THE EXCITATORY EFFECT OF THE NEW HISTAMINE H₂-RECEPTOR ANTAGONIST NIZATIDINE (LY 139037) ON THE GUINEA PIG ILEUM

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The histamine H₂-receptor antagonist, nizatidine (LY 139037), was tested for its effect on the intestinal smooth muscle. Isolated segments of guinea pig ileum were used in Tyrode solution at 37 °C. Nizatidine (from 3.2 × 10⁻⁸ to 3.2 × 10⁻⁴ M) caused a concentration-dependent contractile response by the guinea pig ileum. The average maximum response to nizatidine (3.2 × 10⁻⁴ M) was about 89% of the average maximum response to eserine (3.2 × 10⁻⁶ M). The contractile responses induced by nizatidine were not modified by pyrilamine (10⁻⁸ and 10⁻⁷ M). On the other hand, atropine (10⁻⁸ and 3.2 × 10⁻⁸ M) significantly prevented, while eserine (10⁻⁸ and 3.2 × 10⁻⁸ M) significantly enhanced the nizatidine-induced responses in a concentration-dependent manner. These findings suggest that nizatidine exerts an excitatory effect on the guinea pig ileum which seems to be associated with the cholinergic system, probably through a direct and/or an indirect mechanism (inhibition of acetylcholinesterase and/or increased release of acetylcholine).

Keywords — nizatidine; pyrilamine; atropine; eserine; isolated guinea pig ileum

INTRODUCTION

Nizatidine (LY 139037) is a new potent histamine H₂-receptor antagonist. This agent (Fig. 1) is an analogue of other potent histamine H₂-receptor antagonists with ring side chains of ranitidine and the thiazole ring of tiotidine (ICI 125 211). The latter drug has been removed from further testing following reports of malignant lesions in rats with chronic use.¹,²) Nizatidine has been reported to have a potency similar to that of ranitidine in reducing gastric acid production.³) It has also been shown in previous studies that ranitidine elicits a marked excitatory effect on different parts of the gastrointestinal tract from several species.⁴⁻¹⁰) The present study was designed to examine the effect of nizatidine on the intestinal smooth muscle.

MATERIALS AND METHODS

Preparation of the Isolated Guinea Pig Ileum

---Hartly strain albino guinea pigs of either sex (weighing 400 to 500 g) were killed by a blow on the head and exsanguinated. Ileal segments (3 cm long) were taken 15 to 30 cm from the ileocecal junction. The segments were suspended in 15 ml organ baths, containing Tyrode solution which was bubbled constantly with a mixture of 95% O₂-5% CO₂ gas and maintained at 37 °C. The composition (mmol/l) of the Tyrode solution was: NaCl, 136.90; KCl, 2.68; CaCl₂, 1.80; MgCl₂, 1.05; NaHCO₃, 11.90; NaH₂PO₄, 0.42 and glucose, 5.55. The ileal preparations were loaded (500 mg) and allowed to be stabilized for a period of 30 min before any compound was added. During this period, the preparations were washed with fresh Tyrode solution every 10 min. The isotonic muscle responses of the preparations were recorded by means of isotonic myograph transducers (NARCO Co., U.S.A.) and a Physiograph (desk model type DMP-4A, NARCO Co., U.S.A.).

Drugs --- The following compounds were used: eserine sulfate (Calbiochem-Behring, U.S.A.), nizatidine (LY 139037, Eli Lilly, U.S.A.), pyrilamine maleate (Sigma Chem. Co., U.S.A.) and atropine sulfate (Chropee, Greece). The solutions of these compounds were freshly prepared, before each experiment, using Tyrode solution as a solvent.

FIG. 1. Chemical Structure of Nizatidine
Construction of Concentration-Response Curves — After the 30 min stabilization period, the preparations were exposed to cumulatively increasing concentrations of eserine (from $10^{-8}$ to $3.2 \times 10^{-6}$ M) and nizatidine (from $10^{-6}$ to $10^{-5}$ M) to obtain full concentration response curves.

In another series of experiments the preparations were exposed to cumulatively increasing concentrations of nizatidine (from $10^{-6}$ to $3.2 \times 10^{-4}$ M) and then were exposed to nizatidine 3 min after pretreatment with pyrilamine ($10^{-8}$ and $10^{-7}$ M), atropine ($10^{-8}$ and $3.2 \times 10^{-8}$ M) or eserine ($10^{-8}$ and $3.2 \times 10^{-8}$ M). The contact time of each cumulatively increasing concentration of the above compounds with the preparations was 2 min.

Statistical Analysis — The results were expressed as a percentage of the maximum response induced by the control and the other responses were calculated as a percentage of the maximum response. Statistical evaluation of the data was performed using Student’s t-test. The data were expressed as the mean ± S.E.M. p-values of < 0.05 were considered to be significant.

RESULTS

Responsiveness to Eserine and Nizatidine

The addition of eserine (from $10^{-8}$ to $3.2 \times 10^{-6}$ M) and nizatidine (from $3.2 \times 10^{-6}$ to $3.2 \times 10^{-4}$ M) to the organ bath fluid caused a concentration-dependent contractile response by the guinea pig ileum. The average maximum response (mean ± S.E.M.) caused by nizatidine at the concentration of $3.2 \times 10^{-4}$ M was 89.44 ± 5.40% of the average maximum response of eserine at the concentration of $3.2 \times 10^{-6}$ M (Fig. 2).

Responsiveness to Nizatidine after Pretreatment with Pyrilamine, Atropine or Eserine

Pyrilamine (at concentrations of $10^{-8}$ and $10^{-7}$ M) did not modify the concentration-response curve of nizatidine (Fig. 3). Atropine (at

![Fig. 2. Cumulative Concentration-Response Curves to Eserine (▲) and Nizatidine (●) by Isolated Guinea Pig Ileum](image)

The ordinate is expressed as a percentage of the maximum response induced by eserine (control). Each point represents the mean ± S.E.M. obtained from 30 preparations for eserine and 48 for nizatidine. All values from the concentration of $3.2 \times 10^{-8}$ M for eserine, and from the concentration of $3.2 \times 10^{-8}$ M for nizatidine were significant ($p < 0.05$).

![Fig. 3. Cumulative Concentration-Response Curves to Nizatidine Alone (●) and to Nizatidine in the Presence of Pyrilamine at Concentrations of $10^{-8}$ (□) and $10^{-7}$ M (△)](image)

The ordinate is expressed as a percentage of the maximum response induced by nizatidine alone (control). Each point represents the mean ± S.E.M. obtained from 48 preparations for nizatidine alone and 6 for nizatidine in the presence of each concentration of pyrilamine.
concentrations of $10^{-8}$ and $3.2 \times 10^{-8}$ M) significantly prevented the nizatidine-induced responses in a concentration-dependent manner. This prevention produced a right-ward shift of the concentration–response curve for nizatidine with marked depression of the maximum response (Fig. 4). On the other hand eserine (at concentrations of $10^{-8}$ and $3.2 \times 10^{-8}$ M) significantly enhanced the nizatidine produced contractile responses (Fig. 5).

**DISCUSSION**

Smooth muscle can be stimulated by direct or indirect activation of various excitatory receptors. In our investigation it was shown that the new histamine $H_3$-receptor antagonist nizatidine exerts a concentration-dependent excitatory effect on the smooth muscle of the guinea pig ileum. This effect is similar to that of the histamine $H_3$-receptor antagonist ranitidine on different parts of the gastrointestinal tract from several species.\textsuperscript{4-10} It is worth mentioning here that in a previous investigation concerning isolated segments of guinea pig ileum under the same experimental conditions, it was found that the ranitidine maximum response (at the concentration of $3.2 \times 10^{-4}$ M) was about 90% against eserine (at the concentration of $3.2 \times 10^{-6}$ M),\textsuperscript{10} while in the present study it was found that the nizatidine maximum response (at the concentration of $3.2 \times 10^{-4}$ M) was about 89% against eserine. These data indicate that the maximum activity of nizatidine is similar to that of ranitidine and both of them resemble eserine in their activity but in a weaker manner.

It is generally accepted that receptors mediating the histamine induced stimulating effect on the guinea pig ileum are of the $H_1$ type.\textsuperscript{11} In this study it was found that pyrilamine, an $H_1$-receptor antagonist, did not modify the responses of the guinea pig ileum caused by nizati-
dine. These results rule out the possibility that nizatidine may be stimulating the H\textsubscript{2}-receptors. On the other hand atropine, an antimuscarinic agent, significantly prevented the responses caused by nizatidine, in a concentration-dependent manner. This prevention of nizatidine caused by atropine is similar to that of ranitidine as it was noticed in previous studies performed by us and other workers.8,10 These data suggest that nizatidine, like ranitidine, could be acting through a cholinergic mechanism. Eserine, an anticholinesterase agent, significantly enhanced the nizatidine-induced excitatory effect, in a concentration-dependent manner, by inhibiting the action of acetylcholinesterase enzyme. Thus a greater availability of acetylcholine at the receptor sites produced enhancement of the guinea pig ileum responses.

The conclusion which may be drawn is that nizatidine exerts an excitatory effect on the guinea pig ileum which seems to be associated with the cholinergic system, probably through a direct and/or indirect mechanism (inhibition of acetylcholinesterase and/or increased release of acetylcholine).

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REFERENCES