Effect of chronic renal failure on foregut smooth muscle reactivity: an experimental study

Firuzan Yildiz\textsuperscript{a}, Melih Tugay\textsuperscript{b,*}, Tijen Utkan\textsuperscript{a}, Yusufhan Yazirc

\textsuperscript{a}Department of Pharmacology, Kocaeli Medical School, Kocaeli, Turkey
\textsuperscript{b}Department of Pediatric Surgery, Kocaeli Medical School, Kocaeli, Turkey
\textsuperscript{c}Department of Histology and Embryology, Kocaeli Medical School, Kocaeli, Turkey

Abstract

**Purpose:** An association between chronic renal failure (CRF) and gastroesophageal reflux (GER) is well known. The aim of this study was to pharmacologically characterize and investigate the possible contribution of smooth muscle reactivity pathways involving GER on the CRF rat model.

**Material and Methods:** Chronic renal failure was created in Sprague-Dawley rats by 5 of 6 nephrectomy. The rats were divided into 2 groups: the CRF-induced group (CRF group) and the sham-operated group (control group). Esophageal smooth muscle strips were studied in vitro for their contractile (KCl, carbachol) and relaxant (isoproterenol, serotonin, and papaverine) response to receptor activation in the organ chambers set up. Subsequently, the in vitro lower esophageal sphincter (LES) smooth muscle study was generated by KCl, carbachol, isoproterenol, nicotine, sodium nitroprusside (SNP), and papaverine.

**Results:** Compared with controls, esophageal strips taken from CRF-induced rats associated with decreased smooth muscle responses to carbachol, serotonin, and increased response to KCl. Isoproterenol- and papaverine-induced relaxant responses were not affected. Contractility of the isolated LES strips were significantly increased to KCl and carbachol in the CRF group compared with the control group. Similar relaxant responses were obtained in LES strips stimulated by isoproterenol, SNP, and papaverine in the CRF and control group. Nicotine-induced relaxant responses were decreased in the CRF group compared with the control group.

**Conclusions:** Our study revealed alterations of receptor-dependent esophageal and LES smooth muscle reactivity in the CRF-induced rats. Impaired foregut smooth muscle reactivity may contribute to the development of GER-related functional abnormalities in patients with CRF.

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Gastrointestinal disorders occur frequently in children and adults with chronic renal failure (CRF) [1-3]. It was reported that, even though patients with CRF generally report a good quality of life, the prevalence of gastrointestinal symptoms and of general symptoms is high, and many dialysis patients consider these symptoms to cause major impairment of daily life [4]. Nausea and vomiting are common in the uremic patient, but gastric emptying studies have yielded conflicting results [3,5]. Patients undergoing
renal transplantation are at increased risk of development of esophagitis, complicated peptic ulcer, intestinal ulceration, and perforation [6]. Manometric evidence of esophageal dysfunction is very common in such patients [7]. Few data are available on the mechanisms of symptoms originating from the gastrointestinal tract in this group of patients. Gastroesophageal reflux (GER) is often associated with esophageal and lower esophageal sphincter (LES) motor dysfunction; the causes of this abnormal motility are not well understood. The aim of our study was to assess the relationship between smooth muscle reactivity and CRF by using organ chamber study. We previously reported impaired esophageal smooth muscle reactivity in the GER rat model [8]. Therefore, we suspected that similar mechanisms might also play a role in the CRF-induced GER. In the present study, we wished to define the CRF-induced alterations in esophageal and LES smooth muscle reactivity and their modulation by pharmacologic interventions in the rat model. These experiments provide insight into the new mechanisms involved in the defective foregut peristalsis.

1. Materials and methods

Fifty-two Wistar rats weighing 200 to 250 g were obtained from Experimental Medical Research Center (DETAB, Kocaeli University Medical Faculty, Kocaeli), kept in a controlled environment with temperature and humidity control, and with 12-h light-dark cycles. The rats were starved of food for 8 h before and 24 h after surgery and allowed to drink water. In this study, we examined the alteration of esophageal and LES smooth muscle reactivity in 5 of 6 nephrectomized rats, extensively used as a model of CRF [9]. Experiments were carried out under general anesthesia, which was accomplished by an injection of ketamine hydrochloride (20 mg/100 g body weight). One week after right nephrectomy, left subtotal nephrectomy was (2/3) performed (n = 31). Similar surgical manipulations were carried except nephrectomy in the sham-operated group (n = 21). Two months after the second surgery, the rats were harvested. Blood samples were collected for creatine and urea values, and approximately 1.5-cm thoracic esophageal segment and LES were excised, freshly opened, and macroscopically evaluated. The specimens were fixed in 10% formaldehyde solution for 24 h, and serial transversal 5-μm-thick sections from paraffin blocks were stained with hematoxylin and eosin. A blind observer microscopically assessed the sections.

1.1. Organ chamber experiments

The experiments have been made in the pharmacology laboratory of the Kocaeli Medical School. Experimental procedures were approved by the Kocaeli University Ethics Committee. Esophageal and LES smooth muscle strips were prepared and studied in 20 mL of water-jacketed organ baths for isometric tension recording [10,11]. Exactly the same experimental design and study protocol were used with the previous study [8]. The strips were tied to a force transducer (MAY-COM FDT 10-A; COMMAT Iletisim Co, Ankara, Turkey). The tissue baths contained Tyrode’s solution, which gassed with 95% O2 and 5% CO2, and temperature was maintained at 37°C. The strips were allowed to equilibrate under a resting tension of 0.5 g for 90 minutes. Agonists were added directly to the organ bath. At the completion of each experiment, tissues were lightly blotted and weighted. Isometric tension was recorded on the 4-channel transducer data acquisition system (TDA-94 COMMAT; COMMAT Iletisim Co) using a software (Polywin 95 ver 1.0 COMMAT; COMMAT Iletisim Co), which also had the capacity to analyze 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 to 3.10–4 mol/L), cumulative concentration-response curve was 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 further for 60 minutes and precontracted with a submaximal concentration of carbachol (10–6 to 3.10–6 mol/L). After the contractions reached a plateau, concentration-response relationships for serotonin (10–10 to 10–4 mol/L), isoproterenol (10–10 to 10–4 mol/L), and papaverine (10–5 to 10–4 mol/L) were obtained in a cumulative manner.

In a series of preliminary experiments, LES strips were stimulated with 80 mmol/L of KCl. In examining the contractile response to the muscarinic agonist carbachol (10–10 to 3.10–4 mol/L), cumulative concentration-response curve was 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 further for 60 minutes and precontracted with a submaximal concentration of carbachol (10–6 to 3.10–6 mol/L). After the contractions reached a plateau, concentration-response relationships for serotonin (10–10 to 10–4 mol/L), isoproterenol (10–10 to 10–4 mol/L), nicotine (10–10 to 10–4 mol/L), sodium nitroprusside (SNP) (10–10 to 10–4 mol/L), and papaverine (10–5 to 10–4 mol/L) were obtained in a cumulative manner.

1.2. Analysis of data

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 precontracting to carbachol. Concentration-response curves were fitted by nonlinear regression with simplex algorithm, and EC50 (maximum response) and EC50 values were calculated using the software of transducer data acquisition system.
2. Statistical analysis

Statistically significant differences among groups were calculated by 1-way Student’s t test. Probabilities of less than 5% (P < .05) were considered significant.

3. Drugs

The following chemicals were obtained from Sigma Chemical (St Louis, Mo): carbachol (carbamylcholine chloride), isoproterenole bitartrate, serotonin (serotonin creatinine sulfate), nicotine, SNP, 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.

4. Results

Histologic studies for esophagus and LES showed no evidence of inflammatory infiltrate in the circular muscle layer, which appears normal under light microscopy.

Table 1 Actions of different compounds on the contractions (mg/mg) and relaxations (% of carbachol) on the rat esophagus

<table>
<thead>
<tr>
<th></th>
<th>Control esophagus</th>
<th>CRF esophagus</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>8.03 ± 0.6</td>
<td>9.58 ± 0.743*</td>
</tr>
<tr>
<td>Carbachol</td>
<td>37.5 ± 2.2</td>
<td>19.09 ± 3.1*</td>
</tr>
<tr>
<td>Isoproterenole</td>
<td>96.52 ± 0.92</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Serotonin</td>
<td>100 ± 0</td>
<td>74.3 ± 5*</td>
</tr>
<tr>
<td>Papaverine</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>EC50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>1.34.10⁻⁷ ± 0.26</td>
<td>2.4.10⁻⁷ ± 1.43</td>
</tr>
<tr>
<td>Isoproterenole</td>
<td>3.74.10⁻⁹ ± 0.31</td>
<td>4.4.10⁻⁹ ± 0.65</td>
</tr>
<tr>
<td>Serotonin</td>
<td>1.86.10⁻⁷ ± 1.12</td>
<td>2.95.10⁻⁸ ± 1.92*</td>
</tr>
<tr>
<td>Papaverine</td>
<td>1.95.10⁻⁵ ± 0.05</td>
<td>1.06.10⁻⁵ ± 0.06</td>
</tr>
</tbody>
</table>

* P < .05 compared with the control esophagus.

4.1. Isolated smooth muscle reactivity for esophagus

Contraction elicited by 80 mmol/L of KCl and E_max values were significantly increased in the CRF group compared with the control group (Fig. 1, Table 1).

The cumulative addition of carbachol (10⁻¹⁰ to 3.10⁻⁸M) produced concentration-dependent contractions of the esophageal strips. Contractility significantly decreased in strips obtained from the CRF group compared with the control group (Fig. 2). There was a significant decrease in maximum responses (E_max) (Table 1).

Isoproterenol produced concentration-dependent relaxation in submaximally (55%-60% of maximal contraction) precontracted (3.10⁻⁶ mol/L of carbachol) esophageal strips obtained from each group. When tissues were contracted with carbachol to assess relaxant responses to isoproterenole,
tension induced by carbachol did not significantly change in the groups. Relaxation in response to isoproterenol was not changed significantly in both groups (Table 1).

Serotonin produced concentration-dependent relaxation in submaximally precontracted (3.10⁻⁶ mol/L of carbachol) esophageal strips obtained from the 2 groups. Relaxation elicited by serotonin was decreased in the CRF group compared with the control group (Fig. 3, Table 1).

In precontracted strips, papaverine-induced relaxation response was similar in the 2 groups (Table 1).

### 4.2. Isolated smooth muscle reactivity for LES

$E_{\text{max}}$ and $EC_{50}$ values for KCl and carbachol were significantly increased in the CRF LES group compared with the control group (Table 2, Figs. 4 and 5). Isoproterenol, SNP, and papaverine-induced relaxant responses were similar in the CRF LES and control group (Table 2). However, nicotine-induced relaxant responses were decreased in the CRF LES group compared with the control group (Table 2, Fig. 6).

### 5. Discussion

The factors predisposing to the development of GER in patients with CRF are unclear. Chronic renal failure-induced GER may exhibit a wide spectrum of motor disturbances in the distal part of the esophagus even before the presence of the tissue damage. Using the GER rat model, we have previously discovered strong evidence that the impaired smooth muscle reactivity may actually play a role in GER [8]. Gastroesophageal reflux has been found in about 70% of infants and children with CRF suffering from vomiting and feeding problems, and thus appears to be a major problem in these patients [12,13]. Dysmotility and delayed

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**Table 2** Actions of different compounds on the contractions (mg/mg) and relaxations (% of carbachol) on the rat LES

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control LES</th>
<th>CRF LES</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>19.37 ± 2.4</td>
<td>27.2 ± 1.4*</td>
</tr>
<tr>
<td>Carbachol</td>
<td>28.9 ± 1.7</td>
<td>48.77 ± 3*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>53 ± 2.2</td>
<td>55 ± 7</td>
</tr>
<tr>
<td>SNP</td>
<td>57.70 ± 1.2</td>
<td>50 ± 3.9</td>
</tr>
<tr>
<td>Papaverine</td>
<td>98.88 ± 1.12</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Nicotine</td>
<td>91.29 ± 3.6</td>
<td>69.6 ± 2.9*</td>
</tr>
<tr>
<td>$EC_{50}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>1.23.10⁻⁷ ± 0.2</td>
<td>5.45.10⁻⁸ ± 1.16*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>2.62.10⁻⁷ ± 0.7</td>
<td>1.4.10⁻⁷ ± 0.86</td>
</tr>
<tr>
<td>SNP</td>
<td>2.36.10⁻⁷ ± 2.1</td>
<td>6.10⁻⁷ ± 2.9</td>
</tr>
<tr>
<td>Papaverine</td>
<td>3.51.10⁻⁵ ± 0.3</td>
<td>1.09.10⁻⁵ ± 0.05</td>
</tr>
<tr>
<td>Nicotine</td>
<td>4.80.10⁻⁴ ± 0.5</td>
<td>4.10⁻⁴ ± 0.62</td>
</tr>
</tbody>
</table>

* $P < .05$ compared with the control LES.
emptying of the stomach have been reported in patients with CRF [14-16]. Gastric antral electrical control activity was abnormal in several studies [17,18]. In a number of symptomatic patients with CRF, gastric dysrhythmias and delayed gastric emptying have also been found; hence, there appears to be a complex disorder of gastrointestinal motility in CRF. By directly affecting the smooth muscle of the gut or stimulating particular areas within the central nervous system, all these humoral alterations may well play a major role in the gastrointestinal dysmotility, anorexia, nausea, and vomiting in patients with CRF. Serum levels of several polypeptide hormones involved in the modulation of gastrointestinal motility (glucagon, gastrin, cholecystokinin, and neurotensin) and the regulation of hunger and satiety are significantly raised as a consequence of renal insufficiency, and can be reverted to normal by renal transplantation [13]. Furthermore, several other metabolic abnormalities (eg, hypercalcemia, hypokalemia, and acidosis) are not uncommon in CRF. In this background, we hypothesized that impaired esophageal smooth muscle reactivity may be one of the causative factors in GER etiology in the CRF patients. Therefore, we investigated whether foregut smooth muscle reactivity was impaired also in the CRF animal model.

According to the previous study, normal rats do not have acid reflux [19]. In fact, rat esophageal epithelium is keratinized and highly resistant to acid exposure. Although the pathogenesis is unclear, uralic neuropathy of the vagus nerve as well as smooth muscle myopathy caused by chronic uremia could be causative factors. However, on the contrary to abnormal smooth muscle reactivity, no histologic changes were observed after CRF. Surgically created rat models can induce acid reflux and mucosal ulceration [20,21]. In the present study, in spite of smooth muscle reactivity changes, no lesions were seen after 2 months. However, this was expected like its similar studies [22,23]. Finally, our aim was to investigate smooth muscle reactivity in CRF if any changes occur. Therefore, CRF may not damage esophageal and LES smooth muscle. But other signal transduction mechanisms responsible for release of excitatory neurotransmitters may be affected and cause abnormal smooth muscle reactivity.

Evidence from both in vitro and in vivo studies supports an essential role of cholinergic receptor activation in esophageal peristalsis and LES tonus [24,25]. Esophageal smooth muscle contraction was decreased by carbachol (receptor mediated) and increased by KCl (nonreceptor mediated) LES relaxation response in the CRF group. These data suggest that increased smooth muscle contraction in LES after CRF could be related to specific and/or nonspecific mechanisms. Details of the signaling cascade initiated by muscarinic receptors in this tissue remain to be determined.

Smooth muscle relaxation plays an important role in gastrointestinal motility [28]. Coordinated esophageal and LES peristaltic waves are major barrier of GER. Functionally, LES is specialized with muscle strips that relax during food bolus passing. 5-Hydroxytryptamine (serotonin) and β-adrenoceptor–mediated (isoproterenol) functional responses have been detected in a rat esophagus [29,30]. The present study demonstrated that relaxant response to 5-hydroxytryptamine receptor was impaired, whereas adrenergic and receptor-independent (papaverine) mediated response of the esophageal smooth muscle was unchanged. The exact mechanism underlying this impaired relaxation is not known yet. It was previously shown that 5-hydroxytryptamine receptor activation is induced and associated in raising tissue cyclic adenosine monophosphate levels reflecting their relaxant activities in rat esophagus [30]. Because we have not assessed tissue cyclic adenosine monophosphate levels, we cannot comment on that. However, receptor activation as well as secondary signal transduction may play a role on the impaired serotonin-mediated response in this model. We also observed decreased NO-mediated (receptor-dependent) LES relaxation response in the CRF group. However, SNP-mediated (receptor-independent) response was unaffected in the CRF group. These data indicate that impaired relaxation mechanisms on the receptor level may play a role in abnormal LES response. However, further studies are needed to elucidate the biochemical and cellular mechanisms for these effects.

This is the first report detailing the expression of foregut smooth muscle reactivity in the CRF model. These smooth muscle reactivity changes may reflect primary or secondary abnormalities seen in CRF patients with GER.

References


