Inhibitory Effect of Leminoprazole on Acid Secretion in Parietal Cells Isolated from Guinea Pig Gastric Mucosa

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ABSTRACT—Isolated guinea pig parietal cells, the function of which is similar to that of human parietal cells, were used in this study. The accumulation of ^{14}C-aminopyrine (^{14}C-AP) was measured to study the inhibitory mechanism of leminoprazole in cells. Stimulation by 10 μM histamine, 0.1 mM carbachol, 1 μM gastrin or 1 mM db-cAMP brought about satisfactory incorporation of ^{14}C-AP, and leminoprazole concentration-dependently inhibited acid secretion induced by these stimulants. At 10^{-5} M, almost 100% inhibition was observed. The IC_{50} values of leminoprazole obtained from its inhibitory action on histamine, carbachol, gastrin and db-cAMP-stimulated acid secretion were 4.0×10^{-7} M, 3.5×10^{-7} M, 2.5×10^{-7} M and 5.6×10^{-7} M, respectively. Thus the extent of inhibition was the same for the responses to all the secretagogues. These results indicate that the site of action of leminoprazole is intracellular and distal from cAMP (intracellular second messenger), but not at the receptor sites. The results also strongly suggest that the inhibitory action of leminoprazole on H^+,K^+-ATPase may contribute to the inhibitory effect of this drug on gastric acid secretion.

Keywords: Leminoprazole, ^{14}C-Aminopyrine, Parietal cell, Acid secretion

Leminoprazole has been reported to inhibit H^+,K^+-ATPase activity isolated from dog and rabbit gastric mucosa in vitro (1, 2) and to inhibit gastric acid secretion in rats and dogs (1, 3). Furthermore, it has been reported that leminoprazole markedly suppresses the development of experimental gastroduodenal lesions and protects the gastric mucosa (1). Its remarkable healing effect on acetic acid-induced gastric ulcers in rats has also been demonstrated (4). At present, leminoprazole is the only known drug that shows anti-ulcer effects as a result of a direct action on the gastric lumen to suppress acid secretion.

We have recently established a highly sensitive and rapid isolation method for guinea pig parietal cells, and we found that the responses of guinea pig parietal cells to secretagogues are very similar to those of isolated human parietal cells (5). The aim of the present study is to clarify the inhibitory mechanism of leminoprazole on acid secretion and infer the effect of this agent in humans. Therefore, we used guinea pig parietal cells isolated from the gastric mucosa as a substitute model for human parietal cells. Thereby the action of this drug on acid secretion stimulated by various compounds such as histamine, carbachol and gastrin was examined by measuring ^{14}C-aminopyrine accumulation (6-13).

MATERIALS AND METHODS

Test materials

The following chemicals were obtained from the indicated sources: Leminoprazole and omeprazole (Nippon Chemiphar, Tokyo); cimetidine, carbamylcholine chloride, collagenase (type I) and 2,4-dinitrophenol (Sigma, St. Louis, MO, USA); histamine·2HCl (Nacalai Tasque, Kyoto); gastrin I (human) (Peptide Laboratories, Minoh); N^6,2′-*O*-dibutyryladenosine-3′:5′-cyclic monophosphate sodium salt (db-cAMP) (Wako Pure Chemical Industries, Osaka); dispase (Godo Shusei, Tokyo); and ^{14}C-AP (New England Nuclear (NEN), Boston, MA, USA).

Preparation of isolated parietal cells

Male Hartley guinea pigs (250-300 g) (Nippon Bio.-Supp. Center, Tokyo) were used. The animals were killed and their stomachs were removed. After the removal of the fundus, the exfoliated mucosa was minced and digested. The digestion was carried out by incubation in

TCM199 medium containing collagenase (0.1 mg/ml) and dispase (2 mg/ml) for two successive 20-min periods and incubation in 1 mM EDTA solution for 10 min. These treatments with enzymes and EDTA were repeated once more. Isolated mucosal cells obtained were filtered through Nylon 400-mesh, and parietal cells were enriched to over 80% by counterflow centrifugation using an elutriator rotor (Model J2-21; Beckmann, Tokyo).

Measurement of the incorporation of ^{14}C-aminopyrine (^{14}C-AP)

Various stimulants (histamine (10 μM), carbachol (0.1 mM), gastrin (1 μM) and db-cAMP (1 mM)) and drugs (leminoprazole, omeprazole and cimetidine) were added to aliquots of the prepared cell suspension (2×10^6 cells in 2 ml). Then 0.25 μCi of ^{14}C-AP was added to each cell suspension, and the mixtures were incubated at 37℃ for 30 min. Two aliquots (0.8 ml each) were taken out of each tube and centrifuged. Pellets were dissolved in protzol to measure the radioactivity of ^{14}C-AP incorporated into the cells. The amount of specific incorporation was determined by subtracting the amount of non-specific incorporation (i.e., the radioactivity detected in the presence of 1 mM dinitrophenol) from each measured value. Radioactivity was counted in a liquid scintillation counter (Model LSC-2000; Aloka, Tokyo), with an efficiency of 94%.

The drugs were dissolved in dimethylsulfoxide (DMSO). The experiments were performed under conditions in which the final concentration of DMSO was 1%.

The inhibitory effect of leminoprazole on histamine-stimulated acid secretion was compared with those of omeprazole and cimetidine, and the inhibitory effect on carbachol stimulated acid secretion was compared with that of omeprazole.

Statistical analyses

Four tubes were used for each concentration of each drug and the experiments are repeated. The results are expressed as the means±S.E. IC_{50} values were calculated using the least squares method from the reaction rate when the radioactivity of the control group was taken to be 100%.

RESULTS

Inhibitory effect of leminoprazole on acid secretion stimulated by various secretagogues

The addition of 10 μM histamine induced ^{14}C-aminopyrine incorporation of about 8%, and 1 mM db-cAMP

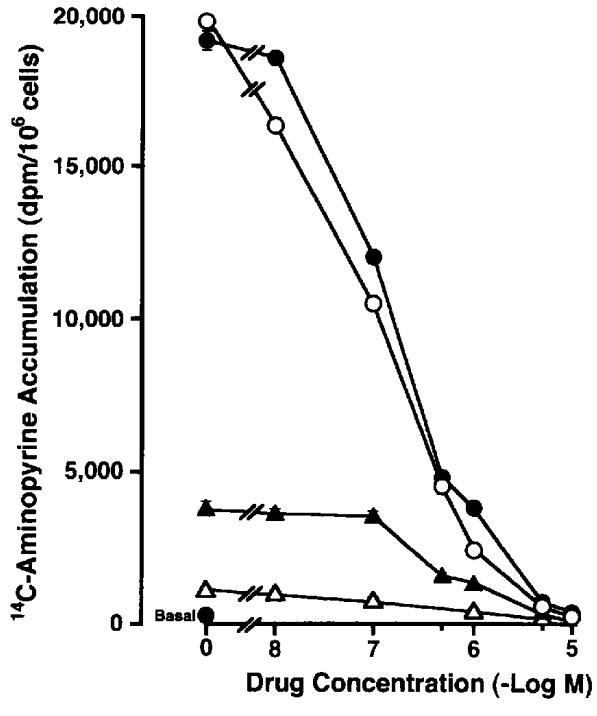

Fig. 1. ^{14}C-Aminopyrine accumulation stimulated by secretagogues and inhibitory effect of leminoprazole. The secretagogues were 10 μM histamine (●), 1 mM db-cAMP (○), 0.1 mM carbachol (▲) and 1 μM gastrin (△). Values are means±S.E. of 4 subjects.

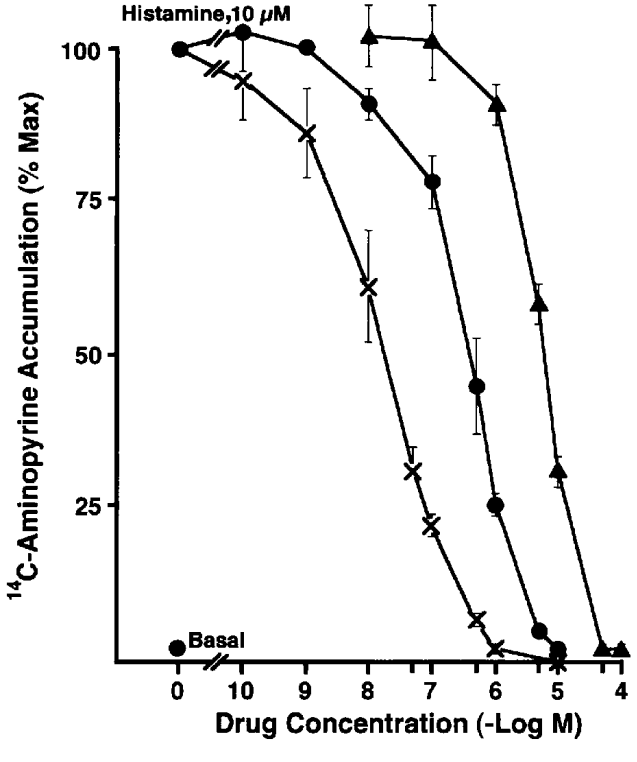

Fig. 2. Inhibitory action of leminoprazole (●), omeprazole (×) and cimetidine (▲) on the histamine (10 μM) stimulated ^{14}C-aminopyrine accumulation by isolated guinea pig parietal cells. The control value without drug has been set to 100%. Values are means±S.E. in 6 preparations.

showed similar results. Stimulation by 0.1 mM carbachol and 1 μM gastrin also produced satisfactory incorporation (about 2% and 0.5%, respectively). On these acid secretions stimulated by the various secretagogues, leminoprazole showed a concentration-dependent inhibitory effect, and also 100% inhibition was observed at 10^{-5} M (Fig. 1).

Inhibitory effect on histamine-stimulated acid secretion

The inhibitory effect of leminoprazole on histamine (10 μM) stimulated acid secretion first appeared at 10^{-8} M, and this drug at 10^{-5} M inhibited the secretion to the basal level. The IC_{50} values of leminoprazole, omeprazole and cimetidine were 4.0×10^{-7} M, 1.8×10^{-8} M and 5.9×10^{-6} M, respectively (Fig. 2).

Inhibitory effect on carbachol-stimulated acid secretion

The inhibitory effect of leminoprazole on carbachol (0.1 mM) stimulated acid secretion first appeared at 10^{-7} M, and this drug at 5×10^{-6} M inhibited the secretion to almost the basal level. The IC_{50} values of leminoprazole and omeprazole were 3.5×10^{-7} M and 2.0×10^{-8} M, respectively (Fig. 3).

Inhibitory effect on gastrin-stimulated acid secretion

The inhibitory effect of leminoprazole on gastrin (1 μM)-stimulated acid secretion first appeared at 10^{-7} M, and this drug at 10^{-5} M inhibited the secretion to the basal level. The IC_{50} value of leminoprazole was 2.5×10^{-7} M (Fig. 4).

Inhibitory effect on db-AMP-stimulated acid secretion

The inhibitory effect of leminoprazole on acid secretion stimulated by 1 mM db-cAMP first appeared at 10^{-7} M, and this drug at 10^{-5} M inhibited the secretion to almost the basal level. The IC_{50} value of leminoprazole was 5.6×10^{-7} M (Fig. 5), and its inhibitory curve was almost the same as that on histamine stimulation.

DISCUSSION

Parietal cells isolated from the gastric mucosa of guinea pigs, which act in a manner similar to human parietal cells, were used in this study, and ^{14}C-aminopyrine accumulation was measured to study the inhibitory effect of leminoprazole in parietal cells. Leminoprazole showed concentration-dependent inhibitory action on histamine-, carbachol- and gastrin-stimulated acid secre-

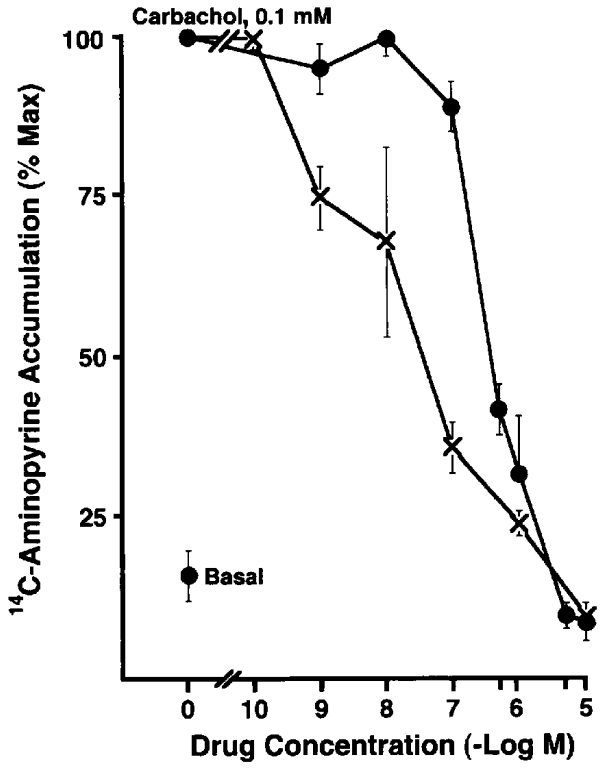

Fig. 3. Inhibitory action of leminoprazole (●) and omeprazole (×) on the carbachol (0.1 mM) stimulated ^{14}C-aminopyrine accumulation by isolated guinea pig parietal cells. The control value without drug has been set to 100%. Values are means±S.E. in 5 preparations.

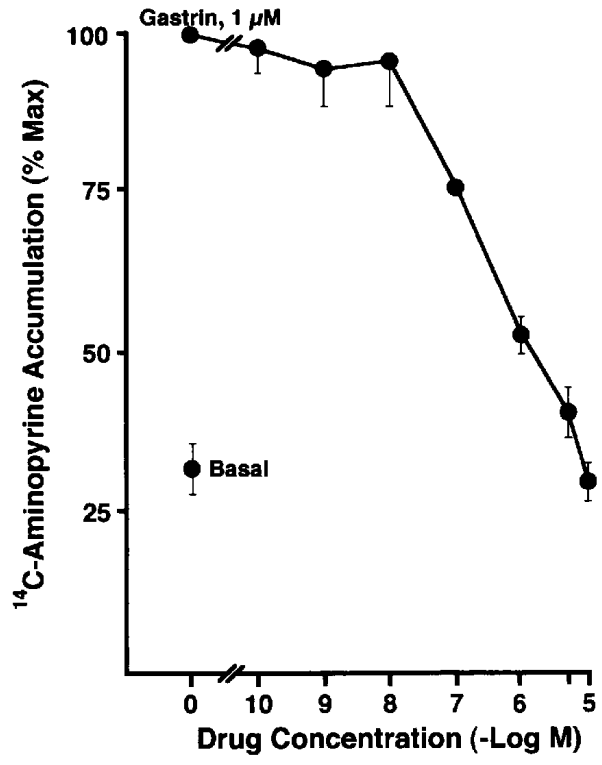

Fig. 4. Inhibitory action of leminoprazole on the gastrin (1 μM) stimulated ^{14}C-aminopyrine accumulation by isolated guinea pig parietal cells. The control value without drug has been set to 100%. Values are means±S.E. in 3 preparations.

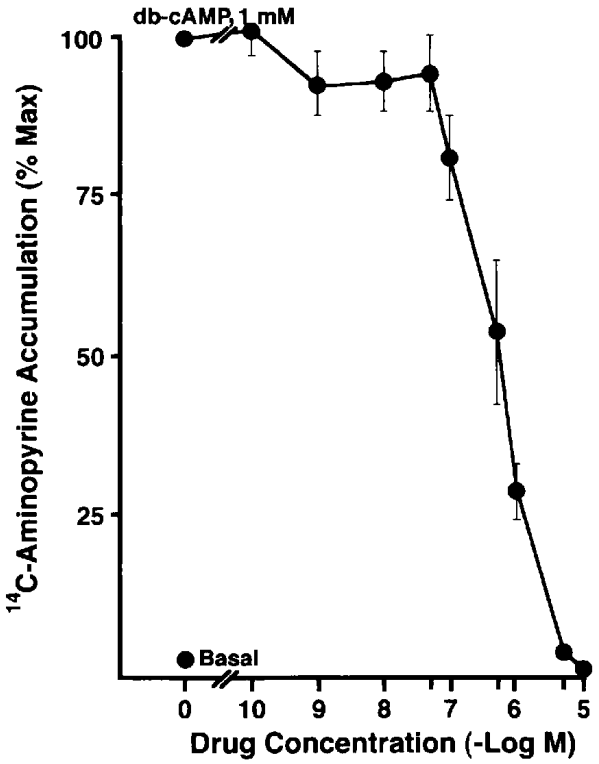

Fig. 5. Inhibitory action of leminoprazole on the db-cAMP (1 mM) stimulated ^{14}C-aminopyrine accumulation by isolated guinea pig parietal cells. The control value without drug has been set to 100%. Values are means±S.E. in 6 preparations.

tion as well as on db-cAMP-stimulated acid secretion. The IC_{50} values for the acid secretions induced by these stimulants were similar. Therefore, leminoprazole inhibited gastric acid secretion in the parietal cells probably in a manner similar to that of other proton pump inhibitors such as omeprazole (14–17). This is in contrast to H_2-receptor antagonists that specifically inhibit the histamine-stimulated acid secretion at the receptor sites in parietal cells. Consequently, these results suggest that leminoprazole acts to suppress acid secretion at cAMP (intracellular second messenger) or in the pathway after cAMP, not at the receptor sites in parietal cells. Considering that leminoprazole inhibits H^+,K^+-ATPase activity, the results of this study strongly suggest that leminoprazole enters into the parietal cells and inhibits gastric acid secretion by inhibiting the H^+,K^+-ATPase activity, which has been reported to play an important role in acid formation in the parietal cells (18, 19).

It has been reported that the IC_{50} values are 5.2×10^{-6} M and 5.3×10^{-6} M, respectively, when leminoprazole inhibits the activity of H^+,K^+-ATPase isolated from dog and rabbit gastric mucosa at pH 6.0 or pH 6.1 (1, 2). The proton pump inhibitors such as omeprazole have been presumed to penetrate into the cytosol of the parietal cells and are transformed into the active sulfenamide form within the acidic compartment, and then react with enzyme SH groups (16, 20–22). Likewise, it has also been demonstrated that the inhibitory effect of leminoprazole on H^+, K^+-ATPase is observed more strongly at a lower pH, and it is speculated that leminoprazole combines with enzyme SH groups through some activated reactions under acidic conditions (2). In general, it has been indicated that there was a great difference in the inhibitory potencies against acid secretion in isolated parietal cells and H^+,K^+-ATPase activity (14, 15). This may be due in part to differences in the experimental conditions, where H^+,K^+-ATPase activity was determined under weak acid pH, but other possibilities may exist. In fact in this study, although the species were different, there was a large ten-fold difference between the concentration of leminoprazole showing inhibitory effects on acid secretion in isolated parietal cells and that on H^+,K^+-ATPase.

In a study using isolated human gastric glands, omeprazole showed an IC_{50} value of 50 nM for acid secretion induced by various stimulants (23), and this was similar to the IC_{50} value obtained in this study using parietal cells isolated from guinea pig gastric mucosa. Therefore, it is considered that when leminoprazole was administered to humans, the agent inhibits gastric acid secretion by entering into parietal cells and then inhibiting H^+,K^+-ATPase activity, but the potency may be slightly weaker than that of omeprazole.

It has been considered that the proton pump inhibitors can not inhibit gastric acid secretion when those agents exist in the stomach lumen, because those agents are transformed into the sulfenamide form that can not penetrate the cell membrane of parietal cells under a strong acid condition and/or are degraded in the stomach. However, leminoprazole has been demonstrated to have intraluminal inhibitory action on acid secretion (3). In this study, we could not examine the effect of leminoprazole by preparing the parietal cell suspensions under the acidic condition, and therefore further studies are needed to determine how leminoprazole enters into parietal cells under acidic condition and then exhibits direct action on them. Moreover, the intracellular mechanisms such as the inhibitory action on myosin light chain kinase, which has been reported to play a role in the proton pump activation in the parietal cells (24), remain to be elucidated.

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