Secretory function of the esophageal mucosa in opossum: the role of cholinergic, peptidergic and histaminergic pathways

Namiot Z[1], Sarosiek J[2], McCallum RW[2]

[1] Department of Medicine and Gastroenterology, J. Śniadecki District Hospital, Białystok, Department of Physiology, Medical University of Białystok, Poland

[2] University of Kansas Medical Centre, Department of Medicine, Division of Gastroenterology and Hepatology, Gastroenterology Research Laboratory, Kansas City, USA

Abstract

Purpose: Esophageal histology in the opossum reveals numerous submucosal mucous glands closely resembling those commonly found in humans. The aim of the study was to examine the secretion of these glands using the commonest secretagogues.

Material and methods: The esophageal lumen in 5 male opossums was continuously perfused with saline during sixteen consecutive 5 min perfusion periods. After four initial equilibrating periods, each animal was injected (s.c.) with pentagastrin (6 μg/kg), bethanechol chloride (100 μg/kg) or histamine dihydrochloride (0.5 mg/kg). All sixteen (5 min) perfusates were assayed for protein by Lowry, mucin by PAS and viscosity using a cone/plate digital viscometer. Results were expressed as mean ± SE. Statistical analysis was performed using paired Student's t-test.

Results: Administration of bethanechol resulted in a significant increase in esophageal mucin release from 2.4 ± 0.4 to 8.0 ± 1.2 μg/cm²/min ($p < 0.01$); enhancement of protein output from 8.9 ± 2.0 to 20.4 ± 2.9 μg/cm²/min ($p < 0.01$) and potentiation of specific viscosity from 7.5 ± 0.6 to 14.4 ± 0.8 ($p < 0.01$). Pentagastrin-induced release of mucin reached the maximal value of 5.5 ± 0.7 μg/cm²/min ($p < 0.01$), protein output increased to 20.0 ± 2.7 ($p < 0.01$) and viscosity expanded to 11.7 ± 0.9 ($p < 0.05$). Histamine evoked an increase in mucin release to 3.9 ± 0.4 μg/cm²/min ($p < 0.01$), protein output to 24.1 ± 3.3 μg/cm²/min ($p < 0.01$) and viscosity to 12.8 ± 1.1 ($p < 0.05$).

Conclusions: The significant influence of cholinergic, histaminergic and peptidergic stimulation on physical and chemical properties of the esophageal secretion provides evidence for the role of these pathways in the pathophysiology of the esophageal mucosa.

Key words: esophageal secretion, bethanechol, pentagastrin, histamine.

ADDRESS FOR CORRESPONDENCE:
Zbigniew Namiot
Department of Medicine and Gastroenterology
J. Śniadecki District Hospital
ul. M. Skłodowskiej-Curie 25
15-950 Białystok, Poland
Tel: +48 85 7488448 Fax: +48 85 7488502
e-mail: namiotz@poczta.onet.pl

Received 29.09.2003 Accepted 4.05.2004

Introduction

Gastroesophageal reflux disease usually develops either when excessive gastroesophageal reflux cannot be compensated by normal protective mechanisms or when the reflux remains within normal limits but protective mechanisms are severely compromised. The gastric content refluxed into the esophagus can damage the mucosa through the presence of hydrochloric acid, pepsin, bile salts, and pancreatic enzymes. Since the refluxate pH is most frequently acidic, the major injuries factors are hydrochloric acid and pepsin. Esophageal mucosa, due to the anatomical proximity to the deleterious gastric milieu, developed effective mechanisms preventing its damage by aggressive factors. The esophageal mucosa in the cat, opossum and man, although covered by nonkeratinized squamous epithelium, contains numerous submucosal mucous glands [1-4]. The secretion of these glands, although histochemically proven to be rich in mucins, has never been explored in detail. By using an esophageal perfusion catheter specially designed for testing the esophageal secretory function in vivo [5,6], we were able to study the physical and chemical characteristics of the esophageal secretion in the opossum. Knownig that acetylcholine, histamine and gastrin are the commonest secretagogues governing the secretory function within the gastrointestinal mucosa, we explored their influence on the major components of esophageal secretion.

Material and methods

Preparation of animals

Five opossums (3.5 kg, Didelphis virginiana) were used in the study according to a protocol approved by Animal Research Committee. After overnight fasting opossums were anesthetized with ketamine (20 mg/kg i.m.) and infused with sodium pentobarbital (30 mg/kg/h i.v.) throughout the entire experimental procedure.

Esophageal perfusion catheter

Esophageal perfusion in opossums was performed using a specially designed 6-channeled catheter equipped with two balloons (balloons spaced 10 cm apart, and were 20 mm in length) as described previously [5]. Briefly, to ensure an excellent recovery rate of esophageal perfusate, an optimal diameter of the inflated lower balloon was 25 mm (inflated with 8 ml of air) whereas the optimal diameter of the upper balloon was 23 mm (inflated with 7 ml of air).

Catheter channels were designated as follows: the first and largest diameter (3 mm) channel was utilised for aspiration of esophageal secretion elaborated during perfusion. The second channel (1.5 mm) served for gastric aspiration to determine any loss of esophageal perfusate into the gastric lumen from the measurement of [^{14}C] polyethylene glycol. The third and fourth channels (1 mm) were connected directly with the lower and the upper balloons, respectively, to control their pressure and volume. The fifth channel was designed to aspirate saliva and esophageal secretion potentially collecting within the esophageal lumen above the upper balloon. The sixth channel was used as an infusion port for esophageal perfusion.

Esophageal perfusion model

The location of the lower esophageal sphincter was identified through an initial esophageal motility recording using a standard perfusion catheter in a pilot study based on similar size and weight of animals. Therefore, we could identify the optimal location for the subsequent placement of our perfusion catheter. Based on motility, the esophageal perfusion catheter was inserted into the esophagus with the proximal balloon located 14 cm and distal balloon 24 cm from incisors. Such placement of the catheter compartmentalized a 10 cm segment of esophagus extending from below the upper esophageal sphincter to just above the lower esophageal sphincter. This segment of the esophagus, comprising most of its length, was sealed between two balloons and exposed to perfusing solutions during the experimental procedure. In all animals, the placement of the esophageal catheter was performed with the same technique so that the same areas of the esophageal mucosa were exposed to perfusate.

Esophageal perfusion procedure

Esophageal perfusion in each opossum was performed using NaCl (0.15 M). [^{14}C] polyethylene glycol was used as a non-absorbable marker of the esophageal volume. The experimental procedure involved sixteen 5 min periods (80 min). In each 5 min period, fresh perfusion solution was infused into the esophageal lumen and circulated with the flow rate of 10 ml/min. The selection of the perfusion rate was based on preliminary experiments indicating that this rate would not cause distention of the esophagus or tissue damage from shear force. After 5 minutes, the entire volume of perfusate was aspirated, secured for analyses, and replaced with the next solution.

After four initial 5 min periods, when equilibration of esophageal secretory function was obtained, an injection (s.c.) of saline (control) or secretagogue was implemented. Each secretagogue was injected (s.c.) in a separate experimental setting allowing animals to fully recover from the previous procedure; a 7-day wash-up period was applied. Bethanechol chloride (Merck), pentagastrin (Ayerst) and histamine hydrochloride (Sigma) were administered (s.c.) in a dose of 100 μg/kg, 6 μg/kg and 0.5 mg/kg, respectively.

Measurements

The evaluation of mucin in esophageal perfusate was based on the method developed by Mantle et al. [7] and protein on the method developed by Lowry et al. [8]. The viscosity of freshly recovered esophageal perfusates was measured using a cone/plate digital viscometer as described previously [9]. Results are for the lowest shear rate (2.25 sec^{-1}) at CP-40 cone, applying the most physiological shear stress to the samples, and were expressed in specific viscosity units.

Data analysis

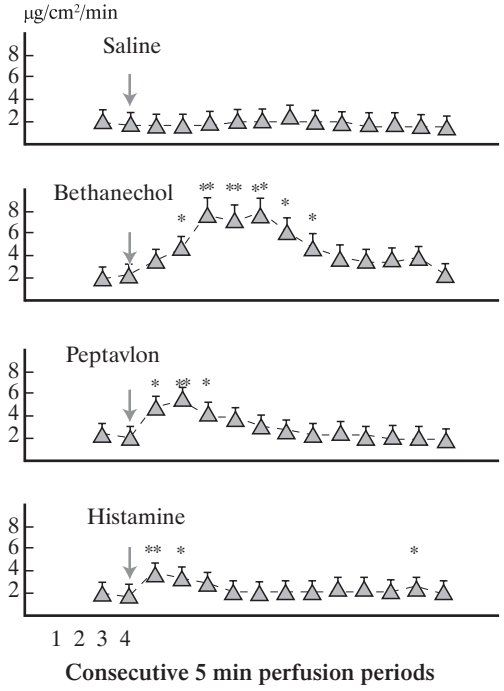
In order to eliminate potential contamination of esophageal perfusate with salivary proteins adsorbed to the esophageal mucosa, we discarded the first two 5 min esophageal perfusate samples from the current analysis. As it can be clearly seen from the control figures, the rate of luminal release of esophageal secretion components reached a plateau during the third and fourth perfusion periods. Therefore, in all samples statistical analysis was performed to compare data obtained after injection of secretagogue (between the fifth and sixteenth perfusion periods) with that recorded in the fourth perfusion period (the last before injection of secretagogues). Results are expressed as mean ± SE. Statistical analysis was performed using paired Students t-test.

Results

Esophageal mucin release

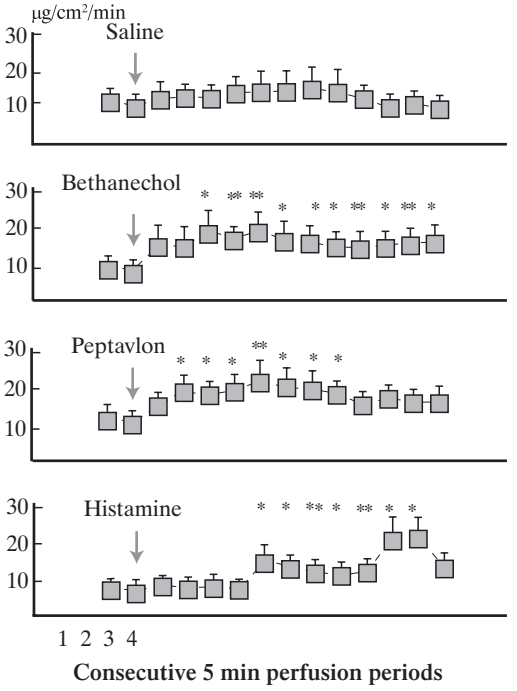
The basal rate of esophageal luminal mucin release was 1.9 ± 0.4 μg/cm^2/min and remained steady throughout the entire control esophageal perfusion procedure with saline (*Fig. 1*). Administration of bethanechol resulted in a significant increase in esophageal mucin release in a step-wise fashion reaching its maximum in 15 minutes from the moment of injection (8.0 ± 1.2 μg/cm^2/min; $p < 0.01$). A significant effect of bethanechol on esophageal mucin release lasted 30 minutes, although at the end of the experimental procedure (60 min), mucin release was still higher than that observed in control animals. The maximal secretory effect of pentagastrin was achieved 5 min faster than that after bethanechol and was also statistically significant (5.5 ± 0.7 vs 2.2 ± 0.4 μg/cm^2/min; $p < 0.01$). The effect of pentagastrin lasted only 15 minutes,

Figure 1. **Effect of bethanechol, peptavlon and histamine on the luminal release of mucin**



* $p < 0.05$; ** $p < 0.01$ as compared to the corresponding values recorded during 4th perfusion period. Arrow indicates the time of injection (s.c.) of the investigated agent

Figure 2. **Effect of bethanechol, peptavlon and histamine on the luminal release of protein**



* $p < 0.05$; ** $p < 0.01$ as compared to the corresponding values revealed during 4th perfusion period. Arrow indicates the time of injection (s.c.) of the investigated agent

although some sustained elevation of mucin release was observed for 30 minutes. Administration of histamine resulted in the fastest secretory response of all secretagogues since it occurred within the first 5 minutes (3.9 ± 0.4 vs 2.0 ± 0.4 μg/cm²/min; $p < 0.01$). This effect, however, was short-lived, although some potentiation of mucin release also took place near the end of perfusion procedure.

Esophageal protein output

Esophageal perfusion with saline resulted in a continuous release of esophageal protein into the perfusate (*Fig. 2*), reaching steady-state phenomenon (12.4 ± 2.8 μg/cm²/min) within the third and fourth perfusion periods in control experiments. Administration of bethanechol resulted in a profound and significant increase in the luminal protein release, reaching its maximum within fifteen minutes of the stimulation (20.4 ± 2.9 vs 8.9 ± 2.0 μg/cm²/min; $p < 0.05$). This stimulatory effect of bethanechol remained significant to the end of the experimental procedure. Pentagastrin also evoked a well sustained stimulatory effect on the luminal release of protein reaching a significantly higher value of 20.0 ± 2.7 μg/cm²/min ($p < 0.05$) after 10 minutes from its injection. The effect of pentagastrin lasted 40 minutes. The effect of histamine on protein release was evidently biphasic with the first peak during the ninth perfusion period and the second at the end of the perfusion procedure.

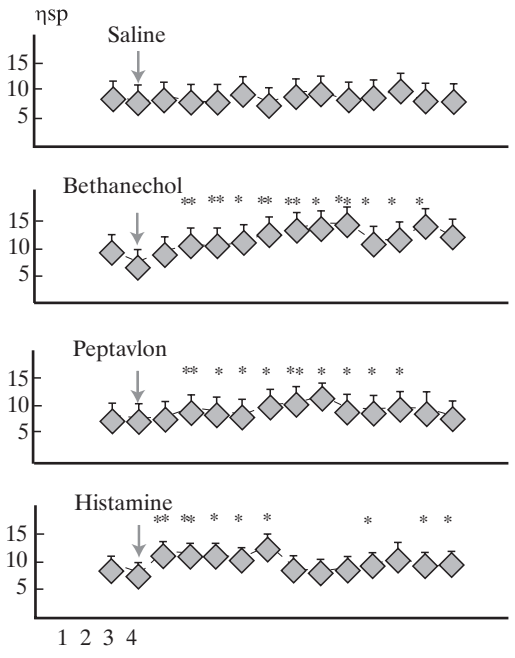
Viscosity of esophageal secretion

The viscosity of the esophageal secretion (*Fig. 3*), measured at the lowest shear rate (2.25 sec^{-1}), mimicking the naturally existing physical forces in vivo, revealed that its value remained unchanged throughout the entire perfusion procedure in control experiments. Administration of bethanechol resulted in a step-wise potentiation of esophageal secretion viscosity reaching its maximal value of 14.4 ± 0.8 vs 7.5 ± 0.6 ($p < 0.01$) during the twelfth consecutive perfusion period and was maintained almost throughout the entire perfusion procedure. Also, pentagastrin evoked a long-lasting stimulatory effect on esophageal secretion viscosity, reaching its maximum during the eleventh consecutive perfusion period (11.7 ± 0.9 vs 7.8 ± 0.7; $p < 0.05$). Even histamine administration resulted in a significant and long-lasting effect on esophageal secretion viscosity, reaching its maximum of 12.8 ± 1.1 vs 8.0 ± 0.5 ($p < 0.05$) at the ninth consecutive perfusion period.

Discussion

The integrity of the alimentary tract mucosa depends upon equilibrium between aggressive factors and protective mechanisms. Although the role of gastric acid and pepsin in the pathophysiology of the esophageal mucosa has been well explored, the role of esophageal mucosal secretion as an

Figure 3. **Effect of bethanechol, peptavlon and histamine on the viscosity of esophageal perfusate**



* $p < 0.05$; ** $p < 0.01$ as compared to the corresponding values measured during 4th perfusion period. Arrow indicates the time of injection (s.c.) of the investigated agent

essential protective factor against damaging agents has not been studied in detail. It has been previously shown that the cat and human esophageal mucosa, sharing similar mucosal and submucosal morphology, have a secretory potential to continuous luminal release of mucin [5,10], epidermal growth factor [6], PGE_2 [11], and bicarbonate [12]. Since numerous submucosal mucous glands are also histologically proven in the opossum [3,4], we have studied their secretion after stimulation with the main secretagogues governing the secretory function of the gastrointestinal tract.

In previous study pentagastrin administered i.v. in a dose of 2 μg/kg/h significantly enhanced gastric mucus secretion in humans [13]. Also histamine (0.05 mg/kg s.c.) was reported to increase gastric mucin output in both controls and patients with gastritis [14]. Furthermore, it has been shown that the biosynthesis of canine gastric mucin was significantly enhanced in response to carbamylcholine, gastrin and histamine [15]. This indicates that gastric mucin synthesis and secretion remain under the significant impact of cholinergic, histaminergic and peptidergic pathways. Our present data suggest that the same pathways exist within esophageal mucosal compartment in opossum.

We have found significant enhancement of esophageal protein release under the impact of all applied stimuli. The origin of this protein remains unknown. We may assume that submucosal mucous glands are the most likely source of these components. Some proteins may have originated from the serum (predominantly albumin) due to an increase in permeability of the mucosa after secretagogues [16], however the contribution of this source of protein may be smaller than that from submucosal glands.

The reason for biphasic effect of secretagogues on protein and mucin release in the fasted state may be explained by coexistent periodic secretion related to the motility patterns. It has been shown that during fasting, the propagation of the migrating motor complex is associated with an increase of secretion in the stomach, pancreas, and intestines [17]. Is it the case also in the esophagus, so far there are no data.

Despite the relatively large volume (10 ml) of the circulating for 5 minutes solution of saline, we were able to record quite high specific viscosity of the esophageal perfusate. Although the mucin is the strongest contributing factor to the viscosity of esophageal perfusate, total protein affects its value as well; this was particularly evident after histamine administration. Considering the fact that viscosity of the esophageal secretion is decisive in the maintenance of the mucus layer on the surface of the epithelium [18], we are convinced that such a layer is able to inhibit hydrogen ion diffusion, and thus contribute significantly to the maintenance of the esophageal mucosal integrity [19].

The results of the present study indicate that esophageal secretion in the opossum remains under the significant influence of cholinergic, peptidergic, and histaminergic pathways and that the strongest secretory response was observed during cholinergic stimulation, followed by pentagastrin and histamine. These data, if confirmed in humans may open new avenues for advanced understanding of the esophageal mucosa pathophysiology. It may also help to design more efficacious treatment for some patients with reflux esophagitis.

References

1. Hopwood D, Coghill G, Sanders DS. Human oesophageal submucosal glands. Their detection mucin, enzyme and secretory protein content. Histochemistry, 1986; 86: 107-12.

2. Chiasson RB, Booth ES. Digestive system. In: Chiasson RB, Booth ES, editors. Laboratory anatomy of the cat. Dubuque, Iowa: WCB Co; 1977, p. 63-70.

3. Turner KS, Powell DW, Carney CN, Orlando RC, Bozymski EMT. Transmucosal electrical potential difference in the mammalian esophagus in vivo. Gastroenterology, 1978; 75: 286-91.

4. Boyd DD, Carney CN, Powell DW. Neurohumoral control of esophageal epithelial electrolyte transport. Am J Physiol (Gastrointest Liver Physiol), 1980; 239: G5-G11.

5. McCallum RW, Feng T, Frierson HF, Sarosiek J. A new experimental model esophagitis in the cat: The role of esophageal mucus and bicarbonate in mucosal protection against peptic erosion and back diffusion of hydrogen ion. Exper Clin Gastroenterol, 1992; 2: 90-6.

6. Sarosiek J, Yu Z, Hetzel DP, Rourk RM, Piaścik R, Li L, McCallum RW. Evidence on secretion of EGF by the esophageal mucosa in humans. Am J Gastroenterol, 1993; 88: 1081-7.

7. Mantle M, Allen A. A colorimetric assay for glycoprotein based on the Periodic Acid/Schiff stain. Biochem Soc Transac, 1978; 6: 607-9.

8. Lowry OH, Rosenbrough NJ, Farr AL, Randall RI. Protein measurement with folin phenol reagent. J Biol Chem, 1951; 193: 265-75.

9. Murty VLN, Sarosiek J, Slomiany A, Slomiany BL. Effect of lipids and proteins on the viscosity of gastric mucus glycoprotein. Biochem Biophys Res Commun, 1984; 121: 521-9.

10. Namiot Z, Sarosiek J, Rourk RM, Hetzel DP, McCallum RW. Human esophageal secretion: Mucosal response to luminal acid and pepsin. Gastroenterology, 1994; 106: 973-81.

11. Sarosiek J, Yu Z, Namiot Z, Rourk M, Hetzel DP, McCallum

RW. Impact of acid and pepsin on human esophageal prostaglandins. Am J Gastroenterol, 1994; 89: 588-94.

12. Meyers RL, Orlando RC. In vivo bicarbonate secretion by human esophagus. Gastroenterology, 1992; 103: 1174-78.

13. Gabryelewicz A, Sarosiek J, Łaszewicz W. The effect of ranitidine on gastric mucus and pepsin secretion in patients with peptic ulcer. Acta Med Pol, 1981; 16: 226-34.

14. Badurski J, Sarosiek J, Gabryelewicz A, Zarzycki W, Głowacka D, Musiatowicz B, Zimnoch L. Changes in gastric secretory function in patients with chronic cholecystitis. Przegl Lek, 1973; 30: 259-62.

15. Scheiman JM, Kraus ER, Boland CR. Regulation of canine gastric mucin synthesis and phospholipid secretion by acid secretagogues. Gastroenterology, 1992; 103: 1842-50.

16. Greenwood B, Mantle M. Mucin and protein release in the rabbit jejunum: Effects of bethanechol and vagal nerve stimulation. Gastroenterology, 1992; 103: 596-605.

17. Davison JS. The enteric nervous system. In: Greger R, Windhorst U, editors. Comprehensive human physiology. Berlin-Heidelberg: Springer; 1996, p. 1415-23.

18. Sarosiek J, Namiot Z, Piaścik R, Hetzel DP, Rourk RM, Edmunds MC, Daniel TM, McCallum RW. What part do the mucous cells of submucosal mucous glands play in the esophageal pre-epithelial barrier? In: Giuli R, Tytgat GNJ, DeMeester TR, Galmiche JP, editors. The esophageal mucosa. Amsterdam-Lausanne-New York-Oxford-Shannon-Tokyo: Elsevier; 1994, p. 278-90.

19. Quigley EMM, Turnbereg LA. PH of the microclimate lining human gastric and duodenal mucosa in vivo: Studies in control subjects and in duodenal ulcer patients. Gastroenterology, 1987; 92: 1876-84.