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

Straathof, J.W.A.

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EFFECT OF GASTRIN-17 ON LOWER ESOPHAGEAL SPHINCTER CHARACTERISTICS IN MAN

J.W.A. Straathof, C.B.H.W. Lamers, A.A.M. Masclee

Department of Gastroenterology-Hepatology
Leiden University Medical Center, The Netherlands

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ABSTRACT

Background: We studied the effect of gastrin-17 on lower esophageal sphincter (LES) characteristics in man.
Methods: Nine healthy volunteers participated in two experiments performed in random order during continuous infusion of saline (control) or gastrin-17 (15 pmol/kg/h). LES pressure (LESP) and transient lower esophageal sphincter relaxations (TLESR), as most important reflux mechanism, were measured with intraesophageal sleeve manometry combined with pH metry.
Results: Infusion of gastrin-17 resulted in plasma gastrin levels comparable to those reached after a mixed meal. During continuous gastrin infusion LESP decreased significantly ($P<0.05$) compared to control. The rate and duration of TLESR was not influenced by gastrin-17. Gastroesophageal reflux and the number of TLESR associated with reflux were significantly ($P<0.05$) increased during gastrin infusion.
Conclusions: These results suggest that in humans gastrin at physiological postprandial plasma concentrations decreases LESP, does not influence TLESR, but increases the percentage of TLESR associated with reflux.

INTRODUCTION

The role of gastrin in the occurrence of gastroesophageal reflux is still contradictory. On the one hand gastrin may increase reflux by stimulating acid secretion whereas on the other hand reflux of acidic gastric content might be prevented because previous studies indicate that gastrin increases lower esophageal sphincter pressure (LESP) (1-7). An intravenous bolus injection of gastrin or continuous infusion of gastrin in supraphysiological doses results in an increase in LESP (1,4). However, when gastrin is administered in doses similar to those reached seen after ingestion of a protein meal, no effect on LESP is observed or LESP even decreases (2,3,5). These results are not conclusive about the effect of gastrin on LESP under physiological conditions.

Transient lower esophageal sphincter relaxations (TLESR) are nowadays recognized as the main mechanism underlying gastroesophageal reflux (GER), both in healthy subjects and in patients with GER disease (8). The neural and hormonal mechanisms controlling TLESR are poorly understood (9). Ingestion of a meal increases the frequency of TLESR (10,11) and stimulated gastrin release. It is not known whether gastrin influences the frequency of TLESR because the effect of gastrin on TLESR has not been investigated previously.

The present study was conducted to investigate the effect gastrin, through i.v. infusion to plasma gastrin levels in physiological postprandial range, on LES characteristics including LESP and TLESR and acid reflux in nine healthy volunteers.

METHODS

Subjects

Nine healthy volunteers (five males, four females; age 22 - 29 years) participated in the 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 polyvinyl tube (outer diameter 5.0 mm) with seven side holes and a 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). Side holes are at 27, 18, 12, 9, 6, 3 and -3 cm from the center of the sleeve. 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.) with a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via a eight channel 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. After identification of LES and upper esophageal sphincter (UES) the catheter was positioned so that the sleeve sensor covered the esophagogastric junction by centering the high-pressure zone on the sleeve sensor. The proximal side hole was positioned in the pharynx, proximal of the UES and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference for intragastric pressure. A second catheter, 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 two tests performed in random order on separate days with an interval of at least seven days. The subjects were fasting since 10.00 p.m the night before the test. The experiments were started at 8.30 a.m. 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 gastrin-17 or saline (control).

Two intravenous cannulas were inserted into the antecubital vein of each arm, one for intravenous infusion, the other for blood sampling. The synthetic unsulfated heptadecapeptide of human gastrin (gastrin-17-I) was continuously infused intravenously in a dose of 15 pmol/kg/h for 180 min. This dose was chosen because plasma gastrin levels are reached in the postprandial range. In the control experiment saline (NaCl 0,9 %) was administered intravenously. Blood samples for determination of plasma gastrin were taken from -60 min to

180 min at 15 min intervals. Plasma gastrin concentration was determined by radioimmunoassay as described previously (12).

To confirm that plasma gastrin levels reached during gastrin infusion were in the postprandial range, a third experiment was performed on a separate day to determine postprandial plasma gastrin levels. After an overnight fast one intravenous cannula was inserted in an antecubital vein of one arm for blood sampling. A liquid mixed meal, containing 400 ml, was drunk within 10 min (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 100 ml Nutridrink contains 6.5 g fat, 17.9 g carbohydrates and 5.0 g protein, 150 kcal = 630 kJ, osmolality: 390 mOsmol/l). At regular intervals blood samples were taken for determination of plasma gastrin (-15, 0, 30, 60, 90, 120, 150, 180 min).

Lower esophageal sphincter data analysis

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. Spontaneous LESR, also known as transient LES relaxation (TLESR) are divided in non-swallow related TLESR, and swallow related TLESR. Spontaneous, non swallow related TLESR are defined as decreases in LES pressure 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 and the duration of LESR is ≥ 10 sec (9).

pH analysis data 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 ≥ 10 sec)
(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR ≤ 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

Pressure 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 gastrin

Ingestion of a mixed liquid meal resulted in a significant postprandial increase of plasma gastrin from 30 ± 4 (basal) to 82 ± 15 ng/l at 60 min postprandial. Three hours after ingestion plasma gastrin was 58 ± 5 ng/l (Figure 1a).

In the study for evaluation of the effect of intravenous gastrin infusion, basal plasma gastrin concentrations were not significantly different among the two experiments: 30 ± 15 ng/l (saline) and 33 ± 17 ng/l (gastrin). Infusion of 15 pmol/kg/h gastrin resulted in significant ($P<0.05$) increases in plasma gastrin over basal level and compared to the control experiment from 15 min after start of the intravenous infusion (Figure 1b).

Lower esophageal sphincter pressure

LESP in the basal period (-60 to 0 min) was not significantly different between the two experiments. During infusion of saline no significant alterations in the LESP were observed. Gastrin infusion significantly ($P<0.05$) decreased LESP from 45 to 180 min compared to the control experiment (Figure 2).

Figure 2. *Lower esophageal sphincter pressure (LESP; mmHg; mean ± SEM) during continuous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in nine healthy volunteers. Small squares represent the control experiment, closed rhomboids represent the intravenous infusion of gastrin. Asterisks denote significant (P< 0.05) differences compared to control.*

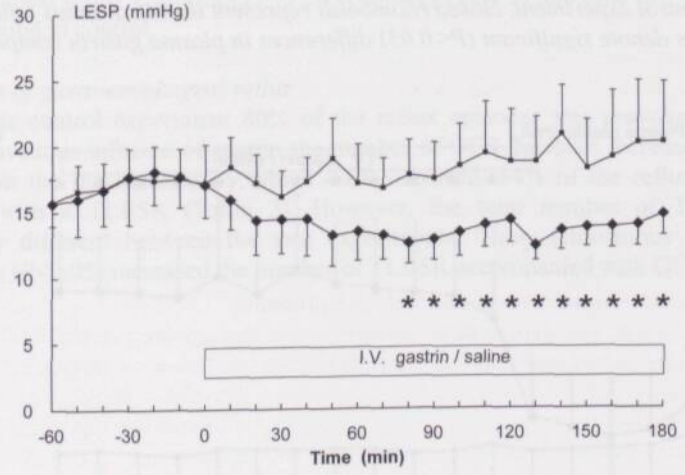


Figure 1a. *Plasma gastrin levels (ng/l; mean ± SEM) after ingestion of a 400 ml meal (100 ml contains 6.5 g fat, 17.9 g carbohydrates and 5.0 g protein) in nine healthy volunteers. Asterisks denote significant (P<0.05) differences in plasma gastrin compared to basal fasting levels.*

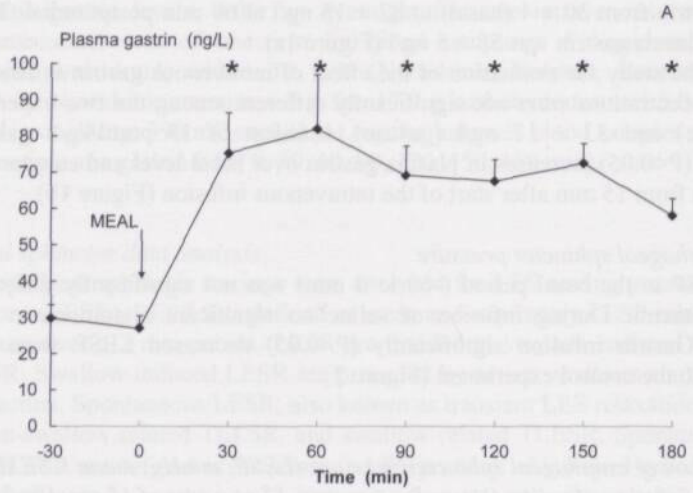
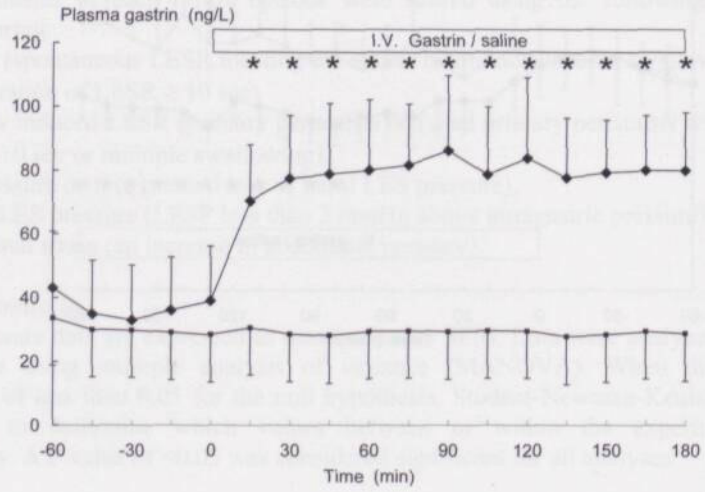


Figure 1b. *Plasma gastrin levels (ng/l; mean ± SEM) during continuous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in nine healthy volunteers. Small squares represent the control experiment, closed rhomboids represent the intravenous infusion of gastrin. Asterisks denote significant (P<0.05) differences in plasma gastrin compared to control.*



Transient lower esophageal sphincter relaxation

The mean frequency of TLESR in the basal state was 3.0 ± 0.9 per hour. Infusion of gastrin did not significantly alter the frequency of TLESR (Table 1). The duration of TLESR did not significantly change during three hours of gastrin infusion.

Table 1. *Frequency and duration of TLESR 1 hour before (fasting) and during 3 hours of intravenous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in 9 healthy subjects.*

TLESR	Control		Gastrin	
	Frequency (N/h)	Duration (sec)	Frequency (N/h)	Duration (sec)
Fasting	3.0 ± 0.9	14.9 ± 1.8	3.0 ± 0.7	14.9 ± 1.0
Infusion				
I	2.3 ± 0.5	15.7 ± 1.0	3.5 ± 1.0	13.0 ± 1.0
II	3.2 ± 0.7	17.1 ± 1.8	2.7 ± 0.5	17.3 ± 1.9
III	3.2 ± 0.6	17.3 ± 0.9	2.6 ± 0.8	16.6 ± 1.7

Gastroesophageal reflux

Acid exposure to the esophagus was of short duration in this group of healthy subjects. In the control experiment the percentage of time pH < 4 was 0.40% (range 0 - 0.75%). Intravenous infusion of gastrin resulted in significant ($P<0.05$) increases in gastroesophageal reflux episodes (55 vs 20 episodes; Table 2) and time pH<4 (2.9%, range 0 - 6.2) compared to control.

Mechanisms of gastroesophageal reflux

In the control experiment 80% of the reflux episodes was provoked by a TLESR. During intravenous infusion of gastrin the number of GER episodes increased but there was no change in the mechanisms by which GER occurred, 84% of the reflux episodes were associated with a TLESR (Table 2). However, the total number of TLESR was not significantly different between the two experiments. Thus, intravenous gastrin infusion significantly ($P<0.05$) increased the number of TLESR accompanied with GER. (Table 3)

Table 2. *Mechanisms of gastroesophageal reflux during 3 hours of intravenous infusion of either saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in 9 healthy subjects. The asterisk denotes, a significant (P<0.05) difference compared to control.*

Mechanism of GER	Control	Gastrin
TLESR	16 (80%)	46 (84%)
Swallow induced LESR	1 (5%)	8 (14%)
LES pressure drift	2 (10%)	0 (0%)
Absent LES pressure	0 (0%)	0 (0%)
Abdominal strain	1 (5%)	1 (2%)
Total reflux episodes	20 (100%)	55* (100%)

Table 3. *Total number of TLESR and the number of TLESR accompanied with GER during 3 hours of intravenous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in nine healthy volunteers. Asterisks denote a significant (P<0.05) difference compared to control.*

	Control	Gastrin
TLESR	79 (100%)	75 (100%)
TLESR with pH<4	16 (20%)	46 (61%)*
Total reflux episodes	20	55*

DISCUSSION

Lower esophageal sphincter pressure is influenced by neural and hormonal mechanisms. Various gastro-intestinal polypeptides affect LES resting tone. Cholecystokinin, glucagon and secretin decrease LESP, while motilin and gastrin are supposed to increase LESP (13). Gastrin is thought to be a stimulator of the LES by increasing LESP. Intravenous injection of the synthetic analogue pentagastrin in pharmacological doses increases LESP (1), but in physiological postprandial doses gastrin does not affect LESP or even decreases LESP (2). Walker et al (2) have reported about a biphasic LES response to pentagastrin. At first, continuous infusion of pentagastrin caused a contraction of the LES but secondly at higher pentagastrin infusion rates LESP transiently decreased. In normal subjects gastrin-17 increases LESP, when given as a rapid intravenous injection. But, continuous infusion of gastrin-17 leading to serum gastrin concentrations in the physiological range did not result in significant changes in LESP (5). Others have observed a significant increase in LESP during continuous administration of gastrin-17 (3,6). We observed a small but significant decrease in LESP in response to gastrin infusion.

How do results from our study relate to those of other studies reporting no effect or even significant increments in LESP in response to gastrin infusion? First, differences in the doses of gastrin infused are probably relevant. Second, in all previous studies LESP was measured either continuously with side-hole manometry or intermittently measured using a pull-through technique. These methods are not well suited for continuous measurements of

the LES. Because the position of the side hole catheter is changing during respiration and swallowing, continuous measurement of LES resting pressure by single side hole manometry is not reliable. Pull through manometry may cause mechanical triggering and is not sufficient for identification of TLESR. Continuous registration of LES motility is best performed using a sleeve device that enables continuous registration of LES pressure and of LES relaxations including TLESR (14).

Ingestion of a meal (11) increases the number of TLESR. Food is also a stimulus for endogenous gastrin release. In the present study gastrin reaching postprandial levels did not affect the frequency nor the duration of TLESR. These results suggest that gastrin at physiological postprandial concentrations is not involved in the postprandial increase in TLESR.

TLESR is a neural reflex that is mediated through the brain stem. It is not clear whether TLESR are under hormonal control (9). The influence of other gastro-intestinal polypeptides on TLESR has not been investigated in humans except for cholecystokinin. Intravenous CCK-33 did not affect the frequency of TLESR under fasting conditions (15). However, CCK may increase the frequency of postprandial TLESR and CCK receptor antagonists possibly decrease TLESR (16,17).

Intravenous gastrin resulted in an increase of gastroesophageal reflux (GER) in healthy subjects. TLESR are the major motor mechanism by which GER occurs. Gastric distension by a meal or by intragastric air increases the number of TLESR (18). The number of TLESR was not affected by intravenous infusion of gastrin-17. The increase in GER is explained by an increase in the percentage of TLESR with acid reflux resulting from an increased gastric acid load due to gastrin infusion.

Do patients with elevated gastrin levels as in Zollinger Ellison syndrome (ZES) or pernicious anemia have abnormal LESP? Patients with elevated plasma gastrin levels due to ZES were studied by Strader et al (19). Esophageal manometry revealed normal LESP in 88% of these patients. No significant correlation was found between fasting plasma gastrin levels and the LESP. On the other hand, over 70% of patients had moderate to severe GER symptoms. Since LESP is not reduced in ZES patients the increased gastroesophageal acid reflux will result predominantly from reflux induced by TLESR. The effect of prolonged elevations in plasma gastrin levels on TLESR frequency is not known.

In conclusion, we have shown that gastrin-17 decreases LESP when infused at physiological postprandial plasma levels. Gastrin-17 did not influence the occurrence of TLESR. However, gastrin increased GER and increased the percentage of TLESR associated with reflux.

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