Effect of pyloroplasty and fundectomy on the delay of gastric emptying and gastrointestinal transit of liquid elicited by acute blood volume expansion in awake rats

M.C.V. Régo,
J.R.V. da-Graça,
F. de-A.A. Gondim,
R.B. de-M. Gondim,
R.P. Dantas and F.H. Rola

Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará,
Fortaleza, CE, Brasil

Abstract

We evaluated the effects of fundectomy and pyloroplasty on the delay of gastric emptying (GE) and gastrointestinal (GI) transit of liquid due to blood volume (BV) expansion in awake rats. Male Wistar rats (N = 76, 180-250 g) were first submitted to fundectomy (N = 26), Heinecke-Mikulicz pyloroplasty (N = 25) or SHAM laparotomy (N = 25). After 6 days, the left external jugular vein was cannulated and the animals were fasted for 24 h with water ad libitum. The test meal was administered intragastrically (1.5 ml of a phenol red solution, 0.5 mg/ml in 5% glucose) to normovolemic control animals and to animals submitted to BV expansion (Ringer-bicarbonate, iv infusion, 1 ml/min, volume up to 5% body weight). BV expansion decreased GE and GI transit rates in SHAM laparotomized animals by 52 and 35.9% (P<0.05). Fundectomy increased GE and GI transit rates by 61.1 and 67.7% (P<0.05) and prevented the effect of expansion on GE but not on GI transit (13.9% reduction, P<0.05). Pyloroplasty also increased GE and GI transit rates by 33.9 and 44.8% (P<0.05) but did not prevent the effect of expansion on GE or GI transit (50.7 and 21.1% reduction, P<0.05). Subdiaphragmatic vagotomy blocked the effect of expansion on GE and GI transit in both SHAM laparotomized animals and animals submitted to pyloroplasty. In conclusion 1) the proximal stomach is involved in the GE delay due to BV expansion but is not essential for the establishment of a delay in GI transit, which suggests the activation of intestinal resistances, 2) pyloric modulation was not apparent, and 3) vagal pathways are involved.

Key words
• Gastric emptying
• Gastrointestinal transit
• Blood volume expansion
• Fundectomy
• Pyloroplasty
• Vagotomy

Introduction

The gastrointestinal (GI) tract, in addition to performing its function of nutrient digestion and absorption, is vitally important in maintaining water, electrolyte and acid-base regulation (1,2).

Acute blood volume (BV) expansion by intravenous infusion of isotonic saline decreases the intestinal absorption of sodium and water, while increases secretion (3). Conversely, the intestinal absorption of sodium
and water increases during acute BV retraction (4).

Since GI motility is finely regulated to provide adequate absorptive and secretory patterns - as GI contractile activity is related to absorption and secretion rates (5) - the response of the GI tract to liquid volume excess may involve a coupling of fluid/electrolyte transport and GI contractile activity adjustments, as proposed in previous reports (6,7).

We have shown that acute BV expansion modifies the gastroduodenal flow of liquid in anesthetized dogs and rats (7,8) and delays gastric emptying (GE) and the gastrointestinal (GI) transit of liquid in awake rats (9; Gondim F de-AA, Oliveira GR, Graça JRV da, Cavalcante DIM, Sousa MAN, Santos AA and Rola FH, unpublished results). In the present study we performed fundectomy (10) or Heinecke-Mikulicz pyloroplasty (11) to evaluate the role of the proximal stomach and pyloric segment in the delay of GE and GI transit of liquid elicited by BV expansion in awake rats (12).

Material and Methods

Animals and surgical procedures

Male Wistar rats (N = 76) weighing 180-250 g were used in this study. The animals were fasted for 16-24 h, with water ad libitum, anesthetized with thiopental and submitted to fundectomy, N = 26 (10), Heinecke-Mikulicz pyloroplasty, N = 25 (11) or SHAM laparotomy (N = 25) 7 days before GE and GI transit measurements. The gastric fundus was easily identified visually after midline laparotomy. Its excision (fundectomy) was performed by cutting the anterior and posterior walls, beginning just below the esophagogastic junction and extending aborally to the inferior limit of the gastric fundus (see Figure 1A). The incision was carefully closed with a transverse layer of suture (6.0 silk). Heinecke-Mikulicz pyloroplasty was performed after laparotomy through a 1-cm seromucosal longitudinal incision extending 0.5 cm in the antrum and 0.5 cm in the duodenum. The incision was carefully closed with a transverse layer of suture (6.0 silk) which joined together the surrounding cut walls (Figure 1B). Both pyloroplasty and fundectomy were closely inspected after surgery to assure that the gastroduodenal junction was not obstructed. At autopsy, the presence of possible gastroduodenal stenosis due to inappropriate scarring was also evaluated.

Six days after surgery (one day before the GE/GI transit measurements) the animals were anesthetized with ether and a polyethylene catheter (PE 50) was placed into the left external jugular vein. The distal end of the catheter was tunneled subcutaneously and its free end secured with suture after a dorsal skin incision between the shoulders. The animals were fasted for 24 h and water was allowed ad libitum until 2 h before the experiment.

All surgical procedures and animal treatments were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” (DHEW Publication No. 85-23, NIH, Bethesda, MD).

GE measurement

The method used to measure GE is a
Fundectomy, pyloroplasty and gastrointestinal transit in hypervolemic rats

First, 1.5 ml of the test meal containing a non-absorbable marker (0.5 mg/ml phenol red solution in 5% glucose) was given orally into the stomach through a stainless steel tube that was removed immediately after delivering the solution intragastrically. The animals were sacrificed with an iv thiopental overdose, the stomach was exposed by laparotomy, quickly clamped at the pyloric and cardiac ends and removed.

Stomachs were then placed in 100 ml of 0.1 N NaOH, cut into small pieces and homogenized for 30 s. The suspension was allowed to settle for 30 min at room temperature and 10 ml of the supernatant was centrifuged for 10 min at 2800 rpm (about 1400 g). Proteins in 5 ml of homogenate were precipitated with 0.5 ml of trichloroacetic acid (20% w:v), centrifuged for 20 min at 2800 rpm and 3 ml of the supernatant was added to 4 ml of 0.5 N NaOH. The absorbance of the sample was read at a wave length of 560 nm.

Percent gastric emptying (%GE) for each rat was calculated according to the following formula: %GE = 1 - amount of phenol red recovered from test stomach x 100/average amount of phenol red recovered from “standard” stomachs.

Rats sacrificed immediately after test meal administration were used to establish “standard” stomachs (100% phenol red in the stomach) in animals submitted to SHAM laparotomy (N = 5), fundectomy (N = 5) or pyloroplasty (N = 5).

Measurement of GI transit

GI transit measurements were performed according to the well-known concept of Green (14). As the test meal is administered intragastrically, GE as well as intestinal propulsion rates influence the final transit of the marker (GI transit). After quickly clamping the pyloric and cardiac ends to perform GE measurements, the small intestine - from the gastroduodenal junction to the cecum - was carefully removed and lightly stretched along a ruler on a flat table top. Tiny scissor cuts were then performed along the small intestine and 0.1 N NaOH solution was dribbled over the leaking phenol red solution to visualize the farthest point reached by the test meal front (pink color). The total length of the small intestine and the distance travelled by the marker along the small intestine were then measured. Since the intestines were all of quite similar length (mean 110.3 ± 4.1 cm), the GI transit index was defined as distance the marker traveled/total length of intestine x 100.

Experimental design and treatments

The animals were divided into 4 main groups, subdivided according to the various experimental conditions and sacrificed 10 min after test meal administration.

In the first group (group 1), SHAM laparotomized rats were used to evaluate the GE and GI transit rates in normovolemic (N = 5) and in expanded animals (N = 5). BV expansion was performed by iv infusion of a volume up to 5% body weight of Ringer bicarbonate solution (Na+ = 140, K+ = 4, Cl− = 124, HCO3− = 20 mmol/l) at the rate of 1 ml/min. In group 2, GE and GI transit rates were determined in normovolemic (N = 6) and in BV expanded animals (N = 5) previously submitted to fundectomy as described previously and in group 3 in animals previously submitted to pyloroplasty (N = 5 for each). In the last group (group 4), a subdiaphragmatic vagotomy was performed following jugular vein cannulation 6 days after SHAM laparotomy, fundectomy or pyloroplasty. A complete esophageal transection 1 cm from the gastroesophageal junction was performed and the esophageal lumen was reconstituted by inserting a 0.7-cm plastic tube (0.4 cm ID) to avoid obstruction. The cut ends were
joined and sutured. One day after subdia-
phragmatic vagotomy and jugular vein can-
nulation, the animals previously submitted
to SHAM laparotomy, fundectomy or py-
loroplasty were submitted to BV expansion
or not (normovolemic control, N = 5 for
each experimental variation). The test meal
was then intragastrically administered and
the animals were sacrificed 10 min after
test meal administration.

**Mean arterial pressure, central venous
pressure and hematocrit**

In a separate group, mean arterial pres-
sure (MAP) was monitored in awake rats
before, during and after the 5% BV expan-
sion. For this purpose a catheter was placed
into the carotid artery and connected to a
mercury (Hg) manometer. Intracardiac blood
samples were also collected for hematocrit
determination. Central venous pressure
(CVP) values were measured in awake rats
before and after 5% expansion by inserting a
PE50 catheter into the right jugular vein. The
catheter was positioned near the right atrium
and connected to a low pressure transducer
(NarcoByo 3, Narco Byo-Systems, Houston,
TX), which was coupled to a physiograph
Desk Model DMD 4B (Narco Byo-Systems).

**Statistical analysis**

The results are reported as means ± SEM.
Descriptive statistics were applied to each
group of experiments. One-way analysis of
variance and the Student-Newman-Keuls test
were then used to compare the various groups.
Differences were considered significant at
P<0.05.

**Results**

Effect of BV expansion on GE of liquid in
SHAM laparotomized animals and in animals
previously submitted to fundectomy or
pyloroplasty

The reservoir capacity of the stomach
after fundectomy was decreased by 33.2%
when we compared the fundectomy stan-
dards with SHAM standards. Pyloroplasty
also decreased gastric capacity by 12.3%
(pyloroplasty standards vs SHAM standards).
As can be seen in Figure 2A and B, fundec-
tomy and pyloroplasty significantly increased
GE of liquid rates by 37.9 and 25.4%, re-
spectively (P<0.05). BV expansion decreased
GE of liquid rates in SHAM laparotomized
animals and in animals submitted to pyloro-
plasty by 52.4 and 49.3%, respectively
(P<0.05), but produced a minor and not sta-
tistically significant GE decrease in animals
previously submitted to fundectomy (2.3%,
NS), as can be seen in Figure 2A.

Effect of BV expansion on GI transit of liquid
in SHAM laparotomized animals and in
animals previously submitted to fundectomy
or pyloroplasty

Fundectomy and pyloroplasty significant-
ly increased the rates of GI transit of liquid

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**Figure 2** - A, Rates of gastric emptying (GE) of liquid in normovolemic (Control) SHAM
laparotomized (N = 5) and in laparotomized expanded animals (N = 5), as well as in normovole-
ic and expanded (Expansion) animals previously submitted to fundectomy (N = 6 and 5, re-
spectively) or pyloroplasty (N = 5 and 5, respectively). B, Rates of gastrointestinal (GI) transit of liq-
uid in the same sequence. Blood volume (BV) expansion was performed by intravenous infusion
of Ringer-bicarbonate in a vol-
ume up to 5% body weight, 1
ml/min. *P<0.05 vs SHAM con-
trols (Student-Newman-Keuls
test); †P<0.05 vs intragroup con-
trol (Student-Newman-Keuls
test).
by 39.6 and 30.9% (P<0.05). BV expansion decreased the rates of GI transit of liquid in SHAM operated animals and in animals submitted to fundectomy or pyloroplasty by 36.7, 21.1 and 42.3% (P<0.05). Thus, as can be seen in Figure 2B, neither fundectomy nor pyloroplasty prevented the effect of BV expansion on GI transit of liquid.

**Effect of vagotomy on GE and GI transit in SHAM laparotomized animals and in animals previously submitted to fundectomy or pyloroplasty**

Subdiaphragmatic vagotomy prevented the effect of BV expansion on the rates of GE and GI transit of liquid in SHAM laparotomized animals. BV expansion also did not modify the rates of GE and GI transit of liquid in vagotomized animals previously submitted to fundectomy or pyloroplasty. Thus, vagotomy prevented 1) the effect of BV expansion on GE and GI transit in SHAM laparotomized animals, 2) the effect of BV expansion on GE and GI transit in animals submitted to pyloroplasty, and 3) the effect of BV expansion on GI transit in animals submitted to fundectomy, as can be seen in Figure 3.

**Effect of BV expansion on MAP, CVP and hematocrit**

MAP before BV expansion was 112.2 ± 3.2 mmHg. BV expansion did not change MAP either during expansion or after expansion was completed (117.8 ± 4.4 and 115.7 ± 7.1, respectively, N = 4, NS). However, after BV expansion, CVP levels increased from 2.4 ± 3.6 to 7.3 ± 2.1 cmH$_2$O (P<0.05, N = 5) and the mean hematocrit values decreased from 49.6 ± 1.6% (N = 5) to 34 ± 1.1% 10 min after expansion (P<0.05, N = 5).

**Discussion**

Acute blood volume expansion delays the gastric emptying and gastrointestinal transit of liquid in awake rats (9; Gondim F de-AA, Oliveira GR, Graça JRV da, Cavalcante DIM, Sousa MAN, Santos AA and Rola FH, unpublished results). In the present study we demonstrated that, as observed in intact controls (9), GE and GI transit rates were decreased in SHAM laparotomized animals after BV expansion, as well as in animals previously submitted to pyloroplasty. However, fundectomy prevented the effect of BV expansion on GE but not on GI transit.

The effect of acute BV expansion on GE and GI transit of liquid appears to be related to alterations in GI motility since BV expansion increases the gastroduodenal resistance offered to saline flow in anesthetized dogs and rats (7,8). In addition, we have previously demonstrated that BV expansion also decreases GE rates in animals submitted to ip administration of ranitidine (9; Gondim F de-AA, Oliveira GR, Graça JRV da, Cavalcante DIM, Sousa MAN, Santos AA and Rola FH, unpublished results), which minimizes the possible interference of a high level of gastric secretion induced by BV expansion.
expansion with the evaluation of actual gastric emptying (13). Thus, the changes in the GE of liquid observed here did not appear to be related to secretory pattern modifications.

The proximal stomach has an important reservoir function, receiving and storing ingesta (15) and is important for GE control (10). Fundectomy decreases the gastric volume capacity, increases postprandial intragastric pressure and accelerates GE, as could be observed in our experiments. The present findings indicate that the proximal stomach is an essential element for the development of the GE delay due to BV expansion, since fundectomy prevented it. This contrasts with our results obtained in anesthetized rats (16). This difference can be explained by the inability of the previous perfusion system (8,16) to separate fundic from antral function. Another point to consider is the possible activation of cardiopulmonary receptors (17) which can increase fundic relaxation and cannot be easily evidenced from our previous experimental protocols (8,16).

The role of the pylorus in GE control is still obscure. However, the pylorus has been proposed as an effective resistance to transpyloric flow of liquid by increased localized pyloric contractions (18), and delays in GE due to changes in pyloric pressure waves, obstructing the flow through the pylorus, have also been reported (19). Pyloric resistance was not essential to trigger the effect of BV expansion on GE, in contrast to previous results in anesthetized animals, which clearly demonstrated its participation (16). In fact, pyloroplasty increased GE and GI transit, but did not block the effect of BV expansion.

In our experimental protocol, changes in GI transit of liquid were influenced by GE as well as by the small intestine propulsion, since the test meal was intragastrically delivered. Concerning the delay in GI transit of liquid, neither fundectomy (which prevented GE delay), nor pyloroplasty prevented it. Thus, since GE rates were markedly decreased by BV expansion, it would not be surprising to find a decrease in GI transit, which could be entirely related to the decrease in GE. This statement is valid considering the experiments in animals submitted to SHAM laparotomy or pyloroplasty, since both GE and GI transits were significantly decreased by BV expansion. However, in animals submitted to fundectomy, GE rates were not decreased by BV expansion (indicating that fundectomy prevented the effect of BV expansion on GE), while GI transit rates were significantly decreased. In this case, the delay in GI transit could not be explained only by the delay in GE, indicating that the GI transit delay due to BV expansion may occur without gastric participation, probably due to small intestine motility activation, which has been suggested previously in anesthetized animals (6). In addition, it has been demonstrated that changes in small intestine transit time could occur independently of changes in GE (20) since the rate of GE can influence the transit of food down the initial intestinal portions, but has a more limited influence down the whole small intestine (20) and other studies have also demonstrated that GE can be increased, but the final GI transit delayed (21).

A relationship between delayed GE and MAP changes was not found, since MAP levels did not change significantly after BV expansion. However, CVP levels were significantly increased after BV expansion. Hematocrit values decreased significantly 10 min after BV expansion. Thus, besides delayed GE, acute BV expansion also modified hematocrit and CVP, but not MAP levels. The GE delay due to acute BV expansion also appears to be influenced by the infused volume but not necessarily by the composition of the expanding solution (8).

Concerning the investigation of the neural pathways, subdiaphragmatic vagotomy blocked the BV expansion effect on GE and GI transit of liquid in SHAM laparotomized animals.
animals and in animals submitted to pyloroplasty, in agreement with our previous observation (9) indicating that vagal pathways are involved in the phenomenon. In fact, BV expansion activates cardiopulmonary receptors which release their signals through vagal pathways (17). However, despite these findings, yohimbine, an α-2 blocker, has also been reported to be effective in blocking the BV expansion effect on GE and GI transit (9).

Our findings suggest that the proximal stomach participates in the GE delay due to BV expansion but is not essential for the establishment of delayed GI transit, indicating a possible activation of intestinal resistances, an absence of pyloric participation and an involvement of vagal pathways.

References