Pharmacological Studies of 2-(3-(3-(1-Piperidinylmethyl)phenoxy)propylamino)-4 (3H)-Quinazolinone (NO-794), a New Histamine H2-Receptor Antagonist

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Abstract—The pharmacological profile of a new histamine H2-receptor antagonist, 2-(3-(3-(1-piperidinylmethyl)phenoxy)propylamino)-4 (3H)-quinazolinone (NO-794), was studied. NO-794 was a potent and selective histamine H2-receptor antagonist in the guinea-pig atria and gastric mucosal cells. NO-794 (1 X 10^-5 M) did not interact with H1-, muscarinic and y1-receptors. In guinea-pig atria, antagonism of NO-794 was unsurmountable. The onset of action of NO-794 was slow, and this antagonism was apparently irreversible not only on the guinea-pig atria but also on the gastric mucosal cells. In addition, NO-794 inhibited gastric acid secretion in pylorus ligated rats when administered intraduodenally. These results indicate that NO-794 is a powerful and unique histamine H2-receptor antagonist and may be useful in the treatment of peptic ulcer.

It is well-known that gastric acid secretion by histamine is mediated by histamine H2-receptor, and blockade of histamine H2-receptor by specific histamine H2-receptor antagonists reduces the gastric acid secretion in the physiological state (1, 2). Thus histamine H2-receptor antagonists (cimetidine, ranitidine and famotidine) are useful for treatment of peptic ulcer disease. Recently, new histamine H2-receptor antagonists that have very potent and long lasting activity were reported (3). These compounds (L-643441, lamtidine (AH22216), loxtidine (AH 23844) and BMY25368) contain a piperidinomethylphenoxypropyl moiety and produced unsurmountable antagonism in guinea-pig atria, and they were found to be difficult to remove from the tissue by washing (3).

2-(3-(3-(1-Piperidinylmethyl)phenoxy)propylamino)-4 (3H)-quinazolinone (NO-794) is one of these compounds that contain the piperidinomethylphenoxy moiety (Fig. 1). In this paper, we investigated the effect of NO-794 on the histamine H2-receptors of guinea-pig atria and gastric mucosal cells and also on the gastric acid secretion in vivo in comparison with ranitidine.

Materials and Methods

Guinea-pig atria: Atria from male guinea-pigs were suspended in an organ bath containing 10 ml of Krebs-Henseleit solution of the following composition (mM): NaCl, 118; KCl, 4.7; CaCl2, 2.6; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25; Glucose, 11.1. Tissues were attached under 0.5 g tension to an isometric force displacement transducer for rate recording and were allowed to stabilize for at least 60 min before the experiments. The bath medium was maintained at 31°C, pH 7.4, and it was equilibrated with a gas mixture consisting of 95% O2 and 5% CO2 during preincubation and experimental periods. Histamine was added cumulatively.

Guinea-pig gastric mucosal cells: Dispersed gastric mucosal cells from guinea-pig stomach were prepared according to
Sewing et al. (4). Guinea-pig gastric mucosal cells were isolated by collagenase and pronase digestion. The cells were tested for viability by the trypan blue dye exclusion test, and 95% of the cells in each preparation were found to be viable.

Change in cellular cyclic AMP was measured in the presence of 5 mM theophylline plus the indicated agents at 37°C. Cyclic AMP was determined by radioimmunoassay with a Yamasa cyclic AMP assay kit.

Gastric secretion in pylorus ligation: Male Wistar rats (180–210 g) fasted 24 hr were prepared according to Shay (5). Drugs were administered intraduodenally immediately before pylorus ligation. Four hr after ligation, the animals were sacrificed and gastric juice was collected; the volume of gastric juice was measured, and acid concentration was titrated to pH 7 with 0.1 N NaOH.

Statistical analysis: Experimental values were given as the mean±S.E. The pA2 values for ranitidine were obtained according to the method of Arunlakshana and Schild (6). Because NO-794 showed apparent irreversible antagonism, we determined the dissociation constant (K_b) for NO-794 according to Kenakin (7), under conditions which were described as a hemi-equilibrium state. The reciprocals of the concentration of agonist before antagonist treatment were plotted against the corresponding effective concentration of agonist after antagonist treatment. From the slope of the straight line fitting the data points, K_b values were calculated by

\[ K_b = \frac{(B)}{\text{Slope} - 1} \]

where (B) is the concentration of the antagonist.

The effects of drugs were considered significant if P<0.05, according to Student's t-test for unpaired data.

Drugs: 2-(3-(3-(1-Piperidinylmethyl)phenoxy)propylamino)-4 (3H)-quinazoline (NO-794) was synthesized in our laboratory. The following drugs were purchased: histamine dihydrochloride (Nakarai), (±)-isoproterenol hydrochloride (Sigma), collagenase (Type I, Sigma), theophylline (Wako), pronase E (Merck), acetylcholine chloride (Ovisot, Daichi) and the Yamasa cyclic AMP assay kit (Yamasa Shoyu). Ranitidine was prepared in our laboratory from zantac (Sankyo).

Results

Effects of NO-794 and ranitidine on the histamine H2-receptor of guinea-pig atria: NO-794 antagonized the positive chronotropic action of histamine concentration-dependently in guinea-pig atria. When NO-794 was incubated with guinea-pig atria for 60 min, NO-794 displaced the histamine

![Fig. 2.](image-url) Effects of NO-794 (A) and ranitidine (B) on the histamine-induced positive chronotropic response in the guinea-pig atria. NO-794 and ranitidine were applied 60 min before histamine application. Responses induced by 3×10⁻⁶ M histamine before drug treatment were taken as 100%. O: before; ●, ▲, ■, ●: after treatment with 3×10⁻⁶ M, 1×10⁻⁷ M, 3×10⁻⁸ M and 1×10⁻⁹ M of each drug. Each value is the mean±S.E. of 4 experiments.
concentration-response curve to the right in a nonparallel fashion (Fig. 2A). Maximal response was reduced by 20–30% at 1 \times 10^{-6} M NO-794. Because NO-794 did not fulfill the criteria for being a competitive antagonist in this respect, we determined the dissociation constant (K_d) for NO-794. The -log K_d for NO-794 was 7.54±0.19. In contrast, ranitidine antagonized the action of histamine concentration-dependently and shifted the concentration-response curve to the right in a parallel fashion (Fig. 2B). The pA_2 and the slope of the Schild plot were 7.1±0.05 and 0.9±0.07, respectively. As shown in Fig. 3, inhibition of NO-794 was time-dependent. When incubation was carried for 60 min, antagonism of NO-794 was stronger than it was for the 1 min incubation. Ranitidine showed the same antagonism for the 1 min and 60 min incubations. Figure 4 shows the reversibility of inhibition of NO-794 and ranitidine. After incubation of NO-794 or ranitidine with atria for 30 min, atria were washed with normal Krebs-Henseleit solution. Sixty min later, NO-794 still showed the inhibition on the response to histamine, although NO-794 was not present in the organ bath. In contrast, after washing out the ranitidine, inhibition of the response to histamine was not detected.

NO-794 (1 \times 10^{-5} M) did not antagonize the responses to acetylcholine and histamine.

Fig. 3. Effects of 3 \times 10^{-7} M NO-794 (A) and 3 \times 10^{-7} M ranitidine (B) on the histamine-induced positive chronotropic response with 1 min (●) or 60 min (▲) preincubation in the guinea-pig atria. (○): control response. Responses induced by 3 \times 10^{-5} M histamine before drugs treatment were taken as 100%. Each value is the mean±S.E. of 4 experiments.

Fig. 4. Effects of washout on the inhibitory actions of 3 \times 10^{-7} M NO-794 (A) and 1 \times 10^{-6} M ranitidine (B) in the guinea-pig atria. (○): control response; (●): response in the presence of drugs for 30 min, (▲): response after washout. Each value is the mean±S.E. of 4 experiments.
in guinea-pig ileum and the response to isoproterenol in guinea-pig atria (data not shown).

Effects of NO-794 and ranitidine on the histamine H2-receptor of guinea-pig gastric mucosal cells: When gastric mucosal cells were incubated with histamine, cellular cyclic AMP increased and showed a plateau phase at 10–30 min (Fig. 5). When NO-794 (1 x 10^{-7} M) was added at 0 time with histamine, the rise in cyclic AMP was reduced by 30% at 5 min and 75% at 30 min. In the presence of ranitidine (3 x 10^{-6} M), the content of cyclic AMP increased gradually to 30 min, and it reduced by 50% at 30 min (Fig. 5). When NO-794 and ranitidine were incubated with histamine for 20 min, respectively, the increase of cyclic AMP was inhibited concentration-dependently by these drugs, and concentration response curves of histamine were displaced to the right (Fig. 6A, B). The pA2 and slope of the Schild plot for ranitidine were 6.7±0.09, 0.9±0.10, respectively. The -log K_B for NO-794 was 8.4±0.11, which was calculated by the same method in the atria. When NO-794 was preincubated for various times before the addition of histamine, inhibition of NO-794 was time-dependent as in the atria (Fig. 7). The inhibition by NO-794 of the response to histamine was very weak for the 1 min preincubation, and maximal inhibition was achieved for the 10 min preincubation, while ranitidine showed the maximal inhibition for only the 1 min preincubation.

Because NO-794 showed apparent irreversible antagonism for the guinea-pig atrial histamine H2-receptor, we investigated the recovery of NO-794 and ranitidine from gastric histamine H2-receptor antagonism. After preincubation of cells with antagonists...
Fig. 7. Effects of NO-794 (A) and ranitidine (B) preincubation on the histamine-induced cyclic AMP production in the guinea-pig gastric mucosal cells. ○: absence of drugs; ●, △, ■: after preincubation for 1, 5 and 10 min with 1 x 10^{-7} M NO-794 or 3 x 10^{-6} M ranitidine. Gastric mucosal cells were incubated for 20 min with histamine. Antagonists were present both in the preincubation and incubation periods. The results represent the mean of two experiments. Each experiment was carried out in duplicate.

Fig. 8. Reversibility of histamine H2-antagonistic activities of NO-794 and ranitidine in the guinea-pig gastric mucosal cells. Gastric mucosal cells were preincubated in the absence (A) or presence of 1 x 10^{-7} M NO-794 (B) and 3 x 10^{-6} M ranitidine (C) for 10 min. After washout, subsequent incubation with histamine was carried out for 20 min without antagonists. The results represent the mean of two experiments. Each experiment was carried out in duplicate.

Fig. 8. Reversibility of histamine H2-antagonistic activities of NO-794 and ranitidine in the guinea-pig gastric mucosal cells. Gastric mucosal cells were preincubated in the absence (A) or presence of 1 x 10^{-7} M NO-794 (B) and 3 x 10^{-6} M ranitidine (C) for 10 min. After washout, subsequent incubation with histamine was carried out for 20 min without antagonists. The results represent the mean of two experiments. Each experiment was carried out in duplicate.

Effects of NO-794 and ranitidine on the gastric acid secretion in the pylorus ligated rats: Because it has been shown that NO-794 is a potent and selective histamine H2-receptor antagonist, we examined the antisecretory effect of NO-794 in vivo. When administered intraduodenally, NO-794 and ranitidine reduced dose-dependently gastric acid secretion in pylorus-ligated rats. The ED50 values of antagonists for reduction of gastric acid secretion were: NO-794, 0.9 (0.55-1.49, 95% Confidence limits) mg/kg; ranitidine, 12.5 (6.58-23.75) mg/kg. Thus, NO-794 was about 14 times as potent as ranitidine.

Discussion

NO-794 was a potent histamine H2-receptor antagonist on the guinea-pig atria and gastric mucosal cells. This antagonism was specific because NO-794 (1 x 10^{-6} M) did not show any remarkable interaction with histamine H1, muscarinic acetylcholine receptors of guinea-pig ileum and cardiac β1-adrenergic receptors of the guinea-pig. In the guinea-pig atria, NO-794 inhibited the response to histamine in an unsurmountable fashion. When the preparation was incubated with NO-794 for 60 min before histamine application, the maximal response to histamine was depressed by 20-30%. On this point, NO-794 differed from L-643441 in its effect on the guinea-pig atria; L-643441 depressed the maximal response by 90% when dimaprit was used as the agonist instead of histamine (8). In a preliminary
Fig. 9. Effects of NO-794 and ranitidine on the basal acid secretion in the pylorus-ligated rats. NO-794 and ranitidine were intraduodenally administered immediately after pylorus ligation and the gastric acid secretion was measured after 4 hr. Each value is the mean±S.E. of 10 animals. *: P<0.05, **: P<0.01, significantly different from the control.

experiment by us. NO-794 showed the same antagonism when dimaprit was used as the agonist; the maximal response was reduced by only 20–30%. Because the cause of this difference between NO-794 and L-643441 was unknown, NO-794 may interact with the same receptors as the agonist, but is unsurmountable by increasing the concentration of the agonist because of its own relatively slow or absent dissociation rate from the active site like in the case of L-643441 (8).

On the other hand, inhibition of NO-794 to the histamine H2-receptor of guinea-pig atria was time-dependent and apparently irreversible. Its inhibition profile was the same as other new histamine H2-receptor antagonists like as L-643441 (8), and it was different from the classical histamine H2-receptor antagonist ranitidine (9).

Gastric mucosal cells are suitable for evaluation of histamine H2-receptor antagonist, because they are easy to set up and devoid of the time-dependent factors which can influence the response of intact tissue (10). Thus we also examined the interaction of NO-794 on the gastric histamine H2-receptor of isolated guinea-pig gastric mucosal cells.

In guinea-pig gastric mucosal cells, NO-794 was a potent histamine H2-receptor antagonist as it was in atria. NO-794 was about 50 times more potent than ranitidine and 8 times more potent than L-643441. The pA2 value of ranitidine in this report (6.7) was in agreement with the value from a previous report (6.82) (11). In addition, NO-794 was an apparently irreversible antagonist, and the onset of inhibition was slow. Lamtidine (AH22216) showed same irreversible inactivation of histamine H2-receptors in the human gastric cancer cell line HGT-1 (12). In this report, it was shown that NO-794 is an apparently irreversible histamine H2-receptor antagonist not only in atria but also in gastric mucosal cells.

L-643441 inhibited the response mediated by histamine H2-receptor by the same order of magnitude in guinea-pig atria and gastric mucosal cells; pA2 values were 7.7 and 7.5, respectively (8, 10). In contrast, antagonism of NO-794 was stronger in gastric mucosal cells than in atria: -log KA values were 7.5 in the atria and 8.4 in gastric mucosal cells. The reason for this is unknown, but this profile may be beneficial for use in treating peptic ulcer.

In addition, NO-794 inhibited gastric acid secretion in pylorus ligated rats. This result showed that NO-794 has a potent antisecretory effect when administered intra-
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duodenally. Since at the present we do not know whether the action of NO-794 is long lasting in vivo, further studies should be done.

In conclusion, NO-794 is a powerful and unique histamine $H_2$-receptor antagonist and may be useful in the treatment of peptic ulcer disease.

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