**Inhibition of Acetylcholinesterase by the H₂-Receptor Antagonist Nizatidine**

Georgios KOUNENIS, Dimitrios VOUTSAS, Maria KOUTSOVITI-PAPADOPOULOU, and Vassilios ELEZOGLOU

Department of Pharmacology, Veterinary Faculty, Aristotelian University of Thessaloniki, 54006 Thessaloniki, Greece

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The purpose of the present investigation was to study if the stimulating effect of nizatidine on the intestinal smooth muscle is related to the acetylcholinesterase activity. Isolated segments of guinea pig ileum were used in Tyrode solution at 37 °C. Nizatidine (from 3.2 × 10⁻⁴ to 3.2 × 10⁻⁶ M) exerted a concentration-dependent contractile effect on the guinea pig ileum. The average maximum activity (mean ± S.E.M.) of nizatidine (3.2 × 10⁻⁴ M) was 96.21 ± 6.19% of the average maximum activity of neostigmine (3.2 × 10⁻⁷ M). The acetylcholine-induced contractions (from 10⁻⁶ to 3.2 × 10⁻⁴ M) were augmented by nizatidine (from 10⁻⁵ to 10⁻⁴ M) in a similar way to that by neostigmine (from 10⁻⁷ to 10⁻⁸ M). The acetylcholine-induced contractions were prevented by acetylcholinesterase (0.1 unit/ml). This acetylcholinesterase activity was inhibited by nizatidine (from 10⁻⁸ to 10⁻⁴ M) and this inhibition was similar to that of neostigmine (from 10⁻⁸ to 10⁻⁷ M). These findings suggest that the stimulating effect of nizatidine on the intestinal smooth muscle of the guinea pig ileum could be explained by the inhibition of acetylcholinesterase activity.

**Keywords** — acetylcholinesterase activity; isolated guinea pig ileum; nizatidine; neostigmine

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**Introduction**

Nizatidine is a new histamine H₂-receptor antagonist. This agent has been synthesized and shows a potency in reducing gastric acid production.¹⁻⁴ In our previous studies it was found that nizatidine elicits a marked excitatory effect on the isolated segments of the guinea pig ileum⁵ and on the longitudinal and circular muscle strips from the rabbit ascending colon.⁶ This excitatory effect of nizatidine on the intestinal smooth muscle was prevented by atropine, an antimuscarinic agent, while it was enhanced by eserine, an anticholinesterase agent.⁵ These data suggest that nizatidine could be acting through a cholinergic mechanism. It has also been reported that histamine antagonists, either the H₁-receptor antagonists, promethazine⁷ and mepyramine,⁸ or the H₂-receptor antagonists, cimetidine,⁸,⁹ oxmetidine⁸ and ranitidine,⁸,¹⁰,¹¹ inhibit the acetylcholinesterase activity.

On the basis of these findings we decided to investigate if the observed excitatory effect of nizatidine on the intestinal smooth muscle is related to an inhibition of the acetylcholinesterase activity. Neostigmine was used as the classical standard anticholinesterase agent for comparison.

**Materials and Methods**

**Preparations of the Guinea Pig Ileum** — Harlt strain albino guinea pigs of either sex weighing approximately 500 g were killed by a severe blow on the head and exsanguinated. Segments of ileum (3 cm long) were taken 15 to 30 cm from the ileocecal junction. The segments were mounted in 15 ml organ baths containing Tyrode solution of the following composition (mM): NaCl, 136.90; KCl, 2.68; CaCl₂, 1.80; MgCl₂, 1.05; NaHCO₃, 11.90; NaH₂PO₄, 0.42 and glucose 5.55. The solution was bubbled constantly with a mixture of 95% O₂ - 5% CO₂ gas and maintained at a temperature of 37 °C. A resting tension of 500 mg was applied to the ileal preparations and they were allowed to stabilize for a period of 30 min before any compound addition. 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 recorder (desk model type

Drugs — The following compounds were used: nizatidine (LY 139037, Eli Lilly, U.S.A.), neostigmine methyl sulfate (F. Hoffmann-La Roche S.A., Switzerland), acetylcholine chloride (E. Merck, Darmstadt) and an aqueous solution of acetylcholinesterase (EC 3.1.1.7) from electric eel (0.65 mg protein/ml, 970 units/mg protein, Sigma Chemical Co., U.S.A.). The solutions of nizatidine, neostigmine and acetylcholine were freshly prepared, before each experiment, using Tyrode solution as a solvent. All solutions were added directly to the organ baths in a volume of up to 100 μl.

Concentration-Response Curves — After the 30 min stabilization period, the preparations were exposed to cumulatively increasing concentrations of neostigmine (from 10⁻⁸ to 3.2 × 10⁻⁶ M) and nizatidine (from 10⁻⁶ to 10⁻³ M) to obtain full concentration-response curves.

In a second series of experiments, the preparations were exposed to cumulatively increasing concentrations of acetylcholine (from 10⁻⁸ to 3.2 × 10⁻⁶ M) and then they were exposed to acetylcholine 3 min after pretreatment with nizatidine (at concentrations of 10⁻⁵, 3.2 × 10⁻⁵ and 10⁻⁴ M) and neostigmine (at concentrations of 10⁻⁸, 3.2 × 10⁻⁸ and 10⁻⁷ M).

In a third series of experiments, the preparations were exposed to cumulatively increasing concentrations of acetylcholine (from 10⁻⁸ to 3.2 × 10⁻⁶ M) and then they were exposed to acetylcholine 3 min after pretreatment with acetylcholinesterase (at the concentration of 0.1 unit/ml of the organ bath fluid), nizatidine (at concentrations of 10⁻⁵, 3.2 × 10⁻⁵ and 10⁻⁴ M) plus acetylcholinesterase (at the concentration of 0.1 unit/ml) and neostigmine (at concentrations of 10⁻⁸, 3.2 × 10⁻⁸ and 10⁻⁷ M) plus acetylcholinesterase (at the concentration of 0.1 unit/ml).

The contact time of each cumulatively increasing concentration of neostigmine, nizatidine and acetylcholine with the preparations was 2 min.

Statistical Analysis of Results — The maximum response induced by the control agent was designated 100% and the other responses were calculated as a percentage of this maximum response. Statistical evaluation of the data was performed using Student’s t-test for paired or unpaired data when appropriate. The data were expressed as mean ± S.E.M., and p values of <0.05 were considered to be significant.

Results

Responsiveness to Neostigmine and Nizatidine

The addition of neostigmine (from 10⁻⁸ to 3.2 × 10⁻⁶ M) and nizatidine (from 10⁻⁶ to 3.2 × 10⁻⁴ M) in the organ bath fluid exerted a concentration-dependent stimulating effect on the guinea pig ileum. The significant stimulating effect started from a threshold concentration of 10⁻⁸ M for neostigmine and 3.2 × 10⁻⁶ M for nizatidine. The average maximum response (mean ± S.E.M.) caused by nizatidine at the concentration of 3.2 × 10⁻⁴ M was 96.21 ± 6.19% of the average maximum activity of neostigmine at the concentration of 3.2 × 10⁻⁶ M (Fig. 1).

Responsiveness to Acetylcholine after Pretreatment with Nizatidine and Neostigmine

The contractile responses induced by acetyl-
Acetylcholinesterase and Nizatidine

Fig. 2. Cumulative Concentration-Response Curves to Acetylcholine Alone (●) and to Acetylcholine in the Presence of Nizatidine at the Concentrations of $10^{-5}$ (○), $3.2 \times 10^{-5} (\triangle)$ and $10^{-4} \text{M} (□)

The ordinate is expressed as a percentage of the maximum response induced by acetylcholine (control). Each point represents the mean ± S.E.M. obtained from 15 preparations for acetylcholine alone and 5 for acetylcholine in the presence of each concentration of nizatidine. a) Shows significant augmentation of response to acetylcholine caused by nizatidine ($p < 0.05$).

choline (from $10^{-8}$ to $3.2 \times 10^{-6} \text{M}$) were augmented by the pretreatment with nizatidine (at concentrations of $10^{-5}$, $3.2 \times 10^{-5}$ and $10^{-4} \text{M}$) and neostigmine (at concentrations of $10^{-8}$, $3.2 \times 10^{-8}$ and $10^{-7} \text{M}$) in a concentration-dependent manner. This augmentation of response to acetylcholine caused by nizatidine was similar to that of neostigmine at the above-mentioned concentrations (Figs. 2 and 3).

Responsiveness to Acetylcholine after Pretreatment with Acetylcholinesterase, Nizatidine plus Acetylcholinesterase and Neostigmine plus Acetylcholinesterase

The contractile responses induced by acetylcholine (from $10^{-8}$ to $3.2 \times 10^{-6} \text{M}$) were significantly prevented by the pretreatment with acetylcholinesterase (at the concentration of 0.1 unit/ml of the organ bath fluid). This acetylcholinesterase activity on the acetylcholine-induced

Fig. 3. Cumulative Concentration-Response Curves to Acetylcholine Alone (●) and to Acetylcholine in the Presence of Neostigmine at the Concentrations of $10^{-5}$ (○), $3.2 \times 10^{-5} (\triangle)$ and $10^{-4} \text{M} (□)

The ordinate is expressed as a percentage of the maximum response induced by acetylcholine (control). Each point represents the mean ± S.E.M. obtained from 18 preparations for acetylcholine alone and 6 for acetylcholine in the presence of each concentration of neostigmine. a) Shows significant augmentation of response to acetylcholine caused by neostigmine ($p < 0.05$).

Fig. 4. Cumulative Concentration-Response Curves to Acetylcholine Alone (●); to Acetylcholine in the Presence of Acetylcholinesterase at the Concentration of 0.1 unit/ml (■) and to Acetylcholine in the Presence of Acetylcholinesterase at the Concentration of 0.1 unit/ml plus Nizatidine at the Concentrations of $10^{-5}$ (○), $3.2 \times 10^{-5} (\triangle)$ and $10^{-4} \text{M} (□)

The ordinate is expressed as a percentage of the maximum response induced by acetylcholine (control). Each point represents the mean ± S.E.M. obtained from 18 preparations for acetylcholine alone; 18 for acetylcholine in the presence of acetylcholinesterase and 6 for acetylcholine in the presence of acetylcholinesterase plus each concentration of nizatidine. a) Shows significant decreases of the acetylcholinesterase inhibition caused by nizatidine ($p < 0.05$).
excitatory effect of nizatidine is in accordance to those which were reported in our previous studies on the isolated segments of guinea pig ileum\(^5\) and on the longitudinal and circular smooth muscle strips from the rabbit ascending colon.\(^6\) In the present study it was also found that nizatidine augmented the action of acetylcholine and this augmentation was similar to that caused by neostigmine. Besides, in a previous investigation concerning isolated segments of guinea pig ileum under the same experimental conditions, it was indicated that atropine, an antimuscarinic agent, produced a substantial prevention of the responses caused by nizatidine, while eserine, an anticholinesterase agent, augmented the nizatidine-induced contraction-s.\(^5\) These findings suggest that nizatidine could be acting through a cholinergic mechanism. According to our previous hypothesis,\(^5,6\) that nizatidine may indirectly act through the cholinergic pathway causing the inhibition of acetylcholinesterase, which modulates the reactivity of intestinal smooth muscle, we studied the effect of this compound on the acetylcholinesterase activity and we found that this activity was clearly inhibited by nizatidine in a concentration-dependent manner. This inhibitory action of nizatidine was similar to that of neostigmine; therefore these data verify the above-mentioned hypothesis. It has also been demonstrated spectrophotometrically that histamine antagonists, either the H\(_1\)-receptor antagonists, promethazine\(^7\) and mepyramine,\(^8\) or the H\(_2\)-receptor antagonists, cimetidine,\(^6,9\) oxmetidine\(^6\) and ranitidine,\(^8,10\) have such an anticholinesterase activity as well. Additionally, the ranitidine anticholinesterase activity has also been revealed on isolated segments of guinea pig ileum.\(^11\) It is worth mentioning here that the anticholinesterase activity of nizatidine has a potency comparable to that of ranitidine, which was examined in a previous study.\(^11\)

The conclusion, which may be drawn, is that the contractile effect of nizatidine on the intestinal smooth muscle could be explained by the inhibition of acetylcholinesterase activity. This substantial anticholinesterase activity of nizatidine is a side effect and may modify the intestinal motility leading to diarrhea and constipation, as

**Discussion**

The H\(_2\)-receptor antagonist nizatidine was found to possess a concentration-dependent stimulating effect on the isolated segments of guinea pig ileum with a maximum response not significantly lower than that caused by neostigmine, but the threshold concentration of nizatidine was higher than that of neostigmine. This concentration-responses was inhibited by pretreatment with nizatidine (at concentrations of \(10^{-5}, 3.2 \times 10^{-5}\) and \(10^{-4}\) M) and neostigmine (at concentrations of \(10^{-8}, 3.2 \times 10^{-8}\) and \(10^{-7}\) M) in a concentration-dependent manner. This inhibition of acetylcholinesterase activity caused by nizatidine was similar to that of neostigmine at the above-mentioned concentrations (Figs. 4 and 5).
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has been observed in a very small percentage of patients after treatment with a single oral dose of ranitidine.  

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References


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