Low esophageal mucosal blood flow in patients with nutcracker esophagus

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Jiang Y, Mittal RK. Low esophageal mucosal blood flow in patients with nutcracker esophagus. Am J Physiol Gastrointest Liver Physiol 310: G410–G416, 2016. First published December 23, 2015; doi:10.1152/ajpgi.00359.2015.—Nutcracker esophagus (NE) is characterized by high-amplitude peristaltic esophageal contractions, and these patients often present with symptoms of “angina-like” or noncardiac chest pain. Tissue ischemia is a known cause of visceral pain, and the goal of our present study was to determine whether esophageal wall blood perfusion (EWBP) is reduced in patients with NE. Fourteen normal subjects (mean age 51 yr, 11 men) and 12 patients (mean age 53 yr, 9 men) with NE and noncardiac chest pain were investigated. The EWBP was measured continuously using a custom-designed laser Doppler probe tethered to a Bravo capsule, which anchored it to the esophageal wall. The baseline EWBP in normal subjects was 651 ± 27 perfusion units. In patients with NE, the baseline EWBP was significantly lower than in the normal subjects (451 ± 32 perfusion units). The EWBP decreased after injection of edrophonium (which increases muscle contractions) and increased following sublingual nitroglycerin administration (which relaxes muscle) in normal subjects, as well as in NE patients. Spontaneous pain events during the recording period were often associated with drops in the EWBP. We propose that low EWBP leads to hypoxia of the esophageal tissue, which may be a mechanism of esophageal pain in patients with NE.

“ANGINA-LIKE” NONCARDIAC esophageal chest pain (NCCP) is extremely common: 25% of adults may suffer from it (5). It is one of the most common causes of visits to the medical emergency room. These patients often undergo extensive cardiac testing to rule out coronary artery disease and consume a large amount of health care resources. Motor disorders of the esophagus are a known cause of “angina-like” chest pain; however, the precise relationship between abnormal motility and pain is not known. Work conducted during 1980s and 1990s focused on the high-amplitude contractions [nutcracker esophagus (NE)] as a mechanism of esophageal pain; however, prolonged esophageal motility and pH recordings revealed poor correlation between pain events and high-amplitude esophageal contractions (16, 29). Work conducted since then suggests that the esophageal pain is related to “hypersensitivity” of the esophagus, which implies that a number of stimuli, i.e., contractions, distension (30), acid, and possibly other factors that do not elicit pain in normal subjects do so in patients (6, 20, 26). Esophageal hypersensitivity is also referred to as allodynia and hyperalgesia.

Several investigators have suspected ischemia of the esophageal wall as a possible mechanism of esophageal pain. MacKenzie et al. (22) studied rewarming rate of perfused water in the esophagus as a measure of esophageal wall blood flow and found it to be lower in patients with esophageal pain compared with normal subjects. We recently reported the relationship between esophageal wall blood perfusion (EWBP) and esophageal muscle contraction in normal healthy subjects using a novel laser Doppler technique (17, 24). We found that contraction of the circular and longitudinal muscle of the esophagus reduce esophageal mucosal perfusion (17, 24). The goal of our present study was to determine EWBP in patients with NE, an esophageal motility disorder associated with NCCP. We measured EWBP using the laser Doppler technique under baseline condition and following administration of pharmacological agents that are often used to induce and relieve esophageal pain.

METHODS AND EXPERIMENTAL DESIGN

Subjects. Studies were conducted in 14 healthy volunteers (mean age = 51 yr, 11 men) and 12 patients (53 yr, 9 men) with NE and NCCP. The protocol for this study was approved by the University of California San Diego Institutional Review Board for the Protection of Humans, and each subject signed a written consent before enrollment in the study. Patients were recruited from the esophageal clinic/gastrointestinal function laboratory at the University of California San Diego. Cardiac etiology of pain was excluded by appropriate standard of care testing in each subject, before their inclusion in the study. All patients had a prior esophageal high-resolution esophageal manometry (HRM) study with the diagnosis of high-amplitude contractions (NE, mean contraction amplitude of >180 mmHg in the distal esophagus). These patients had also failed a trial of acid inhibition therapy, i.e., double-dose proton pump inhibition therapy. An important inclusion criterion for our study was the presence of frequent/daily chest pain symptoms. Protocol for the studies was approved by the University of California San Diego Institutional Review Board for the Protection of Humans. Subjects fasted and stopped smoking for 6 h before the study.

Monitoring technique. The EWBP was monitored using a custom-designed laser Doppler probe (Periflux system 5000, AB, Box 564 SE-175 26 JärFälla, Stockholm, Sweden) anchored to the esophageal wall (Fig. 1, A and B), as described previously (17). Briefly, the laser Doppler probe was firmly taped to a Bravo pH capsule using paraffin film (Fig. 1B). The laser beam exits from the laser Doppler probe in the direction and at the level of the suction cup of the Bravo pH capsule. The laser Doppler signal was calibrated in vitro using PF 1001 calibration device [0–250 perfusion units (PU)] (from Perimed) before placement in each subject. In the majority of subjects, laser Doppler probe was first passed through the nose and pulled out from the mouth. It was then taped to the side of the Bravo pH capsule, and assembly was then introduced through the mouth into the esophagus. Bravo capsule was deployed at 5–6 cm above the lower esophageal sphincter. Following placement of the Bravo pH capsule and laser Doppler catheter probe, a HRM catheter (Sierra Scientific, Los Angeles, CA) was passed through the nose and placed into the esophagus. Thus simultaneous and continuous recordings of the esophagus, lower esophageal sphincter and stomach pressures, esophageal pH,
and EWBP were obtained. All recordings were synchronized manually using event markers on the three recorders (laser Doppler, pH, and HRM).

**Experimental design.** Following an accommodation period of 10 min, 8–10 wet swallows (5 ml water) were recorded. A baseline period of 50–60 min was then recorded, following which subjects ate a standard 1,000-kcal meal. Recordings were performed for several hours, at the end of which edrophonium hydrochloride (tensilon, 80 mg/kg) was injected as an intravenous bolus (19). Ten minutes after tensilon injection, subjects received sublingual nitroglycerin (NTG; 0.4 mg), and recordings were performed for another 10 min. The total study period ranged from 3 to 8 h per subject, depending on the subject’s tolerability and the detachment of laser probe from the Bravo capsule. Blood pressure was monitored continuously following tensilon and NTG administration. At the end of the study, the laser Doppler probe was pulled out by applying a gentle tug to the catheter, which detached it from the Bravo pH capsule.

**Data analysis and statistics for EWBP.** In addition to the laser Doppler PU, laser Doppler monitor also provides a continuous record of the total backscatter (TB) values. The TB is a reading of the amount of light reflected back to the laser Doppler probe. A TB signal reading of ≥1 indicates adequate contact between the Doppler probe and esophageal mucosa. Mean EWBP values during 50- to 60-min baseline period before meal represented the baseline EWBP value. These time periods included spontaneous contractions. The effect of edrophonium on EWBP was determined by comparing the values under 400 PU during 5 min before and 5 min after the injection. Similarly, mean EWBP values 5 min before and 10 min after NTG administration were determined. In addition to EWBP, the manometry record was assessed for any abnormal esophageal contractions, including contractions of ≥180 mmHg and simultaneous contractions during 2 min before the onset of pain. Esophageal pH recordings were scored for reflux events (pH < 4). Data are reported as means ± SE. Data were analyzed using one-way ANOVA (parametric and nonparametric) and Student’s paired or unpaired t-test, as appropriate. Statistical significance equals P < 0.05.

**RESULTS**

**Patient demographics.** Fourteen normal subjects [11 men, median age 50 yr (35–59 yr)] and 12 patients with NE [9 men, median age 49 yr (35–74 yr)] participated in the study. The majority of our patients reported continuous symptoms (pain/heartburn) during the study. In addition to continuous pain, some patients also described increase in symptoms during the study. Three patients did not report any pain during the study, and in others there were only brief episodes of pain in the absence of continuous pain during the study, as shown in Table 1.

**Esophageal wall blood perfusion.** Figure 1C is an example of the baseline EWBP recording superimposed on the esoph-
effect on the EWBP of patients with NE (P with before injection in all subjects. Furthermore, percent time traction amplitude and durations were increased by tensilon (P after the meal (P 4. In normal subjects, the EWBP values increased significantly (P 2 yr apart during which time the patient was treated with botox injections with some improvement in symptoms. His perfusion values improved from 365 to 446 PU. In patient 3, the two studies were conducted 3 mo apart. This patient’s pain improved significantly with diltiazem-SR, 240 mg/day. His perfusion values were 418 and 508 PU. In normal subjects, the EWBP values increased significantly (P 1 yr apart, with no change in symptoms during this period. His perfusion values were 358 and 353 PU. In patient 1, the two studies were done 1 yr apart, with no change in symptoms during this period. His perfusion values were 358 and 353 PU. In patient 2, studies were conducted 2 yr apart during which time the patient was treated with botox injections with some improvement in symptoms. His perfusion values improved from 365 to 446 PU. In patient 3, the two studies were conducted 3 mo apart. This patient’s pain improved significantly with diltiazem-SR, 240 mg/day. His perfusion values were 418 and 508 PU on the 2 study days.

In three NE patients, the EWBP measurements were repeated several months apart. In patient 1, the two studies were done 1 yr apart, with no change in symptoms during this period. His perfusion values were 358 and 353 PU. In patient 2, studies were conducted 2 yr apart during which time the patient was treated with botox injections with some improvement in symptoms. His perfusion values improved from 365 to 446 PU. In patient 3, the two studies were conducted 3 mo apart. This patient’s pain improved significantly with diltiazem-SR, 240 mg/day. His perfusion values were 418 and 508 PU on the 2 study days.

The effects of a meal on the EWBP values are shown in Fig. 4. In normal subjects, the EWBP values increased significantly after the meal (P < 0.02). On the other hand, meal had no effect on the EWBP of patients with NE (P = 0.147).

Effect of tensilon on EWBP. As expected, esophageal contraction amplitude and durations were increased by tensilon administration. The EWBP was lower after tensilon compared with before injection in all subjects. Furthermore, percent time perfusion values < 400 PU were greater in NE patients compared with normal subjects (Fig. 5).

Effect of NTG on EWBP. The 9 normal subjects and all 12 NE patients received sublingual 0.4-mg NTG. The typical effect of NTG on the EWBP in one normal subject is shown in Fig. 6. The NTG did not affect blood pressure, but it caused an increase in EWBP values in both normal subjects and NE patients. The mean increase in EWBP was 83 and 53 PU in normal subjects and NE patients, respectively (Fig. 6). In four

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Mean DCL, mmHg·cm⁻¹·s⁻¹</th>
<th>EWBP Baseline, PU</th>
<th>Pain (Severity) Range 1–10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>M</td>
<td>11,777</td>
<td>349</td>
<td>Continuous pain, fluctuating</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>M</td>
<td>4,435</td>
<td>347</td>
<td>Continuous burning</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>M</td>
<td>2,718</td>
<td>479</td>
<td>Continuous pain with 9 discrete pain events, only 3 with drops in perfusion of 135, 115, 115 PU</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>M</td>
<td>4,518</td>
<td>462</td>
<td>No pain during the recording</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>F</td>
<td>6,161</td>
<td>446</td>
<td>Continuous burning, 4 discrete pain events with drops in perfusion of 82, 172, 133, 106 PU</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>M</td>
<td>4,058</td>
<td>542</td>
<td>No pain during the study</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>M</td>
<td>6,534</td>
<td>417</td>
<td>Continuous pain, 7 discrete pain events, only 2 accompanied by drop in perfusion of 18, 41 PU</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>2,281</td>
<td>640</td>
<td>Continuous pain, 7 discrete pain events, only 2 accompanied by drop in perfusion of 18, 41 PU</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>M</td>
<td>5,250</td>
<td>446</td>
<td>Continuous pain</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>F</td>
<td>13,008</td>
<td>542</td>
<td>No pain during the study</td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>M</td>
<td>5,984</td>
<td>587</td>
<td>Continuous pain</td>
</tr>
<tr>
<td>12</td>
<td>74</td>
<td>F</td>
<td>10,790</td>
<td>548</td>
<td>One pain event with drop in perfusion of 295 PU</td>
</tr>
</tbody>
</table>

DCI, distal contractile integral; EWBP, esophageal wall blood perfusion; PU, perfusion units; M, male; F, female.

Fig. 2. A–C: EWBP and HRM in 3 PTs with continuous pain. These 3 recordings were obtained from 3 different PTs with continuous symptoms of chest pain (A) or heartburn (B and C). Note that the baseline EWBP is low in each of these PTs, compared with the normal subject shown in C. Also note, unlike the normal subject, the change in EWBP associated with esophageal contraction was quite variable.

Table 1. Spontaneous pain events during the recording
patients who had no symptoms before NTG administration and in other patients who complained of pain before NTG, it disappeared in three patients, improved in four, and there was no change in one.

Spontaneous pain events during the recording. Some patients described increase in pain, superimposed upon the continuous pain or in the absence of continuous pain during the study period. Some but not all of these pain events were associated with further drops in perfusion values, as shown in Table 1. Despite clear instructions to the patients, the majority of patients did not describe discrete onset and cessation of pain during the recording period, and, therefore, we could not determine precise relationship between changes in EWBP and pain during all events.

Manometry and pH before the pain events. In two subjects with continuous pain symptoms, esophageal pH was low for extended period of time. In the remainder, the esophageal pH was >4, and no acid reflux event was identified during the entire monitoring period. Manometry recordings were evaluated for esophageal contractions of >180 mmHg during the 2-min window before the increase in pain (both in the setting of continuous pain or no continuous pain); six patients reported such events. High-amplitude esophageal contractions were seen in association with 19 of the 35 such events (median 4, range 1–9 pain events/subject). In the other 16 events, either no contraction or relatively normal-looking contraction was observed. Examples of these events are shown in the Fig. 7.

DISCUSSION

The laser Doppler perfusion technique provides average blood perfusion values in the area of tissue illuminated by the laser light beam (21). It registers blood flow values in PU rather than the actual volume of blood flow and, therefore, is ideally suited to measure relative changes in the blood flow. Since the Doppler probe was calibrated in vitro before placement in each subject, it is reasonable to assume that the comparison of baseline values among different subjects is valid. The depth of the penetration of the laser beam is generally accepted to be 1–2 mm, but in the gastrointestinal application it can be up to 5 mm (18). The depth of penetration depends on several factors, 1) darker tissue generally has a lesser depth of penetration compared with white or transparent tissue, and 2) reduction in the blood perfusion of tissues makes them relatively more transparent and therefore increases the depth of penetration. We observed that the TB values, a measure of the laser light returned to the Doppler probe, increased during manometrically identified contractions.

This is the first study to measure EWBP using a unique method, in which we anchored a laser Doppler probe to the esophageal wall (17), in the NE patients. This assembly is

Fig. 3. A and B: baseline EWBP in normal subjects and PTs. A: mean baseline EWBP is significantly lower in PTs compared with normal subjects. B: the frequency histogram shows that the EWBP curve is shifted to the left in PTs, suggesting lower perfusion values. Values are means ± SE.

Fig. 4. Effect of meal on the EWBP in normal subjects (A) and nutcracker esophagus PTs (B). Note an increase in perfusion values following meal in normal subjects (P < 0.05), but not in the PTs. Values are means ± SE.
crucial for obtaining artifact-free continuous recordings of the EWBP. However, this is not the first study to implicate low EWBP as a possible mechanism of esophageal pain. MacKenzie et al. (22) found significantly longer warming rate of injected water into the esophagus (as a measure of EWBP) in 9 patients with esophageal spasm compared with 20 controls and proposed esophageal wall ischemia as the cause of pain symptom. Gustafsson et al. (11) used a computerized thermistor recording and found an increase in the mucosal blood flow during acid infusion into the esophagus in patients responding to acid-infusion with heartburn. They also studied patients who responded to edrophonium injection with/without pain (a provocative test that increases the strength and intensity of muscle contractions and esophageal pain) (12), and no difference in the rewarming rates was detected between pain-positive and pain-negative patient groups. We suspect that the use of thermistor technique is not ideal to study EWBP, because retention of even a small amount of water in the esophagus due to poor clearance may affect esophageal rewarming significantly. Furthermore, the thermal capacity of thermistor is large and may be incapable of detecting subtle changes in the EWBP. More recently, Hoff and colleagues (13–15) used the laser Doppler perfusion monitoring to determine distension-related esophageal pain and found that esophageal wall stress and strain, rather than mucosal ischemia, is the cause of distension-related pain. It is not clear though if they monitored adequacy of contact between the laser Doppler probe and mucosa during their study using TB values.

Even though our laser Doppler recording technique measures esophageal mucosal perfusion, we suspect it is representative of blood perfusion in the entire wall of the esophagus, i.e., submucosa and muscularis propria. Our reasoning is that the blood vessels traverse through muscle layers to reach the esophageal mucosa. In our earlier studies in normal subjects, we found that circular and longitudinal muscle contractions of the esophagus reduce esophageal mucosal perfusion (24), which is similar to the concept that blood perfusion to the myocardium occurs during diastole rather than systole. In normal subjects, the duration of reduction in EWBP is relatively brief and is similar to the duration of muscle contraction. A continuously low baseline EWBP value unrelated to manometrically identifiable contractions was an unexpected finding in our present study of the NE patients. The reasons for low baseline EWBP may be several. 1) All types of intraluminal manometry register circular and not the longitudinal muscle contraction (25). Furthermore, both circular and longitudinal muscles of the esophagus have sustained tone, which is not identifiable by manometry. Therefore, it is possible that patients with esophageal pain have esophageal contractions that are not identified by the HRM. 2) All types of spastic motor disorders, i.e., achalasia, diffuse spasm, NE, and nonspecific motor disorders, have a thicker muscularis propria/muscular hypertrophy (4, 25). Left ventricular hypertrophy is well known to be associated with ischemia of the myocardium and cardiac pain (3, 23); it is very possible that esophageal muscle hypertrophy leads to a low blood flow state of the esophageal wall that causes the symptoms of chest pain in the NE patients.

We expected to find discrete pain events with discrete drops in blood perfusion in all of our patients for all spontaneous pain events, which we indeed saw in association with several but not all pain episodes. In addition, there were also periods of sharp drop in perfusion values during which patients did not report pain. Based on the above, one may argue that we have not demonstrated a tight relationship between pain and low blood perfusion, which is true. However, we believe that there are several reasons for the above. 1) It is well known that patients do not report symptom precisely and accurately, and thus it is difficult to demonstrate close temporal correlation between the pain and reduction in wall perfusion. 2) In the setting of an already continuous low perfusion, it was difficult at times to discern discrete changes in the EWBP. 3) Tissue hypoxia is more likely to be related to the degree and duration of low perfusion, and precise strategy how one can quantitate low perfusion has not been still established and finally. 4) It is possible that different patients have different threshold for hypoxia-related tissue injury and pain.

Myocardial ischemia causes angina and in some cases “dyspepsia and heartburn like symptoms.” Studies by Longhurst et al., spanning over 30 yr, show that intestinal ischemia results in generation of lactic acid (27), bradykinin (31), histamine (9), serotonin (10), prostaglandins (8), endothelins (7), and possibly other chemicals that are potent stimuli of the nociceptive nerve endings of the sympathetic nervous system (7). Bulmer et al. reported activation of both spinal and vagal visceral nerve endings in response to intestinal ischemia (2). In our laboratory’s earlier studies, we found strong temporal correlation between esophageal pain (1) and heartburn (28) with the sustained longitudinal muscle contraction of the esophagus. We propose that the mechanism by which long-duration longitudinal muscle contraction induces pain may be related to the long-duration reduction in the EWBP. Edrophonium (tensilon)
is a provocative agent to induce esophageal pain, and we observed that it causes a fall in the EWBP. On the other hand, NTG is often used to relieve esophageal pain, and we found that it increases the EWBP. Therefore, both tensilon and NTG data support our hypothesis that low EWBP may be an important mechanism of esophageal pain.

There are several limitations of our study. 1) How can we be sure that esophagus was indeed the cause of NCCP in our patient population? Cardiac etiology of pain was excluded in all of our patients, and none was responsive to what is considered as the adequate acid inhibition therapy. Esophageal manometry revealed evidence of high-amplitude contraction that is considered to be a marker of esophagus pain in the NE patients. 2) We studied small number of patients with severe symptoms, which is not representative of the bigger population of NCCP patients seen in the clinics and emergency rooms with “angina-like” esophageal pain. Our current technique of EWBP can only be performed in the clinic/hospital setting because the laser Doppler monitor is relatively bulky. Esophageal symptoms usually are intermittent, and measurement would require ambulatory long-term EWBP monitoring, like pH monitoring, that is not currently available. 3) How hypoxic injury induces NCCP cannot be fully determined from our study.

In summary, it is possible that, similar to myocardial hypoxia that causes angina, NCCP is related to low EWBP, which leads to activation of the hypoxic pathway, culminating in activation of nociceptive nerve terminals in the esophagus. Improvements in the EWBP technique are needed to study a larger number of patients in the ambulatory setting (similar to pH/Bravo pH techniques) to determine whether NCCP and heartburn nonresponsive to proton pump inhibition therapy are indeed related to low EWBP and activation of the chronic hypoxia/ischemia perfusion pathway. Furthermore, we antici-
pate that identification of hypoxia-generated factors in the esophageal wall that could be the cause of pain and muscle dysfunction will result in better therapeutic strategies for this extremely prevalent condition.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES