ANTISECRETORY EFFECT OF IMIDAZOLE AND ITS DERIVATIVES IN AN ISOLATED GASTRIC MUCOSA PREPARATION AND AN ANESTHETIZED YOUNG CHICKEN PREPARATION: COMPARISON WITH A HISTAMINE H2-RECEPTOR ANTAGONIST

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Accepted October 3, 1977

Abstract—We investigated the influences of imidazole on the basal and the secretagogue-stimulated gastric acid secretion in isolated bullfrog gastric mucosa preparations and in anesthetized young chickens. Imidazole (1 × 10^{-4} g/ml) readily depressed the basal acid secretion in gastric mucosa in vitro. The inhibitory effect of imidazole was diminished considerably after washing out of the drug. The maximum acid secretion elicited by tetragastrin or bethanechol was completely antagonized by imidazole (1 × 10^{-4} g/ml). The stimulatory action of histamine or dibutyryl cyclic AMP was also remarkably depressed in the presence of imidazole (3 × 10^{-4} g/ml). After dibenamine pretreatment (5 × 10^{-5} g/ml) for 60 min, the isolated gastric mucosa preparation became refractory to tetragastrin, bethanechol and histamine, but responded to dibutyryl cyclic AMP. Imidazole protected the histamine sensitivity against dibenamine blockade in the concentration of 5 × 10^{-4} g/ml. In anesthetized young chickens, imidazole (200 mg/kg, s.c.) depressed tetragastrin- and histamine-stimulated gastric acid secretion. The effects of the imidazole derivatives and several antagonists (metiamide, atropine, diphenhydramine, acetazolamide and 2,4-dinitrophenol) on acid production were compared with that of imidazole. From these results, it is concluded that imidazole has a potent antisecretory effect on the basal and the secretagogue-stimulated acid secretion.

It is generally accepted that histamine is involved in the excitation mechanism of the parietal cells and is one of the possible chemomediators in gastric secretory processes (1-4). A new class of histamine antagonists (histamine H2-receptor antagonists) capable of abolishing the secretagogue action of histamine not counteracted by conventional anti-histaminics has recently been introduced by Black and coworkers (5).

Both histamine and histamine H2-receptor antagonists are imidazole derivatives. It seemed therefore, worthwhile to study the effect of imidazole itself on gastric acid secretion. Only a few reports on such effects of imidazole have been published, although many investigations of the effects of imidazole on other pharmacological properties have been done (6-8). Alonso et al (9) compared the effect of imidazole with that of xanthine analogues in frog bladder preparations with respect to active transport, and, in part, mentioned the anti-histaminic effect of imidazole on acid secretion. Their investigation was, however, performed before the concept of the histamine H2-receptor in the stomach wall was established by Ash and Schild (10). The present paper reports the antagonistic interaction between
imidazole and several secretagogues on gastric acid secretion in isolated frog stomach preparations and in anesthetized young chicken preparations with acute gastric fistula. Another aim of this experiment was to compare the antisecretory effect of imidazole with that of histamine $H_2$-receptor antagonist and other inhibitors.

**MATERIALS AND METHODS**

*Isolated gastric mucosa preparation*

*Rana catesbeiana* was used in this study. The experiment was carried out according to the procedure described by Davidson *et al* (11) with some modification (12). The gastric mucosa of the isolated stomach was immediately separated from the muscular layer and was mounted between two lucite chambers. The working area of the mucosa was 4 cm$^2$. The amount of the secreted acid was determined by titration with N/500 NaOH solution to pH 6 using an automatic titrator with a DC recorder. All test drugs except for dibenamine were dissolved in Ringer's solution and added to the serosal side chamber. Dibenamine was dissolved in saline solution.

*Acute gastric fistula preparation of young chicken*

Our recently reported procedure was employed for studying gastric acid secretion in anesthetized young chickens (13). White leghorns, 5–14 days old after hatching, were used. After an 18 hr fast, the birds were anesthetized with urethane (1.2 g/kg, i.p.). After ligation of the lower esophagus, at the position under the crop, a double tube cannula was inserted between the gizzard and the proventriculus. Care was taken to avoid the occlusion of nerves and blood vessels. The stomach was perfused with warm saline solution (2 ml) through the cannula every 15 min. The perfusate was titrated for acid content using phenolphthalein as the indicator. Each drug was dissolved in saline solution and injected subcutaneously. Young chickens weighing 50 to 100 g secreted a large amount of hydrogen ion under deep anesthesia and were considerably sensitive to exogenous tetragastrin or histamine.

*Materials*

Drugs used were as follows: imidazole and dibutyryl cyclic AMP (Daiichi Pure Chem. Indus.), tetragastrin (Nissui Pharmaceutical Co.), bethanechol chloride (Yoshitomi), histamine 2HCl, atropine sulfate, sodium thiocyanate and 2,4-dinitrophenol (Wako Pure Chem.), dibenamine HCl (Nakarai), acetazolamide (Lederle Japan), diphenhydramine HCl (Kowa), and metiamide (kindly provided by Smith, Kline & French Laboratories, England). Student's $t$-test was used for statistical analysis.

**RESULTS**

*Effect of imidazole and other inhibitors on basal acid secretion*

Antisecretory effects on basal acid secretory responses were examined with imidazole and other several antagonists. It was quite evident that imidazole depressed spontaneous $H^+$ secretion concentration-dependently and that the basal acid secretion was restored by
removal of the inhibitor (Fig. 1). Effects of metiamide and atropine are also shown in Fig. 1. The former had a reversible antisecretory effect on non-stimulated acid secretion similar to that seen with imidazole, however, the latter had no influence on basal acid secretion. Effects of several antisecretory agents on basal acid output are summarized in Table 1. The antisecretory activity of diphenhydramine, an H1-receptor antagonist, was less than that of an H2-receptor antagonist. Acetazolamide and 2,4-dinitrophenol abolished acid secretion, irreversibly.

**Table 1. Effect of imidazole and other inhibitors on basal acid secretion in the isolated frog stomach preparation**

| Inhibitor       | g/ml | No. | Acid Output mEq.H+ / 10 min / 4 cm² | Inhibition |
|-----------------|------|-----|------------------------------------|------------|
| Imidazole       | 1 x 10⁻² | 8   | 433.75 ± 20.20                    | 10.4%      |
|                 | 3 x 10⁻² | 8   | 453.75 ± 33.75                    | 27.8%      |
|                 | 1 x 10⁻¹ | 14  | 481.07 ± 31.54                    | 44.5%      |
|                 | 3 x 10⁻¹ | 14  | 508.21 ± 41.42                    | 90.7%      |
| Metiamide       | 2 x 10⁻⁵ | 10  | 325.50 ± 85.43                    | 57.9%      |
|                 | 5 x 10⁻⁴ | 10  | 292.22 ± 53.24                    | 90.8%      |
| Atropine        | 1 x 10⁻³ | 6   | 451.67 ± 44.50                    | 5.2%       |
| Diphenhydramine | 1 x 10⁻³ | 6   | 458.33 ± 20.33                    | 28.0%      |
| Acetazolamide   | 5 x 10⁻⁴ | 6   | 421.67 ± 35.71                    | 32.8%      |
| 2,4-dinitrophenol | 5 x 10⁻⁵ | 4   | 405.00 ± 44.20                    | 97.5%      |

*: P < 0.05.  **: P < 0.01.

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**Antagonistic effect of imidazole and other inhibitors on secretagogue-stimulated acid secretion in vitro**

Gastric acid production *in vitro* was markedly augmented by several secretagogues; tetragastrin, bethanechol, histamine and dibutyryl cyclic AMP. These stimulatory effects were concentration-dependent, and maximum responses by tetragastrin, bethanechol, histamine and dibutyryl cyclic AMP were obtained in the dose of 5 x 10⁻⁷ g/ml, 1 x 10⁻⁶ g/
Fig. 2. Combined effect of secretagogues and inhibitors on gastric acid secretion in isolated frog gastric mucosa. A, effect of four secretagogues on basal acid secretion; B, inhibitory effect of imidazole on secretagogue-stimulated secretion; C, inhibitory effect of metiamide on secretagogue-evoked secretion. T-Gas, tetragastrin; Bet, bethanechol; His, histamine; Bt2cAMP, dibutyryl cyclic AMP. Other abbreviations are as in Fig. 1.

Fig. 3. Competitive inhibition by imidazole and metiamide of histamine-stimulated gastric acid production in isolated frog gastric mucosa.
ml, $1 \times 10^{-3}$ g/ml and $1 \times 10^{-4}$ g/ml, respectively. Fig. 2-A shows that the secretory rate in response to each stimulant was almost equivalent. Typical records of the effects of imidazole and metiamide on three gastric stimulant-evoked acid secretion are also shown in Fig. 2. These two inhibitors effectively inhibited the secretagogue action of histamine as well as those of tetragastrin or bethanechol. The antagonistic effects of these two inhibitors were reversed with an excess dose of histamine. Fig. 3 shows concentration-response relationships of the inhibitory effect of imidazole and metiamide against histamine. Our observations indicate that these two inhibitors competitively antagonize the effect of histamine. $P_{A_2}$ values of imidazole and metiamide against histamine calculated from the result in Fig. 3, were 4.86 and 4.92, respectively. There is, however, a great difference between the effects of these drugs on dibutylryl cyclic AMP-stimulated acid secretion (Fig. 4). The stimulating effect of dibutylryl cyclic AMP was significantly depressed by imidazole but was not influenced by pretreatment with metiamide. Table 2 is a summarization of the antagonistic effects of several agents on acid secretion. Atropine was found to be a more selective inhibitor against bethanechol or tetragastrin than was metiamide and did not affect histamine- or cyclic nucleotide-induced secretory responses. Diphenhydramine had no influence on the activity of oxyntic cells evoked by any secretagogue. Furthermore, it was confirmed that acetazolamide and 2,4-dinitrophenol abolished completely the effects of four gastric stimulants, as well as basal secretion. Janowitz et al (14) have already reported that acetazolamide strongly inhibited the histamine-induced gastric secretion in vivo.

**Histamine receptor protection by imidazole or metiamide against dibenamine blockade**

When the bullfrog gastric mucosa preparation was treated with dibenamine alone ($5 \times 10^{-3}$ g/ml, 60 min), a distinctive irreversible inhibitory effect on histamine-stimulated acid secretion resulted. However, when the preparation was pretreated with either imidazole or metiamide in combination with dibenamine, the acid secretory response to histamine was restored against dibenamine blockade (Fig. 5 and Table 3). The protective effect of these two drugs against dibenamine blockade was selective to histamine but not to tetragastrin or bethanechol. These results are quite coincident with those for burimamide or cimetidine which we have already reported (15-16). This implies that the antagonists directly act on histamine receptor in the isolated gastric mucosa.

**Effect of imidazole derivatives on gastric acid secretion in vitro**

Three imidazole-related compounds were tested to compare their antisecretory activities
| Inhibitor        | g/ml    | Change in Acid Output (μeq.H⁺/10 min/4 cm²) |
|------------------|---------|---------------------------------------------|
|                  |         | tetragastrin | bethanechol | histamine | dibutyryl cyclic AMP |
| None             |         | 580.0 ± 52.7  | 596.7 ± 36.6  | 638.3 ± 66.1  | 455.0 ± 100.9  |
| Imidazole        | 1 × 10⁻¹| 112.5 ± 41.1**(4) | 150.4 ± 42.0***(4) | 320.0 ± 60.0***(4) | 286.7 ± 49.6  |
|                  | 5 × 10⁻⁴| 50.0 ± 21.1***(6) | 71.7 ± 27.6***(6) | 136.7 ± 62.0***(6) | 168.5 ± 64.2***(6) |
| Metiamide        | 2 × 10⁻⁵| 138.3 ± 65.4***(6) | 83.3 ± 34.5***(6) | 593.8 ± 24.3  | 502.9 ± 81.5  |
|                  | 1 × 10⁻¹| 103.5 ± 51.0***(6) | 4.2 ± 4.2***(6) | 337.0 ± 51.0***(6) | 372.5 ± 120.2 |
| Atropine         | 1 × 10⁻³| 26.0 ± 12.4***(10) | 3.0 ± 1.2***(10) | 632.5 ± 64.2  | 604.2 ± 97.4  |
| Diphenhydramine  | 1 × 10⁻⁴| 486.9 ± 94.2  | 514.3 ± 47.7  | 543.1 ± 58.7  | 427.4 ± 69.7  |
| Acetazolamide    | 5 × 10⁻¹| 258.3 ± 49.2***(6) | 286.4 ± 52.3***(4) | 275.0 ± 51.2***(4) | 322.5 ± 27.8  |
| 2,4-dinitrophenol| 5 × 10⁻⁵| 0 ***(4)        | 0 ***(4)       | 0 ***(4)       | 0 ***(4)       |

Tetragastrin (5 × 10⁻⁷ g/ml), bethanechol (1 × 10⁻⁶ g/ml), histamine (1 × 10⁻⁵ g/ml) and dibutyryl cyclic AMP (1 × 10⁻³ g/ml) were administered 10-40 min after the pretreatment with each inhibitor. **: P < 0.01. Numbers in parentheses indicate no. of experiments.
with that of imidazole. As seen in Fig. 6, 2-methylimidazole at the concentration of $5 \times 10^{-1}$ g/ml completely depressed both basal secretion and evoked secretion. This inhibitory effect was found to be more potent than that of imidazole. Both N-methylimidazole and 2-methylimidazole are potent antisecretory agents. The inhibitory effect of urocanic acid was, however, less than imidazole or its methyl analogues.

**Table 3. Protective effects of imidazole and metiamide against dibenamine blockade of the histamine receptor**

| Inhibitor   | g/ml | No. | Acid Output | mEq H+ 10 min/4 cm² | histamine after washing of dibenamine |
|-------------|------|-----|-------------|---------------------|-------------------------------------|
|             |      |     | basal secretion before dibenamine treatment | basal secretion after dibenamine treatment |                                     |
| None        |      | 4   | 87.5 ±36.1  | 10.0 ±5.8          | 5.0 ±5.6                             |
| Imidazole   | $5 \times 10^{-5}$ | 6   | 3.3 ±3.3  | 0 ±0               | 256.7 ±59.8                          |
| Metiamide   | $2 \times 10^{-4}$ | 4   | 153.8 ±39.7 | 0 ±0               | 335.0 ±91.4                          |
| None        |      | 4   | without dibenamine treatment | 62.5 ±22.2          | 602.5 ±152.5                         |

The gastric mucosa preparations were pretreated with dibenamine ($5 \times 10^{-5}$ g/ml) in combination with imidazole or metiamide for 60 min, followed by stimulation with histamine ($1 \times 10^{-3}$ g/ml).

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**Effect of imidazole and metiamide on gastric acid secretion in vivo**

We examined the influence of two antisecretory agents on tetragastrin- or histamine-stimulated acid secretion in acute gastric fistula preparation of anesthetized young chickens.
Fit; 6. Antisecretory effect of three derivatives of imidazole on acid secretion in isolated frog gastric mucosa.

Fig. 6. Antisecretory effect of three derivatives of imidazole on acid secretion in isolated frog gastric mucosa.

Figs. 7 and 8 show the time-course of inhibitory effects of imidazole and metiamide. The premedication of 100 mg/kg to 200 mg/kg of imidazole produced a depressive effect on the secretagogue actions of two stimulants. Metiamide also suppressed the stimulating effects of tetragastrin and histamine. It was also confirmed that metiamide almost completely blocked the stimulatory effect of cholinomimetics in a dose of 1 mg/kg. These results
confirm that imidazole also inhibits gastric acid secretion induced by tetragastrin or histamine in vivo.

**DISCUSSION**

The present study demonstrates that imidazole strongly depressed basal acid secretion in isolated bullfrog stomach preparation, in which the systemic influences were completely eliminated. Imidazole also induced cessation of the production of hydrogen ion evoked by a variety of secretory stimuli such as histamine, tetragastrin, bethanechol and dibutyryl cyclic AMP. These inhibitory effects of imidazole disappeared after its removal. The antisecretory effect of imidazole was compared with those of other inhibitors (atropine, diphenhydramine, acetazolamide and 2,4-dinitrophenol) to search for clues regarding the inhibitory mechanism of imidazole, and we found the inhibitory effect of imidazole to be clearly different from those of atropine and metabolic inhibitors.

An important finding in this investigation was that the inhibitory effect of imidazole, at the lower concentrations ($1 \times 10^{-3}$ to $1 \times 10^{-4}$ g/ml), on histamine-stimulated acid secretion was reversed by addition of excess histamine. The possible inhibitory mechanism of imidazole may therefore be due to the antagonism against histamine $H_2$-receptor. This possibility was examined by the receptor protection experiment against dibenamine blockade. This method is widely accepted for testing the specificity of receptor-antagonist interaction (17-18). We performed the receptor protection experiment in order to elucidate the spe-
sificity of the antisecretory effects of imidazole and metiamide. Pretreatment of the gastric mucosa with \(5 \times 10^{-5}\) g/ml of dibenamine for at least 60 min, was found to abolish the sensitivity to any secretagogues, irreversibly (15). Under these conditions, the sensitivity of the acid secretory cells to histamine was completely protected by pretreatment of the mucosa with a histamine \(H_2\)-receptor antagonist. Imidazole also, to some extent, protected the histamine receptor against dibenamine blockade, providing evidence that the agonist-antagonist relationship between histamine and imidazole on the receptor site of histamine is specific for gastric acid secretion.

On the other hand, it was clear that imidazole, at the higher concentrations (3–5 \(\times\) \(10^{-4}\) g/ml), was different from metiamide in respect to an inhibitory effect on the cyclic nucleotide-induced acid secretion. Pretreatment with imidazole completely abolished the secretagogue activities of all stimulants, while in the case of metiamide, the secretory effect of dibutyryl cyclic AMP remained. Preliminary experiments confirmed that imidazole, even at the high concentration of \(1 \times 10^{-3}\) g/ml, had no effect on axon action potentials of isolated frog sciatic nerves, thus suggesting that imidazole does not have a local anesthetic action.

In the final experiment, we compared the effect of imidazole in \textit{in vivo} preparations with the antisecretory effect of metiamide. Recently, the procedure was established for preparing acute gastric fistula preparations of anesthetized young chickens (13). The preparation may qualify for research of histamine-mediated mechanisms of acid secretion, since it responds more sensitively to histamine than do preparations from rodents. It was demonstrated that imidazole, even though the dosage was rather high, resulted in depression of gastric secretion as did the histamine \(H_2\)-receptor antagonists.

Acknowledgement: This work was supported in part by a Scientific Grant in Aid from the Ministry of Education, Science and Culture, Japan (No. 287180).

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