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Acid Infusion into the Esophagus Increases the Number of Meal-Induced Transient Lower Esophageal Sphincter Relaxations (TLESRs) in Healthy Volunteers

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Abstract

Background—TLESR is the major mechanism of gastroesophageal reflux (GER) but the regulation of TLESR by stimuli in the esophagus is incompletely understood. If stimuli in the esophagus can influence TLESR then such regulation may perpetuate or limit GER. We addressed the hypothesis that acid in the esophagus enhances TLESRs.

Methods—We evaluated the effect of acid infusion into the distal esophagus on TLESRs evoked by a standard meal in a paired randomized study in healthy subjects. TLESRs were evaluated by using high resolution manometry (HRM).

Key Results—We found that acid in the esophagus enhanced meal-induced TLESRs. Compared to control infusion the number of TLESRs (median[interquartile range]) was increased during 2h following the acid infusion (11[9–14] v.s. 17[12.5–20], $p < 0.01$). The average duration of individual TLESRs was not affected. The time course analysis revealed that a robust increase in TLESRs occurred already in the first hour when the number of TLESRs nearly doubled (6[5.5–7.5] v.s. 11[7.5–12.5], $p < 0.05$). In contrast to the enhancement of TLESRs, the number of swallows was not changed.

Conclusions & Inferences—The acid infusion into the esophagus increases the number of meal-induced TLESRs in healthy subjects. Our results provide evidence for the concept that the stimuli in the esophagus can influence TLESRs. The regulation of TLESR by stimuli in the esophagus may contribute to pathogenesis of GER in some patients.

Keywords

transient lower esophageal sphincter relaxation; gastroesophageal reflux; esophagus; acid; vagus nerve

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DISCLOSURES

No conflicts of interest declared.

Transient lower esophageal sphincter relaxations (TLESRs) allow belching to vent the swallowed air from the stomach(1–4). Accordingly, TLESR can be triggered by gastric distension, especially that of the subcardiac region. When dysregulated, TLESRs are thought to be a major contributors to gastroesophageal reflux (GER) in patients with GERD.

The regulation of TLESR has been extensively studied in humans (5–13). The frequency of TLESRs is increased by meal, experimental gastric distension, distension of the esophagogastric junction and by certain body positions. However, it is unknown whether stimuli in the esophagus can influence TLESRs. If esophageal stimuli in the refluxate (such as refluxed acid) can influence TLESRs, then a positive or negative feedback regulation can be established since TLESRs mediate the reflux into the esophagus.

Acid is the most important offensive component of GER (14, 15). The acid suppression therapy relieves the symptoms in many GERD patients. However, it is not known if and how the acid refluxed into the esophagus influences the mechanisms of GER including TLESR and the function of esophago-gastric junction (EGJ).

Here we addressed the hypothesis that acid in the esophagus enhances TLESRs in healthy subjects. We used a meal as a standard physiological stimulus to induce TLESRs (12, 16–20). The acid was delivered by infusion into the distal esophagus following the meal and TLESRs were evaluated by using high resolution manometry (HRM) (21, 22). We found that acid in the esophagus enhanced TLESRs.

MATERIAL AND METHODS

Subjects

Studies were performed in 11 healthy subjects (10 men, 1 woman; mean age 23.3 ± 1.95 ; range 20–27 years) with mean body weight $78.7\text{kg} \pm 10.1\text{kg}$ (BMI 24.6 ± 2.5). None of the subjects had symptoms or history of gastrointestinal disease or upper gastrointestinal surgery, nor were they taking any medication. None of the subjects had any esophageal motility abnormality as defined by Chicago criteria(23). Written informed consent was obtained from each subject and the study protocol has been approved by the Ethics Committee of the Jessenius Medical School, Comenius University in Martin.

Recording method

Subjects were studied in a sitting position. Esophageal manometry was performed by using a high resolution (HR) 4.2 mm outer diameter solid-state catheter with 36 circumferential channels spaced at 1 cm intervals (Given Imaging, Yoqneam, Israel). Before recording, transducers were calibrated at 0 and 300 mmHg using externally applied pressure. After the calibration the catheter was inserted into a disposable sheath (Given Imaging, Yoqneam, Israel) and a polyethylene infusion tube (O.D. = 1mm) for acid/water infusion was attached to the sheath. The manometry/infusion assembly was placed transnasally, and positioned to record esophageal pressure from the hypopharynx to the stomach with approximately 2–3 sensors in the stomach and the opening of the infusion tube 5 cm above the proximal end of LES. The catheter was then tape-fixed to the nose. Addition of impedance/pH catheter to

manometric/acid infusion assembly was poorly tolerated in preliminary studies and we therefore decided not to use it.

Study design

Two HR manometric recordings combined with the distal esophageal infusion were performed at least 1 week apart in each subject. On one occasion the acid infusion and on the other occasion the control infusion was performed in random order.

HR manometric study protocol. After an overnight fasting, the subjects were intubated and, after the positioning of the HR manometric catheter, allowed to accommodate to the assembly for 5–10 min. During the first visit a standard motility testing was also performed (30 s baseline without swallowing, 10 consecutive 5 ml water swallows) prior to the study. After the accommodation period the patient consumed a standardized meal (two chicken sandwiches, total caloric value 740 kcal, 30g protein, 32g fats, 78g carbohydrates) with water (0.3L) over 15 minutes. The meal was followed by 20 min randomized, single-blinded acid or control distal esophageal infusion. The recording continued for 2 h after the completion of the infusion. TLESRs were evaluated during 20 min of infusion and subsequent 2h. This interval was chosen based on the observations that the most robust and consistent induction of TLESRs occurs during 2–3h following the meal (12, 17–20).

Distal esophageal infusion was performed for 20 min with 0.15M HCl, pH=1 (calculated value pH=0.82) or water. The infusion tube was connected to a peristaltic pump (flow rate 8ml/min resulting in total volume of 160 ml during 20 min of infusion, pump type PCD 21M, Kouril, Czech Republic). The acidity, rate and duration of acid infusion was selected based on the thorough studies of acid-induced central sensitization of esophageal pain threshold (24–26). We selected water over buffered saline (pH=7) since a buffered solution would exert additional effects by buffering the acidity in the stomach.

Data analysis

Transient lower esophageal sphincter relaxations—The esophageal pressure tomography (EPT) plots acquired by HRM software ManoScan ESO 2.0 (Given Imaging, Yoqneam, Israel) were analyzed using ManoView 3.0 (Given Imaging, Yoqneam, Israel). The recordings were analyzed primarily for the occurrence of TLESRs by blinded consensus analysis by two observers (16). TLESRs were detected according to following criteria: at least four out of six criteria: 1) absence of a swallow for 4 s before, until 2 s after the onset of the TLESR, 2) LES relaxation rate of ≥ 1 mmHg/s, 3) a time from the onset to complete relaxation of ≤ 10 s 4) nadir pressure of < 2 mmHg, 5) inhibition of the crural diaphragm, 6) prominent after-contraction (16). Each observer analyzed all recordings separately. After completion of separate analyses, the two observers together reviewed all events identified as TLESRs by each observer, and decided by consensus whether the event was TLESR. The same two observers (JH and PB) analyzed all recordings. Recent study found that the number of meal-induced TLESRs evaluated at least a week apart is highly reproducible in healthy subjects (16).

Estimate of lower esophageal sphincter pressure before and after infusion—In order to minimize potential confounding factors we did not include the standard measurement of LES pressure (30s of forced no swallowing) in our protocol. To estimate the LES pressure the LES pressure measurement was averaged (by using ManoView 3.0 Smart Mouse Tool) for a total duration of at least 60s. The swallow-induced relaxations, TLESRs and post-deglutitive LES hypercontraction were excluded (since post-deglutitive LES hypercontraction typically lasts up to 10s (27), the 10s interval after the peristaltic wave ended was excluded). Only the portions of LES pressure without any abnormality (e.g. talking, sneezing, coughing, straining) were included. The LES pressure was estimated immediately after the infusion was completed.

Number of swallows—For analysis, the swallows followed by a peristaltic wave, which are connected with bolus transport with a possibility of air ingestion, were counted manually for each EPT plot. The swallows with only a pharyngeal phase and the opening of upper esophageal sphincter but not followed by a peristaltic wave were also counted for additional analysis.

Statistical analysis

The number of TLESR data are presented as median[interquartile range] and analyzed by the paired Wilcoxon Signed-Rank Test or 2-way ANOVA followed by Bonferroni posttest as appropriate. The duration of TLESRs data are presented as mean $\pm$ SEM and analyzed by the paired T-test or 2-way ANOVA followed by Bonferroni posttest as appropriate. The number of swallow data are presented as mean $\pm$ SEM and analyzed by the paired T-test or 2-way ANOVA followed by Bonferroni posttest as appropriate.

RESULTS

The number of TLESRs evoked by a standardized meal was evaluated on two occasions separated by at least 7 days: 1) following water infusion (control) and 2) following the acid infusion (acid) into the distal esophagus. The subjects were randomly assigned the order of the infusions. 11 subjects completed the study. 5 subjects received the acid infusion first and 6 subjects received the control infusion first.

Subjective outcomes

The subjects described a cold feeling in the nose and pharynx attributable to the room temperature of the fluid perfused through the catheter. During the control infusion the subjects reported no discomfort or pain perceptions from the esophagus. During the acid infusion most subjects (8/11) reported a dull sensation behind the sternum with the intensity increasing over time and possibly developing a mild heartburn (some subjects reported that they were not familiar with the sensation of heartburn). The subjects reported that the location of sensation was difficult to describe, they often reported that it was more pronounced in the epigstric region, but some subjects located the sensation also more proximally. The time to develop the sensation varied from 5 to 15 minutes. Three subjects reported no sensation during acid infusion.

Objective outcomes

TLESRs—TLESRs were detected as described in methods (see Fig. 1A–B for representative traces). The numbers of TLESRs were separately counted during the 20 min of the infusion into the esophagus and for 2h after the completion of the infusion. The numbers of TLESRs during 20 min of the control and acid infusion was not significantly different between the two treatments (2[2–3] vs. 3[2–4.5], respectively, $P>0.1$). The number of TLESRs during 2h after infusion was significantly increased following the acid infusion (Fig. 2A). The numbers of TLESR following the control and acid infusion were 11[9–14] and 17[12.5–20], respectively ($P<0.01$). Figure 2A illustrates that some extent of increase in the numbers of TLESRs was observed in all subjects. The duration of individual TLESRs was not affected averaging 17.7 ± 0.5 s and 17.8 ± 0.4 s in control ($n=125$ TLESRs) and acid ($n=187$ TLESRs) treatment, respectively ($p=0.9$) (Fig. 2B). The overall time that LES was opened due to TLESRs was 202 ± 21 s and 302 ± 31 s following the control and acid infusion, respectively ($p<0.01$).

The analysis of the time course of TLESRs revealed that a robust increase in the number of TLESRs occurred already during the first hour. Compared to the number of TLESRs after the control infusion the number of TLESRs after the acid infusion was nearly doubled in the first hour ($6[5.5-7.5]$ v.s. $11[7.5-12.5]$, $P<0.01$, Fig. 3A). The increase in TLESR in the second hour did not reach statistical significance, nonetheless, the analysis in the second hour was likely underpowered due to decreasing number of TLESR (see below).

LES Pressure Estimate—Although standard measurement of LES pressure was not included in the study protocol, we estimated the LES pressure following the control and acid as described in methods. The estimated LES pressure following the control vs. acid infusion was 12 ± 1 mmHg vs. 12 ± 2 mmHg ($n=11$, $p=0.8$, paired T-test).

Swallowing—The swallows followed by the a peristaltic wave were counted. The numbers of swallows during 20 min of control and acid infusion did not differ between the treatments (38 ± 6 vs. 42 ± 6 , $P>0.1$). In contrast to TLESRs that were enhanced by acid, the number of swallows was not affected during the 2 hours after infusion (Fig. 3B). In both the control and acid infusion condition the number of swallows was higher in the first hour than in the second hour. Similar results were obtained when also the swallows not followed by a peristaltic wave were included in the analysis (data not shown).

DISCUSSION

We found that an acid infusion into the esophagus substantively increased the number of TLESRs evoked by a meal in healthy subjects. This observation provides, to our knowledge, the first evidence for the concept that acid in the esophagus can enhance TLESRs. A positive regulation of TLESR may contribute to the pathogenesis of GERD.

Although studying human subjects precludes many mechanistic experiments, we favor the hypothesis that the increased TLESRs was secondary to acid stimulating vagal afferent (sensory) nerves innervating the esophageal mucosa, leading to changes in central integration and an increase in inhibitory drive to the esophageal sphincter smooth muscle.

This would be similar to observations made in the stomach, where stimulation of vagal afferent tension mechanosensors located in the stomach leads to an increase in TLESR frequency (28). Our speculation that acid in the esophagus similarly enhances the TLESRs reflex arc also leading to increased frequency of TLESRs is based on several largely exclusionary observations.

The effect on TLESRs persisted when acid was no longer infused in the esophagus and the average duration of TLESRs was not affected by acid. These observations argue against a simple direct locally-mediated effect of the acid on the LES smooth muscle tone. The latter finding also indicates that the TLESR motor pattern generator was not affected. The notion of the central origins of the TLESRs in our study is further supported by the observation that the typical time course of the meal-induced TLESRs (high number after the meal that decreases during the second hour) was preserved (12, 17–20).

We also noted that the acid infusion did not alter the number of swallows. This observation indicates that acid does not indiscriminately increase all motor events in the esophagus. It also argues against a possibility that the acid-induced increase in the TLESRs is due to excessive swallowing that could potentially contribute to the increase in TLESRs (for example by swallowing of air) (29).

The effect on TLESRs was unlikely due to acid acting in the stomach, although some contribution of acid acting in the stomach cannot be excluded by the data presented. To our knowledge the effect of gastric pH on the number of TLESRs has not been reported in humans. However, increased number of TLESRs when acidic solution was delivered directly into the empty stomach was reported in fasting dogs (30). Nonetheless, the conditions of the referenced study were different. In our study the acid was infused into the esophagus following the meal, the volume of acid was 5-times smaller (160 ml vs. 700–800ml), delivered more slowly over 3-times longer period (20 min vs. 7–8 min) and the amount of acid relative to body weight (i.e. the buffering capacity of extracellular fluid) in our study was much smaller. We choose not to attempt to eliminate the infused acid from entering the stomach by suction inasmuch as such approach appears to uncontrollably reduce the esophageal exposure to acid (31).

Finally, our data indicate that changes in LES pressure are likely not causative in the acid-induced increase in TLESRs. We noted no significant differences in the LES pressure estimates after the infusion in the two treatment conditions.

The acid-induced enhancement of TLESRs bears certain similarities to acid-induced central sensitization of painful sensations evoked by sensory nerve stimulation in the esophagus (24, 25). It is tempting to speculate that the brainstem TLESR reflex is augmented by acid in a similar manner as the pain-sensing pathways via central sensitization mechanisms. Incidentally, we have previously demonstrated that another brainstem reflex, the cough reflex evoked from the airways, can be similarly sensitized by acid-induced activation of afferent nerves the esophagus (32). Given the brainstem location of TLESR central program pattern generator the effect of esophageal acid on TLESRs could be mediated by vagal afferent nociceptive pathways that have been shown to respond to acid (33–35).

The onset and conclusion of the acid-induced enhancement of TLESR would be difficult to determine given the discrete and infrequent nature of TLESRs. Nonetheless, our results clearly show that the enhancing effect is robust in the first hour following the acid infusion (Fig. 2A). This observation has practical implication for design of similar studies. Although the increase in the second hour did not reach statistical significance, it would be imprudent to conclude that the effect was over by then. Because of the natural decrease in the number of postprandial TLESRs over time, the effect may not be detectable in the second hour in the sample of this size.

Our study raises the possibility that acidic reflux may increase TLESRs. Current consensus is that the number of TLESRs is not increased in GERD (1–4, 36). This conclusion is based on numerous studies in which TLESRs are counted after the meal or nutrient infusion. The majority of these studies found no difference in the number of postprandial TLESRs between healthy controls and GERD patients (13, 18, 19, 37–53). Some of these studies also reported the esophageal acid ($\text{pH} < 4$) exposure time during the postprandial period of TLESRs recording (18, 39, 41, 47, 48, 50). The reported acid exposure time was 0–1% in controls and 5–12% in GERD patients (equals to approximately 3–7 min/1h of acid $\text{pH} < 4$ exposure).

It is possible that such relatively limited average acid exposure time is not sufficient to reveal acid-induced enhancement of TLESRs. Moreover, the majority of acidic reflux episodes commence only after 30 min following the meal (45) further complicating detection of the effect of acid on TLESRs in these type of studies. Intriguingly, the two studies that reported increased number of TLESRs in GERD employed an experimental designs (constant air or fluid infusion into stomach) that resulted in relatively high acid exposure times (44, 54). The first study reported 23% acid exposure time and a 2-fold increase in TLESRs in patients with GERD and hiatal hernia (44). The second study reported 64% acid exposure time and a 3-fold increase in TLESRs in patients with GERD (54). The results of these studies are consistent with the acid-induced enhancement of TLESRs. Our study suggests that the number of TLESRs may deserve a second look by prolonged manometric and pH monitoring with focus on TLESRs and acid exposure time (see also (55)).

Our results also raise the possibility that TLESRs are altered by acid in the esophagus. It has been recognized that TLESRs are more often associated with acidic reflux in patients with GERD (reviewed in (1–4, 36)). The mechanisms of this phenomenon are currently being elucidated and probably include increased EJG compliance, a higher pressure gradient across EJG and differential distribution of acid in the stomach. Our results indicate that acid in the esophagus can lead to altered central integration of the TLESRs. It can be speculated, for example, that in patients with GERD acid in the esophagus changes the dynamics (i.e. motor sequence and coordination of TLESRs) allowing more acid to reflux during a TLESR. Other components of refluxate such as bile and pepsin may potentially also lead to activation of esophageal nerves and contribute to enhancement of TLESR.

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JH, PB, MD and RH performed the manometry studies JH and PB performed consensual TLESR analysis, MH recruited the subjects and performed the infusions, MT performed the analysis and co-wrote the manuscript, MK developed the hypothesis, performed statistical analysis, prepared the figures and wrote the manuscript.

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KEY MESSAGES

- Transient lower esophageal sphincter relaxations (TLESRs) mediate acid reflux into the esophagus, but it is unknown if/how refluxed acid influences TLESRs.
- We evaluated the effect of acid infusion into the esophagus on meal-induced TLESRs by high resolution manometry in healthy volunteers.
- Esophageal acid substantially increased the number of postprandial TLESRs showing that TLESRs can be modulated from the esophagus. Positive regulation of TLESRs from the esophagus may contribute to acid reflux in patients with gastroesophageal reflux disease (GERD).

Figure 1. Examples of TLESRs

TLESRs were recorded during 2h period following the control infusion (upper trace) and acid infusion (lower trace) into the distal esophagus in the same subject. UES, upper esophageal sphincter, LES, lower esophageal sphincter.

Figure 2. The acid infusion into the distal esophagus enhanced meal-induced TLESRs
 TLESRs were evaluated during 2h following the control or acid infusion. (A) Individual data. $**p < 0.01$ Wilcoxon signed rank test. (B) The average duration of TLESRs was not affected (n=125 TLESRs in control, and n=187 TLESRs in acid treatment).

Figure 3. The increase in the number of meal-induced TLESRs was readily detected in the first hour after the acid infusion

(A) TLESRs. * $P < 0.05$ (2-way ANOVA followed by Bonferroni test) (B) The number of swallows was not affected.