

Stimulation by nizatidine, a histamine H₂-receptor antagonist, of duodenal HCO₃⁻ secretion in rats: relation to anti-cholinesterase activity

Koji Takeuchi¹, Shoji Kawauchi¹, Hideo Araki¹, Shigeru Ueki¹ and Osamu Furukawa²

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Abstract

AIM To examine whether nizatidine stimulates duodenal HCO₃⁻ secretion in rats by inhibiting AChE activity.

METHODS Under pentobarbital anesthesia, a proximal duodenal loop was perfused with saline, and the HCO₃⁻ secretion was measured at pH 7.0 using a pH-stat method and by adding 10mM HCl. Nizatidine, neostigmine, carbachol or famotidine was administered i.v. as a single injection.

RESULTS Intravenous administration of nizatidine (3 - 30mg/kg) dose-dependently increased duodenal HCO₃⁻ secretion, and the effect at 10mg/kg was equivalent to that obtained by carbachol at 0.01mg/kg. This nizatidine action was observed at the same dose range that inhibited acid secretion and enhanced gastric motility, mimicked by i.v. injection of neostigmine (0.03mg/kg), and significantly attenuated by bilateral vagotomy and prior s.c. administration of atropine but not by indomethacin, a cyclooxygenase inhibitor, or N^G-nitro-L-arginine methyl ester, a NO synthase inhibitor. The HCO₃⁻ secretory response to acetylcholine (0.001mg/kg) was significantly potentiated by the concurrent administration of nizatidine (3mg/kg, i.v.). The IC₅₀ of

nizatidine for AChE of rat erythrocytes was 1.4×10⁻⁶M, about 12 times higher than that of neostigmine. Neither famotidine (>10⁻³M, 30mg/kg, i.v.) nor cisapride (>10⁻³M, 3mg/kg, i.v.) had any influence on AChE activity or duodenal HCO₃⁻ secretion. Duodenal damage induced by acid perfusion (100mM HCl for 4h) in the presence of indomethacin was significantly prevented by nizatidine and neostigmine, at the doses that increased the HCO₃⁻ secretion.

CONCLUSION Nizatidine stimulates duodenal HCO₃⁻ secretion, in both vagal-dependent and atropine-sensitive manners, and the action is associated with the anti-AChE activity of this agent.

INTRODUCTION

Nizatidine, a histamine H₂-receptor antagonist (N-[2-[[[2-[(dimethylamino) methyl]-4-thiazolyl]-methyl]thio]ethyl]-N"-methyl-2-nitro-1,1-ethenediamine), has been shown to have a potent antisecretory action and clinically ascertained to be effective for peptic ulcers as well as gastroesophageal reflux diseases^[1-3]. Of interest, an additional effect of H₂-antagonists on gastrointestinal motility has been reported, besides the antisecretory activities^[4,5]. Indeed, some H₂-antagonists including nizatidine exhibit a potent anti-acetylcholinesterase (AChE) activity^[6-9] and, as a result of this action, facilitate gastrointestinal motor activity in experimental animals and in humans^[10,11].

On the other hand, duodenal mucosal HCO₃⁻ secretion is a key process that aids in preventing acid-peptic injury^[12,13]. The mechanisms that govern mucosal HCO₃⁻ secretion include neuro-humoral factors and luminal acid^[12-14]. The ability of the mucosa to respond to acid seems especially important in the maintenance of the surface pH gradient and in the protection of mucosa. This process is also mediated by endogenous prostaglandins (PGs) as well as neuronal factors

¹Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan

²CURE, Bldg.114, Suite 217 West LA VAMC 11301 Wilshire Blvd. Los Angeles, CA 90073

Correspondence to: Koji Takeuchi, Ph.D., Department of Pharmacology and Experimental Therapeutics Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan
Tel. 0081-75-595-4680, Fax. 0081-75-595-4774
Email. takeuchi@mb.kyoto-phu.ac.jp

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including vagal-cholinergic mechanisms^[15-17]. Several investigators reported that cholinomimetic drugs increased duodenal HCO_3^- secretion, either directly or indirectly mediated by vasoactive intestinal polypeptide (VIP)^[17]. We also reported that both carbachol and bethanechol stimulated the HCO_3^- secretion, mediated by M_3 but not M_1 receptors^[18]. Because inhibition of AChE activity increases the availability of endogenous acetylcholine, it is possible that nizatidine might increase duodenal HCO_3^- secretion through inhibition of AChE activity. However, the effect of nizatidine on the HCO_3^- secretion has not been studied.

This study was undertaken to confirm the anti-AChE activity of nizatidine *in vitro*, and investigate the effect of this agent on HCO_3^- secretion in the rat duodenum, in comparison with the other H_2 -antagonist famotidine and the AChE inhibitor neostigmine. In addition, we also examined whether the doses sufficient to stimulate the HCO_3^- secretion are comparable to the gastroprokinetic and antisecretory doses.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 200g-230g (Charles River, Shizuoka, Japan), were used in all experiments. The animals kept in individual cages with raised mesh bottoms were deprived of food but allowed free access to tap water for 18h before the experiments. Studies were carried out with 4-6 rats per group under anesthetized conditions induced by pentobarbital Na (30 mg/kg, i.v.), unless otherwise specified. All experimental procedures described here were approved by the Experimental Animal Research Committee of the Kyoto Pharmaceutical University.

Determination of duodenal HCO_3^- secretion

Duodenal HCO_3^- secretion was determined in the duodenal loop according to a previously published method^[16]. In brief, the abdomen was incised, and the stomach and duodenum were exposed. The duodenal loop (1.7cm) was made between the pylorus and the area just proximal to the outlet of the common bile duct, excluding the influences of bile acid and pancreatic juice. Then, the loop was perfused at a flow rate of 0.8mL/min with saline that was gassed with 100% O_2 and kept in a reservoir, and HCO_3^- secretion was measured at pH 7.0 using a pH-stat method and by adding 10mM HCl to the reservoir. Allowing 30-40min for stabilization of basal HCO_3^- secretion, the duodenal HCO_3^- secretory responses were examined for 2h after the following treatment; nizatidine (3-30 mg/kg), carbachol (0.003 mg/kg), neostigmine (0.03 mg/kg), famotidine (10 mg/kg) or

cisapride (3mg/kg). These drugs were administered i.v. as a single injection. In some cases, nizatidine (3 mg/kg) and acetylcholine (0.001 mg/kg) were administered i.v. simultaneously. In some cases, atropine (1mg/kg, s.c.), indomethacin (5 mg/kg, s.c.) or N-G-nitro-L-arginine methyl ester (L-NAME) the NO synthase inhibitor (5mg/kg, i.v.) was given 1h or 10min, respectively, before administration of nizatidine, carbachol or neostigmine. In a separate experiment, bilateral vagotomy was performed at the cervical portion 2h before administration of nizatidine, neostigmine or carbachol.

Determination of gastric acid secretion

Gastric acid secretion was measured in a chambered stomach, according to a previously published method^[19]. Briefly, the abdomen was incised, and both the stomach and duodenum were exposed. Then, the stomach was mounted in an *ex-vivo* chamber and perfused at a flow rate of 0.8mL/min with saline that was gassed with 100% O_2 , heated at 37°C and kept in a reservoir. The acid secretion was measured at pH 7.0 using a pH-stat method (Hiranuma Comtite-8, Tokyo, Japan) by adding 100mM NaOH to the reservoir. After basal acid secretion had well stabilized, the acid secretion was stimulated by continuous i.v. infusion of histamine (4mg/kg/h). Nizatidine (10 and 30mg/kg) and famotidine (10mg/kg) were administered i.v. as a single injection 1h after the onset of histamine infusion, when the acid secretory response to histamine had reached a plateau.

Measurement of gastric motility

Gastric motility was determined using a miniature balloon in conscious rats, according to a previously published method^[20]. Briefly, under ether anesthesia the balloon and the support catheter were placed in the stomach through an incision of the forestomach. The animals were kept in Bollman cages, and gastric motility was monitored on a Hitachi recorder (Model 056, Mito, Japan) using a pressure transducer (Narco Telecare, Model 151-T, Houston, TX., U.S.A.) and a polygraph device (San-ei, Model 6M-72, Tokyo, Japan) after complete recovery from anesthesia. After basal motility had well stabilized, the animals were administered i.v. with nizatidine (30 mg/kg), neostigmine (0.03mg/kg), cisapride (3mg/kg) or famotidine (10 mg/kg), and the motility was measured for 2h thereafter. In some cases, the effect of atropine (1mg/kg, s.c.) was examined on the enhanced gastric motility in response to nizatidine (30mg/kg, i.v.).

Induction of duodenal mucosal damage by acid perfusion

The increased HCO_3^- secretion caused by nizatidine might lead to decrease of the mucosal susceptibility

to acid injury. To test this possibility, we examined the effect of nizatidine on the mucosal ulcerogenic response by perfusing the duodenum with 100mM HCl at the flow rate of 1mL/h for 4h in the presence of indomethacin. The experiment was performed in the duodenal loop preparation, similar to that described for HCO₃⁻ secretion. Animals were first treated with indomethacin (5 mg/kg, s.c.), and 1h later the duodenum was perfused with acid for 4h. Nizatidine (10mg/kg) was administered i.v. 20min before the onset of acid perfusion. In comparison, the animals were treated i.v. with neostigmine (0.03mg/kg) or famotidine (10mg/kg). The duodenums were removed, inflated by injecting 0.5mL of 2% formalin, immersed in 2% formalin for 10min to fix the tissue wall, opened along the mesenteric artery, and examined for lesions under a dissecting microscope with a square grid (×10). The area (mm²) of each lesion was measured, summed per duodenum, and used as a damage score. The person measuring the lesions did not know the treatment given to the animals.

Determination of anticholinesterase activity

Erythrocyte membranes and plasma each from rats were prepared to obtain both AChE and pseudocholinesterase (PChE), according to the method described by Hansen and Bert^[6]. Before the determination of anti-ChE activity, it was confirmed that PChE was not present in the erythrocyte membranes by using the PChE inhibitor, profenamine. The anti-ChE activity of H₂-receptor antagonists and neostigmine was determined by the modified method of Ellman *et al*^[21]. In brief, a reaction mixture was prepared to contain, in a total volume of 1mL, 0.1M sodium phosphate buffer (pH 8.0), 1mM acetylcholine, 0.3mM 5,5-dithio-bis (2-nitrobenzoic acid) and 1mU ChE. The enzyme activity was determined by tracing the changes in absorbance at 412nm at 30°C on a spectrophotometer (Model 320, Hitachi, Ibaragi, Japan) for a period of 70 sec after adding acetylthiocholine. The amount of enzyme required to convert 1μmol of acetylthiocholine within 1min under the above conditions was taken as 1 unit. Anti-enzyme activity was measured by adding 10μL of each test drug solution to the reaction mixture. The concentration of test drug required to inhibit 50% of the enzyme activity (IC₅₀) was calculated from the enzyme inhibition curve.

Preparation of drugs

Drugs used were pentobarbital Na, neostigmine bromide, histamine 2HCl, carbachol, acetylcholine (Nacalai tesque, Kyoto, Japan), nizatidine (Zeria Pharm. Co., Saitama, Japan), famotidine (Gaster R, Yamanouchi Pharm. Co. Tokyo, Japan), atropine, indomethacin, NG-nitro-L-arginine methyl ester (Sigma Chemicals, Saint Louis, Mo.,

USA) and cisapride (Synthesized by Zeria Pharm. Co.). For *in vivo* experiments, each drug except indomethacin was dissolved in or diluted with saline. Indomethacin was suspended in saline with a drop of Tween 80 (Wako, Osaka, Japan). Each drug was administered i.v. in a volume of 1mL/kg or s.c. in a volume of 5mL/kg, or by i.v. infusion in a volume of 1.2mL/h. For *in vitro* experiments, each drug was prepared in purified water or equimolar hydrochloric acid solution. In all experiments, solvents alone were used as controls.

Statistics

Data are presented as the means±SE from 4-6 rats per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of *P*<0.05 were regarded as significant.

RESULTS

Effects of nizatidine on duodenal HCO₃⁻ secretion

Under the present experimental conditions, the rat duodenum spontaneously secreted HCO₃⁻ at a steady rate of 1.0-1.2 μEq/15 min during a 3 h test period. Intravenous administration of nizatidine (3-30mg/kg) caused an increase of the HCO₃⁻ secretion in a dose-dependent manner (Figure 1). At 10 mg·kg⁻¹, the H₂ antagonist nizatidine increased the HCO₃⁻ secretion from 1.2 μEq/15 min to a plateau level of 1.8-2.0 μEq/15 min within 30 min, remaining elevated for 2 h, the ΔHCO₃⁻ output being 5.6 ± 1.4 μEq/2h. Likewise, duodenal HCO₃⁻ secretion was significantly increased in response to i.v. administration of carbachol (0.01mg/kg) and neostigmine (0.03 mg/kg), the ΔHCO₃⁻ output being 4.9 ± 1.1 μEq/2h and 5.2 ± 0.8 μEq/2h, respectively, both of which were almost equivalent to that induced by nizatidine at 10mg/kg (Figure 2). By contrast, neither famotidine the H₂-antagonist (10mg/kg, i.v.) nor cisapride the gastroprokinetic drug (3mg/kg, i.v.) had any influence on basal rates of duodenal HCO₃⁻ secretion (Figures 2 and 3).

The HCO₃⁻ secretory responses induced by both nizatidine (10 mg/kg, i.v.), neostigmine (0.03mg/kg, i.v.) and carbachol (0.01 mg/kg, i.v.). Were all significantly inhibited by prior s.c. administration of atropine (1 mg/kg) (Figure 4). This agent had a minimal effect on the basal HCO₃⁻ secretion without any treatment, but almost totally attenuated the increase of HCO₃⁻ secretion induced by either nizatidine, neostigmine or carbachol; the ΔHCO₃⁻ output remained unchanged before and at all time points after administration of these drugs. Likewise, bilateral vagotomy significantly reduced the HCO₃⁻ secretory response to nizatidine and neostigmine but not carbachol. On the other hand, the pretreatment of tie animals with neither

indomethacin (5mg/kg, s.c.) nor L-NAME (5mg/kg, i.v.) significantly affected the HCO_3^- secretory response induced by nizatidine (not shown).

Effect of the combined treatment of nizatidine and acetylcholine on duodenal HCO_3^- secretion

To further investigate the relation of anti-AChE activity of nizatidine with the HCO_3^- stimulatory action, we examined whether or not the acetylcholine-induced HCO_3^- response was potentiated by co-administration of nizatidine. As shown in Figure 5, acetylcholine (0.001 mg/kg) caused a slight but significant increase in duodenal HCO_3^- secretion, while nizatidine at 3 mg·kg⁻¹ tended to increase the secretion; the ΔHCO_3^- output was $1.8 \pm 0.1 \mu\text{Eq}/2\text{h}$ and $0.7 \pm 0.6 \mu\text{Eq}/2\text{h}$, respectively. However, when nizatidine was administered together with acetylcholine, the HCO_3^- secretion was markedly increased, reaching a peak of about 150% of basal values, the ΔHCO_3^- output being $4.1 \pm 0.7 \mu\text{Eq}/2\text{h}$, which is 2.4 fold greater than that induced by acetylcholine.

Effects of nizatidine on gastric acid secretion and motility

Acid secretion Following intravenous infusion of histamine (4mg/kg/h), gastric acid secretion was increased from $18.6 \pm 3.1 \mu\text{Eq}/10 \text{ min}$ to $39.5 \pm 3.2 \mu\text{Eq}/10 \text{ min}$ -within 60min, and remained elevated during a 2h test period. The acid secretory response to histamine was significantly reduced by i.v. injection of nizatidine (10 and 30mg/kg) in a dose-dependent manner, the inhibition of total acid output for 2h being 58.9 and 86.3%, respectively (Figure 6). A potent inhibition of histamine-induced acid secretion was also observed on i.v. administration of famotidine (10mg/kg), the inhibition of total acid output for 2h being 90.4%.

Gastric motility Normal rat stomachs spontaneously contracted at a frequency of 16-20/10min with an amplitude of $18.6 \pm 3.2 \text{ cm H}_2\text{O}$. Intravenous administration of nizatidine (30mg/kg) enhanced gastric motility, which reached a plateau level (about 2.5 times greater than basal values) within 40min and remained elevated thereafter, and this action was completely inhibited by atropine (1mg/kg, s.c.) (Figure 7). Both neostigmine (0.03mg/kg) and cisapride (3 mg/kg) increased gastric motility, similar to nizatidine, while famotidine (10mg/kg) did not have any effect on spontaneous contractile activity of the stomach (not shown).

Effects of nizatidine on duodenal damage caused by acid perfusion

Perfusion of the proximal duodenum with 100mM

HCl for 4h in indomethacin-treated rats caused severe damage in the mucosa, the lesion score being $49.1 \pm 7.4 \text{ mm}^2$ (Table 1). Pretreatment of animals with nizatidine (10 mg/kg, i.v.) or neostigmine (0.03mg/kg, i.v.) was effective in significantly reducing the severity of duodenal damage in response to acid perfusion, the inhibition being 56.6% or 64.0%, respectively. Famotidine (10mg/kg, i.v.) had no effect on the development of duodenal damage induced by acid perfusion.

Table 1 Effects of nizatidine, neostigmine and famotidine on duodenal damage induced by acid in rats

Group	Dose (mg/kg)	Number of Rats	Duodenal damage (mm ²)	Inhibition (%)
Control		5	49.1±7.4	
Nizatidine	10	5	21.3±5.1 ^a	56.6
Neostigmine	0.03	5	17.7±7.3 ^a	64.0
Famotidine	10	4	40.8±4.5	16.1

Duodenal damage was induced by perfusing a duodenal loop with 100mM HCl for 4h in the presence of indomethacin (5mg/kg, s.c.). Nizatidine (10mg/kg), neostigmine (0.03mg/kg) or famotidine (10mg/kg) was administered i.v. as a single injection 20min before the onset of acid perfusion. Data are presented as the means±SE from 5 rats.

^a Significant difference from control, $P < 0.05$.

Table 2 Inhibition by nizatidine, famotidine, neostigmine and cisapride of acetylcholinesterase activity in rat erythrocyte and plasma

Drugs	IC ₅₀ (M)	
	Rat erythrocytes	Plasma
Nizatidine	1.4×10^{-6}	5.7×10^{-4}
Neostigmine	1.1×10^{-7}	3.3×10^{-6}
Famotidine	$1.0 \times 10^{-3} <$	$1.0 \times 10^{-3} <$
Cisapride	$1.0 \times 10^{-3} <$	$1.0 \times 10^{-3} <$

Values represent the IC₅₀ of AChE activity for each drug. The experiments were performed in triplicate against AChE of rat erythrocyte and plasma.

Effects of nizatidine on acetylcholinesterase activity

Table 2 summarizes the activities of nizatidine for AChE and PChE, in comparison with neostigmine and famotidine. Nizatidine inhibited the AChE activity prepared from rat erythrocytes, and the IC₅₀ was $1.4 \times 10^{-6} \text{ M}$, about 12 times greater than that ($1.1 \times 10^{-7} \text{ M}$) of neostigmine. Likewise, both nizatidine and neostigmine inhibited the PChE activity prepared from rat plasma, the IC₅₀ being $5.7 \times 10^{-4} \text{ M}$ and $3.3 \times 10^{-6} \text{ M}$, respectively. However, neither famotidine nor cisapride had any effect on AChE or PChE activities, the IC₅₀ for these drugs being over $1 \times 10^{-3} \text{ M}$.

DISCUSSION

The present study showed for the first time that nizatidine, an histamine H₂-receptor antagonist, stimulates duodenal HCO_3^- secretion in rats, in both vagal-dependent and atropine-sensitive

manners, and this action is associated with the anti-AChE activity of this agent. Furthermore, the HCO₃⁻ stimulatory property of nizatidine was observed at the dose ranges for both gastric antisecretory and prokinetic actions. Since neither famotidine nor cisapride had any effect on duodenal HCO₃⁻ secretion, it is unlikely that this action of nizatidine is simply resulted from the inhibition of gastric acid secretion due to H₂ receptor blockade or the increased luminal pressure due to enhanced duodenal motility.

It has been shown that several H₂-receptor antagonists are endowed with anti-AChE

activity^[6-10]. This action results in facilitating gastrointestinal motor activity in experimental animals and in humans^[5,11]. Ueki *et al*^[11] reported that nizatidine stimulates gastrointestinal motility and gastric emptying at antisecretory doses, mainly through its anti-AChE activity. In the present study, we confirmed that nizatidine potently inhibited both AChE and PChE activities. On the basis of the K_i values, it was also noted that the anti-AChE activity of nizatidine was much potent as compared with the other H₂-antagonist famotidine, although it was weaker than that of neostigmine, the authentic AChE inhibitor.

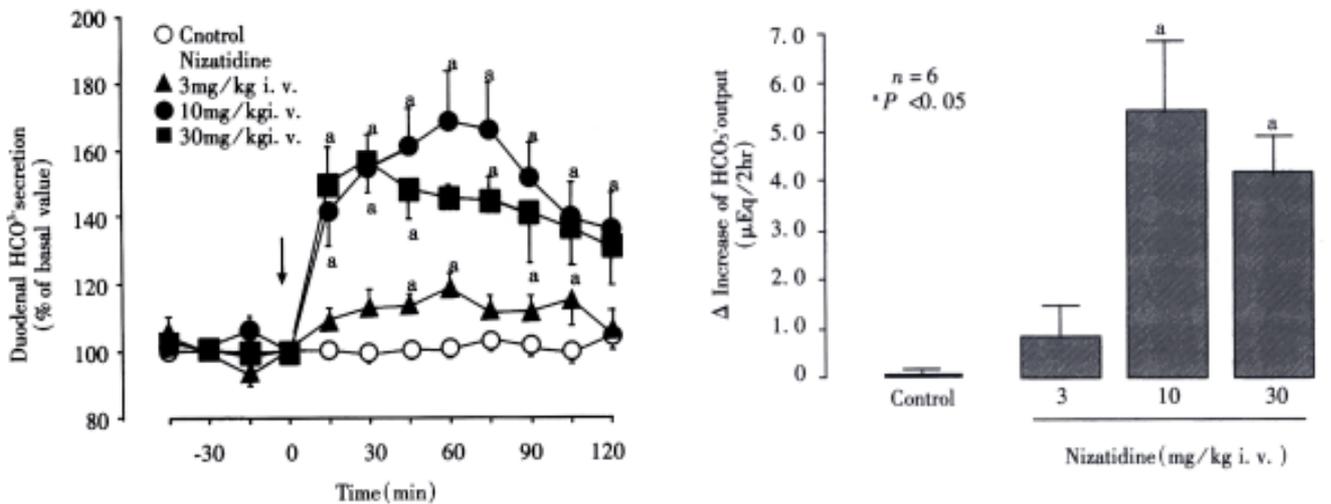


Figure 1 Effect of nizatidine on duodenal HCO₃⁻ secretion in rats. The HCO₃⁻ secretion was measured by perfusing a duodenal loop with saline and by adding 10mM HCl to the reservoir. Nizatidine (3-30mg/kg) was administered i.v. as a single injection after the basal secretion had well stabilized, and the HCO₃⁻ secretion was measured for 2h. Data are expressed as % of basal values and represent the means±SE of values determined every 15min from 6 rats. Lower panel shows Δ increase of HCO₃⁻ output for 2h. Data are presented as the means±SE from 6 rats. *Significant difference from controls, P<0.05.

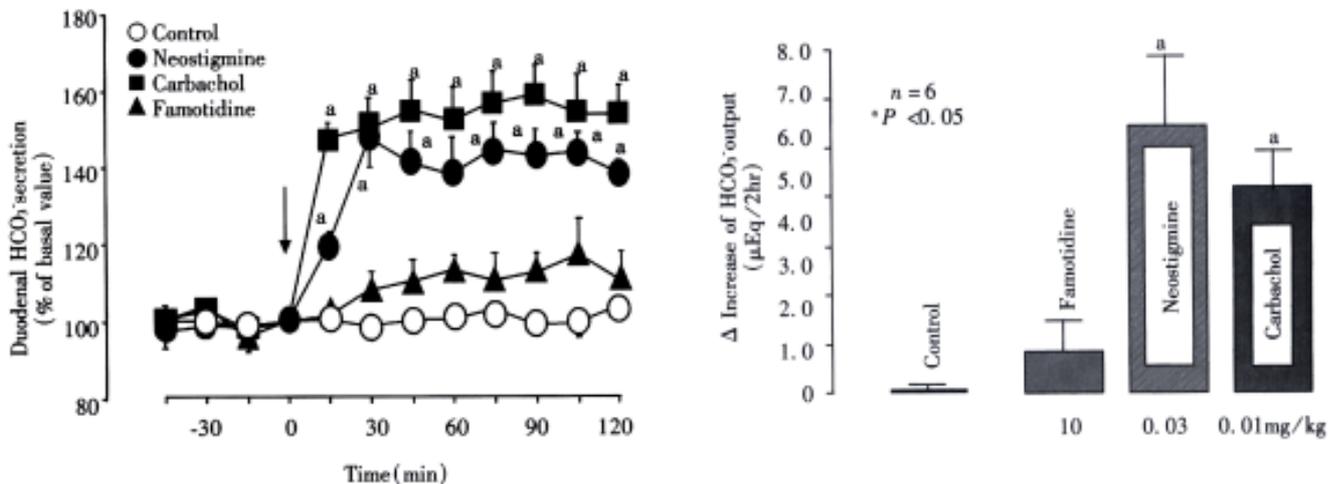


Figure 2 Effects of neostigmine, carbachol and famotidine on duodenal HCO₃⁻ secretion in rats. The HCO₃⁻ secretion was measured by perfusing a duodenal loop with saline and by adding 10mM HCl to the reservoir. Neostigmine (0.03mg/kg), carbachol (0.01mg/kg) or famotidine (10mg/kg) was administered i.v. as a single injection after the basal secretion had well stabilized, and the HCO₃⁻ secretion was measured for 2h. Data are expressed as % of basal values and represent the means±SE of values determined every 15min from 6 rats. Lower panel shows Δ increase of HCO₃⁻ output for 2h. Data are presented as the means±SE from 6 rats. *Significant difference from controls, P<0.05.

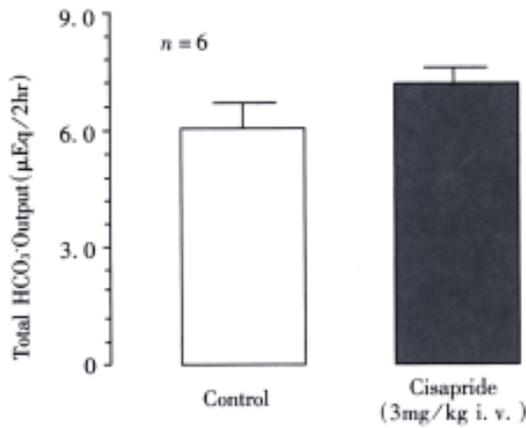


Figure 3 Effect of cisapride on duodenal HCO₃⁻ secretion in rats. The HCO₃⁻ secretion was measured by perfusing a duodenal loop with saline and by adding 10mM HCl to the reservoir. Cisapride (3 mg/kg) was administered i.v. as a single injection after the basal secretion had well stabilized, and the HCO₃⁻ secretion was measured for 2h. Data indicate total HCO₃⁻ output obtained for 2h after administration of cisapride, and represent the means±SE from 6 rats.

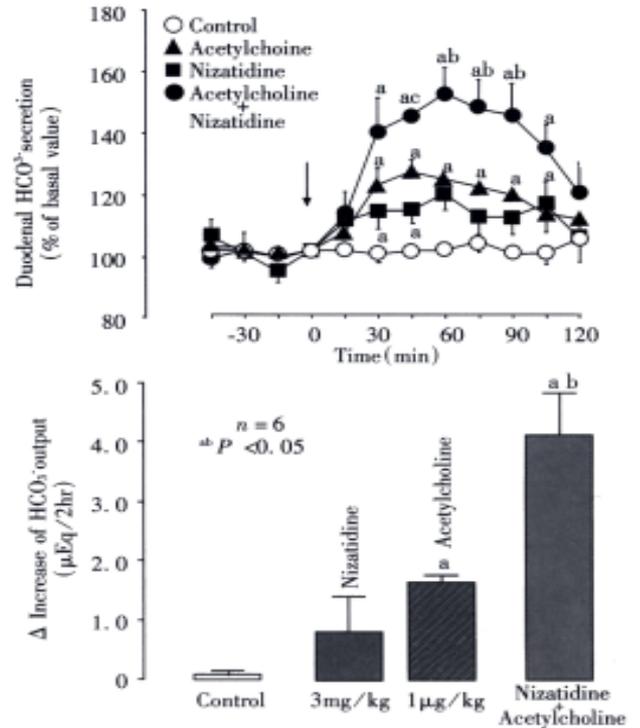


Figure 5 Effects of nizatidine and acetylcholine, either alone or in combination, on duodenal HCO₃⁻ secretion in rats. The HCO₃⁻ secretion was measured by perfusing a duodenal loop with saline and by adding 10mM HCl to the reservoir. Nizatidine (3mg/kg) and acetylcholine (0.003mg/kg), either alone or in combination, were administered i.v. after the basal secretion had well stabilized, and the HCO₃⁻ secretion was measured for 2h. Data are expressed as % of basal values and represent the means±SE of values determined every 15min from 6 rats. Lower panel shows Δ increase of HCO₃⁻ output for 2h. Data are presented as the means ± SE from 6 rats. Significant difference at *P* < 0.05 ^afrom control; ^bfrom nizatidine or acetylcholine.

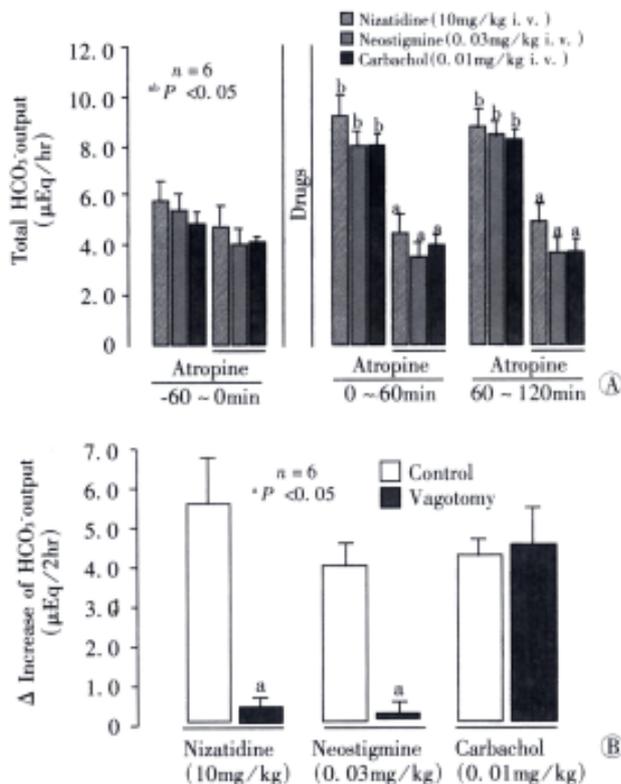


Figure 4 Effects of atropine or bilateral vagotomy on the HCO₃⁻ stimulatory action of nizatidine, neostigmine and carbachol in the rat duodenum. The HCO₃⁻ secretion was measured by perfusing a duodenal loop with saline and by adding 10mM HCl to the reservoir. Neostigmine (0.03 mg/kg), carbachol (0.01 mg/kg) or famotidine (10mg/kg) was administered i.v. as a single injection after the basal secretion had well stabilized, and the HCO₃⁻ secretion was measured for 2h. Atropine (1mg/kg) was given s.c. 1h before administration of the above agents. Bilateral vagotomy was performed acutely at the cervical portion 2h before the administration of the agents. Data are presented as the means ± SE from 6 rats. Significant difference at *P* < 0.05; ^afrom corresponding groups without atropine; ^bfrom corresponding values observed for 1h before the treatment (time: -60-0 min)

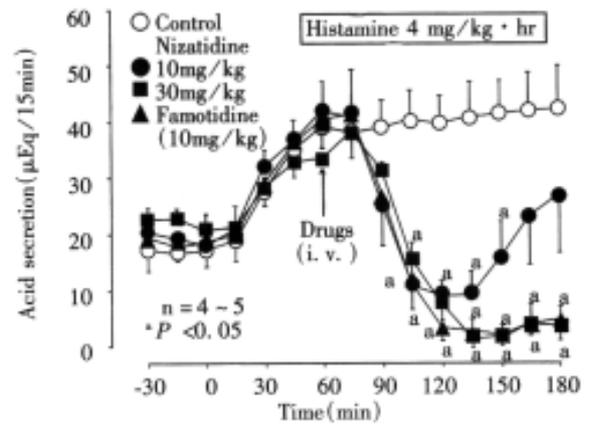


Figure 6 Effects of nizatidine and famotidine on histamine-induced gastric acid secretion in rats. A rat stomach mounted in an *ex-vivo* chamber was perfused with saline, and the acid secretion was measured by adding 100mM NaOH to the reservoir. The acid secretion was stimulated by i.v. infusion of histamine (4mg/kg/h), while nizatidine (10 and 30mg/kg) or famotidine (10mg/kg) was administered i.v. as a single injection 1h after the onset of histamine infusion, when the acid secretion had reached a plateau. Data are presented as the means±SE of values determined every 15min from 5 rats. ^aSignificant difference from controls, *P* < 0.05.

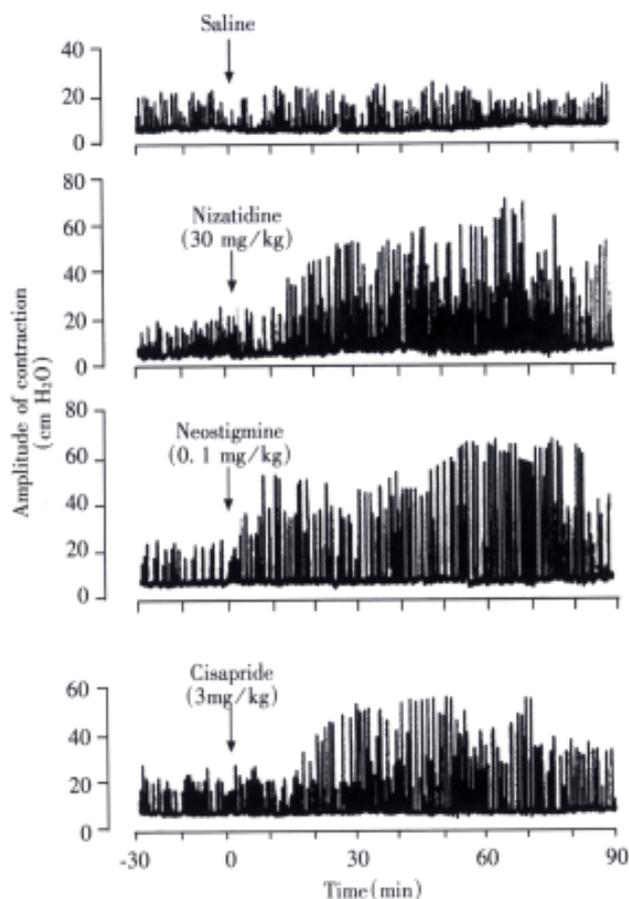


Figure 7 Representative recordings of gastric motor activity in conscious rats, before and after administration of nizatidine. gastric motor activity was monitored as intraluminal pressure recordings, by using a miniature balloon placed in the stomach. Nizatidine (30mg/kg) was administered i.v. as a single injection after the basal motility had well stabilized. Atropine (1mg/kg) was given s.c. 1h after administration of nizatidine. Note that gastric motor activity was apparently enhanced by nizatidine at the doses that stimulate HCO₃⁻ secretion in the rat duodenum, and this action was completely blocked by atropine.

It is widely accepted that acetylcholine is a transmitter released by enteric excitatory neurons to influence gastrointestinal motility. Likewise, several studies showed that duodenal HCO₃⁻ secretion was increased by the vagal-cholinergic excitation^[17,18, 22,23]. Lenz *et al*^[22] reported that the HCO₃⁻ secretion was increased in response to both central and peripheral cholinergic agents. It is also known that the vagal-cholinergic pathway plays a role in the HCO₃⁻ secretory response to acid and sham feeding^[23]. Since the electrical stimulation of the vagus nerves increased duodenal HCO₃⁻ secretion mediated by the atropine-sensitive cholinergic pathway^[24], it is expected that this process is also stimulated by peripheral cholinomimetic agents. The present study clearly showed that nizatidine increased the HCO₃⁻ secretion by stimulating cholinergic excitation

through anti-AChE activity, and this effect was mimicked by neostigmine. These results support the contention that the HCO₃⁻ stimulatory effect of nizatidine in the rat duodenum is mediated by endogenous acetylcholine released from cholinergic neurons. Indeed, this action of nizatidine on the HCO₃⁻ secretion was totally abolished by bilateral vagotomy or prior administration of atropine. Certainly, the antagonism of H₂-receptor does not account for the HCO₃⁻ stimulatory action of nizatidine, because famotidine did not have any effect on this secretion.

In the present study, the HCO₃⁻ secretion induced by nizatidine was not affected by either indomethacin or L-NAME, excluding the possibility for involvement of PG or NO in this response. We previously reported that the HCO₃⁻ response induced by cholinergic agents was not affected by indomethacin^[18]. Furukawa *et al*^[25] reported that NO stimulates the HCO₃⁻ secretion, mediated by endogenous PGs in isolated bullfrog duodenum. Thus, it is reasonable that the HCO₃⁻ response induced by nizatidine is not affected by the NO synthase inhibitor L-NAME. Segawa *et al*^[26] reported that nizatidine did not affect *in vitro* PGE₂ biosynthesis and even doses that markedly inhibit gastric acid secretion had no effect on the mucosal PGE₂ contents in rat stomachs. Hallgren *et al*^[27] found that the NO synthase inhibitors such as L-NAME caused an increase of both luminal alkalization and luminal pressure in the rat duodenum and suggested that the HCO₃⁻ stimulatory action of L-NAME is due to the neural reflex resulting from the rise in luminal pressure through mechano-receptors. The same mechanism might be applied to the HCO₃⁻ stimulatory action of nizatidine, because this agent enhanced gastrointestinal motility, leading to increase of the luminal pressure. However, cisapride at the dose that clearly enhanced gastric motility did not affect the HCO₃⁻ secretion. Thus, it is unlikely that nizatidine stimulates duodenal HCO₃⁻ secretion due to the increase of luminal pressure, resulting from the smooth muscle contraction.

The secretion of HCO₃⁻ in the duodenum is the main defense mechanism against acid. Mucus adherent to the luminal surface of the mucosa provides a zone of low turbulence, allowing the development of a gradient for HCO₃⁻ from the luminal side^[12,13,28]. Small amounts of HCO₃⁻ protect the mucosa against large amounts of acid by neutralizing H⁺ ions that diffuse back into the mucus layer^[13]. A number of studies demonstrated a close relationship between the mucosal ulcerogenic response and the duodenal HCO₃⁻ disorder^[12, 13,29]. Indeed, it is reported that patients with inactive duodenal ulcers have decreased production of HCO₃⁻ in the proximal duodenum during exposure

to acid^[30], though the exact mechanism involved remains unknown. In the present study, perfusion of the proximal duodenum with 100mM HCl for 4h produced extensive hemorrhagic damage in rats in the presence of indomethacin. However, nizatidine reduced the severity of duodenal damage at the dose which stimulated HCO₃⁻ secretion. These results confirmed that nizatidine afforded protection of the duodenal mucosa by increasing HCO₃⁻ secretion, in a PG-independent pathway.

It is known that the mucosal acidification increases the HCO₃⁻ secretion via both humoral and neural factors as well as endogenous PGs^[12,23,31]. Vasoactive intestinal polypeptide (VIP) is the most likely humoral factor mediating the HCO₃⁻ secretory response to acid, because it is a potent stimulant of duodenal HCO₃⁻ secretion and is released from nerve endings during the exposure of duodenal mucosa to acid^[17]. Odes *et al*^[32] reported that acetylcholine increases a release of VIP from enteric nerves via both muscarinic M₁ and M₃ receptors, which in turn stimulates HCO₃⁻ secretion in the duodenum. Since nizatidine significantly potentiated the HCO₃⁻ response to acetylcholine, it is possible that the acid-induced HCO₃⁻ secretion is also enhanced by the anti-AChE activity in the presence of nizatidine. Ueki *et al*^[11] demonstrated that the ED₅₀ values of nizatidine for inhibition of acid output in rats overlapped the effective doses for stimulation of gastrointestinal motility and gastric emptying. We also showed that nizatidine increased duodenal HCO₃⁻ secretion at the doses that were effective in inhibiting histamine-induced acid secretion or in stimulating gastric contractile activity. These results suggest that the HCO₃⁻ stimulatory effect of nizatidine can be expected in duodenal ulcer patients who are treated with this drug at the antisecretory dose.

In summary, the present results clearly showed that the histamine H₂-receptor antagonist nizatidine stimulates duodenal HCO₃⁻ secretion, and this action is associated with its anti-AChE activity and mediated by vagal-cholinergic mechanisms. It is assumed that the HCO₃⁻ stimulatory action of nizatidine is useful for treatment of duodenal ulcer, in addition to its anti-acid secretory and gastric prokinetic effects.

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