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

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EFFECT OF SOMATOSTATIN ON LOWER ESOPHAGEAL SPHINCTER CHARACTERISTICS IN MAN

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ABSTRACT

Background: Somatostatin (SST) is known for its inhibitory effect on the gastrointestinal tract. Transient lower esophageal sphincter relaxations (TLESR), low or absent LES pressure (LESP), swallow induced LES relaxations and are the most important reflux mechanisms.
Methods: We have studied the effect of somatostatin on lower esophageal sphincter (LES) characteristics in man. Nine healthy volunteers participated in four experiments performed in random order and double blind during continuous infusion of somatostatin (250 μg/h) or saline (control) under fasting and postprandial conditions. Esophageal motility was measured with sleeve manometry combined with pH metry.
Results: Under fasting conditions LESP and TLESR frequency were not influenced by somatostatin. Ingestion of the carbohydrate meal significantly ($p<0.01$) decreased LESP. During continuous somatostatin infusion the postprandial decrease in LESP did not occur and LESP was even significantly ($p<0.05$) increased over basal. Somatostatin did not significantly influence TLESR frequency, neither under basal conditions, nor postprandially. The residual pressure during swallow induced LES relaxation was significantly ($p<0.05$) increased by somatostatin.
Conclusions: In humans somatostatin prevents postprandial reduction in LESP, does not affect TLESRs but inhibits swallow induced LES relaxation.

INTRODUCTION

Gastroesophageal reflux (GER) is common and may occur because of low pressure at the esophagogastric junction. Recent studies in both healthy volunteers and patients with GER disease have indicated that transient lower esophageal sphincter relaxations (TLESR) are the major mechanism permitting GER (1-3). After meal ingestion not only lower esophageal sphincter pressure (LESP) decreases but also the frequency of TLESRs increases and thus may lead to a postprandial increase in reflux. Intraluminal nutrients stimulate the secretion of gastrointestinal peptides of which several are involved in regulation of motility including the LES. Gastrin and cholecystokinin (CCK) at postprandial plasma levels decrease LESP (4,5). Somatostatin (SST) is a polypeptide known for its inhibitory effect on the gastrointestinal tract (6-9). Somatostatin inhibits gastric acid secretion and may increase LESP (8,10). Somatostatin is therefore of potential clinical interest to reduce GER. However, the influence of somatostatin on reflux mechanisms, especially TLESRs, is unknown.

We have investigated the effect of somatostatin during continuous intravenous infusion on LES characteristics including LESP, TLESR and acid reflux, under both fasting and postprandial conditions.

METHODS

Subjects

Nine healthy volunteers (five females, four males; age 19 - 53 years) participated a double blind, placebo controlled randomized study. None of the subjects had a history of gastro-intestinal disease or surgery or other illness or was on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

The manometry catheter consisted of a multilumen silicone tube (outer diameter 5.0 mm) with seven side holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia) (11). The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greendale, Wisconsin, U.S.A.) at a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Synectics Medical, Stockholm, Sweden) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Synectics Medical, Stockholm, Sweden).

The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

Each subject participated in four tests performed on separate days in random order with an interval of at least seven days. Two tests were performed under fasting conditions (control vs SST) and two tests during and after ingestion of a meal (control-meal vs SST-meal). The experiments were started at 8.30 a.m. after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced into the esophagus and positioned as described above. Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by three hours (time 0 to 180 min) during infusion of somatostatin or saline (control).

Two intravenous cannulas were inserted into the antecubital vein of each arm, one for intravenous infusion, the other for blood sampling. Somatostatin (Somatostatin, UCB, Brussels, Belgium) was given intravenously starting with a bolus of 250 µg followed by continuous infusion of 250 µg/h for 180 min. These doses are employed when treating patients with gastrointestinal bleeding or to reduce pancreatic exocrine secretion. In the control experiment saline (NaCl 0.9 %) was administered intravenously. In two tests a carbohydrate rich - low fat meal was given 15 min after the start of infusion. A carbohydrate

meal was chosen to avoid postprandial release of CCK, since fat and protein, but not cabohydrates, induce CCK secretion. CCK is known to influece LESP and TLESR frequency. The meal consisted of 200 g bananas blended with 125 ml water and 25 ml Roosvicee (Koninklijke De Ruiter, Baarn, The Netherlands); (2 g protein, 0 g fat and 55 g carbohydrates, 986 kJ). The subjects was asked to consume the meal within 10 min. At regular intervals (-60, 0, 30, 60, 120, 180 min) five wet swallows with 5 ml of water were given to determine swallow induced LES relaxations. Blood samples for determination of plasma hormone levels were taken from -60 min to 180 min at regular intervals (-60, -30, 0, 15, 30, 60, 90, 120, 180 min). Plasma somatostatin, CCK and gastrin concentration were determined by radioimmunoassay (12-14).

Data analysis

The tracings of esophageal motility and pH were analyzed by two investigators who were not aware of the nature of the infusion (somatostatin or placebo). The code was broken when all tracings had been analyzed.

Esophageal body

The amplitude and duration of the peristaltic waves were registered for the proximal (15 cm above the upper margin of the LES), the middle (10 cm above LES), and the distal parts (5 cm above LES) of the esophagus. The propagation velocity was also determined.

Lower esophageal sphincter

Lower esophageal sphincter tracings were analyzed for LES resting pressure (LESP) and LES relaxations (LESR). LESP was defined as mean end-expiratory LESP relative above intragastric pressure over a 2 min period. LESR are divided in swallow induced LESR and spontaneous LESR. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Residual LESP after wet swallows was defined as end-expiratory nadir LESP above intragastric pressure. Spontaneous LESR, better known as transient LES relaxation (TLESR) was divided into non-swallow related TLESR, and swallow related TLESR. Spontaneous, non swallow related TLESR are defined as decreases in LESP of ≥ 5 mmHg with a rate of ≥ 1 mmHg/sec, within 10 sec reaching a pressure of ≤ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related TLESR are defined as spontaneous TLESR, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec (15).

pH analysis

Gastroesophageal reflux episodes are defined as a sudden fall of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted.
The mechanisms of each reflux episode were scored using the following criteria. GER occurred during:
(1) TLESR (spontaneous LESR meeting the earlier mentioned criteria; swallow related LESR with the duration of LESR $\geq$ 10 sec)
(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR $\leq$ 10 sec or multiple swallowing).
(3) LES pressure drift (a gradual loss of basal LES pressure).
(4) Absent LES pressure (LESP less than 2 mmHg above intragastric pressure).
(5) Abdominal strain (an increase in abdominal pressure).

Statistical analysis

Data are expressed as mean values ± SEM. Data were analyzed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A p value of <0.05 was considered significant for all analyses.

RESULTS

Plasma somatostatin, CCK and gastrin

Basal plasma concentrations of somatostatin, CCK and gastrin were not significantly different between the four experiments. Ingestion of the carbohydrate meal did not influence plasma somatostatin levels. Intravenous infusion of 250 µg/h somatostatin resulted in significant ($p<0.001$) increases in plasma somatostatin concentrations starting from 15 min until the end of the experiment (Figure 1A).

Neither infusion of somatostatin nor ingestion of the carbohydrate meal did influence plasma CCK concentrations (Figure 1B). Ingestion of a carbohydrate meal resulted in significant ($p<0.05$) increases in plasma gastrin. Somatostatin significantly ($p<0.05$) reduced plasma gastrin levels compared to basal. During somatostatin infusion no significant changes in plasma gastrin were observed in response to the meal (Figure 1C). Gunshefski et al (10) have shown that somatostatin stimulates esophageal body motility. Our data confirms that somatostatin increases esophageal contraction amplitude and velocity.

Table 1. *Characteristics of esophageal body contractions after wet swallow in nine healty volunteers before and during continuous infusion of somatostatin. *Significant (P<0.01) increases, compared to control.*

	control	SST	control-meal	SST-meal
Amplitude (mmHg)				
Proximal	37 ± 1	53 ± 2*	40 ± 1	47 ± 2*
Mid	49 ± 2	57 ± 2*	54 ± 3	67 ± 1*
Distal	58 ± 2	65 ± 2*	55 ± 2	69 ± 2*
Duration (sec)				
Distal	3.3 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	3.5 ± 0.2
Velcity (cm/sec)				
Proximal	3.3 ± 0.2	4.6 ± 0.2*	4.1 ± 0.2	5.5 ± 0.2*
Distal	3.7 ± 0.1	4.7 ± 0.2*	4.1 ± 0.1	5.2 ± 0.2*

Esophageal body motility

Characteristics of peristaltic contractions of the esophageal body are described in Table 1. The amplitude of esophageal body contractions and the velocity of peristalsis were significantly (P<0.001) increased during somatostatin infusion under both fasting and postprandial conditions.

Lower esophageal sphincter pressure

LESP in the basal period (-30 to 0 min) was not significantly different between the four experiments (control: 22 ± 4 mmHg; SST: 23 ± 4 mmHg; control-meal: 23 ± 3 mmHg; SST-meal: 23 ± 4 mmHg). During three hours of intravenous infusion of somatostatin there were no significant changes compared to the control experiment (Figure 2A). After ingestion of a meal LESP decreased significantly ($p<0.01$) compared to basal level from $t=30$ to $t=75$ min. During somatostatin infusion the meal did not decrease LESP but on the contrary, LESP gradually increased. The LESP was significantly ($p<0.01$) higher compared to the control-meal experiment from 60 to 180 min (Figure 2B). In the control experiment residual LESP after wet swallows was 9 ± 1% and 7 ± 1% after meal ingestion. Intravenous infusion of somatostatin significantly ($p<0.01$) increased the residual pressure after a wet swallow under postprandial (20 ± 2%) but not under fasting (11 ± 1%) conditions.

Transient lower esophageal sphincter relaxation

The frequency of TLESR was not significantly different between the basal periods of the four experiments (Table 2). During the control experiment no significant changes in TLESR frequencies were observed. Intravenous infusion of somatostatin had no significant effect on the frequency of TLESR compared to the control experiment. After ingestion of the carbohydrate meal the frequency of TLESR significantly ($p<0.05$) increased in the first postprandial hour (4.3 ± 0.5 TLESR/h). During somatostatin infusion the postprandial increase in TLESRs was no longer significant compared to basal.

Table 2. *Frequency of non-swallow related TLESR (number/hour; mean±SEM) one hour before (basal) and during three hours (0-180 min) of intravenous infusion of saline (control) or somatostatin (SST) at a rate of 250 μg/h in nine healthy subjects under fasting and postprandial conditions. Asterisks denote a significant ($p<0.05$) increase compared to basal.*

TLESR	control	SST	control-meal	SST-meal
Basal	2.1 ± 0.6	3.3 ± 0.7	2.6 ± 0.6	2.7 ± 0.6
Infusion				
0-60 min	2.1 ± 0.4	2.6 ± 0.6	4.3 ± 0.5*	3.9 ± 0.7
60-120 min	2.4 ± 0.4	3.7 ± 0.7	4.0 ± 0.4	3.0 ± 0.8
120-180 min	3.3 ± 0.6	3.4 ± 0.7	2.9 ± 0.6	4.1 ± 0.9

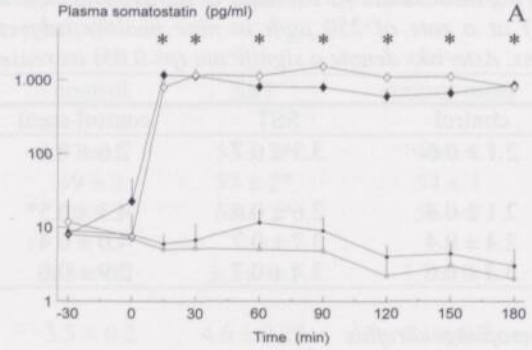
Mechanisms of gastroesophageal reflux

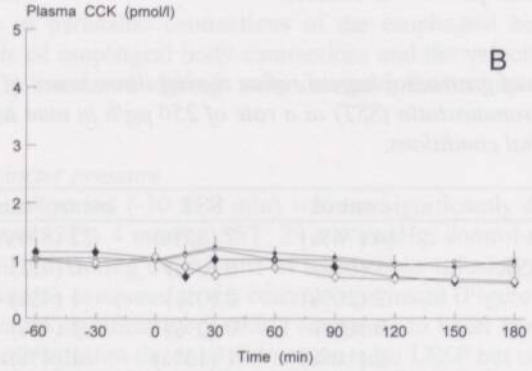
As expected, the number of reflux episodes and the percentage of time pH<4 was very low in the healthy volunteers (Table 3). In all experiments the predominant mechanism of reflux during the infusion period was TLESR.

Table 3. *Mechanisms of gastroesophageal reflux during three hours of intravenous infusion of saline (control) or somatostatin (SST) at a rate of 250 μg/h in nine healthy subjects under fasting and postprandial conditions.*

Mechanism of GER	control	SST	control-meal	SST-meal
TLESR	14 (74%)	7 (87%)	22 (88%)	14 (77%)
Swallow induced LESR	1 (6%)	0 (0%)	0 (0%)	2 (11%)
LES pressure drift	2 (10%)	0 (0%)	1 (4%)	1 (6%)
Absent LES pressure	0 (0%)	0 (0%)	1 (4%)	0 (0%)
Abdominal strain	2 (10%)	1 (13%)	1 (4%)	1 (6%)
% time pH<4	0.19%	0.17%	0.28%	0.14%

Figure 1A-C. *Plasma levels (mean ± SEM) of [A] somatostatin, [B] cholecystokinin and [C] gastrin during control (open squares) or continuous infusion of somatostatin (open diamonds). Somatostatin was given as a bolus of 250 μg followed by a continuous infusion at a rate of 250 μg/h, 15 min after start of the infusion a carbohydrate meal was ingested (closed squares and closed diamonds). Asterisks denote significant ($P<0.01$) differences compared to basal levels.*


Plasma somatostatin (pg/ml)
A
1.000
100
10
1
-30
0
30
60
90
120
150
180
Time (min)


Plasma CCK (pmol/l)
B
5
4
3
2
1
0
-60
-30
0
30
60
90
120
150
180
Time (min)

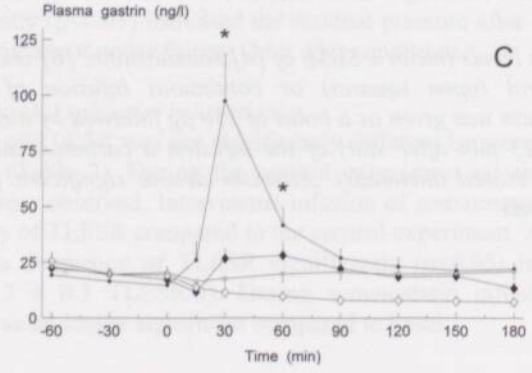
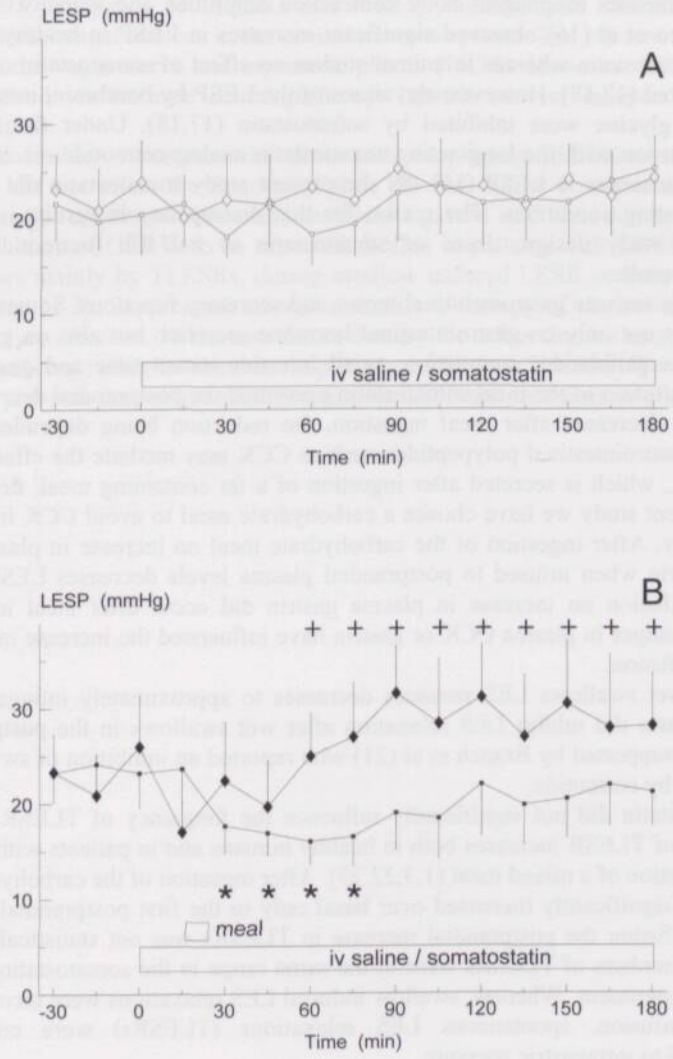

Plasma gastrin (ng/l)
C
125
100
75
50
25
0
-60
-30
0
30
60
90
120
150
180
Time (min)

Figure 2A-B. *LESP (mean ± SEM) during control (open squares) or continuous intravenous infusion of 250 µg/h somatostatin (open diamonds) in nine healthy volunteers under [A] fasting and [B] postprandial conditions. The 400 ml carbohydrate rich meal consisted of 2 g protein, 0 g fat and 55 g carbohydrates (closed squares and closed diamonds). Asterisks and crosses denote significant (P<0.05) differences compared to basal LESP.*



DISCUSSION

We have shown that in healthy subjects under fasting conditions, somatostatin does not affect LESP and TLESR frequency. However, somatostatin prevents the postprandial reduction in LESP and the postprandial increase in TLESR. During somatostatin infusion swallow induced LES relaxations were incomplete, under postprandial conditions.

The effect of somatostatin on LESP has been studied previously. Gunshefski et al (10) have shown that somatostatin stimulates esophageal body motility. Our data confirms that somatostatin increases esophageal body contraction amplitude and velocity. Under fasting conditions Greco et al (16) observed significant increases in LESP in healthy volunteers in response to somatostatin whereas in animal studies no effect of somatostatin on basal LESP has been observed (17,18). However, elevations of the LESP by bombesin, intragastric alkali or intragastric glycine were inhibited by somatostatin (17,18). Under fasting conditions intravenous infusion with the long-acting somatostatin analog octreotide resulted in a small but significant increase in LESP (10). IN the present study somatostatin did not influence LESP under fasting conditions. The reason for this discrepancy in results is not obvious. Differences in study design, doses of somatostatin or half-life (octreotide) may have influenced the results.

Nutrients activate gastrointestinal motor and secretory functions. Somatostatin has an inhibitory effect not only on gastrointestinal hormone secretion but also on gastrointestinal motility such as gallbladder contraction, small intestine transit time and gastric emptying (8,19). After ingestion of the meal somatostatin prevented the postprandial decrease in LESP. Usually, LESP decreases after meal ingestion, the reduction being dependent upon meal composition. Gastrointestinal polypeptides such as CCK may mediate the effect of nutrients on LESP. CCK, which is secreted after ingestion of a fat containing meal, decreases LESP (4). In the present study we have chosen a carbohydrate meal to avoid CCK induced effects on LES motility. After ingestion of the carbohydrate meal no increase in plasma CCK was observed. Gastrin when infused to postprandial plasma levels decreases LESP (5). During somatostatin infusion no increase in plasma gastrin did occur after meal ingestion. It is unlikely that changes in plasma CCK or gastrin have influenced the increase in LESP during somatostatin infusion.

After wet swallows LES pressure decreases to approximately intragastric pressure (20). Somatostatin did inhibit LES relaxation after wet swallows in the postprandial state. This finding is supported by Branch et al (21) who reported an inhibition of swallow induced LES relaxation by octreotide.

Somatostatin did not significantly influence the frequency of TLESR when fasted. The frequency of TLESR increases both in healthy humans and in patients with symptomatic GER after ingestion of a mixed meal (1,3,22,23). After ingestion of the carbohydrate meal the frequency was significantly increased over basal only in the first postprandial hour. During somatostatin infusion the postprandial increase in TLESRs was not statistically significant. However, the numbers of TLESRs were in the same range in the somatostatin-meal and the control-meal experiment. Whereas, swallow induced LES relaxations were incomplete during somatostatin infusion, spontaneous LES relaxations (TLESRs) were complete, with relaxation equal to intragastric pressure.

The mechanism by which somatostatin influences the LES is largely unknown. Somatostatin is widely distributed in the human body both in the central nervous system and

the gastrointestinal tract. However, the esophagus has a pattern of innervation by peptide-containing neurons that is different from the stomach and intestine. Somatostatin immunoreactive nerve fibers have been found in the human LES region but are scarce (24-27). It is not clear whether the effect of somatostatin on the LES is mediated directly, via receptors or by central or peripheral neural pathways (28).

The inhibition of the LES relaxation induced by swallows but not the LES relaxation during a TLESR by somatostatin might be explained by different pathways of stimulation. It has been suggested that TLESRs share a final common pathway with swallow induced LES relaxation (29). Triggering of TLESRs and swallow induced LES relaxations is mediated through the dorsal vagal nucleus in the central nervous system. The efferent stimuli reach the LES through the vagus nerve and myenteric plexus (15,30). However, LES relaxation can also be triggered via intrinsic neural pathways (gastric nerves) independent of extrinsic nerves (15,31,32).

In the healthy volunteers the time with esophageal pH below 4 was minimal. Somatostatin did not affect the fraction of time pH was below 4 neither under fasting nor under postprandial conditions. Somatostatin inhibits gastric acid secretion and when reflux is present a reduction of GER is to be expected. Reflux in patients with GERD and in healthy subjects occurs mainly by TLESRs, during swallow induced LESR or over a very low LES resting pressure (1-3). Suppression of acid secretion is therapy of choice in GERD and has proven to be very effective. In the near future therapy for reflux disease may become focused more on influencing reflux mechanisms especially LESP and TLESRs. We have shown that somatostatin prevents the postprandial decrease in LESP and increases swallow induced LES nadir pressure.

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