Brazilian Journal of Medical and Biological Research (1998) 31: 431-437
ISSN 0100-879X

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. Rêgo,
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.

Correspondence
F.H. Rola
Departamento de Fisiologia e Farmacologia, UFC
Rua Coronel Nunes de Melo, 1127
Caixa Postal 3157
60430-270 Fortaleza, CE
Brasil
Fax: 55 (085) 243-9333

Presented at the Fifth United European Gastroenterological Week, Paris, 1996.

Research supported by CAPES, CNPq, UFC and UNIMED.

Received June 28, 1997
Accepted November 25, 1997

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 esophagogastric 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.

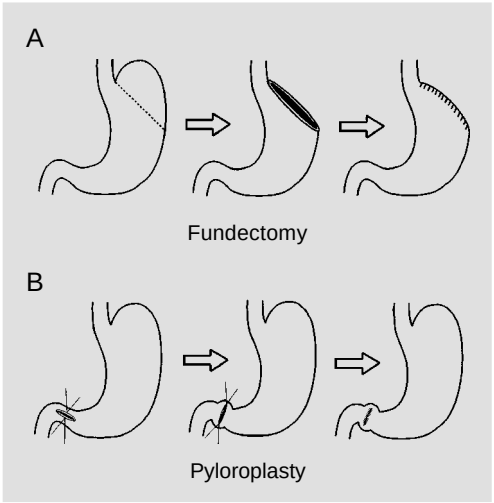

Figure 1 - *A*, Technique of gastric fundus excision (fundectomy), with emphasis on the cut margins and the final sutures (for more details, see Material and Methods). *B*, Technique of Heinecke-Mikulicz pyloroplasty with emphasis on the cut margins and the final sutures (for more details, see Material and Methods).

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

modification of that described by Scarpignato et al. (13).

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, HCO_3^- = 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 subdiaphragmatic vagotomy and jugular vein cannulation, the animals previously submitted to SHAM laparotomy, fundectomy or pyloroplasty 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 pressure (MAP) was monitored in awake rats before, during and after the 5% BV expansion. 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).

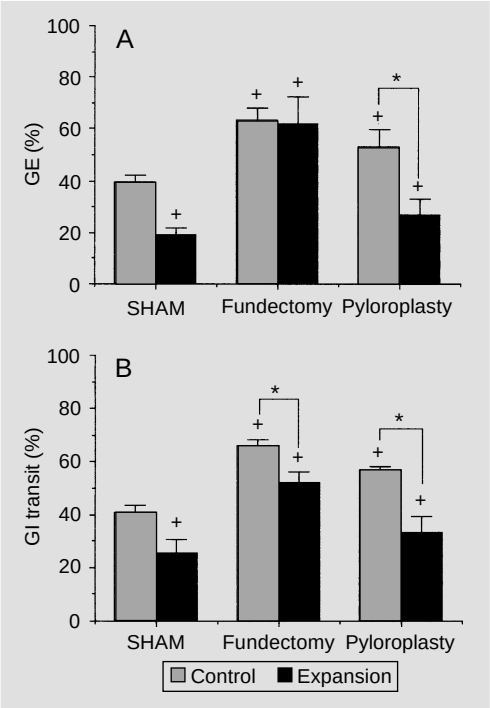

Figure 2 - *A*, Rates of gastric emptying (GE) of liquid in normovolemic (Control) SHAM laparotomized (N = 5) and in laparotomized expanded animals (N = 5), as well as in normovolemic and expanded (Expansion) animals previously submitted to fundectomy (N = 6 and 5, respectively) or pyloroplasty (N = 5 and 5, respectively). *B*, Rates of gastrointestinal (GI) transit of liquid in the same sequence. Blood volume (BV) expansion was performed by intravenous infusion of Ringer-bicarbonate in a volume up to 5% body weight, 1 ml/min. $^{+}P<0.05$ *vs* SHAM controls (Student-Newman-Keuls test); *P<0.05 *vs* intragroup control (Student-Newman-Keuls test).

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 standards 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, fundectomy and pyloroplasty significantly increased GE of liquid rates by 37.9 and 25.4%, respectively ($P<0.05$). BV expansion decreased GE of liquid rates in SHAM laparotomized animals and in animals submitted to pyloroplasty by 52.4 and 49.3%, respectively ($P<0.05$), but produced a minor and not statistically 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 significantly increased the rates of GI transit of liquid

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_2O (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

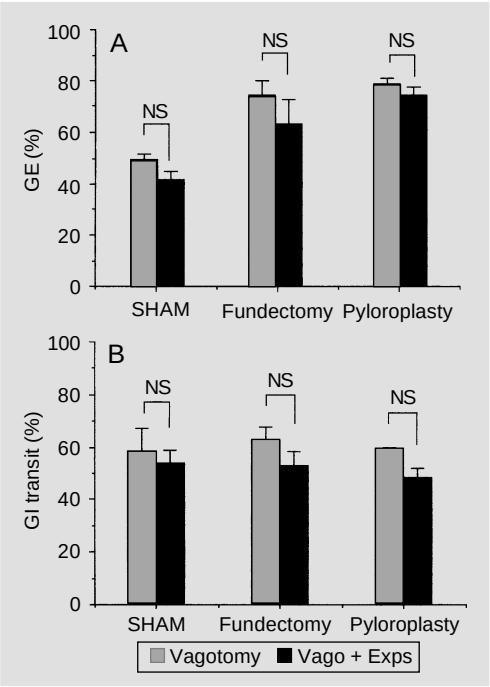

Figure 3 - Effect of subdiaphragmatic vagotomy on the rates of gastric emptying (GE) (A) and gastrointestinal (GI) transit (B) in normovolemic animals (Vagotomy) and in animals submitted to blood volume (BV) expansion (Vago + Exps) and to SHAM laparotomy, fundectomy or pyloroplasty (N = 5 for each experimental variation). BV expansion was performed by intravenous infusion of Ringer-bicarbonate in a volume up to 5% body weight, 1 ml/min. NS, Not significant.

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 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

1. Michel AR (1986). The gut: the unobtrusive regulator of sodium balance. *Perspectives in Biology and Medicine*, 29: 203-213.
2. Sawchenko PE & Fridman MI (1979). Sensory functions of the liver. *American Journal of Physiology*, 236: R5-R20.
3. Duffy PA, Granger DN & Taylor AE (1978). Intestinal secretion induced by volume expansion in the dog. *Gastroenterology*, 75: 413-418.
4. Levens NR (1985). Control of intestinal absorption by the renin-angiotensin system. *American Journal of Physiology*, 249: G3-G15.
5. Lee JS (1983). Relationship between intestinal motility, tone, water absorption and lymph flow in the rat. *American Journal of Physiology,* 345: 489-499.
6. Rola FH, dos-Santos AA, Xavier-Neto J, Cristino-Filho G, Rocha CI, Santiago Jr AT, Gondim FAA, Pereira JM & Capelo LR (1989). Effects of acute volemic changes on jejunal compliance in dogs. *Brazilian Journal of Medical and Biological Research*, 22: 523-531.
7. Santos AA, Xavier-Neto J, Santiago-Jr AT, Souza MAN, Martins AS, Alzamora F & Rola FH (1991). Acute volaemic changes modify the gastroduodenal resistance to the flow of saline in anaesthetized dogs. *Acta Physiologica Scandinavica,* 143: 261-269.
8. Xavier-Neto J, dos Santos AA & Rola FH (1990). Acute hypervolemia increases the gastroduodenal resistance to the flow of saline in rats. *Gut,* 31: 1006-1010.
9. Gondim F de AA, Oliveira GR, Graça JRV da, Cavalcante DIM, Santiago Jr AT, Santos AA & Rola FH (1996). Neural mechanisms involved in gastric emptying delay due to acute expansion of the extracellular fluid volume. *Neurogastroenterology and Motility*, 1: 74 (Abstract).
10. Wilbur BG, Kelly KA & Code CF (1974). Effect of gastric fundectomy on canine gastric electrical and motor activity. *American Journal of Physiology*, 226: 1445-1449.
11. Ormsbee III HS & Bass P (1976). Gastroduodenal motor gradients in the dog after pyloroplasty. *American Journal of Physiology*, 230: 389-397.
12. Rego MCV, Graça JRV, Gondim F de AA, Gondim RBM, Dantas RP & Rola FH (1996). Role of proximal stomach and pylorus on gastric emptying and gastrointestinal transit delays elicited by acute blood volume expansion in awake rats. *Gut*, 39 (Suppl 3): 214 (Abstract).
13. Scarpignato C, Capovilla T & Bertaccini G (1980). Action of caerulein on gastric emptying of the conscious rat. *Archives Internationales de Pharmacodynamie et de Therapie*, 246: 286-293.
14. Green AF (1959). Comparative effects of analgesics on pain threshold, respiratory frequency and gastrointestinal propulsion. *British Journal of Pharmacology,* 14: 26-34.
15. Kelly KA (1980). Gastric emptying of liquids and solids: roles of proximal and distal stomach. *American Journal of Physiology*, 239: G71-G76.
16. Graça JRV, Gondim F de-AA, Cavalcante DIM, Xavier-Neto J, Messias ELM, Marques JAP, Santos AA & Rola FH (1997). Gastroduodenal resistance and neural mechanisms involved in saline flow decrease elicited by acute blood volume expansion in anesthetized rats. *Brazilian Journal of Medical and Biological Research*, 30: 1257-1266.
17. Thames MD (1978). Contribution of cardiopulmonary receptors to the control of the kidney. *Federation Proceedings*, 37: 1209-1213.
18. Treacy PJ, Jamieson GG & Dent J (1994). Pyloric motility and liquid gastric emptying during barostatic control of gastric pressure in pigs. *Journal of Physiology*, 474: 361-366.
19. Houghton LA, Read NW, Hedge R, Horowitz M, Collins PJ, Chatterton B & Dent J (1988). Relationship of the motor activity of the antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal. *Gastroenterology,* 94: 1285-1291.
20. Read NW, Cammack J, Edwards C, Holgate AM, Cann PA & Brown C (1982). Is the transit time of a meal through the small intestine related to the rate at which it leaves the stomach? *Gut*, 23: 824-828.
21. Stewart JJ, Battarbee HD & Betzing KW (1992). Intestinal myoelectrical activity and transit time in chronic portal hypertension. *American Journal of Physiology*, 263: G474-G479.