produce an increase in the uptake of \(^{3}H\) noradrenaline in vitro by synaptosomes isolated from the rat brain (10). Lithium may thus be considered to increase noradrenaline uptake by adrenergic endings in the heart, which would in turn result in a decrease in the transmitter available for interaction with receptors. To rule out such a possibility, isoproterenol was used, as the amine is not a substrate for the neuronal uptake mechanism (11). Peak responses to isoproterenol after lithium perfusion also decreased in parallel with the transmission blockade. The duration of the actions decreased to 8 to 16 min. The results are consistent with the findings that positive inotropism as well as the activation of phosphorylase A by noradrenaline and isoproterenol decreased after perfusion with a 10 mM lithium solution in guinea-pig hearts (12).

Thus it is concluded that the primary blocking action of lithium on the adrenergic transmission is not due to a decrease in the noradrenaline which interacts with receptors but rather to an inhibition of post-synaptic responses of the rabbit heart to the transmitter.

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mucosa. The thin layer of the mucosa is easily oxygenated and the gastric mucosa contains no chief (pepsin-secreting) cells (4). These characteristics imply great advantages of this preparation for use in pharmacological investigations. On the other hand, the difficulty of obtaining concentration-response curves *in vitro* has impeded progress in the study on the mechanisms of acid secretion, as described by Shoemaker et al (5). The present study was carried out to establish the experimental condition required to obtain the concentration-response relationships of tetragastrin, bethanechol and histamine, and to clarify the antagonistic effects of the anticholinergic or antihistaminergic drugs to the secretagogue action of each stimulus using the isolated bullfrog gastric mucosa preparation.

The experiments were carried out according to the procedure of Davidson et al (4) with slight modifications (6). Briefly, the isolated frog stomach preparation was mounted between two lucite chambers, which contained oxygenated Ringer's solution (non-buffered solution in mucosal side). The rate of acid output was measured by titration to pH 6 with N/500 NaOH solution using an automatic titrator with a recorder (Toa Electronics Ltd., HS-2A and EPR-3T). The test drugs were dissolved in the distilled water and added to the serosal side solution. The test drugs used in this experiment did not affect the pH of the Ringer's solution in the serosal side.

Fig. 1 shows the concentration-response curves of three secretagogues, histamine, bethanechol and tetragastrin. The mean secretory rate without any stimulant was 458.63±16.33 mEq.H+ /4 cm²/10 min. The parameter of the secretory response was assessed by taking the differences in the mean secretory rate during the secretagogue-stimulated period and those of the preceding control period. The frog gastric mucosa preparation was concentration-dependently stimulated in response to each secretagogue. The maximal secretory responses to three stimuli were obtained 20 or 30 min after the application and were almost similar in magnitude. The concentrations, which were required to elicit the maximal responses, of tetragastrin, bethanechol and histamine, were $1 \times 10^{-5}$, $1 \times 10^{-6}$ and $1 \times 10^{-8}$.

![Fig. 1. Concentration-response curves for three secretagogues.](image-url)

Ordinate is the net increase of acid output with each secretagogue. Each point indicates the mean value, with the S.E.M. The number near the point indicates the number of preparations.
The combined effects of three secretagogues and anticholinergic or antihistaminics (H₁-receptor antagonist) on gastric acid secretion in vitro are summarized in Table 1. The concentration of each stimulant was selected to produce the maximal or supramaximal response, and three inhibitors were tested in the concentration of $1 \times 10^{-5}$ g/ml. It was noted that the stimulatory actions of tetragastrin and bethanechol were significantly abolished after the preincubation with atropine, while that of histamine was not. Diphenhydramine, a histamine H₁-receptor antagonist, showed little or no antagonistic effect to any secretagogues. On the contrary, burimamide, a specific competitive antagonist to histamine H₂-receptor (7), showed an inhibitory effect on gastric acid secretion stimulated by all of the secretagogues. It is noteworthy that the stimulatory actions of tetragastrin and bethanechol were completely abolished by the rather lower concentration of burimamide ($1 \times 10^{-5}$ g/ml) compared to the concentration required to inhibit the action of histamine. The acid secretion stimulated by the lower concentration of histamine ($3 \times 10^{-6}$ g/ml) was reduced by $1 \times 10^{-5}$ g/ml of burimamide and the decreased acid output by the antagonist was returned to the maximal level by application of a higher concentration of histamine ($1 \times 10^{-5}$ g/ml).

Isolated preparations of gastric mucosa provide experimental advantages for the study of gastric function, as several complex factors affecting gastric secretion can be excluded. However, there is a paucity in the literature regarding the antagonism between the agonist and the antagonist in vitro with regard to acid secretion. Dikstein and Sulman (8) examined the effect of histamine and divalent metal ions on basal secretion using the everted rat stomach preparation, but quantitative data were not shown, since the isolated mucosa could not continue to secrete for more than 2 hours. Recently, the procedure for preparing the mammalian stomach preparation was described by Wan et al (9). They found that acid secretion stimulated by lower concentration of histamine was reduced by $1 \times 10^{-5}$ g/ml of burimamide and the decreased acid output by the antagonist was returned to the maximal level by application of a higher concentration of histamine ($1 \times 10^{-5}$ g/ml).

### Table 1. Combined effects of secretagogues and inhibitors on gastric acid secretion in isolated bullfrog gastric mucosa

<table>
<thead>
<tr>
<th>inhibitor</th>
<th>No. of experiments</th>
<th>tetragastrin $5 \times 10^{-5}$g/ml</th>
<th>bethanechol $1 \times 10^{-4}$g/ml</th>
<th>histamine $1 \times 10^{-5}$g/ml</th>
<th>$\Delta$H⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>10</td>
<td>$245.3 \pm 36.4$</td>
<td>$237.3$</td>
<td>$293.3 \pm 39.0$</td>
<td>$264.7 \pm 19.3$</td>
</tr>
<tr>
<td>atropine</td>
<td>10</td>
<td>$8.0 \pm 23.5$</td>
<td>$71.3 \pm 20.8$</td>
<td>$262.7 \pm 16.1$</td>
<td>$193.4$</td>
</tr>
<tr>
<td>saline</td>
<td>8</td>
<td>$256.7 \pm 44.7$</td>
<td>$271.7 \pm 43.1$</td>
<td>$322.5 \pm 42.3$</td>
<td>$46.7$</td>
</tr>
<tr>
<td>diphenhydramine</td>
<td>8</td>
<td>$210.0 \pm 42.8$</td>
<td>$273.3 \pm 42.8$</td>
<td>$347 \pm 32.6$</td>
<td>$46.7$</td>
</tr>
<tr>
<td>saline</td>
<td>6</td>
<td>$320.0 \pm 31.8$</td>
<td>$306.7 \pm 68.1$</td>
<td>$330.0 \pm 59.0$</td>
<td>$393.3$</td>
</tr>
<tr>
<td>burimamide</td>
<td>6</td>
<td>$53.3 \pm 33.9$</td>
<td>$3.3 \pm 12.0$</td>
<td>$48.3 \pm 19.8$*</td>
<td>$281.7$</td>
</tr>
</tbody>
</table>

Each value is the mean $\pm$ S.E.M., and $\Delta$H⁺ indicates the difference between the acid secretory rate stimulated with each secretagogue and that in combination with the inhibitor (saline-inhibitor). Atropine, diphenhydramine and burimamide were examined in the concentration of $1 \times 10^{-5}$g/ml, except burimamide* in this column was tested in $1 \times 10^{-4}$g/ml.
secretion induced by histamine or pentagastrin was strongly antagonized by metiamide (one of the histamine H2-receptor antagonists). The clear-cut concentration-response relationships of secretagogues, however, were not shown in their report. In our experiment, the frog gastric mucosa preparation responded to each stimulant more quickly than did the rat stomach. Accordingly, the effect of the secretagogues in the same preparation could be determined repetitively. As shown in the results, the stimulatory effects of tetragastrin, bethanechol and histamine were found to be concentration-dependent and the three concentration-response curves were parallel. In addition, the actions of tetragastrin or bethanechol were strongly reduced by both atropine and burimamide. The activation of parietal cells by histamine was inhibited only by the histamine H2-receptor antagonist. These fundamentals may provide a reliable basis for discussion of the peripheral mechanism of action of physiological secretagogues and their inhibitors.

REFERENCES


INDIVIDUAL DIFFERENCES IN THE OCCURRENCE OF A LIPID PEROXIDATION INHIBITOR IN RAT LIVER SOLUBLE FRACTION

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In previous reports from this laboratory (1-5), it has been demonstrated that lipid peroxidation markedly affects the activity of hepatic drug metabolizing enzymes. In addition, an inhibitor(s) of hepatic microsomal lipid peroxidation has been shown to be present in the cytosol of rat liver (3). It was noted, however, that there were great individual differences in the occurrence of this lipid peroxidation inhibitor in the cytosol.

The present study was carried out to examine the individual difference in the occurrence of the lipid peroxidation inhibitor in the cytosol of rat liver, and the effect of these differences