Impaired Gastric Motility in the Gastroesophageal Reflux Rat Model: An in Vitro Study

Melih Tugay,* Firuzan Yildiz,† Zafer Utkan,‡ Tijen Utkan,† and Yusuf Sarioğlu§

*Department of Pediatric Surgery, †Department of Pharmacology, and ‡Department of General Surgery, Kocaeli University, Medical School, Kocaeli, Turkey; and §Department of Pharmacology, Gazi University, Medical School, Ankara, Turkey

Submitted for publication February 10, 2003

Purpose. The present study investigated the effects of acid and mixed reflux on the responsiveness of gastric smooth muscle in the gastroesophageal reflux (GER) rat model.

Material and methods. Three groups of rat were studied encompassing acid reflux, mixed reflux and sham operation. Acid reflux was induced by pyloric ligation (AR group) and mixed reflux was induced by jejunal ligation 1 cm distal to Treitz ligament (MR group). Similar surgical manipulations were carried out in the sham operated rats (SO group). Carbachol-, serotonin-, KCl-induced contractile response and nicotine-, sodium nitroprusside-, papaverine-induced relaxant response in isolated gastric fundus smooth muscle strips were determined using in vitro muscle technique 24 h after surgery.

Results. Isolated gastric fundus smooth muscle contractility to serotonin, carbachol or KCl was significantly reduced in the AR and MR groups with decreased Emax and pD2 values compared with the SO group. Relaxant responses to nicotine was significantly increased in the AR and MR groups with increased Emax and pD2 values compared with the SO group. Sodium nitroprusside and papaverine-induced-relaxant responses were similar in all of the groups and there was no change in agonist potency.

Conclusion. The present study indicates that decreased contractile and increased nicotine-induced relaxant response of the gastric smooth muscle in the surgically created GER model. These findings suggest that impaired gastric smooth muscle reactivity at least in part may play a role in gastric dysmotility in GER. © 2003 Elsevier Inc. All rights reserved.

Key Words: gastroesophageal reflux; gastric motility; smooth muscle; in vitro.

INTRODUCTION

Gastric emptying is a highly coordinated physiological response to the presence of food. Lower esophageal sphincter and gastric fundus probably act as a synergic functional unit, both showing a relaxation reflex in response to swallowing, which is almost entirely mediated by the non-adrenergic non-cholinergic (NANC) nerve fibers [1]. The number of studies has shown that GER disease associated with impaired gastric motor function such as delayed gastric emptying, impaired antral and transpyloric motility [2–12]. Moreover, impaired electrogastrogram parameters were found to predict delayed gastric emptying reflecting smooth muscle motor abnormalities in patients with GER [13, 14]. Based on this, our aim was to investigate gastric smooth muscle reactivity in the GER rat model.

MATERIALS AND METHODS

Twenty-four Sprague-Dawley rats were used weighing 200 to 250 g obtained from Experimental Medical Research Unit (DETAB, Kocaeli University Medical Faculty, Kocaeli, Turkey). The Kocaeli University Ethics Committee approved the procedures. Rats were kept in individual solid bottom plastic cages on sawdust bedding at a temperature and humidity controlled room (22 ± 3°C and 62 ± 7%, respectively) in which a 12–12 h light-dark cycle maintained (08:00–20:00 h light). Rats were deprived of food but allowed free access to water for 8 h before and 24 h after the operation. Experiments were carried out under general anesthesia, which was accomplished by an injection of ketamine hydrochloride intraperitoneally (10 mg/100 g body weight). The acid reflux model was created by ligating of pylorus in the AR group. The mixed reflux model was created by ligating of jejunum 1 cm distal to the Treitz ligament in the MR group as previously described [15, 16]. Reflux enhanced by splitting esophagus longitudinally across the gastroesophageal junction for a distance 1 cm in the both groups. Following laparotomy similar surgical
manipulations carried out except that ligating of pylorus or jejunum in the SO group. Total gastrectomy was performed 24 h after the operation. Gastric fundus smooth muscle strips were prepared for organ bath studies.

**Organ Bath Studies**

The experiments were performed in the Experimental Medical Research Unit (DETAB, Kocaeli University Medical Faculty). Gastric fundus strips were prepared in a manner consistent with the method described by Vane [17]. Gastric fundus strips were mounted in 20 ml organ bath for isometric tension measurement. The organ baths contained Tyrode’s solution composed of (millimoles per liter): NaCl 136.0; KCl 2.7; CaCl 2 1.8; MgCl 2 1.05; NaH 2PO 4.H2O 0.42; NaHCO3 11.9; glucose 5.5. The solution was gassed with 95% O2 and 5% CO2 during the study and the temperature was maintained at 37°C. Each strip was connected to a force transducer (FDT 10-A, May IOSB 99, COMMAT Iletisim Co., Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA) using software (BSL PRO v 3.6.7, BIOPAC Systems Inc.) which also had the capacity to analyze the data. After mounting, each strip was allowed to equilibrate with a basal tension of 1 g for 1 h and during this time Tyrode’s solution was replaced every 15 min with fresh solution. Agonists were added directly to the organ bath. At the completion of each experiment, tissues were lightly blotted and weighted. In a series of preliminary experiments gastric fundus were stimulated with 80 mmol/L KCl. In examining the contractile response to carbachol (10-10 to 10-3 mol/L) and serotonin (10-10 to 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. Following completion of carbachol concentration-response curve, tissues were washed for a further 60 min and then pre-contracted with a submaximal concentration of carbachol (10-6 to 3.10-6 mol/L). After the contractions reached a plateau, concentration-response relationships for nicotine (10-6 to 3.10-3 mol/L), SNP (10-10 to 10-4 mol/L), and papaverine (10-7 to 10-4 mol/L) was obtained in a cumulative manner.

**Analysis of Data**

The results were expressed as mean ± SEM of different experiments. The contractile force was expressed as milligrams of developing force.

**TABLE 1**

<table>
<thead>
<tr>
<th>Maximum Contractile Response (E_max) Values for KCl, Carbachol and Serotonin in Gastric Fundus Strips</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E_max</strong></td>
</tr>
<tr>
<td>KCl</td>
</tr>
<tr>
<td>Carbachol</td>
</tr>
<tr>
<td>Serotonin</td>
</tr>
</tbody>
</table>

Note. Values are arithmetic means ± SEM of 5–8 observations in each group from different animals.

* Compared with the SO group.
* Compared with the AR group.
* * P < 0.05 statistically different from the SO group.

**TABLE 2**

<table>
<thead>
<tr>
<th>Maximum Relaxation Response (E_max) Values for Nicotine, SNP and Papaverine in Gastric Fundus Strips Precontracted with 3.10-8 M Carbachol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E_max</strong></td>
</tr>
<tr>
<td>Nicotine</td>
</tr>
<tr>
<td>SNP</td>
</tr>
<tr>
<td>Papaverine</td>
</tr>
</tbody>
</table>

Note. Values are arithmetic means ± SEM of 5–8 observations in each group from different animals.

* P < 0.05 statistically different from the SO group.
oped tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the precontracting to carbachol. To evaluate the effects of agonists, maximum response (Emax) and pD2 values (i.e., the negative logarithm of the concentration for the half maximal response; EC50) were calculated. Agonist pD2 values were calculated from each agonist concentration response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. Statistical comparison between groups was performed using one-way analysis of variance (ANOVA) with a post-hoc Tukey’s-Kramer test. Probabilities of less than 5% (P < 0.05) considered significant.

Drugs

The following drugs were used (Sigma Chemical, St. Louis, MO): Carbachol chloride (carbamylcholine chloride), serotonin (serotonin creatinine sulfate), SNP (sodium nitroprusside), nicotine (nicotine hydrogen tartrate), and papaverine (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.

RESULTS

The MR group had min rare foci of hemorrhage in gastric fundus. Microscopically correlating with the gross appearance, rare foci of superficial erosion and hemorrhage were seen in the MR group. The AR group did not show any remarkable macroscopic and microscopic lesions in the stomach. Likewise no pathologic lesion was observed in the stomach of the SO group.

Cumulative addition of carbachol (10−10 to 3.10−4 mol/L) produced concentration-dependent contractions of the gastric fundus strips. The contractility was significantly decreased in the gastric fundus strips from the AR and MR groups compared with the SO group (Fig. 1). The Emax and pD2 values for carbachol from the AR and MR strips were significantly less than the SO group (Tables 1 and 3).

The contraction elicited by 80 mM KCl was significantly reduced with decreased Emax value in the AR and MR groups compared with the SO group, respectively (Fig. 2, Table 1).

Serotonin elicited concentration-dependent contraction in the gastric fundus strips. Serotonin-induced contractile responses was significantly reduced in the AR and MR groups with decreased Emax and increased pD2 values compared with the SO group (Fig. 3, Tables 1 and 3).

Nicotine, SNP or papaverine produced concentration-dependent relaxation in submaximally pre-contracted (10−6 to 3.10−6 carbachol) gastric fundus strips obtained from the AR, MR, and SO groups. When tissues were contracted with carbachol for the study of responses to

**TABLE 3**

| pD2 Values for Concentration-Response-Curves in Gastric Fundus Strips |
|-----------------------------|----------------|----------------|
| pD2   | SO     | AR     | MR     |
| Carbachol  | 7.94 ± 0.02 | 6.91 ± 0.01* | 7.14 ± 0.13* |
| Serotonin  | 6.68 ± 0.47 | 7.39 ± 0.47* | 7.74 ± 0.08* |
| Nicotine   | 3.91 ± 0.38 | 3.45 ± 0.21* | 3.37 ± 0.58* |
| SNP       | 4.51 ± 0.21 | 4.57 ± 3.17 | 5.77 ± 1.06 |
| Papaverine | 5.15 ± 0.03 | 5.18 ± 0.01 | 5.11 ± 0.02 |

Note. Values are arithmetic means ± SEM of 5–8 observations in each group from different animals.

* P < 0.05 statistically different from the SO group.

![FIG. 2](image-url) KCl-induced contractile responses to gastric fundus strips. Each point is expressed as mg tension/mg tissue and is given as the mean ± SEM. Number in parenthesis indicates number of preparations used from different animals. *P < 0.001, statistically different from the SO group. **P < 0.01, statistically different from the AR group.
agonists, similar tension was achieved in the three
groups.
In the AR and MR groups, nicotine concentration-
response curve was shifted to the left with significantly
increased Emax and decreased pD2 values compared
with the SO group (Fig. 4, Tables 2 and 3).
The relaxation elicited by SNP or papaverine was
similar in all groups and there were no signifi-
cant changes in the pD2 or Emax values (Figs. 5 and 6,
Tables 2 and 3).

**DISCUSSION**
GER can be part of a complex disorder in which the
gastrointestinal tract distal to the esophagus involved
[2–14]. Impaired gastric motor function in GER has
been described in children and adult with different
studies and methods (ultrasonography, radioscintigra-
phy, impedance electrogastrography, magnetic reso-
nance imaging) [18–20]. In this background, our aim
was to determine whether gastric smooth muscle reac-

![Graph of nicotine concentration-response curves](image1)

**FIG. 3.** Serotonin concentration-response curves. Each point is expressed as mg tension/mg tissue and is given as the mean ± SEM. Number in parenthesis indicates number of preparations used from different animals. *P < 0.05, statistically different from the SO group.

![Graph of nicotine concentration-response curves](image2)

**FIG. 4.** Nicotine-concentration-response curves in gastric fundus strips pre-contracted with carbachol. Number in parenthesis indicates number of preparations used from different animals. Data are expressed as a percentage of the contraction induced by carbachol. *P < 0.05, statistically different from the SO group.
Activity affected by acid and/or mixed reflux in the surgically created rat model. Gastric smooth muscle activity was assessed in vitro using organ bath technique in the presence of acid and mixed reflux. Both type of reflux caused to impaired receptor-dependent (carbachol, serotonin, nicotine) and receptor-independent (KCl) gastric smooth muscle reactivity. Therefore, it is speculated that GER is associated with impaired gastric smooth muscle reactivity. Because the model reflecting dysmotility secondary to gastric and small bowel obstruction; we must be cautious to extrapolate these data to human pathology.

According to the previous studies, acid reflux alone is more common than duodenogastric or mixed reflux. However mixed reflux is considered important because more severe and frequent complications were observed in some patients with GER disease [7, 9–12, 18–19, 21]. Therefore, gastric smooth muscle reactivity was also determined in the presence of mixed reflux. Even though impaired gastric smooth muscle reactivity was more prominent in the AR group, the difference between the AR and MR groups was not significant.

Our aim was to investigate the motor function of the gastric fundus after the induction of esophagitis in the

![FIG. 5. SNP-concentration-response curves in gastric fundus strips pre-contracted with carbachol. Number in parenthesis indicates number of preparations used from different animals. Data are expressed as a percentage of the contraction induced by carbachol.](image)

![FIG. 6. Papaverine-concentration-response curves in gastric strips pre-contracted with carbachol. Number in parenthesis indicates number of preparations used from different animals. Data are expressed as a percentage of the contraction induced by carbachol.](image)
GER model. Even though acid reflux was not induced gastric injury, smooth muscle reactivity was impaired in the AR group. During intestinal inflammation, motility disturbances are not restricted to inflamed regions, but may also occur in remote non-inflamed sites of the gastrointestinal tract [22]. Therefore impaired gastric smooth muscle reactivity might be attributable to inhibited neurotransmission in the non-inflamed gastric fundus. This phenomenon may be mediated by intrinsic connections within the enteric nervous system [22].

Carbachol binds to muscarinic receptors on smooth muscle cells stimulating G protein to activate phospholipase C to hydrolyze phosphoinositide, so initiates contractile machinery [23, 24]. Other endogenous agonist serotonin acts through similar intracellular pathways although it acts at different membrane receptor [23–25]. Abnormalities in one or more of these intracellular pathways might explain the decreased responsiveness to carbachol or serotonin in this study. The reduced contractile response to carbachol and serotonin in AR and MR groups could also be explained simply by non-selective mechanisms, because KCl-induced contraction was decreased in the presence of acid or mixed reflux [23–25]. Contractile response of gastric smooth muscle to K⁺ depolarization depends on an opening of voltage-gated calcium channels spanning the sarcolemmal membrane with the resulting influx of calcium activating the contractile protein assembly. Because reflux caused to decrease KCl-induced responses in gastric fundus of the reflux groups, it seems likely that reflux exerted a major deleterious effect both on voltage-dependent sarcolemmal calcium channels or subsequent calcium dependent activation of gastric fundus smooth muscle contractile filaments. Rather because carbachol and serotonin receptors share activation of phospholipase C with its resulting effects on inositol triphosphate and intracellular calcium, it seems plausible that reflux in some way adversely affects this transduction cascade in gastric fundus smooth muscle following reflux-induced injury.

There is substantial evidence that endogenous Nitric Oxide (NO) plays an essential role in the physiological regulation of gastrointestinal motility [26]. NO directly relaxes gastrointestinal smooth muscle and modulates spontaneous motility by mediating NANC receptor-dependent relaxations or regulating the release of contractile mediators from local neuronal or intestinal sources. In human and animal studies, increased formation of NO has been demonstrated during pathological states such as esophagitis, sepsis and inflammatory bowel disease [27–33]. Nicotine induces NO mediated relaxant response in gastric fundus. Therefore, increased relaxant response to nicotine in the reflux groups might be explained by increased NO production. SNP and papaverine act in smooth muscle in receptor-independent manner. Because the gastric fundus smooth muscle relaxant responses to SNP or papaverine were not changed among the groups, it can be speculated that the increased relaxant response to NO appears to be related to increased production of NO but not to the intracellular mechanisms. It is also important to note that at the concentrations of carbachol used, developed tension was similar for gastric smooth muscle strips from AR, MR, and SO groups, thus, ensuring that any difference in relaxation among the groups was not because of differences in the degree of pre-contraction. Therefore, it is possible that esophagitis increases the synthesis of NO in gastric tissue. This is consistent with the results of previous reports [27–33].

In conclusion, decreased receptor-dependent and receptor-independent gastric smooth muscle reactivity to various agonists and increased NO mediated smooth muscle relaxant response were found in the surgically created GER rat model. These results provide pharmacological evidence that GER affects gastric smooth muscle function. Although it is difficult to extrapolate in vitro data to human pathology, impaired gastric smooth muscle reactivity may play a role at least in part various gastric motor abnormalities in patients with GER requires further study.

REFERENCES


