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# Measurement of Gastric Acid Secretion in Isolated Gastric Mucosa of the Rat: Effects of Secretagogues and Inhibitors including Cyclic Adenosine Monophosphate Related Agents and an $H_2$-Receptor Antagonist[1)]

Kazuo Watanabe, Hiroshi Y. Watanabe, Yoshiaki Goto, and Yōko Kariya

*Department of Pharmacometrics, Research Institute for Wakan-yaku, University of Toyama*[2)]

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An improved method was described for the measurement of gastric acid secretion of isolated rat gastric mucosa. With this method, dose-dependent acid secretory responses were obtained to secretagogues such as histamine, bethanechol, tetragastrin and dibutyryl cyclic adenosine monophosphate (AMP). However, the maximum response to tetragastrin was always smaller than that to the other secretagogues in this preparation. Inhibitory effects on acid secretion of some antisecretory agents were also examined. Antagonistic interactions of atropine against bethanechol, and of metiamide against histamine were clearly confirmed. On the other hand, inhibitions of metiamide on bethanechol and of atropine on tetragastrin or histamine were found rather weak in contrast to the results obtained with other methods. Strong stimulatory effect of theophylline and absence of the influence of dibutyryl cyclic GMP were shown in our preparation. The actions of miscellaneous agents including prostaglandin $E_1$, imidazole and metabolic inhibitors were also described.

**Keywords**——acid secretion; gastric mucosa; histamine receptor; gastrin; cyclic AMP; cyclic GMP; prostaglandin

For a research on direct actions of drugs on gastric acid secretion, isolated gastric mucosa preparation is necessary which is free from complicating systemic control mechanisms. Although amphibians have long been used in this field, mammalians are not commonly employed because of the difficulties in maintaining the secretory activity in the isolated condition. Recently, several papers have been published on the methods for making the isolated gastric mucosa preparation of mouse,[3)] rat,[4,5)] guinea-pig,[6)] rabbit,[7)] and pig.[8,9)] However, reproducibility of the secretory response or sensitivity to certain drugs is not satisfactory in each method. Particularly, the responses to gastrin analogues are not reproducible, and those to theophylline or cyclic adenosine monophosphate (AMP) analogues are different in each experimental condition.

In this paper, we describe an improved experimental method to obtain reproducible dose-response relations of some secretagogues including dibutyryl cyclic AMP and theophylline. The effects of some antisecretory drugs were also studied.

## Methods

The procedures to make an isolated gastric mucosa preparation were carried out principally in accordance

1) A part of this work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1976.
2) Location: *3190, Gofuku, Toyama, 930, Japan.*
3) B.Y.C. Wan, *Brit. J. Pharmacol.*, **56**, 357p (1975).
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with the method described by Wan, *et al.*[4] A few steps were modified to improve the reproducibility of response and the sensitivity to drugs. Male immature Wistar rats weighing 40—110 g were used without fasting. The animal was killed by dislocation of the neck and the stomach was quickly isolated. The muscular layer of the glandular stomach was carefully eliminated with scissors. The extended sheet of the mucosa was mounted onto a 1.77 $cm^2$ opening of a glass tube, with the mucosal surface facing outwards. Composition of the nutrient solution was as described by Sernka and Hogben.[10] The serosal solution contained (mM): NaCl 110, KCl 5.0, $NaHCO_3$ 26, $MgCl_2 \cdot 6H_2O$ 1.2, $CaCl_2$ 3.6 and glucose 16.7. The mucosal solution has the same composition but with 26 mM NaCl replacing equimolar $NaHCO_3$. The volume of mucosal and serosal solution was 5 and 25 ml respectively. The solution incubating the serosal or the mucosal surface was gassed with 95% $O_2$–5% $CO_2$ or 100% $O_2$ respectively, and the mucosal solution was stirred with a magnetic stirrer. The amount of the secreted acid during every 10 min was continuously determined and recorded by means of an automatic titrator connected to a recorder as we previously reported.[11] The pH-stat was set at pH=4.0. The arrangement of the apparatus was shown in Fig. 1. All experiments were performed at 37° and the drugs were applied to the serosa.

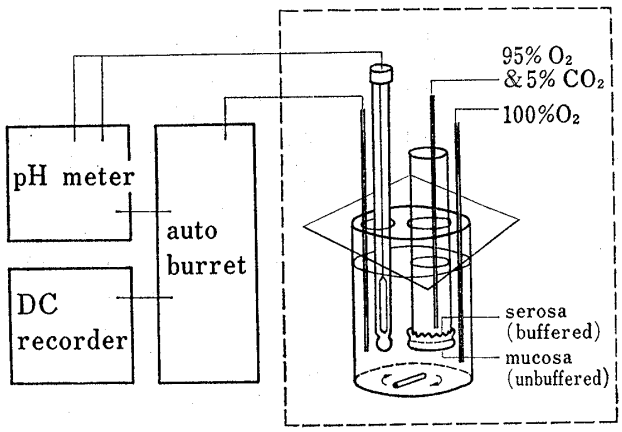


Fig. 1. An Arrangement of Apparatus for Recording Acid Secretion from an Isolated Gastric Mucosa of Rat

Concentration of NaOH in the autoburret was *N*/500. The pH stat was set at pH=4.0. The volume dial of the pH stat was set at zero every 10 min.

Drugs used were as follows; histamine 2HCl (Wako Pure Chem. Indust.=Wako), atropine sulfatemonohydrate (Wako), theophylline (Wako), imidazole (Daiichi Pure Chem. Indust.=Daiichi), cyclic-3′,5′-AMP (Daiichi), dibutyryl adenosine-3′,5′-cyclic monophosphate Na (=dibutyryl cyclic AMP, Daiichi), dibutyryl guanosine-3′,5′-cyclic monophosphate Na (=dibutyryl cyclic GMP, Kyowa Hakko), cyclic GMP (Daiichi), Prostaglandin $E_1$ (Fuji Chem. Indust.), metiamide (S. K. F.), dibucaine HCl (Daiichi Seiyaku Co.), bethanechol (Yoshitomi Pharmaceutical Indust. Co.), tetragastrin (Nissui Pharmaceutical Co.). sodium thiocyanate (Wako) and 2,4-dinitrophenol (Wako). Results are given as the mean with s.e. mean. For statistical analysis, Student's t-test was used.

## Results

### 1. Basal Acid Secretion and Responses to the Standard Secretagogues

Spontaneous hydrogen ion secretion of the isolated rat gastric mucosa preparations varied within the range of 0.3—1.0 μeq/$cm^2$/10 min, and was almost constant for over 8 hr. The stomachs of immature rats (40—50 g) secreted higher amount of acid. One of the working records of the acid secretory responses to four standard secretagogues was shown in Fig. 2. The dose of each secretagogue was selected to induce maximum response. Bethanechol, histamine and dibutyryl cyclic AMP induced remarkable increase in acid secretion, but tetragastrin always induced much smaller response than the other secretagogues did. The response to bethanechol or tetragastrin gradually faded, while the responses to histamine or dibutyryl cyclic AMP were long lasting. The dose-response relations in the repeated experiments were summarized in Fig. 3. The dose dependent increase of acid secretory response was shown for each secretagogue in the following dose range respectively; bethanechol $3\times10^{-8}$—$3\times10^{-6}$ g/ml, tetragastrin $3\times10^{-6}$—$6\times10^{-5}$ g/ml, histamine $1\times10^{-6}$—$1\times10^{-4}$ g/ml, dibutyryl cyclic AMP $1\times10^{-5}$—$1\times10^{-3}$ g/ml. Although the maximum response to each secretagogue was almost the same, tetragastrin did not induce this level of response even with the highest concentration of practically available solution of this drug.

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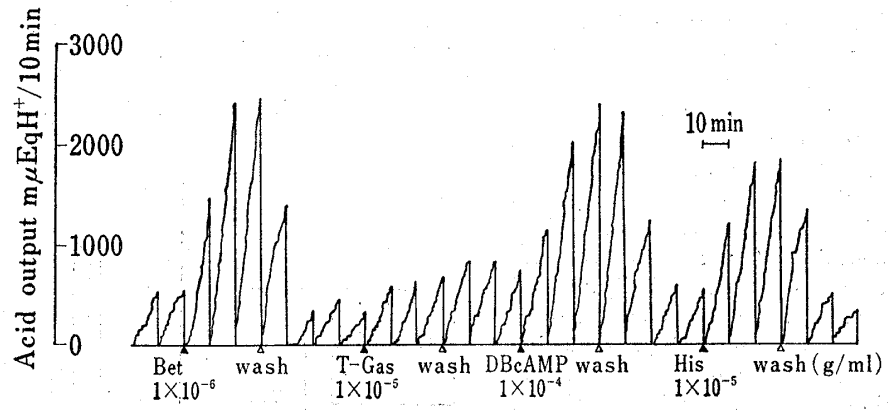


Fig. 2 Secretory Responses of an Isolated Gastric Mucosa Preparation of Rat to the Representative Secretagogues

Ordinate: the rate of acid output in mμ equivalent [H+] per 10 min from 1.77 cm² area of the isolated gastric mucosa.
Abbreviations: Bet=bethanechol, T-Gas=tetragastrin, DBcAMP=dibutyryl cyclic AMP, His=histamine.

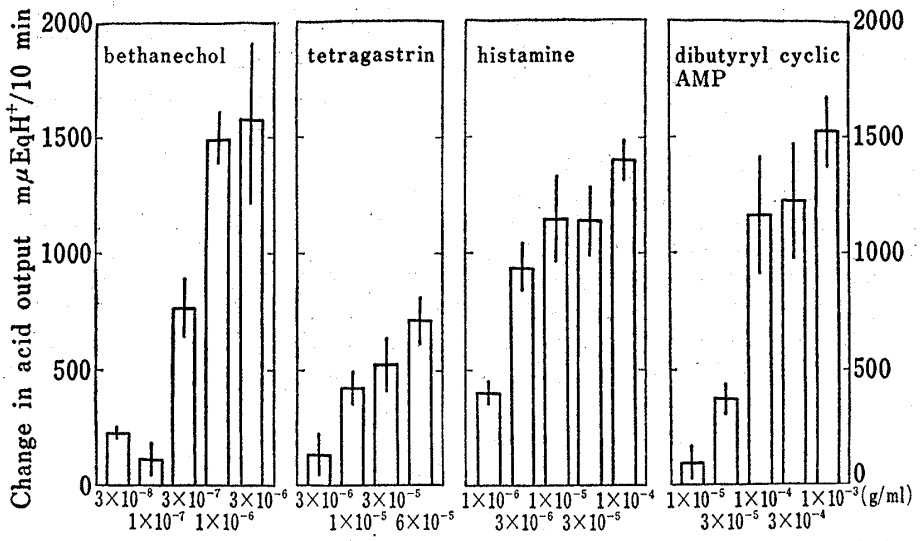


Fig. 3. Dose-response Relations of the Representative Secretagogues in the Isolated Rat Gastric Mucosa Preparations

Ordinate: increase of acid output after drug application in mμ equivalent [H+] per 10 min.
Abscissa: drug concentration in g/ml. $N=6$ for each concentration.

## 2. Responses to the Standard Antisecretory Drugs

A few standard drugs were examined for their antisecretory effects on basal and secretagogue-stimulated acid secretory response. The typical records of the effects of atropine and metiamide were shown in Fig. 4. Atropine completely inhibited the action of bethanechol. The effect of histamine or dibutyryl cyclic AMP was not affected by atropine. Basal secretion was not influenced by this drug. (Fig. 4A) The effect of metiamide was also shown in Fig. 4. It clearly inhibited the secretagogue action of histamine as well as that of tetragastrin (Fig. 4B). The effect of bethanechol was also depressed by metiamide, but not completely. The effect of dibutyryl cyclic AMP was not influenced by the pretreatment with metiamide. Dibucaine, a local anesthetic, blocked all of the secretagogues. Similar general inhibition was observed after the pretreatment with thiocyanate. The effects of these miscellaneous agents on acid secretion were summarized in Table I. In this table, the effect of a metabolic inhibitor, 2,4-dinitrophenol, was also shown. It completely abolished the sensitivity of gastric mucosa to all secretagogues and even the basal secretion was blocked.

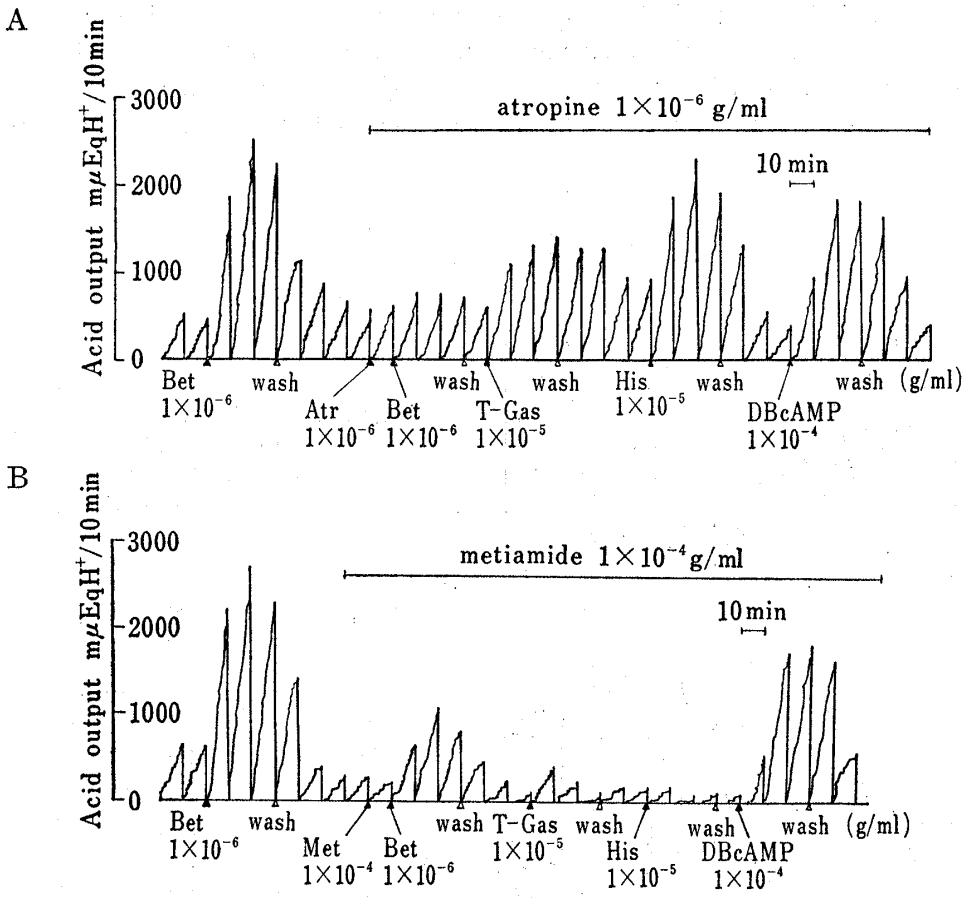


Fig. 4. Patterns of Inhibition of Atropine and Metiamide on Acid Secretory Responses to Secretagogues

A: secretory responses under atropine $1\times10^{-6}$ g/ml.
B: secretory responses under metiamide $1\times10^{-4}$ g/ml.
Abbreviations are the same as those in Fig.2.

TABLE I. Effects of Miscellaneous Antisecretory Drugs on the Secretagogue Stimulated Gastric Acid Secretion in the Isolated Gastric Mucosa Preparation of Rat

| Inhibitor | Dose (g/ml) | Change in acid output (mμ eqH+/10 min) Bethanechol $1\times10^{-6}$ g/ml | Tetragastrin $1\times10^{-5}$ g/ml | Histamine $1\times10^{-5}$ g/ml | Dibutyryl cAMP $1\times10^{-4}$ g/ml |
|---|---|---|---|---|---|
| None | | 1483±123(10) | 468± 94(10) | 1154±131(10) | 1291± 94(10) |
| Atropine | $1\times10^{-6}$ | 187± 60 (4)a) | 202±169 (4) | 845±193 (4) | 1311±237 (4) |
| Metiamide | $1\times10^{-4}$ | 1013±121 (4)b) | 60± 62 (4)a) | −22± 90 (4)a) | 1303±131 (4) |
| Dibucaine | $1\times10^{-4}$ | 309±100 (5)a) | 31± 42 (4)a) | 247±162 (4)a) | 741±114 (4)a) |
| Sodium thiocyanate | $1\times10^{-3}$ | 61± 90 (4)a) | −37± 25 (4)a) | 37± 83 (4)a) | 300±191 (5)a) |
| 2,4-Dinitrophenol | $1\times10^{-4}$ | 0 (4)a) | 0 (4)a) | 0 (4)a) | 0 (4)a) |

a) $p<0.01$. b) $p<0.05$. ( ): No. of experiments.

### 3. Effects of Cyclic AMP and Related Compounds

Some cyclic nucleotide-related agents were examined for their action on gastric acid secretion in the isolated stomach preparation. In Fig. 5 and Table II, the effects of cyclic AMP, dibutyryl cyclic AMP and theophylline were shown. The exogenous cyclic AMP was found ineffective to stimulate acid secretion in contrast to its dibutyryl derivative (Fig. 5A). Theophylline, a phosphodiesterase inhibitor, elicited a marked stimulant effect when added

to the nutrient fluid bathing the isolated gastric mucosa at a concentration of $1\times10^{-3}$ g/ml (Fig. 5B). However, neither cyclic GMP nor dibutyryl cyclic GMP exerted stimulant effect on our preparation (Table II). Our results on cyclic GMP are quite inconsistent with those of Brennan, *et al.*[12] who studied the drug action on isolated whole stomach preparation of rats.

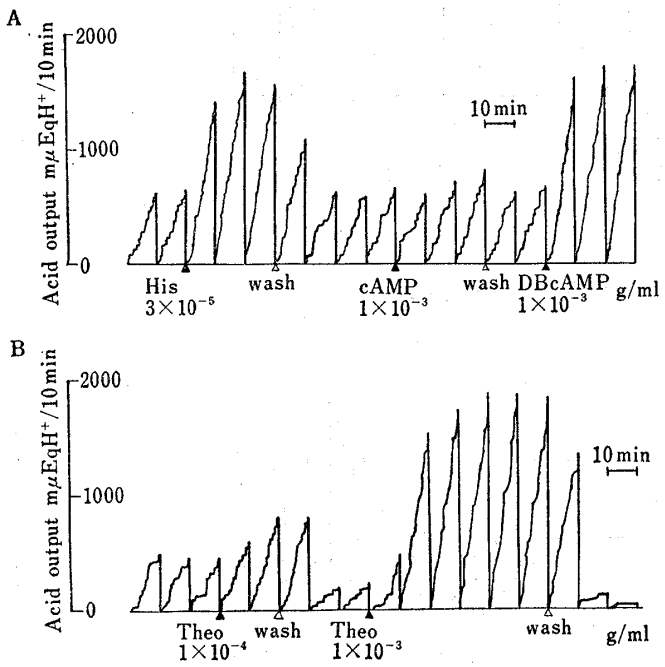


Fig. 5. Secretory Responses to Dibutyryl Cyclic AMP and Theophylline of the Isolated Gastric Mucosa Preparation of Rat

A: effects of cyclic AMP (=cAMP $1\times10^{-3}$ g/ml) and dibutyryl cyclic AMP (=DBcAMP $1\times10^{-3}$ g/ml).
B: effect of theophylline (=Theo $1\times10^{-4}$ and $1\times10^{-3}$ g/ml).
The other abbreviations are the same as those in Fig.2.

Table II. Effects of Cyclic Nucleotides and the Related Compounds on the Acid Secretion of the Isolated Gastric Mucosa Preparation of Rat

| Drug | Dose (g/ml) | No. | Change in acid output (mμeqH+/ 10 min) |
|---|---|---|---|
| Control | | 10 | 94± 54 |
| Cyclic AMP | $1\times10^{-3}$ | 4 | 61±104 |
| Dibutyryl cAMP | $1\times10^{-4}$ | 7 | 1163±245 |
| | $1\times10^{-3}$ | 5 | 1523±146 |
| Cyclic GMP | $1\times10^{-4}$ | 5 | 144± 95 |
| Dibutyryl cGMP | $1\times10^{-4}$ | 5 | 116± 50 |
| Theophylline | $1\times10^{-4}$ | 7 | 459±115 |
| | $1\times10^{-3}$ | 5 | 1200±202 |

## 4. Effects of Imidazole, Dibutyryl Cyclic GMP and Prostaglandin $E_1$ on the Secretagogue-stimulated Acid Secretion

Imidazole is known as a phosphodiesterase activator.[13] As to cyclic GMP and prostaglandins, there is the assumption that they may play some important roles in the regulation of acid secretion.[14,15] There is no report on the effect of cyclic GMP on the drug-stimulated acid secretion in isolated gastric mucosa. On account of these facts, we studied the effects of these three agents on our isolated gastric organ system. Stimulatory action of all secretagogues tested was significantly depressed by imidazole ($1\times10^{-3}$ g/ml) (Table III). Prostaglandin $E_1$ inhibited the action of histamine. However, we could not make the results on the other secretagogues more conclusive, since the higher concentration of prostaglandin $E_1$ was not available because of its poor solubility in nutrient solution. Dibutyryl cyclic GMP ($1\times10^{-4}$ g/ml) did not elicit any effect on the stimulated acid secretion.

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14) I.H.M. Main and B.J.R. White, *Eur. J. Pharmacol.*, **26**, 204 (1974).
15) M.S. Amer, *Am. J. Dig. Dis.*, **19**, 71 (1974).

Table III. The Influences of Imidazole, Prostaglandin $E_1$ and Dibutyryl Cyclic GMP on the Stimulatory Effects of Secretagogues in the Isolated Gastric Mucosa Preparation of Rat

| Inhibitor | Dose (g/ml) | Change in acid output (mμeq$H^+$/10 min) | | | |
|---|---|---|---|---|---|
| | | Bethanechol $1\times10^{-6}$ g/ml | Tetragastrin $1\times10^{-5}$ g/ml | Histamine $1\times10^{-5}$ g/ml | Dibutyryl cAMP $1\times10^{-4}$ g/ml |
| None | | 1383±169(8) | 597±123(12) | 1392±177 (7) | 1569±138(10) |
| Imidazole | $5\times10^{-4}$ | 858±214(4) | 145± 54 (4) | 683±144 (6)[a] | 846± 94 (7)[b] |
| | $1\times10^{-3}$ | 413±229(4)[b] | 19±103 (4)[a] | 151±130 (4)[b] | 705±264 (5)[b] |
| Prostaglandin $E_1$ | $4\times10^{-6}$ | 1021±169(8) | 433±140 (7) | 577±107(10)[b] | 1411±137(10) |
| | $2\times10^{-5}$ | 923±345(5) | 499± 55 (4) | 478± 87 (7)[b] | 1095±175 (4) |
| Dibutyryl cGMP | $1\times10^{-4}$ | 1488±100(4) | 483±176 (4) | 863±144 (4) | 1358± 96 (4) |

*a*) $p<0.05$. *b*) $p<0.01$. ( ): No. of experiments.

## Discussion

Recent devices for measuring acid secretion in isolated mammalian stomach can be classified in three types; whole stomach,[3,16,17] open stomach,[4,6] and isolated gastric mucosa free from muscle layer.[5,7] Although clear augmentation of acid secretion response to selected stimulants was shown by each device, it seemed difficult for any of them to give the reliable dose-response relations for all secretagogues, probably because the reproducibility of the responses was not satisfactory. The report by Wan was the first that showed the quantitative data on the secretagogue action in isolated open stomach preparation of rats. We modified their method in the following points; (1) immature rats were used, (2) the animals were not fasted before experiment, (3) muscle layer of the stomach was eliminated, and (4) pH stat was set at pH 4.0. These modifications, except (4), agreed with those in the short report by Hearn and Main.[5] These modifications much improved the reproducibility of the secretory response of the preparation. Moreover, possible contamination of $CO_2$ or lactic acid in the titrated acid can be excluded by the last modification. Under these conditions clear dose-response relationship was obtained for bethanechol, histamine, tetragastrin and dibutyryl cyclic AMP. However, the maximum response to tetragastrin was about a half of those to the other secretagogues. This is consistent with the observation by Hearn and Main.[5] Wan, *et al.*[4] reported that peak response was obtained with the moderate dose of histamine or pentagastrin and that the acid output rather decreased with higher doses. Such phenomena were not observed in our experiments.

Antagonistic interactions between atropine and bethanechol, and metiamide and histamine were also clearly confirmed. However, the inhibitory effects of atropine against tetragastrin, and of metiamide against bethanechol were found very weak in this preparation. This is in contrast with the results by other authors in intact animals[18] and by ourselves in the isolated bullfrog gastric mucosa[19] in which both antagonists completely inhibited the effect of tetragastrin. The stimulatory effect of dibutyryl cyclic AMP was found refractory to the anticholinergic agent or the histamine $H_2$-receptor antagonist, but was sensitive to local anesthetics, sodium thiocyanate and metabolic inhibitors. This implies that dibutyryl cyclic AMP can be used to differentiate the site of action of inhibitory drugs between receptor function and post receptor mechanisms.

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19) Y. Goto and K. Watanabe, *Jpn. J. Pharmacol.*, **25**, 790 (1975).

Recently, possible role of cyclic GMP as well as cyclic AMP in the control of gastric acid secretion is increasing its importance. It is postulated that acid secretory responses to agonist or antagonist would reflect the change in the intracellular cyclic AMP/cyclic GMP ratio.[15] In the present experiments, cyclic GMP and its dibutyrate did not exert the stimulatory action on our preparation. Brennan, *et al.*[12] reported that dibutyryl cyclic GMP strongly stimulated acid secretion in isolated whole stomach preparation of rat. In their preparations, when the animal was not fed, dibutyryl cyclic AMP did not stimulate acid secretion. Thus, the delicate alterations of the experimental condition seem to affect deeply the sensitivity to cyclic nucleotides. Stimulatory effect of theophylline was found to be easily detectable in this preparation even though the effective concentration was rather high. The effect of theophylline was eliminated soon after washing, but the action was persistent as long as the drug was present in the nutrient solution. This pattern of action was similar to those of dibutyryl cyclic AMP and histamine, but different from those of tetragastrin and bethanechol which showed a rapid fade in acid production after peak response.

Prostaglandins, as well as cyclic GMP, were assumed to be possible mediators of gastric acid secretion.[14,19] In our experiments, prostaglandin $E_1$ weakly depressed the secretagogue-stimulated acid secretion, but dose-dependent or complete inhibition was not obtained within the tested dose range.

We previously found the inhibitory effect of imidazole on the secretagogue-stimulated acid secretion in isolated frog gastric mucosa.[20] This effect was confirmed also in the present study. Details of the action of imidazole are under investigation.

In conclusion, it was found in the present study that the isolated gastric mucosa preparation of the rat is sensitive enough to various kinds of secretagogues and inhibitors including cyclic nucleotides and their related compounds.

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