Effect of esophageal contraction on esophageal wall blood perfusion

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Mittal RK, Bhargava V, Lal H, Jiang Y. Effect of esophageal contraction on esophageal wall blood perfusion. Am J Physiol Gastrointest Liver Physiol 301: G1093–G1098, 2011. First published September 8, 2011; doi:10.1152/ajpgi.00293.2011.—Myocardial blood flow occurs during the diastolic phase of the cardiac cycle, because myocardial contraction during the systolic phase impedes myocardial perfusion. Using laser Doppler perfusion technique, we studied the effect of esophageal contraction on the esophageal wall perfusion. Studies were conducted in rats. A laser Doppler probe was anchored to the esophageal wall, and wall perfusion was studied under various experimental conditions. Increase and decrease in the systemic blood pressure induced by different pharmacological agents was associated with the increase and decrease in the esophageal wall perfusion, respectively. Esophageal contractions induced by electrical stimulation of the vagus nerve and electrical stimulation of the muscle directly resulted in a reduction in the esophageal wall perfusion, in a dose-dependent fashion. Esophageal wall perfusion could be monitored by placing the Doppler probe on the esophageal mucosa or on the outside of the esophageal wall. Esophageal contraction impedes entry of blood into the esophageal wall. Future studies may investigate if ischemia of the esophageal wall induced by sustained esophageal contractions/esophageal spasm is the cause of esophageal pain symptoms in humans.

esophageal ischemia; blood flow esophagus; esophageal pain; laser Doppler flowmetry; esophageal spasm

UNDER PHYSIOLOGICAL CONDITIONS, blood perfusion of all tissues is essential for their normal functioning. Blood flow increases and decreases in the tissues to meet metabolic demands; e.g., following ingestion of food, intestinal blood flow increases to cope with the demands of absorption of fluid, electrolytes, and nutrients. Blood flow homeostasis is also vital to the integrity of intestinal mucosa; decrease in blood flow to less than the critical threshold leads to ischemia and necrosis of the gastrointestinal tract (4).

In animal studies, microsphere injection technique has been used extensively to study blood flow in various tissues (5, 6); however, it cannot be used in humans because of the need of tissue harvesting. Furthermore, it cannot be used to measure changes in the blood flow during muscle contractions. A thermistor (temperature recording device) to record rewarming of the cold water injected into the esophagus, as a surrogate of esophageal blood flow, has been used in humans by several investigators (7, 8, 16). However, technical issues preclude the thermistor technique from being adequate; e.g., lack of clearance of even small amounts of water from the esophagus (e.g., related to peristaltic dysfunction) may affect the rewarming rate of the esophagus. More recently, Hoff et al. used laser Doppler flow probe to determine the relationship between balloon distension-related alteration in esophageal wall perfusion and esophageal pain (9–12).

Blood flow in the myocardium occurs predominantly during the diastolic phase of the cardiac cycle, because myocardial contraction during the systolic phase impedes the entry of blood in the myocardium (1). Similarly, muscle contraction also prevents entry of blood flow in the skeletal muscles (2) and gastric tissues (15). The effect of esophageal contractions on the esophageal wall flow has never been adequately studied. We used the laser Doppler probe system to study the effects of esophageal contractions on esophageal wall perfusion in rats.

METHODS

The protocol was approved by the Animal Safety Committee of the San Diego Veterans Affairs Health Care System (IACUC). Studies were performed in Sprague-Dawley rats, weighing between 250 and 400 g (from Charles River, Wilmington, MA). Animals were anesthetized with an intraperitoneal injection of 4 ml/kg cocktail containing ketamine (3.75 ml, 100 mg/ml), xylazine (0.4 ml, 100 mg/ml), acepromazine (0.75 ml, 10 mg/ml), and distilled water (15.1 ml). A venous cannula and arterial line were placed to administer pharmacological agents and measure blood pressure, respectively. A tracheotomy was performed for ventilation of the lungs. Vagus nerve was isolated on both sides of the neck and transected. The peripheral end of one of the vagus nerve was placed on a pair of platinum electrodes for electrical stimulation at constant currents using a dual-output square-pulse stimulator (Grass S88X, West Warwick, RI).

A midline laprotomy was performed, and esophago-gastric junction was identified. Two silver wire electrodes were sutured on the esophageal wall for local stimulation of esophageal muscles using constant current parameters. Intraluminal pressure of the esophagus was measured by a 2.5 F solid-state pressure transducer catheter (Millar Instruments, Houston, TX) placed directly underneath the stimulating electrodes. The catheter was passed through a small incision, 2 cm below the lower esophageal sphincter, on the front wall of the stomach (Fig. 1). In some experiments, electrodes were also implanted on the stomach wall to study the effects of gastric muscle contractions on gastric wall blood perfusion.

A custom-designed laser Doppler perfusion monitoring probe (AB, Box 564 SE-175 26 JärFälla, Stockholm, Sweden) was used for these studies. Before placement on the esophageal wall, the Doppler probe was calibrated as follows: the Doppler transducer was placed in the calibration chamber and solution to read 0 and 250 perfusion units (PU), respectively. The Doppler probe was then anchored on to the esophageal wall by a suture. In the above setting, the laser beam was directed from outside of the esophageal wall facing toward the esophageal lumen. In another set of experiments, a longitudinal incision was made on the esophageal wall to expose the lumen. The laser Doppler probe was then placed on the esophagus, and the laser beam was directed toward the esophageal wall. Doppler signal was recorded using the Perimed software, with a moving time constant of 0.2 s.

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Study protocol. Esophageal wall perfusion was determined under the following experimental conditions. 1) The first condition was administration of pharmacological agents that increase or decrease systemic blood pressure. Phenylephrine (6 mg/kg) was used to increase systemic blood pressure; phentolamine (2 mg/kg) and sodium nitroprusside (SNP, 10 μg/kg), on the other hand, were used to decrease it. 2) The second was esophageal contractions induced by electrical stimulation of the peripheral end of one of the cervical vagus nerve at various electrical current intensities. 3) The third was muscle contraction induced by locally implanted electrodes on the esophageal and stomach wall. 4) Fourth, in some experiments, laser Doppler probe was first placed on the outside of the esophageal wall and then inside the esophageal lumen. Electrical stimulation parameters used to stimulate vagus nerve and local muscle contraction were as follows: constant current of 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 mA; pulse width 10 ms; pulse frequency 20 Hz; and train duration 10–30 s. 5) Fifth, to determine whether the effects of local electrical stimulation were induced by contraction of vascular smooth muscle or skeletal muscle, an α-blocker, phentolamine, was injected intravenously, followed by direct stimulation of the esophageal muscle.

Data analysis. Changes in wall perfusion were measured in PU. Esophageal pressure was measured as the increase in pressure with vagus nerve and local muscle stimulation. Changes in blood pressure were also recorded. Values are shown as means ± SE, unless stated otherwise. Statistical comparisons were performed using paired or unpaired t-test, as appropriate.

Fig. 1. Schematic of the experimental design. LES, lower esophageal sphincter.

Fig. 2. Effects of alternation of systemic blood pressure on the esophageal wall perfusion (EWBF). A: tracings show effect of sodium nitroprusside (SNP), phenylephrine (PE), and phentolamine on EWBF (n = 8) and arterial pressure. Note that SNP and phentolamine decrease blood pressure and esophageal wall perfusion. B: effect of phenylephrine on stomach wall perfusion (n = 3) and arterial pressures. Note that effects of various pharmacological agents on stomach wall perfusion are similar to those on the esophagus. C: mean ± SE data with regards to the effects of various pharmacological agents on esophageal and stomach wall perfusion. Note that the effects on blood pressure and wall perfusion parallel each other. PU, perfusion units.
RESULTS

Spontaneous fluctuations of esophageal wall blood perfusion. Baseline esophageal perfusion values ranged from 385 to 842 PU (mean ± SD = 547 ± 189 PU) among different animals (n = 11). During 1-h recording period, significant change in the baseline values was seen in all animals. In addition, there were spontaneous fluctuations in the wall perfusion values that occurred with the same frequency as the respiratory rate. These fluctuations ranged from 100 to 150 PU.

Effects of alteration of systemic blood pressure on the esophageal wall perfusion. Administration of intravenous SNP caused a rapid decrease in the blood pressure and a rapid decrease in the esophageal wall perfusion. The changes in blood pressure and wall perfusion were almost mirror images of each other. At the peak effect, systemic blood pressure dropped by 48 ± 12%, and esophageal wall perfusion also dropped by 48 ± 15%. Phenylephrine administration, on the other hand, resulted in an increase in the system blood pressure, and, correspondingly, there was an increase in the esophageal wall perfusion. Percent changes in blood pressure and change in esophageal wall perfusion at the peak effect were not significantly different. Similar to SNP, phentolamine reduced systemic blood pressure and esophageal wall perfusion. The effects on the stomach wall perfusion, in response to the above-mentioned pharmacological manipulations, were similar to the effects on esophageal wall perfusion (Fig. 2).

Effect of esophageal contractions on esophageal wall blood perfusion. Esophageal contractions were induced by two methods (Fig. 3): 1) electrical stimulation of vagus nerve, and 2) electrical stimulation of esophageal muscle using electrodes implanted into the esophagus directly. Vagus nerve stimulation was performed using a 20-Hz tetanic stimulus, and it induced a current intensity-dependent increase in the esophageal contraction amplitude and decrease in esophageal wall blood perfusion (EWBP). Unlike spontaneous changes in EWBP, change with electrical stimulation was rapid, as fast as the muscle contraction. Maximal reduction in EWBP occurred when esophageal contraction amplitude ranged from 80 to 120 mmHg. Increase in contraction amplitude above these values did not cause further decrease in the EWBP. In addition to decrease in EWBP, there was a decrease in the systemic blood pressure during vagus nerve stimulation. Since change in blood pressure also affects EWBP, fall in the EWBP during vagus nerve stimulation could be related to either the drop in the systemic blood pressure, or esophageal muscle contraction, or both. Electrical stimulation of the esophageal muscle using directly implanted electrodes, on the other hand, induced current intensity-dependent increase in the esophageal contraction amplitude and decrease in EWBP without any effect on the systemic blood pressure (Figs. 3 and 4A). Similar to vagus nerve stimulation-induced changes in the EWBP, local muscle stimulation-induced changes were fast and reached peak reduction at contraction amplitude of 80–120 mmHg. Figure 4B shows the second-order polynomial curve fit relationship of esophageal blood perfusion as a function of esophageal contraction pressure. It implies that the maximal reduction in esophageal wall perfusion occurs at contraction pressure of ~100 mmHg. Stimulation of the gastric muscle, similar to esophageal muscle, also reduced gastric wall perfusion (Fig. 5).

To answer the question if direct esophageal muscle stimulation induced contraction of the vascular smooth muscle to decrease EWBP, we used an α-adrenergic blocker (phentolamine) to block the adrenergic vascular smooth muscle contraction. Phentolamine administration reduced the baseline EWBP to ~60% of the baseline values. Direct stimulation of

Fig. 3. Effect of vagus nerve stimulation and local muscle stimulation on esophageal wall perfusion. Electrical stimulus parameter was as follows: constant current 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 mA; pulse width 10 ms; and train duration 10–30 s. Note, vagus nerve stimulation induces esophageal contractions, and the effects are dose dependent, i.e., increase in current intensity increases contraction amplitude. Also note reduction in blood pressure with vagus nerve stimulation. On the other hand, local muscle stimulation induces esophageal wall contraction and esophageal wall perfusion in a dose-dependent fashion without any effect of the blood pressure.
the muscle in the presence of phentolamine induced esophageal contractions and reduced EWBP to near zero values (Fig. 6).

**Effect of laser Doppler probe location on EWBP.** We studied the effect of placement of laser Doppler probe on the esophageal wall, outside and then inside the esophageal lumen. Direct muscle stimulations using electrical stimulation were performed in both settings. Baseline perfusion values were higher when the Doppler probe was located outside the esophagus compared with the mucosa in three of the four rats in which direct comparisons were made. In response to local muscle stimulation, there was similar reduction in the EWBP in both settings (Fig. 7).

**DISCUSSION**

In summary, our data show the following. 1) Changes in blood pressure affect esophageal wall perfusion. Pharmacological agents that increase and decrease blood pressure increase and decrease esophageal wall perfusion, respectively, thus validating laser Doppler perfusion monitoring as an adequate technique to measure esophageal wall perfusion. 2) Esophageal contractions induced by vagus nerve stimulation and local muscle stimulation decrease esophageal wall perfusion in a dose-dependent fashion. Unlike vagus nerve stimulation, local muscle stimulation does not affect blood pressure, and hence the effects are not entirely related to the reduction in blood pressure. 3) Esophageal wall perfusion can be measured by placing the probe inside the esophageal lumen, i.e., on the esophageal mucosa.

In contrast to microsphere injection technique, laser Doppler monitoring is a relative simple technique to monitor blood flow or wall perfusion of an organ and has been used extensively in various animal and human studies. Since it does not require tissue harvesting, laser Doppler can be used in the human settings to monitor changes in the tissue perfusion continuously. How does it measure tissue perfusion? Briefly, an optical fiber leads laser light to the probe tip, which rests against the tissue. A beam of the laser light enters tissue and becomes scattered; blood cells moving within the volume illuminated by the laser beam cause light to change frequency (Doppler shift). Within the tissue, there is a Doppler shifted and Doppler nonshifted light. The proportion of shifted to nonshifted light is related to the number of moving objects or blood cells within the light path. Part of the light within the tissue is scattered back to the probe and returned via an optical fiber to the photo detector, which converts phase shift in the light to an electrical signal. The major advantage of laser Doppler technique is that it allows continuous monitoring of tissue perfusion or blood flow in vivo. The depth of laser beam penetration depends on the laser light wavelength, distance between emitter and detector fibers, and tissue characteristics. On the skin surface, the depth of laser beam penetration is ~2 mm, but, in the gastrointestinal tract, it may be 5–6 mm (13). Studies conducted during last 30 yr have validated laser Doppler perfusion technique against microsphere injection technique (13, 14, 18).
Effects of muscle contraction on the muscle perfusion have been studied in various organ systems. As early as 1935, Anrep and von Saalfeld (2) reported the effects of muscle contraction of the limb skeletal muscle (gastrocnemius) on the arterial blood flow using a hot wire anemometer. They describe four phases of changes in the arterial blood flow with the onset of stimulation:

1) back thrust of blood in the artery;
2) diminution or arrest of the blood inflow;
3) overshoot of blood flow during relaxation; and
4) the hyperemic after-effect. The first two changes in the arterial blood flow are due to a compression of the blood vessels by the contracting muscle fibers. The compression of vessels with contraction has also been observed in the heart, other skeletal muscles, including diaphragm, as well as the smooth muscles of small intestine. Anrep and von Saalfeld (2) also reported that the degree of compression of blood vessels depends on the strength of the muscular contraction. The fourth phase, the period of hyperemia, has been explained on the basis of peripheral vasodilator action of locally produced metabolites. Myocardial perfusion also takes place predominantly in the diastolic phase of cardiac cycle, because myocardial contraction during systolic phase constricts blood vessels. Livingston et al. (15), using in vivo microscopy, provided visual evidence of constriction of capillaries of the gastric corpus and antrum during electrical field stimulation of the gastric corpus and antrum. Changes in capillary size paralleled that of the phasic contractions of gastric corpus and gastric antrum. Decrease in capillary size was much greater in the antrum than the gastric corpus. Interestingly, the corpus showed stronger hyperemia following constriction than the antrum. The significance of their findings was that they hypothesized that contraction-related ischemia could play an important role in the pathogenesis of ulcer disease. Autoregulation of blood flow is an important feature in the gastrointestinal tract, e.g., following ingestion of meal blood flow increases significantly in the stomach and small intestine. We observed parallel changes in the blood pressure and esophageal wall perfusion, suggesting absence of autoregulation (Fig. 4B). Similarly, one may expect autoregulatory phenomena during muscle contraction, which is not the case.

Esophageal blood flow has been studied using various techniques, in animals and humans. Using microsphere injection technique, it was determined that the blood flow to the oesophagus is greater than that to the body of the esophagus (19). Mucosa and submucosa receive greater blood flow than the muscular propria, and there is a gradient of blood flow in the esophagus; the distal part receives greater blood flow than the proximal. Acid instillation in the esophagus increases esophageal mucosal blood flow through the release of histamine, nitric oxide, and calcitonin gene-related peptide (5, 6). A thermistor to record rewarming of the cold water injected into the esophageal lumen, as a surrogate of esophageal blood flow, has been used in the humans. MacKenzie et al. (16) found a significantly longer warming rate of injected water in 9 patients with esophageal spasm compared with 20 controls and proposed esophageal wall ischemia as the cause of pain. Gustafsson et al. (7), using a computerized thermistor, found an increase in the mucosal blood flow during acid infusion into the esophagus, in patients responding to acid

Fig. 6. Effect of phenolamine on local stimulation-induced changes in esophageal wall perfusion. A: note that phenolamine administration reduced blood flow pressure and esophageal wall perfusion. Also note similar reduction in esophageal wall perfusion with local muscle electrical stimulation (current intensity 3 mA) before and after phenolamine (see text for explanation). B: summary data show means ± SE (n = 7).

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infusion with heartburn. They also studied patients who responded to edrophonium injection with/without pain (a test of esophageal pain) (8). However, they found no difference in the rewarming rates between the pain-positive and pain-negative patient groups. The thermistor/rewarming technique may not be adequate to study blood flow in the esophagus, e.g., retention of even a small amount of water in the esophagus due to inadequate clearance may affect esophageal rewarming significantly. More recently, Hoff and colleagues (9–12) used laser Doppler perfusion monitoring to determine distension-related esophageal pain and found that esophageal wall stress and strain, rather than wall ischemia, is the cause of distension-related pain. These investigators, and to the best of our knowledge any others, have not studied the relationship between esophageal contractions and esophageal wall perfusion. Our study is clear in that the esophageal contraction reduces esophageal wall perfusion. Since laser Doppler provides average tissue perfusion in the area of laser illuminated tissue, it cannot distinguish between mucosal vs. muscle blood flow, but it appears that blood flow reduces in both layers because, during muscle contraction, we could see blanching of the mucosa and muscle visually.

Our finding of reduction of esophageal wall perfusion with esophageal wall contraction has considerable clinical relevance. Mechanism of esophageal pain has been an area of significant debate, and it is currently thought to be related to the hypersensitivity of the esophagus. However, we observed a close temporal correlation between esophageal pain and heartburn events with sustained (prolonged) contraction of the longitudinal muscles of the esophagus (3, 17). We propose that a sustained esophageal muscle contraction induces prolonged reduction in the EWBP, thus rendering esophageal wall ischemic, which, in turn, causes pain through the generation ischemia/hypoxia-related chemicals. Our study shows that laser Doppler probe can be placed on the mucosal surface to record changes in the esophageal wall perfusion in humans. Using laser Doppler probe, future studies should investigate the relationship between esophageal symptoms of heartburn and chest pain with EWBP.

REFERENCES