Positive Modulation of Pepsinogen Secretion by Gastric Acidity After Vagal Cholinergic Stimulation

CORRADO BLANDIZZI, ROCCHINA COLUCCI, DIEGO CARIGNANI, GLORIA LAZZERI and MARIO DEL TACCA

Division of Pharmacology and Chemotherapy, Department of Oncology, University of Pisa, Pisa, Italy

Accepted for publication August 26, 1997

ABSTRACT
Parallel increments of gastric acid and pepsinogen secretion generally occur after the application of cholinergic stimuli. However, it still remains to be established whether the changes in acid output associated with cholinergic stimulation play a role in regulation of the concomitant peptic secretory activity. In the present study, an anesthetized rat model was used for the evaluation of pepsinogen secretion in order to pursue a dual purpose: 1) to assess the relative functional relevance of direct and acid-dependent control exerted by cholinergic pathways on pepsinogen output and 2) to characterize the mechanisms through which changes in acidity within the stomach lumen may affect the peptic secretory activity of gastric mucosa. Bethanochol, 2-deoxy-D-glucose or electrical vagal stimulation caused parallel and atropine-sensitive increments of peptic and acid secretions. Omeprazole, a selective inhibitor of gastric secretion, may affect the peptic secretory activity of gastric mucosa. Bethanochol, 2-deoxy-D-glucose or electrical vagal stimulation failed to increase either pepsinogen or acid secretion in omeprazole-pretreated rats. When tested in animals pretreated with both omeprazole and physostigmine (a drug able to prevent the enzymatic breakdown of vagally released ACh through the blockade of acetylcholinesterase), 2-deoxy-D-glucose or electrical vagal stimulation significantly increased pepsinogen secretion without affecting acid secretion. In omeprazole-pretreated rats, perfusion of the gastric lumen with acid solutions caused a pH-dependent and atropine-sensitive increase in pepsinogen output only when applied in combination with electrical vagal stimulation. Functional ablation of capsaicin-sensitive sensory neurons did not modify the gastric secretory responses induced by bethanochol or electrical vagal stimulation. However, after topical application of lidocaine to the gastric mucosal surface, bethanochol stimulated both peptic and acid outputs, whereas electrical vagal stimulation only evoked acid secretion without affecting basal peptic output. The present results indicate that the activation of muscarinic receptors by vagally released ACh is not sufficient by itself to stimulate pepsinogen secretion and that a facilitatory action mediated by acid secretion is necessary to allow an increment of peptic output in response to vagal cholinergic stimuli. It is suggested that such facilitatory input is driven to chief cells by local intramural reflexes that involve capsaicin-insensitive intrinsic nerves.

Vagal cholinergic stimuli and muscarinic receptor agonists evoke parallel increments of gastric acid and pepsinogen secretions in a variety of experimental models as well as in humans (Hersey, 1987; Smith and Torres, 1990; Hirschowitz and Groarke, 1993). However, although a great deal is known about the control of acid secretion mediated by cholinergic pathways (Hirschowitz and Groarke, 1993; Lloyd and Debas, 1994; Welsh et al., 1994), the mechanisms that account for the in vivo regulation of pepsin secretion are still unclear (Basson et al., 1988; Raufman, 1992). In particular, it is matter of debate whether the changes in acid output associated with cholinergic stimulation might play any role in regulation of the concomitant peptic secretory activity (Basson et al., 1988; Smith and Torres, 1990).

Several studies, based on binding, molecular biology and functional techniques, have shown that both gastric chief cells and parietal cells are provided with muscarinic receptors, the activation of which increases intracellular calcium concentration and leads to subsequent stimulation of pepsinogen and acid secretions, respectively (Raufman, 1992; Hersey, 1994; Soll and Berglindh, 1994). In addition, pharmacological experiments performed on isolated rat stomach demonstrated that gastric glands receive direct innervation from cholinergic fibers (Welsh et al., 1994). These findings would suggest that endogenous ACh, released from vagal cholinergic fibers, causes a parallel stimulation of pepsinogen and acid secretions through a simultaneous activation of muscarinic receptors located on chief cells and parietal cells, respectively (Hersey, 1994; Soll and Berglindh, 1994). However, both in vivo and in vitro studies indicated that pepsinogen secretion in response to stimulation of chief cells appears...
to require a flow of water and acid from adjacent parietal cells (Hersey, 1987; Sandvik et al., 1987) and that acidity in the stomach lumen is able to stimulate the pepsinogen output from gastric mucosa (Johnson, 1972; Smith and Torres, 1990). This suggests that changes in local hydrogen ion concentration evoked by cholinergic stimulants might be, at least in part, responsible for the parallel variations of the peptic secretory output.

Previous attempts to discriminate between direct and acid-dependent cholinergic control of pepsinogen secretion were based mainly on the use of in vitro experimental models (Hersey et al., 1983; Sandvik et al., 1987; Basson et al., 1988). However, under such conditions an assessment of the secretory actions mediated by endogenously released ACh is not usually allowed, and the effects of hydrogen ions on chief cells cannot be clearly established because of methodological problems related to the presence of incubation media (Basson et al., 1988). In addition, data concerning the putative changes of peptic output induced by gastric lumen acidification after suppression of endogenous acid production are still lacking.

Therefore, in the present study, an in vivo gastric preparation was used for the evaluation of pepsinogen secretion, under different experimental conditions, to pursue a dual purpose: 1) to assess the relative functional relevance of direct and acid-dependent control exerted by cholinergic pathways on pepsinogen output after the activation of muscarinic receptors by endogenous ACh or an exogenously administered muscarinic agonist; 2) to characterize the mechanisms through which changes in acidity within the stomach lumen may affect the peptic secretory activity of gastric mucosa.

Materials and Methods

Animals

The experiments were carried out on male Wistar rats weighing 200 to 220 g. The animals were fed standard laboratory chow and tap water ad libitum and were not used for at least 1 week after their delivery to the laboratory. The animals were housed, six in a cage, in temperature-controlled rooms on a 12-h light cycle at 22–24°C and 50–60% humidity. Their care and handling were in accordance with the provisions of the European Economic Community (EEC) Council Directive 86-609, recognized and adopted by the Italian government. Twenty-four hours before the experiments, the animals were maintained in single cages provided with wire net bottoms and were deprived of food. Free access to water was allowed until 1 h before the experiment.

Perfusion of the Gastric Lumen in Anesthetized Rats

Continuous perfusion of the rat stomach in situ was carried out, following the procedure previously reported (Blandizzi et al., 1995). The animals were anesthetized with urethane (1.2 g/kg) administered i.p., and the trachea was surgically exposed and cannulated by a polyethylene catheter to ensure a patent airway. A polyethylene catheter was introduced into the esophagus and advanced as far as 5 mm beyond the gastroesophageal junction. After a midline laparotomy, the proximal duodenum was exposed and its wall incised. Then a second polyethylene catheter was introduced into the duodenum and pushed forward until its tip was about 5 mm beyond the pylorus. The stomach lumen was perfused continuously at a rate of 1 ml/min with saline solution (154 mM NaCl) at 37°C (pH = 7.0 ± 0.2) unless otherwise stated, and 15-min effluent fractions were collected. The effluent samples were used for the quantitative evaluation of both pepsinogen and acid secretions.

Evaluation of Pepsinogen and Acid Secretion

Pepsin levels in the gastric effluent were determined as previously reported (Blandizzi et al., 1995). Briefly, 2 ml of 2.5% bovine hemoglobin plus 0.5 ml of 0.3 N HCl and 0.5 ml of gastric effluent were maintained in separate tubes at 37°C for 10 min and then mixed. Mixtures were incubated for 10 min at 37°C, and the reaction was stopped by the addition of 5 ml 0.3 N trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm by an Uvikon 930 Spectrophotometer (Kontron Instruments, Milan, Italy). The results were compared to a standard curve, which was generated in an identical manner using known amounts of porcine pepsin (1 μg = 3 peptic units), and were expressed as micrograms of pepsin. The acidity in the gastric perfusate was measured with an autocitrator pH meter (PHM 85, Radiometer, Copenhagen, Denmark) by automatic potentiometric titration to pH 7.0 with 0.01 N NaOH and was expressed as μEqH+.

After the surgical preparation of animals, basal gastric secretion was allowed to stabilize for 30 min. At the end of this period, we collected two consecutive 15-min effluent fractions to assess basal secretory values. Both pepsinogen and acid secretions were then monitored at 15-min intervals for an additional 120 min. The peptic and acid outputs obtained during the 120-min period after the collection of basal effluent samples were calculated and expressed as μg of pepsin/120 min and μEqH+/120 min, respectively.

Experimental Procedures

Effects of bethanechol, of 2DG and of electrical vagal stimulation under basal secretory conditions. The first set of experiments was carried out on rats with intact vagus nerves. The gastric secretory activity of these animals was evoked by bethanechol (0.3, 1 and 3 mg/kg), a well-known muscarinic receptor agonist, or 2DG (200 mg/kg), a centrally acting stimulant of vagal efferent cholinergic pathways (Eisenberg et al., 1966). Both these drugs were administered i.v. as a bolus immediately after the collection of basal effluent samples.

The second series of experiments was performed on rats whose vagus nerves were carefully separated from the carotid arteries and cut at the cervical level 30 min before the collection of basal effluent samples began. The effects of bethanechol or 2DG on gastric secretions were reassessed in animals that underwent this vagotomy procedure. In addition, in a group of vagotomized animals, the distal end of the left vagus nerve was placed on a bipolar platinum electrode and immersed in paraffin oil. In this case, after the collection of basal effluent samples, the gastric peptic and acid secretions were evoked by continuous electrical stimulation of the left vagus nerve (120 min). The stimulus parameters were square-wave pulses 0.5 ms in duration, delivered at 5 Hz with supramaximal intensity (10 V) by means of a Grass S5 stimulator (Grass Instruments, Quincy, MA) (Blandizzi et al., 1992).

Effects of bethanechol, of 2DG and of electrical vagal stimulation in animals pretreated with omeprazole. A group of experiments was designed to assess the effects of bethanechol, of 2DG and of electrical vagal stimulation on pepsinogen secretion in the presence of a complete blockade of the acid secretory function of gastric parietal cells. For this purpose, 90 min before the collection of basal effluent samples began, animals were pretreated with omeprazole (30 mg/kg i.v.), a benzimidazole derivative that inhibits gastric acid secretion through a selective blockade of H⁺-K⁺-adenosin-triphosphatase (Fellenius et al., 1981) without interfering with receptor or signal transduction pathways of gastric secretory cells (Clissold and Campoli-Richards, 1986). The dose of omeprazole was selected because of its ability to suppress acutely both basal and stimulated gastric acid secretion in anesthetized rats (Blandizzi et al., 1995).

Effects of perfusion of the gastric lumen with acid solutions. The influence exerted on pepsinogen secretion by topical application of acid solutions at various pH on the surface of gastric


mucosa was studied both under basal conditions and in the presence of electrical vagal stimulation. In this case, the anesthetized rats first were pretreated with omeprazole, to suppress endogenous acid production, and then were subjected to bilateral cervical vagotomy. Perfusion of the gastric lumen was performed with saline up to the collection of basal effluent samples and then was continued until the end of the experimental period with one of the following acid solutions: 0.1 mM HCl plus 153.9 mM NaCl (pH 4.0); 1 mM HCl plus 153 mM NaCl (pH 3.0); 10 mM HCl plus 144 NaCl (pH 2.0); 100 mM HCl plus 54 mM NaCl (pH 1.0).

**Effects of bethanechol and of electrical vagal stimulation on animals subjected to systemic capsaiacinization or intra-gastric application of lidocaine.** A group of experiments was designed to assess whether bethanechol and whether electrical vagal stimulation was able to affect gastric pepsinogen and acid secretions from vagotomized rats in the presence of a systemic ablation of capsaiacin-sensitive sensory nerve fibers. For this purpose, some animals were given a dose of 125 mg/kg capsaiacin s.c., as previously reported by Pabst et al. (1993). Ten days after this treatment, the animals were used for the assessment of gastric pepsinogen and acid secretions. One day before the experiment, the efficacy of capsaiacin treatment was checked by instilling a drop of a capsaiacin solution (0.1 mg/ml in saline solution) into one eye of each rat. Capsaiacin-treated rats were expected not to react by wiping their eyes, but whenever an animal responded with wiping, the afflicted eye was immediately and extensively rinsed with water.

An additional series of experiments was performed to investigate the effects of bethanechol and of electrical vagal stimulation on both pepsinogen and acid secretions from vagotomized rats after topical application of the local anesthetic lidocaine to the gastric mucosal surface. In this case, pre-exposure of the gastric mucosa to lidocaine was carried out according to the procedure reported by Mercer et al. (1994) with minor modifications. After the collection of two basal effluent samples, the perfusion was interrupted, and the gastric lumen was gently emptied and then filled with 3 ml of 4% lidocaine. Fifteen minutes later the stomach was emptied, rinsed by gently flushing with 6 ml of saline and filled again with 3 ml of saline. Perfusion of the gastric lumen with saline was then resumed and continued up to the end of the experimental period. An interval of 5 min was allowed to elapse between the restart of gastric perfusion and either bethanechol injection or electrical vagal stimulation.

**Effects of physostigmine and atropine.** In some experiments the enzymatic breakdown of vagally released ACh was prevented in order to promote its accumulation over physiological concentrations. For this purpose, 5 min before they received 2DG injection or electrical vagal stimulation, some animals were treated with the acetylcholinesterase inhibitor physostigmine (1 mg/kg i.v.). In those experiments where the involvement of muscarinic receptors in the gastric secretory responses was assessed, atropine 1 mg/kg i.v. was always administered 5 min before collection of the second basal effluent sample ended.

**Drugs**

The following drugs and reagents were used: urethane ethyl carbamate, crystalline porcine pepsin, lyophilized bovine hemoglobin, bethanechol chloride, 2DG, physostigmine sulfate, capsaiacin, aminophylline, terbutaline, lidocaine (Sigma, St. Louis, MO), omeprazole (kindly provided by Malesci, Florence, Italy) and atropine sulfate (BDH Chemicals, Poole, England). Other reagents were of analytical grade. Bethanechol, 2DG, atropine, physostigmine and lidocaine were dissolved in saline immediately before use. Omeprazole was initially dissolved in polyethylene glycol (PEG, molecular weight = 400) and then diluted with 7 mM NaHCO₃ to a final concentration of 50% PEG (v/v). All drugs administered i.v. were injected in a volume of 0.25 ml/rat. Capsaicin was dissolved (125 mg/ml) in a vehicle composed by 10% ethanol, 10% Tween 80 and 80% saline solution (v/v/v). The total dose of capsaicin (125 mg/kg s.c.) was administered under ether anesthesia in four injections over two consecutive days (first day: 25 mg/kg in the morning and 25 mg/kg in the late afternoon; second day: 25 mg/kg in the morning and 50 mg/kg in the late afternoon). To counteract the respiratory impairment associated with the administration of capsaicin, rats received atropine (0.2 mg/kg i.p.), terbutaline (0.2 mg/kg i.p.) and aminophylline (20 mg/kg i.p.) 10 min before the first and third capsaicin injections.

**Statistics**

Results are given as mean ± S.E. The significance of differences was evaluated by Student's t test or one-way analysis of variance (ANOVA) followed by post-hoc analysis by Student-Newman-Keuls' test, and P values less than .05 were considered significant; n indicates the number of experiments.

**Results**

**Evaluation of basal pepsinogen and acid secretions.** In control animals with intact vagus nerves (n = 6), basal gastric pepsinogen and acid secretions, assessed after a 30-min stabilization, accounted for 59.8 ± 10.1 μg of pepsin/15 min and 3.2 ± 0.8 μEq H⁺/15 min, respectively, and these values remained nearly constant until the end of the experiments (120 min). In addition, when control animals underwent bilateral cervical vagotomy (n = 6), basal pepsinogen and acid secretions accounted for 54.8 ± 9.1 μg of pepsin/15 min and 3.3 ± 0.9 μEq H⁺/15 min, respectively. These values did not differ significantly from those obtained in control rats with intact vagus nerves, and they remained at a steady level throughout the experiment.

In a series of preliminary experiments, both basal pepsinogen and acid outputs were monitored in rats, with or without intact vagus nerves, after pretreatment with omeprazole or atropine (n = 6 for each drug). An almost complete inhibition of basal acid secretion, but not of peptic output, was detected only in rats undergoing pretreatment with omeprazole; atropine did not significantly affect basal peptic or acid secretory activities (fig. 1).

**Effects of bethanechol on pepsinogen and acid secretions.** In animals with intact vagus nerves, bethanechol (0.3, 1 and 3 mg/kg i.v.) caused a dose-dependent and parallel increase in both pepsinogen and acid secretions, the maximal effects occurring at the dose of 1 mg/kg (fig. 2A and B). The
excitatory responses elicited by bethanechol were not significantly affected by bilateral cervical vagotomy, while they were completely prevented by pretreatment with atropine (fig. 2,C and D). When administered to rats pretreated with omeprazole, bethanechol failed to stimulate acid secretion, but it was still able to evoke an atropine-sensitive increase in pepsinogen output that did not differ from the increase observed in omeprazole-untreated rats (fig. 2,C and D).

**Effects of 2DG and of electrical vagal stimulation on pepsinogen and acid secretions.** In animals with intact vagus nerves, the administration of 2DG (200 mg/kg i.v.) induced significant and simultaneous increases in pepsinogen and acid outputs (fig. 3,A and B). Both of these effects were prevented by bilateral cervical vagotomy as well as by atropine (fig. 3,A and B). However, in contrast with the results obtained in the presence of bethanechol, 2DG failed to stimulate either pepsinogen or acid secretion in omeprazole-pretreated animals (fig. 3,A and B).

Both pepsinogen and acid outputs increased simultaneously after the application of electrical stimulation to the left vagus nerve, and these effects were completely prevented by atropine. However, analogously to 2DG, electrical vagal stimulation was also not able to modify basal pepsinogen or acid secretions after pretreatment of animals with omeprazole (fig. 3,C and D).

In animals where the enzymatic breakdown of ACh was prevented by acute pretreatment with physostigmine, 2DG injection or electrical vagal stimulation evoked atropine-sensitive increases in both peptic and acid outputs (fig. 4). However, whereas acid secretion in response to 2DG or vagal stimulation did not differ from that obtained in physostigmine-untreated rats, the increments in peptic values were higher in the presence of physostigmine than in its absence (P < .05), and they were similar to those induced by bethanechol 1 mg/kg i.v. in physostigmine-untreated animals. In addition, when physostigmine was administered to animals pretreated with omeprazole, both 2DG and vagal stimulation were still able to induce marked and atropine-sensitive increments in pepsinogen secretion (fig. 4,A and C), although they did not exert any stimulant action on acid output (fig. 4,B and D). These peptic responses did not differ from that obtained after bethanechol injection to omeprazole-pretreated rats.

**Effects of perfusion of the gastric lumen with acid solutions.** The influence on pepsinogen secretion exerted by topical intragastric application of solutions with different pH values was studied in animals subjected to bilateral cervical vagotomy and pretreatment with omeprazole. Under these
conditions, perfusion of the gastric lumen with solutions at pH 4.0, 3.0, 2.0 or 1.0 did not modify basal pepsinogen secretion (fig. 5A). However, when gastric perfusion with one of these solutions was carried out in combination with electrical stimulation of the left vagus nerve, the pepsinogen output increased in a pH-dependent manner, the maximal increment occurring at pH 2.0 (fig. 5B). Pretreatment with atropine prevented the peptic response evoked by gastric perfusion with acid solution at pH 2.0 combined with electrical vagal stimulation (fig. 5B).

Effects of bethanechol and of electrical vagal stimulation on pepsinogen and acid secretions after pretreatment with capsaicin or lidocaine. The functional ablation of capsaicin-sensitive sensory neurons by systemic pretreatment with capsaicin did not significantly modify basal gastric secretions and failed also to affect the stimulant actions of bethanechol or electrical vagal stimulation on pepsinogen and acid outputs (fig. 6A and B).

In animals whose gastric mucosa was exposed to lidocaine, both basal peptic and acid outputs did not differ significantly from those measured in lidocaine-untreated animals. In addition, after topical application of lidocaine to the gastric mucosal surface, bethanechol increased both pepsinogen and acid secretions to an extent similar to that induced by the same drug in lidocaine-untreated rats (fig. 6A and B). However, under the same conditions, electrical stimulation of the left vagus nerve caused a significant increase in acid secretion without affecting basal pepsinogen output (fig. 6A and B).

Discussion

Current interest in studying the mechanisms underlying the regulation of gastric pepsin secretion arises from recognition of the relevant role played by this enzyme in the pathophysiology of various digestive disorders, including peptic ulcer (Samloff, 1989; Raufman, 1992) and gastritis associated with Helicobacter pylori infection (Cave and Cave, 1991; Lamers, 1992). As far as the cholinergic control of
pepsinogen secretion is concerned, it is known that a parallel increase in peptic and acid outputs generally occurs after the application of cholinergic stimuli (Smith and Torres, 1990; Hirschowitz and Groarke, 1993), but it remains to be established whether peptic hypersecretion depends mainly on a direct activation of chief cells or on a stimulant action exerted on these cells by the concomitant increase in acid secretory output (Basson et al., 1988; Kaufman, 1992). In the present study, it was possible to dissociate direct from acid-dependent cholinergic regulation of pepsinogen secretion, and we obtained evidence that the secretory response of chief cells to endogenous ACh is driven by the parallel increment of acid output.

The present results showing that, despite a total inhibition of acid output, the basal pepsinogen secretion continued unchanged after treatment of animals with omeprazole, are in agreement with findings obtained from fundic mucosal sheets (Basson et al., 1988) and suggest that in the present model, the basal pepsinogen output is independent of acid secretion. It must be noted also that in this preparation, the anesthesia with urethane depresses the vagal cholinergic outflow to the stomach (Maggi and Meli, 1986). Accordingly, atropine or bilateral cervical vagotomy did not modify basal peptic and acid outputs, which indicates that the anesthetized animal is not subjected to an endogenous tonic cholinergic control, and therefore it represents an useful model to test the influence of applied cholinergic stimuli on gastric secretory functions. It has also been reported that urethane stimulates both synthesis and release of gastric somatostatin (Yang et al., 1990). However, with regard to putative interferences elicited by this peptide in the present study, it should be considered that muscarinic receptors mediate inhibitory effects on somatostatin secretion (Del Tacka et al., 1987), so the application of cholinergic stimuli was expected to counterbalance the somatostatin-releasing action of urethane. In addition, because an increase in gastrin release may occur as a consequence of omeprazole administration (Wilde and McTavish, 1994), the presence of a somatostatin background might have counteracted the possible interference of gastrin in the excitatory effects evoked by cholinergic stimuli.

Putative differences in the peptic response of chief cells to the application of an exogenous cholinergic agonist or to the release of endogenous ACh were investigated in experiments where gastric secretion was elicited by bethanechol, 2DG or electrical vagal stimulation, either in the absence or in the presence of omeprazole. In the absence of omeprazole, these stimulants induced parallel and atropine-sensitive increments of both peptic and acid outputs, which indicates the involvement of muscarinic receptors. However, quite surprising results were obtained when the same stimuli were applied after blockade of acid secretion by omeprazole. Under these conditions, bethanechol was still able to evoke an atropine-sensitive increase in pepsinogen secretion, whereas neither 2DG nor electrical vagal stimulation affected basal peptic output. These findings are compatible with the hypothesis that below a threshold level of muscarinic receptor activation, acid secretion may exert an excitatory influence on the peptic secretory response of chief cells to cholinergic stimuli. In particular, the results obtained in the presence of omeprazole suggest that a background of acid secretion plays a pivotal role in facilitating peptic hypersecretion promoted by endogenous ACh concentrations, which are likely to lead to submaximal and/or short-lasting occupation of muscarinic receptors. In contrast, according to our data as well as those yielded by previous in vitro studies (Hersey et al., 1983; Basson et al., 1988), acid secretion does not appear to be necessary to increase the pepsinogen secretion when muscarinic receptors are activated by pharmacological concentrations of exogenous agonists, such as bethanechol or carbachol, and it rather appears that parietal cells and chief cells can be stimulated simultaneously by these agents only by virtue of sharing similar receptors (Basson et al., 1988). In support of this view, in animals where endogenous ACh concentrations were raised to pharmacological levels by pretreatment with phystostigmine, both the injection of 2DG and the stimulation of vagus nerve induced marked and atropine-sensitive increments in pepsinogen secretion regardless of whether omeprazole-induced acid inhibition was present.

In the present study, a series of experiments was designed in the attempt to gain more insight into the mechanisms underlying the in vivo interaction between hydrogen ions and chief cells. For this purpose, we took care to suppress endogenous acid production by pretreatment with omeprazole, and the gastric mucosal surface was continuously irrigated with iso-osmotic solutions at pH ranging from 4 to 1. Because under these peculiar conditions a pH-dependent and atropine-sensitive increment of pepsinogen output could be achieved only after electrical stimulation of vagus nerve, it is conceivable that hydrogen ions are not able to promote by themselves a direct stimulation of pepsinogen secretion but that, rather, they facilitate the activation of chief cells in response to the release of endogenous ACh. This conclusion is in line with our data showing that basal pepsinogen secretion remained unchanged after suppression of acid secretion by omeprazole, and it is consistent with the findings of previous studies indicating a lack of direct stimulant influence by gastric acidity on the secretory function of chief cells (Puurunen, 1979; Kleveland et al., 1987). Indeed, no significant changes in basal pepsinogen secretion from urethane-anesthetized rats were observed after perfusion of the gastric lumens with iso-osmotic NaCl solutions containing HCl from 0.01 to 100 mM (Puurunen, 1979). Analogously, intragastric perfusion with 1 mM HCl failed to affect basal pepsinogen secretion from isolated and vascuarily perfused rat stomach (Kleveland et al., 1987).

It must be noted also that although observations arguing in favor of a direct pepsigic action exerted by luminal hydrogen ions have been previously reported (Johnson, 1972; Smith and Torres, 1990), they probably reflected the occurrence of methodological bias. For instance, significant increments of pepsinogen secretion were detected after topical application of acid solutions to the gastric mucosa in humans (Bynum and Johnson, 1975; Smith and Torres, 1990), dogs (Johnson, 1972), cats and rabbits (Descroix-Vagne et al., 1993). In all these cases, however, the peptic response did not appear clearly to be related to pH changes, but rather to concomitant gastric distension or osmotic stimulation by test solutions (Descroix-Vagne et al., 1993). Accordingly, distension of the stomach wall and osmolarity of the gastric contents are well recognized as stimulants of pepsin secretion in different mammalian species, acting by gastrin release or local cholinergic reflexes (Descroix-Vagne et al., 1993; Sen-gupta and Gebhart, 1994). In addition, when incubated in the
presence of acidified media, isolated gastric gland preparations increased their pepsinogen release, but under those conditions, chief cells were subjected to alterations in intracellular pH that occur only after marked back-diffusion of acid through a damaged gastric mucosal barrier (Norris and Hersey, 1983).

There is evidence in the literature to support the view that changes in hydrogen ion concentration might modulate pepsinogen secretion indirectly by interacting with pH-sensitive mucosal chemoreceptors, which in turn would sensitize chief cells to the stimulant action of endogenous cholinergic pathways. Indeed, electrophysiological studies demonstrated the existence of gastric mucosal receptors that respond to low pH in various species (Norris and Hersey, 1983; Yamamoto et al., 1994a). In addition, the rat stomach is provided with two distinct populations of sensory fibers, belonging to either capsaicin-sensitive afferent nerves or capsaicin-insensitive intrinsic neurons, which can affect some gastric functions in response to changes in intraluminal pH (Geppetti et al., 1991).

Because capsaicin-sensitive nerves of the GI mucosa have been implicated in the control of various digestive functions (Sharkey et al., 1991; Pabst et al., 1993), we attempted first to ascertain whether capsaicin-sensitive fibers might be involved in the sensitizing action exerted by hydrogen ions on chief cells. The present data, which show that bethanecol or vagal stimulation was still able to induce simultaneous increments of acid and peptic outputs after pretreatment with capsaicin, indicate that systemic capsaicinization did not influence the functional status of chief cells and suggest that capsaicin-sensitive sensory nerves are not implicated in the mechanisms linking pepsinogen secretion to changes in hydrogen ion concentration. In accordance with these findings, Sharkey et al. (1991) showed that in urethane-anesthetized rats subjected to vagal stimulation at 4 Hz, the gastric secretory response is totally independent of the activation of capsaicin-sensitive afferent fibers.

In the present study, different results from those obtained after systemic capsaicinization were obtained when gastric mucosa was acutely exposed to lidocaine in order to induce a local surface anesthesia. Lidocaine-pretreatment did not impair the ability of parietal or chief cells to respond to secretory inputs, as demonstrated by the significant and parallel increase in both acid and pepsinogen secretions after bethanecol administration. However, under the same conditions, vagal stimulation evoked acid secretion but did not affect basal peptic output, which suggests that mucosal afferent fibers, probably belonging to capsaicin-insensitive intrinsic neurons, drive an acid-mediated facilitatory input to chief cells, allowing a peptic secretory output in response to the activation of mucaric receptors by endogenous ACh. In support of this view, previous studies indicated that capsaicin-insensitive intrinsic neurons of rat stomach can be activated by low pH levels to promote a reflex increase in mucosal blood flow (Geppetti et al., 1991).

Although the existence of a local intramural reflex linking pepsinogen output to changes in acid secretion may represent a possible explanation for our findings, two points remain to be clarified: 1) how gastric intrinsic neurons can detect pH variations and 2) how these neurons can transmit their information to chief cells. With regard to the first point, it is hard to believe that under normal conditions, hydrogen ions can penetrate the gastric epithelium to reach the free endings of intrinsic sensory fibers, and it is generally considered that such nerve terminals may be connected to special sensory structures sensitive to acidic pH (Yamamoto et al., 1994a). Accordingly, enteroendocrine or enterochromaffin cells of the mucosal epithelium are currently regarded as possible transducer elements for pH variations (Sengupta and Gebhart, 1994; Yamamoto et al., 1994a), and it has been postulated that, once activated by gastric acidification, these cells release a specific factor that, in turn, stimulates local sensory nerve terminals (Yamamoto et al., 1994b). As far as the second issue is concerned, the activation of a local acid-dependent reflex might theoretically result either in an enhancement of ACh release from cholinergic terminals or in the release (or co-release) of a distinct transmitter acting on chief cells to facilitate the pepsipogic action of ACh. However, further investigations are needed to clarify this issue.

In conclusion, the present results suggest that the activation of muscarinic receptors on chief cells by vagally released ACh is not sufficient to increase peptic output and that an acid-mediated facilitatory action is necessary to allow pepsinogen hypersecretion in response to stimulation by endogenous cholinergic pathways. According to our findings, it may be proposed that such acid-dependent facilitatory input is driven to chief cells by a local gastric intramural reflex that involves capsaicin-insensitive intrinsic neurons. Overall, the hypothesis emerging from these findings would reconcile the conflicting results of previous reports that have indicated, on the one hand, a close parallel between acid and peptic hypersecretory responses to cholinergic stimulation in vivo and, on the other, the ability of chief cells to be activated by muscarinic agonists in vitro independently of acid secretion.

Acknowledgments
The experiments were carried out with the technical assistance of Mr. Bruno Stacchini.

References