Reflux Mechanisms in Gerd : Analysis of the role of transient lower esophageal sphincter relaxations

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INTRODUCTION:

TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS

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Gastro-esophageal reflux

Heartburn, resulting from gastro-esophageal acid reflux, is one of the most common gastro-intestinal symptoms in the general population (1). In the past decades we have gained a better insight into the mechanisms that cause gastro-esophageal reflux (Table 1). Nowadays gastro-esophageal reflux is considered a motility disorder. The main barrier for gastro-esophageal reflux is the esophago-gastric junction. A constant elevated pressure barrier at the esophagogastric junction is maintained by the lower esophageal sphincter (LES) and the crural diaphragm. A hiatal hernia separates the two pressures of lower esophageal sphincter and crural diaphragm and thereby diminishes the synergistic pressure effect reading to a more weak anti reflux barrier (2). The lower esophageal sphincter is able to relax to a pressure equal to intragastric pressure. Short-lasting, swallow-induced relaxations occur during ingestion of liquid or solid food. Prolonged relaxation of LES also occurs. These phenomena are not related to swallowing and have been described as transient lower esophageal sphincter relaxations (TLESRs). There are also other gastrointestinal factors that influence gastroesophageal reflux. The content of the refluxate is of importance for damaging esophageal mucosa. Not only acidic content of the stomach has an erosive effect on the esophagus. Bile mixes with acid after duodenal-gastric reflux. Bile can be measured using fotospectrometry (Bilitec 2000). Patients with Barrett esophagus have excess of biliary duodeno-gastro-esophageal reflux.

Table 1. *Factors involved in the pathogenesis gastro-esophageal reflux*

- Low LES pressure
- Transient LES relaxation
- Hiatal Hernia
- Ineffective esophageal peristalsis
- Delayed gastric emptying
- Content of refluxate
- Saliva
- Esophageal mucosal resistance

Delayed gastric emptying increases gastroesophageal reflux. Not only by increasing the time of intragastric content but also by prolonged gastric distension. High caloric or fat containing meals delay gastric emptying. In the esophagus several mechanisms are involved in clearance of gastroesophageal reflux. Salivary flow can neutralize acidic refluxate. Esophageal motility performs mechanical clearance through primary and/or secondary peristalsis. The last defense mechanism is the esophageal mucosa.

Gastroesophageal reflux may lead to specific symptoms like heartburn, retrosternal pain, regurgitation, belching and dysphagia. Long-lasting gastroesophageal reflux of acidic gastric content may lead to erosive esophagitis, esophageal ulceration, esophageal intestinal metaplasia (Barrett) and even strictures of the esophagus. With endoscopy one is able to objectify the severity of esophagitis. Long-term ambulatory pH-metry is of clinical value in the detection of pathologic reflux in patients with GERD (3; 4). The grade of esophagitis is

correlated to the amount of reflux measured by 24-hour pH metry. Percentage of time with esophageal pH below 4 and number of reflux episodes lasting longer than 5 minutes are the best parameters to indicate the severity of gastroesophageal reflux (5). Combining esophageal pH- and manometry may give information on mechanisms of gastroesophageal reflux.

Transient lower esophageal sphincter relaxations

Transient lower esophageal sphincter relaxations are abrupt decreases of lower esophageal sphincter pressure to intragastric pressure. These relaxations are not related to swallowing. TLESR probably are a mechanism to evacuate excess of intragastric air. Therefore the TLESR may function as a safety valve of the esophagogastric junction. (6; 7). TLESR are identified by esophageal manometry. Water perfused manometry with catheter with small side-hole openings is not adequate, since the position of the catheter orifice at the esophagogastric junction changes during respiration. The standard method for continuous LES registration is the water perfused sleeve device. The sleeve has been first described by Dent in 1978 (8). The water perfused sleeve has been used extensively both in animal as in human studies and is considered as the golden standard technique for lower esophageal sphincter manometry. Miniature devices were able to detect TLESR at very early age (9; 10).

Prolonged recording of LES pressure was possible using a portable manometric system (11). A solid-state catheter has been developed for sphincter manometry. This sphinctometer is not dependent on water perfusion, but it consists of an oil filled cylinder incorporating a solid state transducer. The sphinctometer records LES pressure and identifies TLESR. However due to the technique LES pressures are expressed lower and the LES pressure is dependent on the length of the sleeve exposed to the LES. Because of low LES pressure output, it may prove more difficult to identify TLESR with sphinctometer than with standard sleeve manometry(12; 13).

Holloway described objective criteria for TLESR. Based on analysis in healthy subjects and patients with GERD, TLESR were defined by 1) absence of swallowing for 4 s before to 2 s after the onset of LES relaxation, 2) relaxation rate of $\geq$1 mmHg/s 3) time from onset to complete relaxation of $\leq$10 s and 4) nadir pressure of $\leq$2 mmHg. Prolonged relaxations by multiple swallows are excluded. LES pressure falls that fulfill the last three criteria but have a duration of >10 s can also be classified as TLESR irrespective of timing of LES relaxation to swallowing (14)

LES tracing are analyzed visually in order to detect TLESRs. Although there are precise criteria for TLESR interobserver variation may lead to differences in detection of TLESR. Computer analysis of LES tracings is feasible however computer assisted detection of TLESR until now, has not been satisfactorily accomplished (15; 16).

The frequency of TLESR has been investigated extensively. First, they have been identified in animals, healthy human subject and patient with GERD. Secondly mechanisms that increase TLESR frequency were studied, and third factors that inhibited the frequency of TLESR were studied. Main factors that influence TLESR a summarized in Table 2.

Even a catheter in the pharynx affects TLESR. Subjects with catheters placed via the pharynx have been compared with manometric catheters placed via a gastrostomy tube. The frequency of TLESR was significantly higher with a catheter positioned in the pharynx and therefore it was concluded that the pharynx may mediate the induction of TLESR (17). Stimulation of laryngopharyngeal mechanoreceptors induces LES relaxation but not crural

diaphragm relaxation. (7; 18; 19). Gastro-esophageal reflux was found only during simultaneous LES and crural diaphragm relaxations.

Body posture affects the frequency of TLESR. The frequency of TLESR was higher in the vertical position than in the horizontal position (20-22). Sleep inhibits the frequency of TLESR (23).

The frequency of TLESR varies during the day. Under fasting conditions the frequency of TLESR ranges from 0-3 TLESR per hour. Ingestion of a meal results in an increase in the frequency of TLESR. The frequency of TLESR after meal ingestion is dependent on both the volume and content of the meal. After meal ingestion the number of TLESR may increases to about 3-6 TLESR per hour (24; 25). The frequency of TLESR after a fat containing meal persists for several hours, while after ingestion of a carbohydrate, low fat containing meal the frequency of TLESR more rapidly returns to basal level (26). Another study on composition of the meal reported no significant differences between a balanced meal and a high fat meal, neither in patients with GERD nor in healthy subjects (27)

Gastric distension is a potent trigger of TLESR. Distention of the proximal stomach with intragastric air or an intragastric balloon increases the frequency of TLESR (28). It has been described in dogs that the subcardiac region of the stomach in dogs is primarily responsible for triggering TLESR (29). Intragastric distension with air provoked TLESR with shorter duration than those seen after distension with liquid. At high intragastric pH fewer TLESR occurred compared to low pH. Triggering of TLESR is therefore dependent both on physical and chemical nature of the stimulus (30).

Table 2 *Factors that influence TLESR*

Increase in frequency of TLESR
• Meal composition
• Gastric distension
• Cholecystokinin
• *Cholestyramine*
Decrease in frequency of TLESR
• Muscarine receptor antagonist (atropine)
• Opiod alkaloid (morphine)
• Nitric-oxide synthase inhibitor (L-NAME; L-NMMA)
• CCK-receptor antagonist (loxiglumide)
• 5HT3 receptor antagonist (ondansetron, granisetron)
• 5HT4 receptor partial agonist (tegaserod)
• GABA-B receptor antagonist (baclofen)
• Body posture
• Sleep
• Fundoplication
• Radiofrequency energy at esophagogastric junction

In asymptomatic recumbent subjects gastroesophageal reflux is related to TLESR rather than to low basal LES pressure (31). The mechanisms of gastroesophageal reflux have repeatedly been evaluated in patients with reflux esophagitis. The predominant reflux mechanism varied in individual patients: some had normal resting LES pressure and reflux that occurred primarily during TLESR, whereas others with low resting sphincter pressure had spontaneous free reflux over a defective or insufficient LES or reflux that occurred during an increase in abdominal pressure (32). It is not clear whether patients with GERD differ from controls with respect to the frequency of TLESRs. It has been suggested that patients with GERD have an increased frequency of TLESR compared to healthy subjects (32), but others reported that the frequency of TLESR is not significantly different between patients with GERD and controls (33). After a mixed meal the rate of TLESR showed a trend towards higher values (not significant) in patients with GERD compared with healthy subjects (27).

Mechanisms of reflux

Absence of LES pressure or very low residual LES pressure is a prerequisite for the occurrence of gastroesophageal reflux. During intervals of negligible LES pressure, increments of intra-abdominal pressure results in gastroesophageal reflux, but intra-abdominal increases of LES pressure alone do not increases gastroesophageal reflux (34). During TLESR gastroesophageal reflux may occur. Not only the frequency but probably the number of TLESRs associated with reflux is significantly higher in patients with GERD compared to controls (32). In patients with a hiatus hernia excess of gastroesophageal reflux is not caused by TLESR but by other mechanisms like low LES pressure, swallow associated normal LES relaxations or abdominal straining during low LES pressure (35).

In children TLESRs are the most common mechanism of reflux (10; 36; 37). Ambulatory manometry studies have confirmed results of studies with stationary manometry that TLESRs are the main mechanism of GER in healthy subjects (38; 39).
Gastroesophageal reflux of air, belches, are associated with TLESR, as is the case with acid reflux episodes (40) Belching produces a characteristic noise that is preceded by a common cavity. A common cavity phenomenon is defined as a sharp rise in esophageal pressure to the level of gastric pressure occurring within 1 sec and is accompanied by complete LES relaxation (41).

Patterns of gas and liquid reflux during TLESR were studied using intraluminal electrical impedance. Both liquid and gas reflux occurs during TLESR although acid reflux occurs as a primary event (42). Patients with GERD have more acid reflux and less nonacid reflux compared to controls. Difference in the air-liquid composition of the refluxate may contribute to higher rate of acid reflux in patients with GERD (43).

Neural regulation

A neural pathway underlying TLESRs has been suggested. The afferent pathway of the reflex arc consists of stretch sensitive vagal afferent fibers presumably located within the muscle layers of the proximal stomach. The vagal afferent fibers, activated during gastric distension, terminate in the nucleus tractus solitarius and the dorsal motor nucleus of the vagal nerve. The motor neurons located in the nucleus tractus solitarius synapse with dendrites from the motor neurons located in the dorsal motor nucleus of the vagal nerve and

the nucleus ambiguus, which in turn project to the enteric nervous system of the esophagus, LES and stomach. This circuitry enables fast vago-vagal reflexes and is presumably involved in mediating TLESR (7; 44).

In dogs, cooling of the vagal nerve decreased the number of TLESR (45). In dogs, TLESR were abolished general anesthesia (46), but another study showed that in cats anesthetized using ketamine (light anesthesia) TLESR were still present. The muscarine receptor antagonist atropine inhibited gastric distension induced TLESR compared with placebo (47) in healthy controls and in patients with GERD (48). In patients with achalasia TLESR do not occur after gastric distention supporting neural mediation of TLESR (49). Morphine decreases the number of TLESR (50).

Nitric oxide (NO) is a nonpeptide transmitter produced in the gut. The nitric oxide synthase inhibitor N^G-nitro-L-arginine-methyl esther (L-NAME) inhibited gastric distension induced TLESR in dogs (51). Nitric oxide synthesis inhibition by N^G-monomethyl-L-arginine (L-NMMA) reduced the number of TLESR triggered by meal induced or gastric balloon gastric distension in human (52; 53). The neurotransmitter nitric oxide is involved in the occurrence of LES relaxation.

Gastrointestinal peptides and transmitters

Gut peptides function as messenger molecules in the gastrointestinal tract. They may function as hormones, neuropeptides, paracrine or autocrine agents. Patients with GERD may have an altered serum hormonal profile. The hormonal abnormalities are more marked in patients with low LES pressure. It is not known whether these changes are primary or secondary (54). Gastrointestinal peptides may be involved in the regulation of lower esophageal sphincter.

Cholecystokinin-A receptors are located on vagal afferents. Cholecystokinin-33 decrease LES pressure only when infused at plasma levels comparable with those reached after ingestion of high fat meals. Cholecystokinin-33 had no effect on the frequency of TLESR (26). Cholecystokinin-8 increases the number of TLESR (51; 55). Endogenous cholecystokinin, stimulated by cholestyramine, increased the frequency of TLESR (56). Devazepide, a cholecystokinin-8 receptor antagonist, reduces the number of TLESR after gastric distension in dog. Loxiglumide another cholecystokinin-A receptor antagonist also inhibits TLESR (56-58).

Gastrin may increase reflux by stimulating acid secretion. Previous studies indicated that gastrin may increases LES pressure, however when gastrin is administered in doses similar to those reached after ingestion of a protein meal, no effect on LES pressure was observed or LES pressure even decreased. The effect of gastrin on TLESR had not been investigated (59-65).

Somatostatin is a polypeptide known for its inhibitory effect on the gastrointestinal tract (66-70). Somatostatin inhibits gastric acid secretion and may increase LES pressure (68; 70). The effect of somatostatin on reflux mechanisms, especially TLESR, was unknown.
The tetradecapeptide bombesin affects gastrointestinal secretion and motility. Bombesin increases LES pressure. This effect of bombesin is not influenced by atropine or somatostatin (71). The effect of bombesin on TLESR had not been investigated.

Glutamate is thought to be a major transmitter of vagal afferents. It has been suggested that glutamate may be involved in the neurocircuitry underlying TLESR. However

in healthy subjects riluzole, a glutamate release inhibitor, reduces the number of TLESR evoked by gastric distension.(72)

Serotoninergic 5-HT3 receptors are located on vagal fibers. The 5-HT3 antagonists ondansetron and granisetron, reduced the number of TLESR induced by i.v. cholecystokinin-8 in dogs (73).

Baclofen, a $GABA_B$ receptor antagonist, inhibits gastric distension induced TLESR in the ferret (74; 75) and in humans (48; 76).

In GERD patients tegaserod, a 5HT4 receptor partial agonist with prokinetic effects, decreases frequency of TLESR (77).

Esophageal body motility

Gastroesophageal acid reflux occurs predominately during TLESR and when LES pressure is very low (78). The severity of reflux esophagitis is related not only to the frequency but also to the duration of gastroesophageal reflux episodes (79; 80). Therefore another factor like esophageal body motility plays an important role in GERD. Clearance of acid refluxate from the esophagus is dependent on the efficacy of esophageal peristalsis. Previous studies using stationary manometry have shown that esophageal motility is impaired in patients with GERD. No improvement in peristaltic activity after healing of esophagitis has been observed (81; 82). It has been suggested that impaired motility in reflux esophagitis is an irreversible consequence of esophageal inflammation but others have also pointed to impaired motility as a preexistent factor in the pathogenesis of reflux. Sleep may impair esophageal acid clearance, but arousal from sleep ensures normal swallow responses to acid reflux and characteristics of peristaltic contractions are not altered by the level of consciousness (83; 84).

Management of gastroesophageal reflux disease

Acid suppression is the therapy of choice in patients with GERD. Prokinetic drugs may have additional benefit in patients with delayed gastric emptying. In patients refractory to prolonged and intensive medical therapy surgical treatment should be considered. Fundoplication of the esophagogastric junction is the most used surgical procedure. Complete (360^o wrap) Nissen fundoplication or partial (180^o-270^o wrap) fundoplication (Toupet, Belsey Mark IV) have been performed. Fundoplication either by open or by laparoscopic route increases pressure at the esophagogastric junction to levels in the normal range (85). After fundoplication the frequency of TLESR is decreased (86-89). Postfundoplication acid reflux is diminished to values in the normal range (90). Also symptoms of reflux improve. Dysphagia is a known complication of fundoplication which may be treated with endoscopic dilatation. However some patients need surgical reintervention by changing the wrap from complete to partial fundoplication, or even undo the fundoplication. Damage of the vagal nerve may lead to symptoms of diarrhea.

Recently, a number of endoscopic techniques have been developed aimed at improving the esophagogastric junction to prevent gastroesophageal reflux. Reduction of GERD symptoms, reduction of requirement for acid suppressant medication and reduction in acid reflux has been reported in studies using delivery of radiofrequency energy (91), endoscopic deep mural implantation of polymer (92) or endoscopic plication at esophagogastric junction (93). Delivery of radiofrequency energy reduced the rate of postprandial TLESR (91). However, convincing results have been obtained in only two-thirds of patients with median follow up of 6 months. Moreover, several inconsistencies have

emerged between the efficacy of this form of treatment in improving symptoms and quality of life ad a lack of improvement of objective parameter such as lower esophageal sphincter pressure and esophageal acid exposure (94).

In conclusion, several studies have shown that TLESR play a major role in gastroesophageal reflux. Gastric distension is a potent trigger for TLESR. Inhibition of TLESR frequency is considered as a therapeutic goal in GERD patients and substances that affect TLESRs are being explored for their efficacy. Knowledge on mechanisms of reflux is relevant for control of gastroesophageal reflux especially in patients with GERD.

AIMS AND OUTLINE OF INVESTIGATION

The studies presented in this thesis have been designed to gain more insight into reflux mechanisms. Especially the role of TLESR in gastroesophageal reflux was investigated. Esophageal motility was studied under standardized conditions using esophageal manometry combined with pH-metry. Healthy subjects, patients with GERD and patients after antireflux surgery participated in these studies.

In *chapter 2* we studied LES characteristics in a large cohort of patients with GERD. LES pressure, TLESR and mechanisms of reflux were investigated before and after ingestion of a meal. A meal was used to trigger TLESR. We have investigated whether the frequency of TLESR is increased in patients with GERD compared to healthy controls. Secondly we investigated whether the frequency of TLESR is related to the severity of GERD, presence of a hiatal hernia or LES pressure.

Gastric distension is considered a potent trigger for TLESR. In *chapter 3* we explored the effect of an acute gastric distension by intragastric insufflation with air. The intragastric air will leave the stomach by belching. TLESR and mechanisms of belching were studied in patients with GERD and patients after fundoplication and results were compared with those obtained in healthy controls.

In *chapter 4* we explored the effect of a prolonged gastric distension using an intragastric bag. The intragastric bag is controlled by a barostat system to maintain constant intragastric pressures with variable intragastric bag volumes. The aim of the study was to explore the relation between gastric distension and TLESR.

We studied in *chapter 5* the effect of intravenous gastrin. Ingestion of a meal increases the frequency of TLESR and stimulates gastrin release. Supra-physiological doses of gastrin influence LES pressure. Since gastrin induces acid secretion, gastrin may also be a cause of acid reflux, however the effect of gastrin on TLESR is not known. We have investigated LES pressure and TLESR during infusion of gastrin at doses comparable with postprandial levels of gastrin. Healthy volunteers were investigated under basal, fasting conditions.

We studied in *chapter 6* the effect of intravenous somatostatin. Somatostatin has an inhibitory effect on several functions in the gastrointestinal tract. Somatostatin inhibits gastric acid secretion and may increase LES pressure. Somatostatin is therefore of potential clinical interest to reduce gastroesophageal reflux. The effects of somatostatin were investigated in healthy subjects under basal fasting conditions and after ingestion of a meal. Carbohydrate-rich, low fat meals were used to trigger TLESR.

Chapter 7 deals with the effect of bombesin on TLESR. An increased LES pressure inhibits gastroesophageal reflux. Patients with GERD may have low LES pressure. The LES pressure can be surgically increased by fundoplication. Bombesin also elevates LES pressure, but the effect of bombesin on TLESR and mechanism of reflux is not known. We studied the

effect of bombesin on LES characteristics and reflux mechanisms in patients with GERD, patients after antireflux surgery and healthy controls. Gastric distension with air was used to trigger TLESR.

Non-adrenergic non-cholinergic (NANC) nerves mediate inhibitory responses such as relaxation of the LES after swallowing. Nitric oxide (NO) is known as an inhibitory neurotransmitter of NANC nerves. In *chapter 8* we investigated the influence of l-arginine, a NO precursor, on TLESR in healthy subjects. Carbohydrate-rich, high fat meals were used to trigger TLESR.

The sphinctometer is a solid state manometry system designed for sphincter manometry under ambulatory conditions. In *chapter 9* we have compared the sphinctometer with the water perfused sleeve device in healthy subjects. LES characteristics especially TLESR and mechanisms of reflux were measured simultaneously with sleeve and sphinctometer.

In *chapter 10* we performed prolonged ambulatory LES pressure measurements using a sphinctometer. LES pressure, TLESR frequency and mechanisms of reflux were studied during upright and supine episodes in twenty-four hour registrations. Patients with GERD and healthy controls participated in the study.

Esophageal body motility is another factor involved in gastroesophageal reflux, in addition to TLESR and LES pressure. The role of esophageal motility on the clearance of acid reflux was studied in *chapter 11*. Esophageal clearance of acid is performed by peristaltic esophageal contractions. Clearance of acid may be dependent on characteristics of esophageal contractions and the efficacy of peristalsis. Esophageal motility was analyzed during episodes of gastroesophageal reflux. Ambulatory esophageal pH and manometry was performed in healthy subjects and patients with GERD.

The effect of surgical therapy on patients with GERD was studied in *chapter 12*. In a prospective study the effects of laparoscopic Nissen fundoplication on TLESR and LES pressure and reflux mechanisms were investigated.

Finally, the results of the various studies presented in this thesis are summarized and discussed.

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