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Effect of Esophageal Distention on Basal and Stimulated Gastric Acid Secretion in Rats

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ABSTRACT

Background: It is well established that the esophageal distention (ED) leads to gastric relaxation, partly by vago-vagal reflex, but till now, the effect of ED on gastric acid secretion has not been investigated. The aim of this study was to investigate the effect of ED on basal and stimulated gastric acid secretion. **Methods:** Adult male Wistar rats (200-240 g) were deprived of food but not the water 24 h before the experiments. Under urethane anesthesia (1.2 g/kg, i.p.), animals underwent tracheostomy and laparotomy. A catheter was inserted in the stomach through duodenum for gastric distention and gastric washout and the esophagus was cannulated with a distensible balloon orally to distend esophagus (0.3 ml, 10 min). Gastric acid secretion was stimulated by gastric distention, carbachol (4 µg/kg, i.p.) or histamine (5 mg/kg, s.c.). Effects of vagotomy, N^G–nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, i.v.) and also hexamethonium were investigated. **Results:** Basal and gastric distention- and carbachol, histamine-stimulated acid secretion were reduced by the ED ($P<0.05$, $P<0.0001$, $P<0.01$ and $P<0.02$, respectively). L-NAME (10 mg/kg, i.v.) elevated the acid output ($P<0.002$). Vagotomy reduced the inhibitory effect of the esophagus distention on gastric distention-induced acid secretion ($P<0.01$). **Conclusion:** These results indicate that the vagus nerves are involved in the inhibitory effect of the ED on the basal and stimulated gastric acid secretion. Furthermore, nitric oxide could be involved. *Iran. Biomed. J. 11 (3): 177-183, 2007*

Keyword: Esophageal distention, Gastric acid secretion, Rat, Vagus nerve, nitric oxide (NO)

INTRODUCTION

It has long been known that the esophageal distention (ED) produced by swallowing elicits a powerful proximal gastric relaxation [1]. This reflex was termed the "receptive relaxation reflex" and is an important mechanism which increases gastric volume and reduces intragastric pressure to ensure that swallowed food is efficiently transport to the stomach. The vagus nerve provides an essential role in the mediation of this relaxation reflex via the non-adrenergic non-cholinergic (NANC) inhibitory innervation as well as inhibition of gastric cholinergic excitatory fibers [2]. It has been shown that this potent proximal gastric relaxation is triggered by the activation of low-threshold vagal afferent mechanosensors in the esophagus [3]. This reflex requires intact vago-vagal connections among the esophagus, brain stem and stomach [2]. Also the vago-vagal reflex pathways participate in the control of gastric secretion [4]. Activation of gastric and ED-sensitive afferent fibers can also produce a potent gastroinhibition through the activation of vagal NANC pathways to the gastric fundus [2, 5].

On the other hand, a growing body of evidence suggests that nitric oxide (NO) acts as a transmitter in some NANC nerves in the gastrointestinal tissue and modulates various functions, including acid secretion [6, 7]. Recent evidence suggests that NO can serve as a key NANC inhibitory transmitter in the gastrointestinal tract [8, 9]. Other studies indicate that distension induced gastric receptive relaxation and gastric adaptive relaxation in both the isolated guinea-pig stomach [8, 10] and in the anesthetized dogs mediated by NO via vagal NANC inhibitory nerves [11]. Although, early studies have shown that the ED generates inhibitory signals related to the gastric motility functions, however, the effect of these inhibitory signals on the gastric secretory function has not been elucidated.

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MATERIALS AND METHODS

Animals. All animal experiments were carried out in accordance with the Ahwaz Jundishapur University of Medical Sciences guidelines (Ahwaz, Iran) for the care and use of laboratory animals. Adult male Wistar rats (n = 74, 200-240 g) were maintained on a 12 h light/dark cycle with free access to food and water.

Surgery. Rats were fasted 24 h before the experiments but had free access to water and were anesthetized with urethane (1.2 g/kg, i.p.) [12]. Depth of anesthesia was checked throughout the experiment by the pedal withdrawal (toe pinch) reflex every 30-45 min. If the pedal withdrawal reflex was observed, a supplemental dose of urethane (0.4 g/kg, i.p.) was administered to maintain adequate anesthesia. Animal body temperature was measured with a rectal thermometer and maintained at 37°C using a homeothermic blanket control system (Harvard, UK). The trachea was surgically exposed and cannulated by a polyethylene catheter (O.D. 2 mm) to ease respiration**.** After a midline laparotomy, both of stomach and the duodenum were exposed. A polyethylene catheter (O.D. 3 mm) was inserted into the stomach through duodenum and held in place by ligature around the pylorus. At the beginning of each experiment, the lumen of the stomach was gently rinsed with isotonic saline (pH 7) until gastric washout was clear. In a group of animals, subdiaphragmatic vagus nerves were sectioned as the vagotomized group.

Esophageal distention. Esophagus distention was performed by a distensible balloon (0.3 ml of saline for 10 min) through a esophageal catheter (O.D. 1 mm) inserted orally into the distal part of the esophagus (7-8 cm from incisors). The center of the balloon was positioned in the thoracic portion of the esophagus as conformed by autopsy. Inflation of the balloon with 0.3 ml distended the esophagus to a cylindrical shape with an outer diameter of 4 mm and a length of 9 mm. This range of distention has been shown to activate the low-threshold vagal mechonoceptors excited by the peristaltic esophageal contraction of swallowing and not spinal nociceptors [3].

Stimulation of gastric acid secretion. Gastric acid secretion was stimulated by distention (1.5 ml/100 g b.w. or 6 ml by isotonic saline, pH 7 and 37°C) [13], carbachol (4 µg/kg, i.p.), histamine (5 mg/kg, s.c.) and by L-NAME (N^G–nitro-L-arginine methyl ester), 10 mg/kg (i.v.) into a tail vein [14-16]. To determine the role of NO on the inhibitory effect of ED on acid secretion, the stomach was distended by 6 ml of isotonic saline with 10 min intervals. Latest effluent before L-NAME administration considered as the control of distention-stimulated acid secretion and thereafter L-NAME was administrated (10 mg/kg, i.v.). The effect of L-arginine (500 mg/kg, i.p.) was also investigated [17]. In another set of experiment, hexamethonium (10 mg/kg i.v. bolus, followed by 10 mg/kg/h i.v. from a tail vein) [17], was administered at the plateau of distension-induced acid secretion.

Vagotomy. To investigate whether the inhibitory effect of ED is mediated via an extrinsic pathway, subdiaphragmatic vagotomy was performed by cutting the vagal trunks around the abdominal esophagus [18].

Evaluation of gastric acid secretion. The acidity in the gastric washout was measured with an autotitrator pH meter (Radiometer, Copenhagen, Denmark) by automatic potentiometric titration to pH 7 with 0.01 N NaOH and was expressed as μEqH^+/10 min.

Basal acid secretion. After the surgical preparation, basal gastric acid secretion was allowed to stabilize for at least 30 min. At the end of this period, two consecutive 10-min of gastric effluent were collected to assess basal acid secretion and the acid output was expressed as μEqH^+/10 min.

Materials. L-NAME, carbachol (carbamoylcholine chloride), hexamethonium bromide, histamine 2HCl, L-arginine monohydrochloride and urethane (Ethyl carbamate) were purchased from Sigma (USA).

Statistical analysis. All results are expressed as mean ±SEM and statistical analysis was performed by Student's *t*-test. *P* values <0.05 were considered significant.

RESULTS

Effect of ED on basal and gastric distension-stimulated acid secretion. In the urethane anesthetized rats, the ED 0.3 ml for 10 min reduced the basal acid secretion significantly (n = 10,

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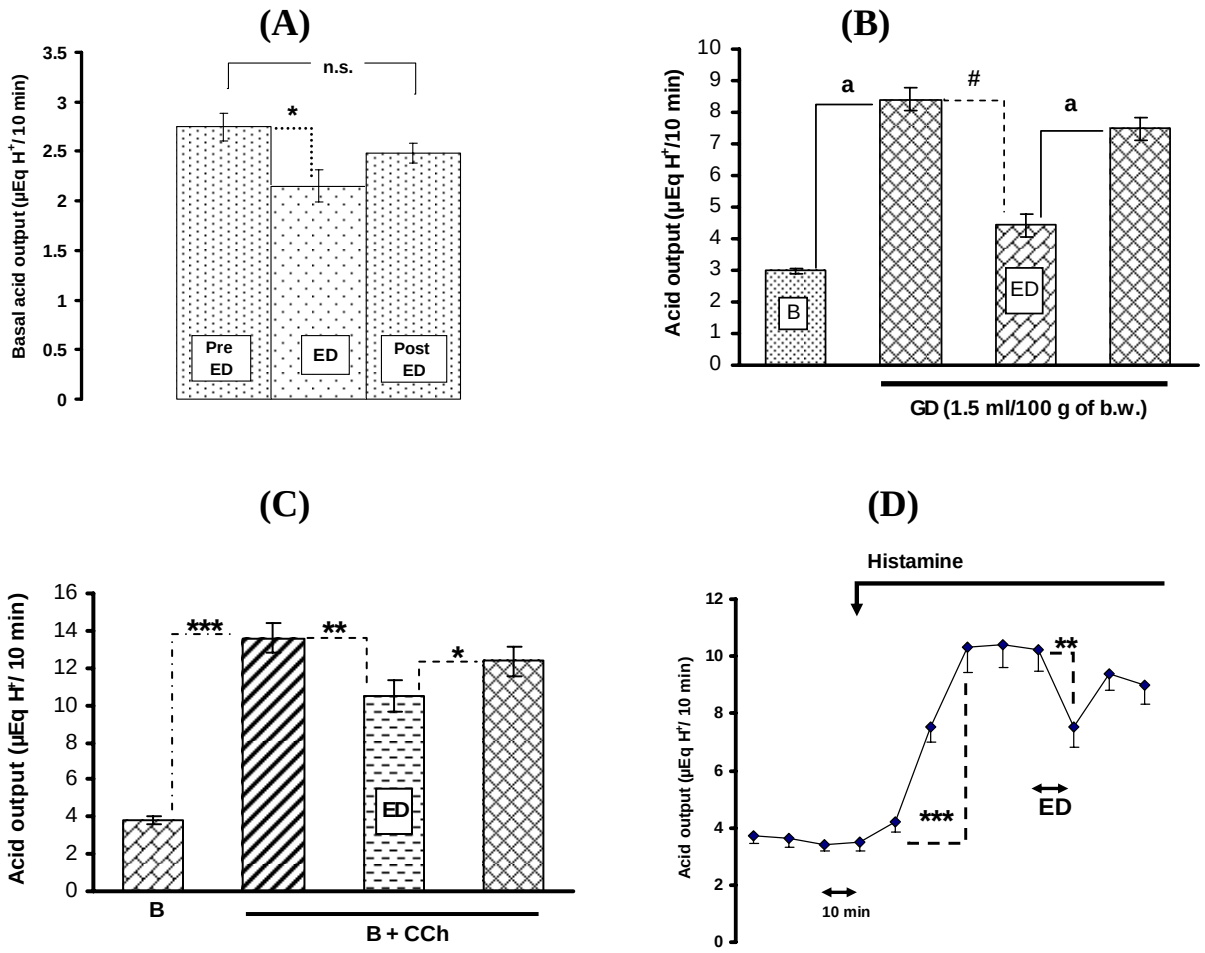

Fig. 1. **(A)** The effect of ED (0.3 ml) on basal acid secretion in rats (n = 10, **P*<0.05); **(B)** The effect of ED (0.3 ml) on gastric distention-induced acid secretion in rats (n = 10, ^{a}P<0.001, $^{\#}P$<0.0001); **(C)** The effect of ED (0.3 ml) on carbachol-induced acid secretion in rats (n = 10; **P*<0.05, ***P*<0.01, ****P*<0.001), cch (4 μg/ kg, i.p.); **(D)** The effect of ED (0.3 ml) on histamine (5 mg/kg, s.c.)-stimulated gastric acid secretion (n = 10, ****P*<0.001, ***P*<0.02). ED, esophageal distention; n.s..= non-significant; B, basal; GD, gastric distention; CCh, carbachol.

P<0.05) as shown in the Figure 1A. Gastric distension-stimulated acid secretion was also significantly reduced by the esophagus distension (n = 10, *P*<0.0001). The results of this section are presented in the Figure 1B.

Effect of ED on carbachol-induced gastric acid secretion. Carbachol administration (4 μg/kg, i.p.) elevated the gastric acid secretion significantly in compare to basal acid secretion (*P*<0.001). However, the esophagus distension (0.3 ml, 10 min) lowered this elevation significantly (n = 10, *P*<0.01) as it is shown in Figure 1C.

Effect of ED on histamine-stimulated gastric acid secretion. Administration of histamine (5 mg/kg, s.c.) elevated the gastric acid secretion significantly in compare to basal acid secretion (*P*<0.001). However, the esophagus distension (0.3 ml, 10 min) lowered this elevation significantly (n = 10, *P*<0.02) as it is shown in Figure 1D.

Effect of L-NAME on distention-stimulated gastric acid secretion. L-NAME (10 mg/kg, i.v.) as a NO synthase inhibitor potentiated the acid secretion response to the gastric distension (*P*<0.002) as shown in Figure 2.

Effect of ED on distension-induced gastric acid secretion in the presence of L-NAME and L-arginine. Gastric distension-induced acid secretion was reduced (*P*<0.001, n = 7) by L-arginine

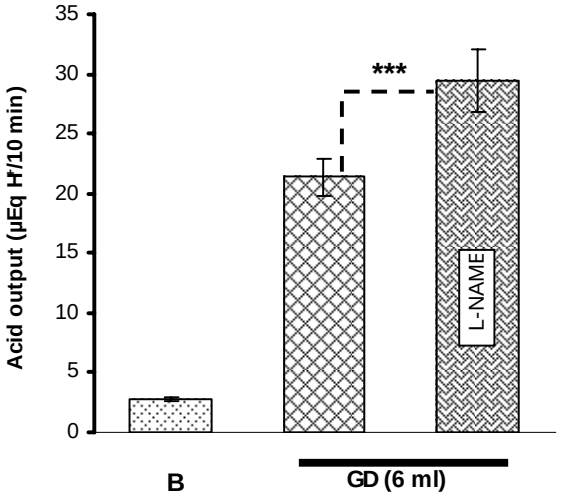

Fig. 2. The effect of L-NAME (10 mg/kg, bolus) on distention-induced gastric acid secretion (n = 10, ***P<0.002). B, basal; GD, gastric distention.

administration (500 mg/kg, i.p.), however, the inhibitory effect of L-arginine was attenuated (P<0.001) by L-NAME (10 mg/kg, i.v.). In the presence of L-arginine and L-NAME, ED had no effect on acid secretion as depicted in Figure 3.

Effect of vagotomy on the gastric distension-induced acid secretion and on ED inhibitory effect. To evaluate the involvement of vagal reflexes in the inhibitory effect of the ED on acid secretion, two groups of rats were vagotomized or received hexamethonium (10 mg/kg, i.v and 10 mg/kg/h, i.v.).

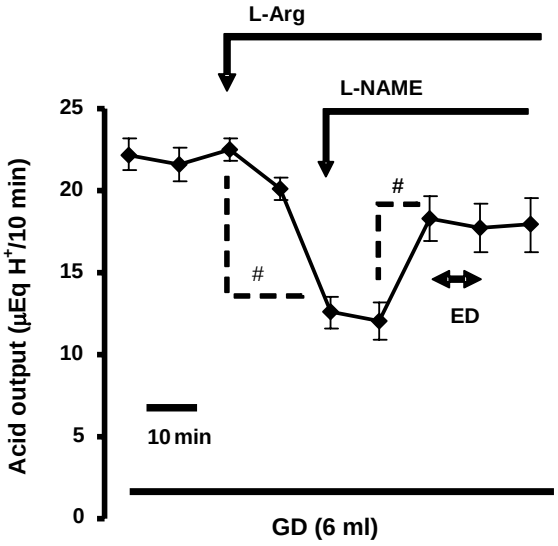

Fig. 3. Effect of ED on L-NAME-stimulated gastric acid secretion in presence of L-arginine (500 mg/kg, i.p.). ED, esophageal distention; GD, gastric distention; L-arg, L-arginine (n = 7), #P<001.

In these animals, the effect of ED on gastric distension-stimulated acid secretion was investigated. The results showed that subdiaphragmatic vagotomy reduced the gastric distension-induced acid secretion response (P<0.0001). Furthermore, after vagotomy, ED decreased the acid secretion in response to gastric distension (P<0.05, n=10). These results are presented in Figure 4. The inhibitory effect of ED in animals with intact vagus nerve was greater than in vagotomized rats (45 ± 3% vs 10 ± 0.59%).

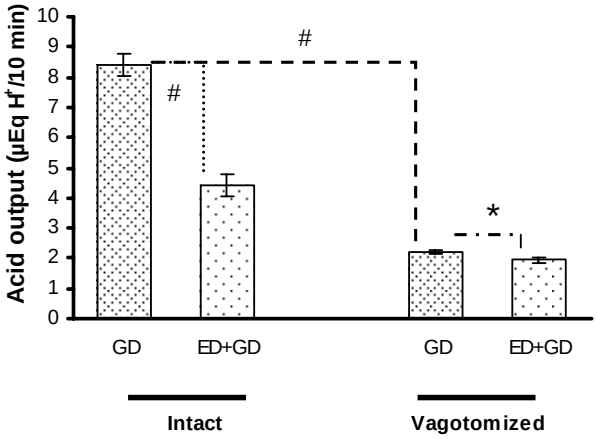

Fig. 4. Comparison of the inhibitory effect of esophageal distention on acid secretion in the animals with intact vagus nerve (n = 10) and (n = 10) vagotomized rats. GD, gastric distention (1.5 ml/100 g b.w.); ED, esophageal distention. (*P<0.05, #P<0.0001)**.**

Effect of hexamethonium on the gastric distension-induced acid secretion and on ED inhibitory effect. Distention-induced gastric acid secretion was reduced by hexamethonium administration (10 mg/kg, i.v followed by 10 mg/kg/h, i.v.) in vagal intact rats (P<0.001). However, in the presence of hexamethonium, the ED also induced reduction in acid secretion (P<0.01, n = 7). In addition, the deflation of esophageal balloon reversed the inhibitory effect of ED as shown in Figure 5.

DISCUSSION

The present study showed that the ED decreases the basal and carbachol-, histamine- or distention-induced gastric acid secretion. L-NAME, as a NO synthase inhibitor, potentiated the acid secretory

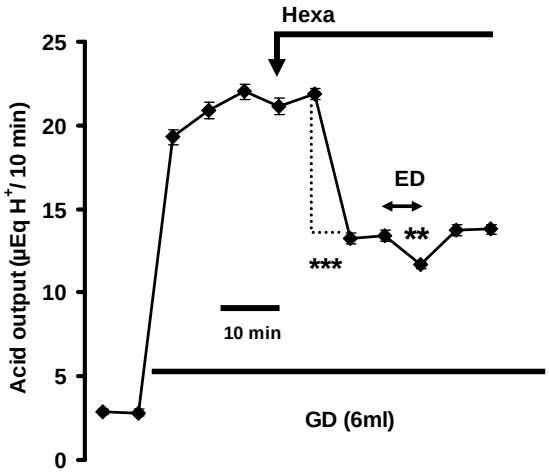

Fig. 5. Effect of hexamethonium on the acid secretion stimulated by distention and on the inhibitory effect of esophageal distention (n = 7, ***P*<0.01, ****P*<0.001); ED, esophageal distention; GD, gastric distention; Hexa, hexamethonium (10 mg/kg i.v. bolus, followed by 10 mg/kg/h continuous infusion), was administered at the plateau of distention-induced acid secretion.

response to distention. Vagotomy reduced, but not abolished, the inhibitory effect of ED on gastric distention-stimulated acid secretion.

It has been reported that the presence of background amounts of acetylcholine, gastrin and histamine account for at least part of basal acid secretion [19]. The present study showed that the ED decreases gastric basal acid secretion which may be, at least partially, due to inhibiting vagal excitatory fibers or inhibition of the background releasing of histamine or gastrin.

Activation of gastric and ED-sensitive afferent fibers can also produce a potent gastroinhibition through the activation of the vagal NANC pathways to the fundus [2, 5]. Furthermore, it is well known that gastric relaxation is triggered by the activation of low-threshold afferent mechanosensors in the esophagus [3].

Studies have shown that stomach distention stimulates gastric acid secretion [20, 21]. Furthermore, a number of investigators have shown that distention of the stomach stimulated acid secretion, mainly mediated by a vagocholinergic mechanism [16, 21].

Our results in this section demonstrated that ED attenuated this stimulatory effect of gastric distension on acid secretion. This inhibitory effect of ED could be due to inhibition of excitatory fibers and/or activation of inhibitory vagal fibers. Since, the reversibility of inhibitory effect of ED by deflation of esophageal balloon suggests that the ED inhibitory effect should be carried out by a neural pathway.

The reduction of acid secretion (in response to gastric distension) by vagotomy supports involvement of vagal fibers. Furthermore, the reduction of the inhibitory effect of ED on acid secretion induced by vagotomy also suggests that the mechanism underlying inhibition of acid secretion by ED is partly mediated by vagus nerve.

In addition, carbachol directly [22] and indirectly [23] stimulates the gastric acid secretion and on the other hand, histamine elevates acid secretion directly. Our results indicated that the ED reduces these stimulatory effects. This inhibitory effect could be due to activation of an inhibitory pathway such as releasing somatostatin at D cells in the gastric glands or releasing neurotransmitter such as NO. Additionally, it has been reported that NO has an inhibitory effect on carbachol- and histamine-stimulated acid secretion [24].

It has been shown that NO attenuates gastric acid secretion in response to gastric distension [16], YM-14673 (an analogue of TRH) and pentagastrin [7] and this conclusion is supported by reversing this inhibitory effect with L-NAME (as a NO synthase inhibitor) [16]. In this study, we have shown that the acid secretory response to gastric distention augmented by L-NAME as reported by Kitamura and his colleagues [16].

Therefore, it seems that during gastric distension, although acid secretion is elevated by distension simultaneously, endogenous NO has released by gastric distension and modulated the acid secretion and L-NAME by inhibiting the NO generation caused elevation in acid secretion as seen in Figure 2.

The inhibitory role of NO on gastric acid secretion has been also reported by Kato *et al.* [7]. In addition, it has been shown that NO decreases acid secretion and protect gastric mucosa lesions induced by aspirin and hypertonic NaCl via inhibition of histamine release [25, 26].

It has been shown in Figure 3 that L-NAME prevents the inhibitory effects of NO (resulting from L-arginine) and ED on gastric distension induced acid secretion. This part of results again supports the involvement of NO in the ED inhibitory effect on acid secretion. In addition, in the present study, it was shown that hexamethonium as a ganglionic blocker reduced the elevated acid secretion caused by gastric distension. This gastric acid secretion mediated by vagal fiber has already been well

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documented [12, 27, 28].

In the presence of hexamethonium, the ED reduced acid secretion. This inhibitory effect, however, was reversed by deflation of esophageal balloon. The incomplete abolishing effect of hexamethonium on ED inhibitory effect may indicate that in ganglionic level, in addition to nicotinic receptors, the other non- nicotinic receptors such as serotonergic receptors should be involved [29, 30].

In conclusion, the results of the present study show that, the gastric stimulated acid secretion by some stimulants reduced by ED and in this reduction, the vagal fibers and NO may be involved. Furthermore, in the parasympathetic ganglionic level, in addition to the nicotinic receptors, other receptors such as serotonergic may be involved.

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