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

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BOMBESIN INDUCED CHANGES IN LES CHARACTERISTICS IN REFLUX DISEASE AND POST-FUNDOPLICATION

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ABSTRACT

Background: In healthy controls bombesin affects gastrointestinal motility including a rise in lower esophageal sphincter (LES) pressure. Little is known on the effect of bombesin on LES pressure and reflux mechanisms such as transient lower esophageal sphincter relaxations (TLESRs) in reflux patients (GERD) with low LES pressure and patients post-fundoplication.
Methods: We studied LES characteristics (sleeve manometry) in 10 healthy controls, 22 patients with GERD and 24 patients post-fundoplication. All subjects were studied twice, in random order during intravenous bombesin or placebo. LES pressure, TLESRs and reflux were scored under based condition and after gastric air distension.
Results: Basal LES pressure was 16 ± 2 mmHg in controls, 11 ± 1 mmHg in GERD ($p<0.05$ vs controls) and 18 ± 2 mmHg post-fundoplication. Peak increments in LES pressure in response to bombesin were 20 ± 4 mmHg (controls), 17±3 mmHg (GERD) and 12±2 mmHg (post-fundoplication; $p<0.05$ vs controls and GERD). Basal TLESR frequency was significantly ($p<0.05$) reduced post-fundoplication (0.6±0.1/20min) versus controls (1.6±0.3 TLESR/20min) and GERD (1.2±0.3 TLESR/20min). Gastric distension significantly ($p<0.05$) increased TLESR to 4.0 ± 1.0 TLESR/20 min (controls); 3.4 ± 0.4 TLESR/20 min (GERD); 1.7 ± 0.5 TLESR/20 min (post-fundoplication). Bombesin did not affect TLESR frequency neither under basal conditions nor after gastric distension.
Conclusions: Bombesin significantly increases LES pressure not only in healthy subjects but also in patients with GERD and post-fundoplication. TLESR frequency is not influenced by bombesin.

INTRODUCTION

The tetradecapeptide bombesin, originally isolated from the skin of the European amphibian Bombina Bombina, is the mammalian counterpart of gastrin releasing peptide [1]. Bombesin has a wide range of biological effects that includes the release of gut hormones, stimulation of gastric and pancreatic secretion and gastrointestinal motility [2-6]. Bombesin like immunoreactivity has been demonstrated in gastrointestinal endocrine cells and in nerve fibres innervating mucosa and muscle layers [7-9]. Specific bombesin binding sites have been identified in fibres innervating both circulated and longitudinal muscle tissue [10]. Recent studies have provided evidence for a direct myogenic action of bombesin-like peptides in the gastrointestinal tract, including the esophagus [10].

With respect to the esophagus: bombesin, when given intravenously increases lower esophageal sphincter (LES) pressure in healthy subjects [1-3]. Bombesin does not act on LES pressure via cholinergic pathways nor through release of gastrointestinal hormones since the effect of bombesin is not influenced by atropine or somatostatin [3]. Because of the effect on LES pressure, bombesin-like substances deserve further evaluation in conditions with LES dysfunction such as in patients with gastroesophageal reflux disease (GERD). Reflux of acid occurs when LES pressure is low or during transient lower esophageal sphincter relaxations (TLESRs). These TLESRs have been well recognized as the most important mechanism through which reflux occurs [12-15].

Aim of the present study was to investigate the effect of bombesin on LES characteristics especially on TLESRs in healthy controls, in patients with GERD who usually

have a low LES pressure and patients after fundoplication. After fundoplication LES pressure at the esophagogastric junction increases significantly and the frequency of TLESRs is significantly reduced [16-18]. It is not known whether bombesin will influence LES characteristics in patients post-fundoplication. TLESRs were studied under basal conditions and after provocation through gastric distension with air.

METHODS

Subjects

Three groups of subjects were studied. A total of 56 subjects agreed to participate:
1) healthy volunteers (n=10; 6 females, 4 males; age 20 - 48 years). None of them had a history of gastro-intestinal disease or previously underwent surgery or was on chronic medication.
2) patients with GERD (n=22; 8 females, 14 males; age 27 - 59 years). Gastroesophageal acid reflux in these patients was documented by abnormal result during 24 hour ambulatory pH metry and/or endoscopy showing erosive reflux disease. Results of 24 hour pH metry: total time pH<4: 10.5±2.6%; (normal value <4.0%).
3) patients after antireflux surgery (n=24; 11 females, 13 males; age 31 - 76 years). Fourteen patients underwent a complete (360o) Nissen fundoplication. Ten patients underwent a partial (180°-270°) fundoplication. Postoperatively the percentage of total time with esophageal pH<4 was 3.7±0.8%. 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

A manometry catheter consisting 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) was used. The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system at a rate of 0.5 ml/min. The external pressure transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Medtronic, Denmark) to a personal computer system. The data were displayed continuously on a monitor and stored on a personal computer system for later analyses.

The manometry catheter was 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

Two experiments were performed (bombesin, placebo) in random order and on separate days. The experiments were started at 9.00 a.m. after an overnight fast. Subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced and positioned as described above. Infusion of bombesin or placebo

was started at time 0 min until 20 min and recording was continued for 60 min. Air was insufflated twice into the stomach in 750 ml portions through the distal intragastric side-hole of the manometry assembly with a 20 min interval. Bombesin was given i.v. a dose of 5 ng/kg/min for 20 min. When subjects had not belched or were unable to belch at the end of the experiment, the air was removed from the stomach.

Lower esophageal sphincter data analysis

Lower esophageal sphincter tracings were analyzed for LES resting pressure and TLESRs. LES pressure was defined as mean end-expiratory LES pressure relative above intragastric pressure over a 2 min period. LES pressure was scored with 10 min interval. TLESRs defined as decreases in LES pressure of $\geq$ 5 mmHg with a rate of $\geq$ 1 mmHg/sec, within 10 sec reaching a pressure of $\leq$ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LES relaxation. 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 [13, 14]. The occurrence of TLESRs was scored in three subsequent 20 min periods: 1) fasting, bombesin vs placebo i.v. and 2) twice with air insufflation, bombesin vs placebo i.v.

Gastroesophageal acid reflux

Gastroesophageal acid 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 acid reflux episode were scored using previously described criteria [14].

Statistical analysis

Data are expressed as mean ± SEM. Data were analyzed for statistical significance using (multiple) analysis of variance. 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. The Mann-Whitney U test for comparison of nonparametric data was used for statistical analysis to compare results between the groups of patients and the controls. A p value of <0.05 was considered significant for all analyses.

RESULTS

Lower esophageal sphincter pressure

Basal LES pressure was 16 ± 2 mmHg in controls, 11 ± 1 mmHg patients with GERD ($p<0.05$ vs controls) and 18 ± 2 mmHg in patients after fundoplication (Figure 1). Basal LES pressure was significantly ($p<0.05$) higher in patients after fundoplication compared to patients with GERD. Intravenous bombesin significantly ($p<0.05$ - $p<0.001$) increased LES pressure in all subjects starting from 10 min until 60 min. Peak increments in LES pressure in response to bombesin were 20 ± 4 mmHg in controls, 17 ± 3 mmHg in patients with GERD and 12 ± 2 mmHg in patients after fundoplication ($p<0.05$ vs controls and GERD).

Transient lower esophageal sphincter relaxations

In the placebo experiment, under basal conditions the frequency of TLESR was not significantly different between controls and patients with GERD (1.6 ± 0.3 vs. 1.2 ± 0.3 TLESR/20 min). Patients after fundoplication had a significantly ($p<0.05$) lower frequency of TLESR (0.6 ± 0.1 TLESR/20 min) compared to controls. (Figure 2 upper panel). Bombesin did not affect TLESR frequency.

The frequency of TLESRs increased significantly ($p<0.05$) after gastric distension in controls (to 4.0 ± 1.0 TLESR/20 min), patients with GERD (to 3.4 ± 0.4 TLESR/20 min) and patients after fundoplication (to 1.7 ± 0.5 TLESR/20 min; Figure 2, lower panel). The number of TLESRs after gastric distension in patients post-fundoplication was significantly ($p<0.05$) lower compared to controls. After infusion of bombesin, gastric distension again significantly ($p<0.05$) increased the frequency of TLESRs in controls, patients with GERD and patients after fundoplication (Figure 2, lower panel). Bombesin did not influence the effect of gastric air distension with air on the frequency of TLESRs.

Table 1. *Gastroesophageal reflux (GER) in healthy controls, patients with gastroesophageal reflux disease (GERD) and patients post-fundoplication under fasting conditions in response to placebo i.v. and in response to bombesin i.v. Results are expressed as group during the 60 min period accumulated data of each group during 60 min period.*

	Control (n=10)	GERD (n=22)	Fundoplication (n=24)
Placebo			
GER episodes (in 60 min) total	9	24	24
Time pH<4 (%)	0.4%	0.8%	0.9%
GER during TLESR (%) /GER episodes	78%	63%	71%
Bombesin			
GER episodes in 60 min (total N)	19	70	24
Time pH<4 (%)	1.4%	4.4%*	1.1%
GER during TLESR (%)/GER episodes	74%	79%	71%

Gastroesophageal acid reflux

The number of reflux episodes and the percentage of time with pH < 4 under basal conditions in response to placebo i.v. was low in healthy volunteers. This was also the case for patients with GERD and patients after fundoplication (Table 1). In patients with GERD acid reflux increased significantly ($p<0.05$) in response to bombesin infusion. The majority of gastroesophageal acid reflux occurred during TLESRs.

Figure 1. *Lower esophageal sphincter pressure (mean ± sem) in 10 healthy controls (small squares), 22 patients with gastroesophageal reflux disease (diamonds) and 24 patients with fundoplication (triangles). The upper panel shows LES pressure under fasting conditions in response to i.v. placebo.. The lower panel shows LES pressure in response to bombesin infusion.*

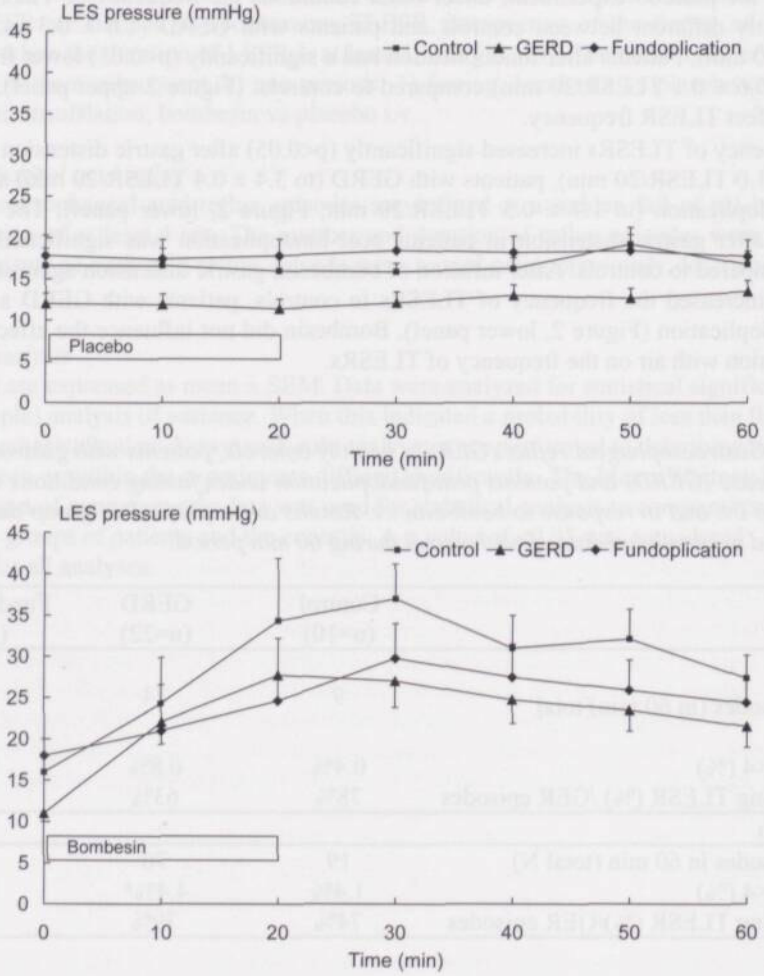
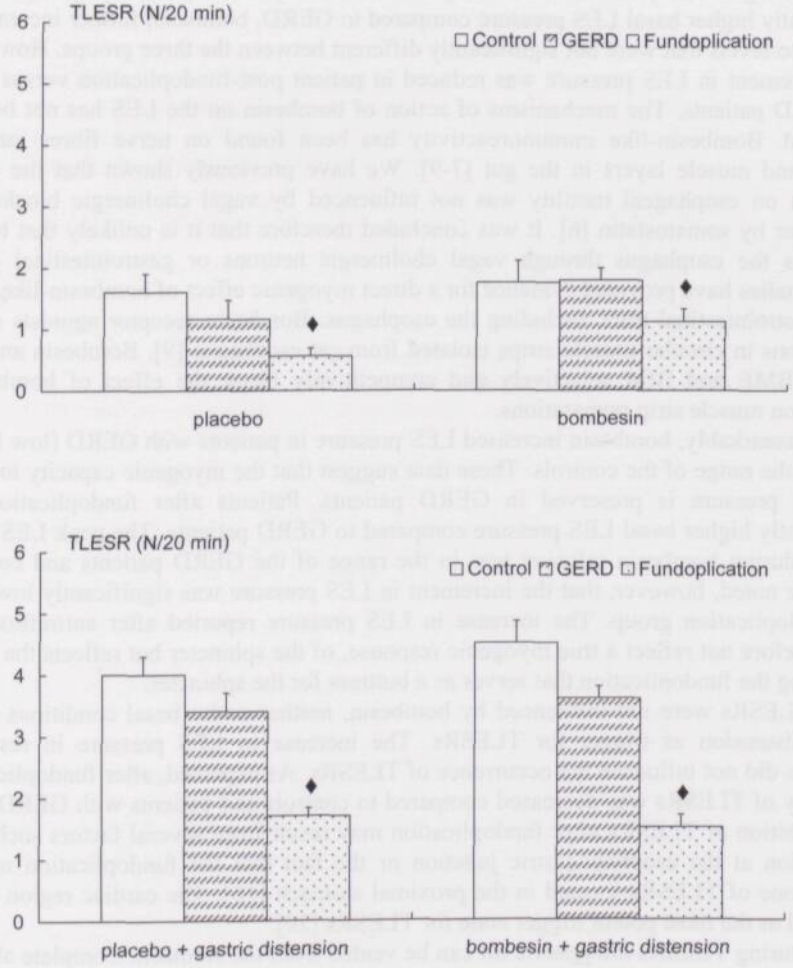


Figure 2. *Frequency of transient lower esophageal sphincter relaxations (mean ± sem/20 min) in 10 healthy controls (open bars), 22 patients with gastroesophageal reflux disease (shaded bars) and 24 patients post-fundoplication (dotted bars). The upper panel shows TLESR frequency under fasting conditions during i.v. placebo and i.v. bombesin. The lower panel shows TLESR frequency in response to gastric distension during i.v. placebo and during i.v. bombesin. Diamonds denote significant ($p < 0.05$) differences compared to control.*



DISCUSSION

It was shown that bombesin significantly increases LES pressure in controls, GERD patients and patients post-fundoplication. Bombesin did not influence the frequency of TLESR neither under basal conditions nor after gastric air distension.

With respect to the effect of bombesin on LES pressure, our results are in line with previous observations that bombesin significantly increases LES pressure in healthy subjects [2, 6, 19]. Bombesin increased LES pressure to a same extend in the patients with GERD, who had a significantly lower basal LES pressure. In the patients post-fundoplication, with a significantly higher basal LES pressure compared to GERD, bombesin further increased LES pressure to levels that were not significantly different between the three groups. However, the peak increment in LES pressure was reduced in patient post-fundoplication versus controls and GERD patients. The mechanisms of action of bombesin on the LES has not been fully elucidated. Bombesin-like immunoreactivity has been found on nerve fibres innervating mucosa and muscle layers in the gut [7-9]. We have previously shown that the effect of bombesin on esophageal motility was not influenced by vagal cholinergic blockade with atropine or by somatostatin [6]. It was concluded therefore that it is unlikely that bombesin influences the esophagus through vagal cholinergic neurons or gastrointestinal peptides. Recent studies have provided evidence for a direct myogenic effect of bombesin-like peptides in the gastrointestinal tract, including the esophagus. Bombesin receptor agonists stimulate contractions in circular muscle strips isolated from cat esophagus [9]. Bombesin antagonists such as BME and BIM selectively and competitively block the effect of bombesin-like peptides on muscle strip preparations.

Remarkably, bombesin increased LES pressure in patients with GERD (low LESP) to levels in the range of the controls. These data suggest that the myogenic capacity to increase sphincter pressure is preserved in GERD patients. Patients after fundoplication had a significantly higher basal LES pressure compared to GERD patients. The peak LES pressure reached during bombesin infusion was in the range of the GERD patients and controls. It should be noted, however, that the increment in LES pressure was significantly lower in the post-fundoplication group. The increase in LES pressure reported after antireflux surgery may therefore not reflect a true myogenic response, of the sphincter but reflects the response to creating the fundoplication that serves as a buttress for the sphincter.

TLESRs were not influenced by bombesin, neither under basal conditions nor after gastric distension as trigger for TLESRs. The increase in LES pressure in response to bombesin did not influence the occurrence of TLESRs. As expected, after fundoplication the frequency of TLESRs was decreased compared to controls and patients with GERD [16-18]. The inhibition of TLESRs after fundoplication may result from several factors such as local denervation at the esophagogastric junction or the fact that the fundoplication masks the trigger zone of TLESRs located in the proximal stomach [16]. The cardiac region has been identified as the most potent trigger zone for TLESRs [20].

During TLESRs intragastric air can be vented from the stomach. Complete absence or a very low frequency of TLESRs may lead to symptoms as gas bloat. When LES pressure was elevated, after bombesin infusion, TLESRs occurred at the same frequency as during placebo i.v. Thus, bombesin did not negatively affect LES relaxation, neither during swallowing as shown previously, nor during TLESRs.

Despite an increase in LES pressure, acid reflux was not reduced, but even increased after bombesin infusion. Bombesin is known to stimulate gastrin release and gastric acid secretion [2, 3]. The results of the present study do not support a further exploration of the role of bombesin or bombesin agonists in reflux related disorders.

In conclusion: bombesin significantly increases LES pressure not only in healthy controls, but also in patients with GERD and patients after fundoplication. TLESR frequency is not influenced by bombesin.

REFERENCES

1. Anastasi A, Erspamer W, Baca M. Isolation and amino acid sequences of alytestin and bombesin, two analogous active tetradecapeptides from the skin of the European discoglossid frogs. Arch Biochem Biophys 1972;148:443-6.
2. Delle Fave G, Annibale B, De Magistris L, et al. Bombesin effects on human GI functions. Peptides1985;6:113-6.
3. Jansen JBMJ, Lamers CBHW. The effect of somatostatin on bombesin-stimulated serum gastrin and gastric acid secretion in man. Digestion 1981;21:193-7.
4. Jebbink MCW, Jansen JMBJ, Mooy DM, Rovati LC, Lamers CBHW. Effect of the specific cholecystokinin-receptor antagonist loxiglumide on bombesin-stimulated pancreatic enzyme secretion in man. Regul Pept 1991;32:361-8.
5. Douglas BR, Jebbink MCW, Tjon a Tham RTO, Jansen JBMJ, Lamers CBHW. The effect of loxiglumide (CR-1505) on basal and bombesin-stimulated gallbladder volume in man. Eur J Pharmacol 1989;166:307-10.
6. Masclee AAM, Lam WF, Lamers CBHW. Effect of bombesin on esophageal motility in humans. Diseases of the Esophagus 1999:12:54-9.
7. Polak JM, Bloom SR, Hobbs S. Distribution of a bombesin-like immunocreactivity in the rat gastrointestinal tract. Lancet 1976;307:1109-10.
8. Dockray GJ, Vaillant C, Walsh JH. The neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract. Neuroscience 1979;4:1561-8.
9. Milusheva EA, Kortezova NI, Mizhorkova ZN, Papsova M, Voy DH, Balint A, Vizi ES, Varga G. Role of different bombesin receptor subtypes mediating contractile activity in cat upper gastrointestinal tract. Peptides 1998;19:549-56.
10. Moran TH, Moody TW, Hostetler AM, Robinson PH, Goldrich M, McHugh PR. Distribution of bombesin binding sites in the gastrointestinal tract. Peptides 1988;9:643-9.
11. Dent J, Dodds WJ, Friedman RH, et al. Mechanisms of gastroesophageal reflux in recumbent asymptomatic human subjects, J Clin Invest 1980;65:256-67.
12. Dent J, Holloway RH, Toouli J, et al. Mechanism of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988;29:1020-8.
13. Mittal RK, Holloway RH, Penagini R, et al. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-10.
14. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268:G128-33.
15. Trudgill NJ, Riley SA. Transient lower esophageal sphincter relaxations are no more frequent in patients with gastroesophageal reflux disease than in asymptomatic volunteers. Am J Gastroenterol 2001;96:2569-74.
16. Ireland AC, Holloway RH, Toouli J, et al. Mechanisms underlying the antireflux action of fundoplication. Gut 1993;34: 303-8.
17. Masclee AAM, Horbach JM, Ledeboer M, et al. Prospective study of the effect of the Belsey Mark-IV fundoplication on reflux mechanisms. Scand J Gastroenterol 1998;33:905-10.
18. Straathof JWA, Ringers J, Masclee AAM. Prospective study on the effect of laparoscopic Nissen fundoplication on reflux mechanisms. Br J Surg 2001;88:1519-24.
19. Corazziari E, Delle Fave G, Pozzessere C, et al. Effect of bombesin on lower esophageal sphincter pressure in humans. Gastroenterology 1982;83:10-4.
20. Franzi SJ, Martin CJ, Cox MR, et al. Response of canine lower esophageal sphincter to gastric distension. Am J Physiol 1990:259:G380-5.