Esophageal smooth muscle reactivity is impaired in chronic reflux esophagitis by both receptor- and nonreceptor-mediated mechanisms

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Abstract

Aim: Esophagitis is associated with an impaired esophageal peristalsis. A few studies have been aimed at understanding the pathophysiology of abnormal peristaltic activity. The mechanism of impaired esophageal smooth muscle reactivity in the chronic gastroesophageal reflux (GER) model is investigated in vitro for the first time.

Materials and Methods: The chronic GER rat model was created by partial gastric outlet obstruction. The histopathological findings related to esophagitis were evaluated. Smooth muscle strips of the tunica muscularis mucosa of esophagus were studied in standard organ chambers. Carbachol- and KCl-induced contractile responses and serotonin- and papaverine- induced relaxant responses in both reflux and sham-operated control groups were determined.

Results: Histopathologically, chronic reflux esophagitis was observed in all specimens of the reflux group. Contractile (carbachol- and KCl-induced) smooth muscle responses were significantly decreased in the reflux group. When compared to control group, relaxant response of smooth muscle to serotonin was also significantly decreased in the reflux group. However, there was no difference in papaverine-induced relaxant responses between 2 groups.

Conclusions: Our study describes the effects of chronic GER on rat esophageal smooth muscle contractility in vitro. We found that both receptor- (carbachol, serotonin) and nonreceptor-mediated (KCl) esophageal smooth muscle reactivity were impaired in chronic reflux esophagitis. These changes may correspond to the functional motor abnormalities of the esophagus seen in patients with chronic reflux esophagitis.

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In vitro;
Esophagus

Esophageal motility abnormalities, common in gastroesophageal reflux (GER) disease, are likely to be related to esophagitis. Impaired esophageal smooth muscle reactivity
is a major determinant in the pathogenesis of abnormal esophageal motor activity [1-6]. The question of the effect of GER disease on esophageal motor function is extremely important and well worth studying. Previously, within the limitations imposed by the animal models, it was suggested that impaired smooth muscle response was a contributing factor in the impaired esophageal motor function [7]. However, in that study, esophagus was exposed to acid reflux for only 24 hours. Although this could explain to some extent the abnormal esophageal smooth muscle reactivity, it was not in accordance with the human situation. Therefore, we investigated the effect of reflux-induced esophagitis on the smooth muscle reactivity of the esophageal body in the chronic GER model. During the study, the following compounds acting at different sites were employed: (1) carbachol acts predominantly on cholinergic muscarinic receptors and leads to $\mathrm{Ca}^{2+}$ influx in esophageal muscle and excitation-contraction coupling mechanism; (2) 5-hydroxytryptamine (5-HT) stimulates cyclic adenosine monophosphate (cAMP) formation via adenylyl cyclase in the tunica muscularis mucosa via 5-HT_{4} receptors leads to esophageal smooth muscle relaxation; (3) KCl-related depolarization activates voltage-gated $\mathrm{Ca}^{2+}$ channels and initiates receptor-independent smooth muscle contraction; (4) papaverine is a phosphodiesterase inhibitor that directly relaxes smooth muscle via cAMP synthesis receptor-independent mechanism. The effect of chronic GER was studied on (1) concentration-response curves of full agonists and (2) the histology of the tissue.

### 1. Materials and methods

#### 1.1. Animals

Male Sprague-Dawley rats weighing between 200 to 250 g (n = 53) were housed in an air-conditioned room with 12-h light/dark cycles, where the temperature (22°C ± 2°C) and relative humidity (65%-70%) were kept constant. Standard laboratory chow was withdrawn 16 hours before surgery with free access to water. All experimental protocols were approved by the Animal Care and Use Committee of the Marmara University School of Medicine.

#### 1.2. Surgery and experimental design

Ketamine hydrochloride (20 mg/kg) and Rompun (100 mg/kg) were administered intraperitoneally as anesthetics for the operation. Under anesthesia, the rats were placed in supine position, and the skin was washed with 10% povidine-iodine solution. The operation was performed through an upper abdominal midline incision. In the reflux group, chronic acid reflux esophagitis was induced by pyloric stenosis plus limiting ridge ligation method [8]. Pyloric stenosis was made by covering the duodenum near the pyloric ring with a small piece of an 18F Nelaton catheter, whereas the transitional region between the forestomach and the glandular portion (limiting ridge) was ligated by a nonabsorbable suture. In the sham-operated group, the abdomen was opened through a midline incision, and similar surgical manipulations were performed as described above. All the animals were decapitated 2 months after surgery.

### 1.3. Histopathological examination

Evidence of GER was examined through histologic evaluation in all animals. Approximately 1.5 cm of thoracic esophageal segment was excised and opened longitudinally and fixed in 10% formalin for 24 hours. Each specimen was examined macroscopically to detect signs of esophagitis. One longitudinal strip along the entire length of the esophagus was taken and processed routinely, embedded in paraffin, sectioned at 3 to 5 μm, and stained with H&E. The histopathological findings of GER were evaluated by an experienced pathologist who was unaware of the experimental groups using a light microscope. Thickness of esophageal mucosa, elongation of the lamina propria papillae, inflammatory cells infiltration, interruption of lamina muscularis mucosa, and amount of collagen fibers in lamina propria and submucosa were accepted as typical histopathological features of chronic reflux esophagitis. Thickness of esophageal mucosa was assessed by an ocular micrometer.

### 1.4. Organ chamber experiments

The experiments were made in the Experimental Research Laboratory (DETAB) of the Kocaeli Medical School. Esophageal smooth muscle strips were prepared and studied in 20-mL water-jacketed organ baths for isometric tension recording [9]. Silk ligatures were applied at both ends of the preparations and the strips were then mounted between 2 metal prongs in thermostatically controlled (37°C) organ baths bubbled with a mixture of 95% oxygen and 5% carbon dioxide (pH 7.4). As a standard procedure, the fluid in the organ baths was replaced every 20 minutes with fresh solution. The tissue baths contained Tyrode’s solution composed of (mmol/L): NaCl, 136.0; KCl, 2.7; CaCl_{2}, 1.8; MgCl_{2}, 1.05; NaH_{2}PO_{4H_{2}O}, 0.42; NaHCO_{3}, 11.9; glucose, 5.5. After mounting, an initial tension of 0.5 g was applied, and an equilibration period of approximately 90 minutes was allowed during which tension was adjusted to attain final stable tension level for optimal force development. To verify the contractile ability of the preparations, 80 mmol/L of KCl was added to the organ baths. Agonists were added directly to the organ bath. At the completion of each experiment, tissues were lightly blotted and weighed. Isometric tension was recorded on a computer via 4-channel transducer data acquisition system (TDA-94 COMMAT, COMMAT Iletisim Co Ankara, Turkey) using a software (Polywin 95 ver 1.0 COMMAT, COMMAT Iletisim Co), which also analyzed the data. In a series of preliminary experiments, esophageal strips were stimulated with 80 mmol/L of KCl. In examining the
contractile response to the muscarinic agonist carbachol (10^{-10}-3.10^{-4} \text{ mol/L}), cumulative concentration-response curves were constructed in a stepwise manner after the response to the previous concentration had reached a plateau. After completion of carbachol dose-response curve, tissues were washed for a further 60 minutes and precontracted with a submaximal concentration of carbachol (10^{-6}-3.10^{-5} \text{ mol/L}). After the contractions reached a plateau, concentration-response relationships for serotonin (10^{-10}-10^{-4} \text{ mol/L}) and papaverine (10^{-7}-10^{-4} \text{ mol/L}) were obtained in a cumulative manner.

1.5. Analysis of data

The data are expressed in concentration-response curves. Results are expressed as mean ± SEM where n equals the number of animals. The contractile force was expressed as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the tension precontracted with carbachol. Concentration response curves were fitted by nonlinear regression with simplex algorithm, and $E_{\text{max}}$ and $EC_{50}$ values were calculated using the software of transducer data acquisition system. $E_{\text{max}}$ is the maximal response that can be produced by the drug. $EC_{50}$ is the concentration of drug produces 50% of maximal effect.

1.6. Statistical analysis

Statistically significant differences among groups were calculated by 1-way analysis of variance with a post hoc Tukey-Kramer test. Probabilities of less than 5% ($P < .05$) were considered significant.

1.7. Drugs

After chemicals were obtained from Sigma Chemical (St Louis, Mo): carbachol (carbamylcholine chloride), serotonin (serotonin creatinine sulfate), and papaverine hydrochloride. In the high K+ solution, NaCl was exchanged for equimolar amounts of KCl. Drugs were prepared daily in distilled water and kept in ice during the course of experiments.

2. Results

In specimens of sham groups, esophageal mucosa consisted of keratinized stratified squamous epithelium with

![Fig. 1](image1.png)

Fig. 1 Microscopic appearance of esophagus of sham group (H&E, original magnification $\times 40$).

![Fig. 2](image2.png)

Fig. 2 Basal cell hyperplasia, elongation of lamina propria and chronic inflammatory cell infiltration as histopathological findings of chronic esophagitis in reflux group (H&E, original magnification $\times 100$).

![Fig. 3](image3.png)

Fig. 3 Carbachol concentration-response curves in isolated rat esophageal strips. All points show the mean ± SEM of responses obtained individual experiments on different tissues from different animals. Data are expressed as milligrams of tension per mg of tissue wet weight. $^*P < .05$, compared with the other group.
thin basal cell zone occupying less than 15% of the total epithelial thickness and lamina propria was made up of loose connective tissue (Fig. 1). There were no histopathological features of esophagitis. In experimentally induced reflux esophagitis group, there were epithelial thickening, manifested by expansion of basal cell zone, which was characterized by increased thickness of basal cell layer exceeding 15% of the total epithelial thickness, elongation of lamina propria papillae, infiltration of lymphocytes, eosinophils, and neutrophils in the epithelial and subepithelial areas (Fig. 2). These findings were considered as typical histopathological features of chronic reflux esophagitis. There was no mucosal erosion or ulceration in this group.

The specific alterations observed in these experiments may be listed as follows: There was no significant difference in the mass of strips used for the contractility studies. The cumulative addition of carbachol (10^{-10} - 3.10^{-4}M) produced concentration-dependent contractions of the esophageal strips. Reflux group had lower contractile responses to carbachol than the sham group (Fig. 3). There was a significant decrease in the maximum responses (E_{max}) but not EC_{50} values of strips obtained from the reflux group compared with the sham group (Table 1). Contraction elicited by 80 mmol/L KCl and E_{max} values were significantly decreased in the reflux group compared with the sham group (Fig. 4, Table 1).

Serotonin produced concentration-dependent relaxation in submaximally (55%-60% of maximal contraction) precontracted (3.10^{-6} mol/L carbachol) esophageal strips obtained from each group. When tissues were contracted with carbachol to assess relaxant responses to serotonin, tension induced by carbachol did not significantly change in the groups. Relaxation in response to serotonin was significantly decreased in the reflux group compared to the sham group (Fig. 5). In addition, E_{max} values were also significantly decreased in the reflux group compared with the sham group (Table 1).

In precontracted strips, papaverine produced concentration-dependent relaxations. Relaxation elicited by papaverine was similar in all groups, and there were no significant changes in EC_{50} or E_{max} values (Table 1).

### Table 1: Comparison of maximal effects (E_{max}) and EC_{50} values in sham and reflux groups

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Sham</th>
<th>Reflux</th>
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</thead>
<tbody>
<tr>
<td>E_{max}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>19.94 ± 1.89</td>
<td>12.36 ± 1.05*</td>
</tr>
<tr>
<td>Carbachol</td>
<td>36.96 ± 0.45</td>
<td>26.05 ± 0.73*</td>
</tr>
<tr>
<td>Serotonin</td>
<td>83.27 ± 10</td>
<td>59.52 ± 0.12</td>
</tr>
<tr>
<td>Papaverine</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>EC_{50}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>2.25.10^{-7} + 0.16</td>
<td>3.64.10^{-7} + 0.63</td>
</tr>
<tr>
<td>Serotonin</td>
<td>1.03.10^{-6} + 0.38</td>
<td>2.67.10^{-6} + 1.28</td>
</tr>
<tr>
<td>Papaverine</td>
<td>7.9.10^{-6} + 0.19</td>
<td>8.12.10^{-6} + 0.18</td>
</tr>
</tbody>
</table>

| E_{max} (% of carbachol) and EC_{50} values for serotonin and papaverine, E_{max} (mg/mg) and EC_{50} values for carbachol, and E_{max} (mg/mg) values for 80 mmol/L KCl in esophageal strips obtained from the 2 groups. Values are arithmetic means ± SE. * P < .05 (statistically significant) when compared with sham values.

**Fig. 4** KCl-induced (80 mmol/L) maximal contractile responses of esophageal strips. Each point is expressed as mg tension/mg tissue and is given as the mean ± SEM. Numbers in parenthesis indicate the number of preparations used from different animals. *P < .05 compared with the other group.

**Fig. 5** Serotonin concentration-response curves in isolated rat gastric fundus strips. All points show the mean ± SEM of responses obtained in individual experiments on different tissues from different animals. Data are expressed as a percentage of the contraction induced by carbachol. *P < .05 compared with the other group.

### 3. Discussion

The esophagus is a very complex structure provided with different receptors supplied by different nervous fibers leads to peristaltic activity. Marked differences, however, have been observed in GER in the outset to investigate the action of different compounds, which affect the cholinergic system in an attempt to throw some light on the behaviors of esophagus and the precise mechanisms of action of the
Esophageal smooth muscle reactivity

examined compounds. Esophageal contraction is neurally mediated, and Ca\textsuperscript{2+} required for neurotransmitter release as well as large phasic contractions. Under conditions of tone induced by cholinergic receptor activation, in rat esophagus, serotonin induced a relaxation mediated by activation of 5-HT\textsubscript{4} receptors. Normal esophageal motor activity results in coordinated propulsion of esophageal contents by peristaltic contractions and acid clearance. Disturbances in this series of events can result in various clinical symptoms and pathologic changes in the esophagus. Important esophageal motor function changes have been reported based on the pH monitoring and manometric, radiological, and scintigraphic studies in such patients [10-13]. For example, decreased peristaltic activity, increased nonpropulsive waves, irregular esophageal contractions, and diminished esophageal peristalsis were observed during GER episodes in previous studies [14,15]. Acid-induced esophageal dysmotility was demonstrated in pediatric patients with symptomatic GER [16]. Symptomatic GER is a common problem in infants and young children, but the mechanical events that lead to its intermittent occurrence are poorly understood. Our previous work in the acutely induced GER model demonstrated an impaired smooth muscle reactivity of the esophageal body. Although the physiological part of that study was convincing, the method of GER induction was too acute, and the esophageal damage was too severe to be a model of clinical GER. Thus, it was not in parallel to the human situation. For this reason, the present investigation undertaken mainly to verify if these alterations still occur after a longer period of chronic GER.

In different animal models of acute esophagitis, acid-induced inflammation alters the contractile pathway of esophagus. The contractility of esophagus is mainly dependent on cholinergic control of the organ [17]. Harnett et al [18] demonstrated that basal levels of phosphatidylinositol hydrolysis are substantially reduced, and intracellular Ca\textsuperscript{2+} stores are functionally damaged, resulting in a reduction of resting tone after induction of experimental acute esophagitis in lower esophageal sphincter circular muscle. The reduction in intracellular Ca\textsuperscript{2+} release causes a switch in the signal transduction pathway mediating contraction in response to acetylcholine (ACh). Therefore, similar mechanisms might play a role in chronic esophagitis. Furthermore, KCl-induced contractile response in reflux group was decreased compared to controls. Taken together with the decreased KCl responses, which are induced by direct depolarization of smooth muscle, the generalized contractile defect suggests the involvement of basic alterations either in Ca\textsuperscript{2+} mobilization or in the contractile apparatus within the esophageal smooth muscle.

It was previously shown that inflammatory cytokines may play a role in the decrease of ACh release in the acute esophagitis model [19]. Proinflammatory cytokines present in inflammatory sites have been shown to alter muscle contractility by suppressing the release of ACh and norepinephrine. Therefore, decreased cholinceptor-mediated contractile responses found in this study suggest that inflammatory processes and related cytokines might play a role in decreased cholinceptor mediated contractile response. Because signal transduction pathways are responsible for the maintenance of esophageal contractility, further studies are required to explain the postreceptor mechanisms of impaired cholinceptor-related contractility after chronic esophagitis.

Coordinated smooth muscle contraction and relaxation play an important role in esophageal peristaltic activity. In rat esophagus, serotonin stimulates cAMP formation in the tunica muscularis mucosae of the rat esophagus via serotonin receptors [20]. We found an impaired relaxant response to serotonin following esophagitis. Therefore changes in mechanical events and intracellular levels of cAMP induced by the activation of the serotonin receptor might be responsible for decreased serotonin response following chronic inflammation. The mechanism underlying this impaired relaxation requires further studies.

We conclude that esophageal inflammation can lead to an increased irritability and decreased stimulus response of the smooth muscle of the esophagus. These changes may correspond to the functional abnormalities of the esophagus seen in patients with reflux esophagitis. Further studies are needed to assess the role of Ach and postreceptor pathways in esophageal motility.

References


