm, CaCl2 0.85 mm, KCl 1.0 mm, NaHCO3 1.2 mm, Glucose 11.1 mm, (b) secretory side—NaCl 104.4 mm, CaCl2 0.85 mm, KCl 1.0 mm, Glucose 11.1 mm. The nutrient solution was gassed with a mixture of 95% O2 and 5% CO2, while the secretory solution was gassed with 100% O2 or air. Incubation was carried out at room temperature (16-22°C). The working area of the mucosa was 4.0 cm². The change of pH in the secretory side was recorded by a Toa Electronics DC recorder (EPR-2TB) using a pH meter (Toa Electronics, HM-5A). The H+ concentration was calculated from the calibration curve. The chemicals, dissolved in 0.6% NaCl solution or frog Ringer’s solution, were added to the serosal solution bathing gastric mucosa.

ii) “Resting” gastric mucosa preparation

The resting gastric mucosa preparation was made following the procedure described by Kasbekar (7). A pair of gastric mucosa was isolated from two frogs of about the same body weights. One of the pair of gastric mucosae was kept in normal frog Ringer’s solution for 14.5 hours, and another mucosa was immersed in the Ringer’s solution containing 1 X 10⁻⁶ g/ml of hemicholinium-3 for the same period. Other experimental conditions were the same as those with fresh mucosae.

Chemicals: Gastrin-like tetrapeptide (Carbobenzoxy-L-tryptophanyl-L-methionyl-L-aspartyl-L-phenylalanine amide; kindly supplied by Nissui Pharmaceutical Co., Ltd.), Hemicholinium-3 (Aldrich), Histamine dihydrochloride (Wako Pure Chem. Industries), Bethanechol chloride (Yoshitomi).

Results and discussion: The effect of hemicholinium-3 on the basal and drug-stimulated acid secretion of the “fresh” gastric mucosa are summarized in Table I. Hemicholinium-3 inhibited basal acid secretion at the concentration of 1 x 10⁻⁴ g/ml–5 x 10⁻³ g/ml. The inhibition was dose-dependent, and at the highest concentration (5 x 10⁻³ g/ml) the acid secretion was reduced to 35% of the control. This effect could not be considered as specific, since the concentration of hemicholinium-3 was too high.

The secretagogue action of gastrin-like tetrapeptide was almost abolished after hemicholinium-3 treatment. Even at the lowest concentration (1 x 10⁻⁴ g/ml), the inhibitory effect of hemicholinium-3 was observed. The effect of histamine or bethanechol, however, was not influenced by the pretreatment with hemicholinium-3. Namely, among the three secretagogues tested, only the gastrin-like tetrapeptide was selectively inhibited by hemicholinium-3.

The preparation of the “fresh” gastric mucosa has some disadvantages for this kind of experiment, since the basal secretion is too active and the large portion of the stimulative action of the secretagogues is surmounted. A technique was described by Kasbekar et al. (7) for preparing “resting” bullfrog gastric
TABLE 1. Effect of hemicholinium-3 on basal acid secretion and on the actions of some secretagogues in the bullfrog "Fresh" gastric mucosa.

| Secretory rate (mEeq·H⁺/cm²/15 min) | Stimulated | Hemicholinium-3 concentration (g/ml) |
|-------------------------------------|------------|-------------------------------------|
|                                     | Basal*     | 0   | 1 × 10⁻⁴ | 5 × 10⁻⁴ | 1 × 10⁻³ | 5 × 10⁻³ |
| Gastrin-like tetrapeptide (MZ-150)   | 798±101    | 100% | -19.9% | -11.4% | -19.3% | -40.9% |
| 1 × 10⁻⁷ g/ml                        | 578±139    | (+220±65) | (-44±23) | (-25±23) | (-43±20) | (-90±29) |
| Betahexachloridechloride             | 520±41     | 100% | +69.3% | +26.8% | +3.6% | -26.2% |
| 1 × 10⁻⁴ g/ml                       | 380±27     | (+140±31) | (+125±55) | (+38±20) | (+3±5) | (-37±17) |
| Histamine 2HCl                      | 1134±159   | 100% | +32.6% | +46.5% | +19.1% | +82.3% |
| 1 × 10⁻³ g/ml                       | 650±76     | (+494±155) | (+158±66) | (+225±33) | (+93±49) | (+398±173) |
| Saline**                            | 672±60     | 0%   | -12.9% | -29.1% | -54.7% | -65.0% |

Each value is the mean of four experiments with the S.E. indicated.
The percentage represents the ratio of net change of secretory response against maximum net increase in response to each secretagogue except saline.
* The figures in this column indicate the net value of secreted acid.
** The figures in this column represent percentage against basal secretion.

Fig. 1. Effect of hemicholinium-3 on the actions of some secretagogues in the bullfrog “resting” gastric mucosa.
A: exposed to frog Ringer’s solution for 14.5 hours.
B: exposed to frog Ringer’s solution containing 1 × 10⁻⁶ g/ml of hemicholinium-3 for 14.5 hours.
Gas.: gastrin-like tetrapeptide (MZ-150) 1 × 10⁻⁷ g/ml.
Bet.: betahexachloride chloride 1 × 10⁻⁹ g/ml.
His.: histamine dihydrochloride 1 × 10⁻⁵ g/ml.

mucosa which secreted acid in response to exogenously added secretagogues. Basal secretion was minimized in this preparation. We examined the effect of hemicholinium-3 using the resting bullfrog gastric mucosa preparation.
A typical response of a pair of the preparations to three secretagogues is shown in Fig. 1. Both preparations responded to histamine (1 \times 10^{-5} \text{g/ml}) and bethanechol (1 \times 10^{-6} \text{g/ml}) to the same degree. As to the gastrin-like tetrapeptide, the situation was quite different. Normal preparation responded to the peptide to the maximum, while the hemicholinium-3-treated preparation did not respond at all. The above mentioned tendency was confirmed in several pairs of the preparations. It is noticeable that the concentration of hemicholinium-3 is much lower than that in the experiments with fresh mucosa.

From all these results, we are lead to the conclusion that the acetylcholine synthesis is an important factor for the secretagogue action of gastrin-like tetrapeptide, including the possibility that acetylcholine would be the mediator itself.

Summary: The effect of hemicholinium-3 on gastric acid secretion was studied in the isolated bullfrog gastric mucosa with special reference to the mode of action of gastrin-like tetrapeptide.

In the “fresh” gastric mucosa, basal acid secretion was inhibited by hemicholinium-3 (1 \times 10^{-6} \text{g/ml}-5 \times 10^{-3} \text{g/ml}). Secretagogue action of gastrin-like tetrapeptide was inhibited by hemicholinium-3 (1 \times 10^{-4} \text{g/ml}-1 \times 10^{-3} \text{g/ml}), whereas the stimulative action of histamine or bethanechol was not influenced. This tendency was also confirmed in the experiment with the “resting” gastric mucosa, in which the basal acid secretion was minimized. From these results, it is concluded that the acetylcholine synthesis is an important factor for the secretagogue action of gastrin-like tetrapeptide.

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EFFECT OF HISTAMINE ON CALCIUM EXCHANGE
IN GUINEA PIG TAENIA COLI

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It has already been shown that high potassium (K) solution or barium (Ba) affected the cellular Ca exchange in the guinea pig taenia coli (1, 2). On the other hand, an influence of histamine, which stimulates the specific receptors sited on the smooth muscle (3), has not been systematically studied on Ca exchange in the smooth muscle. In this paper, it was attempted to investigate an effect of histamine on Ca exchange.