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Atrial stretch delays gastric emptying of liquids in awake rats

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ABSTRACT

Aims: We previously reported that mechanical atrial stretch (AS) by balloon distention increased gastric tonus in anesthetized rats. The present study evaluated the effect of AS on the gastric emptying of a liquid test meal in awake rats and its underlying neural mechanisms.
Main methods: Anesthetized male rats received a balloon catheter into the right atrium and a gastrostomy cannula. The next day, mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), and cardiac output (CO) were continuously monitored. After the first 20 min of monitoring (basal interval), the balloon was either distended or not (control) with 30, 50, or 70 μl saline for 5 min. Fifteen minutes later, the rats received the test meal (glucose solution with phenol red), and fractional gastric dye retention was determined 10, 20, or 30 min later.
Key findings: Heart rate and CVP values were transiently increased by 50 or 70 μl AS but not 30 μl AS, whereas gastric emptying was slower after 30, 50, or 70 μl AS than after sham distention. Subdiaphragmatic vagotomy or splanchnicotomy + celiac ganglionectomy and capsaicin, ondansetron, hexamethonium, L-NAME, and glibenclamide treatment prevented the AS-induced delay in gastric emptying, whereas atropine and guanethidine treatment failed to prevent it.
Significance: Atrial stretch inhibited the gastric emptying of liquid via non-adrenergic and non-cholinergic pathways that activate nitric oxide-K^+_{ATP} channels.

Introduction

Capelo et al. (1983) reported that hypervolemia caused by the infusion of isotonic saline enhanced gastric tonus in anesthetized dogs, whereas hypovolemia induced by bleeding decreased gastric tonus. However, the precise role of blood volume in such a phenomenon is uncertain because saline infusion also induces hemodilution, acidosis, and hypoxemia, which might modulate gut motility (Tack, 2006). Blood transfusion in rats increases gastric tonus for at least 60 min, whereas acute bleeding reduces gastric tonus that is reversed by blood replacement (Graça et al., 2002).

Blood volume homeostasis is achieved via a complex process that involves afferent pathways triggered by low-pressure mechanoreceptors located in several venous territories, including the cardiac atria (Antunes-Rodrigues et al., 2004; Michell et al., 2008). We found that mechanical distention of the right atrium using a balloon catheter in anesthetized rats volume-dependently increased gastric tonus (Palheta et al., 2010). Gastric tonus is the major driving factor for the gastric emptying (GE) of liquid meals (Tack, 2006), and we hypothesized that atrial stretch (AS) also alters GE. The present study investigated the following: (*i*) the effect of mechanical stretch of the right atrium on GE and the small intestinal transit of a liquid test meal in awake rats and (*ii*) the neural pathways that underlie such effects.

Materials and methods

Animals and surgical procedures

Animal handling followed the International Guiding Principles for Research Involving Animals (International Council for Laboratory Animal Science) after approval from the Ethics Committee on Animal Experiments of the Federal University of Ceará (CEPA #02/2009). Male Wistar rats (250–280 g, $n = 266$) were obtained from the central housing station of the Federal University of Ceará and maintained in a temperature-controlled room on a 12 h/12 h light/dark cycle. The animals were then isolated in Bollman's cages and fasted for 18 h with free access to an oral rehydration solution (75 mM Na^+, 65 mM Cl^-, 20 mM K^+, 75 mM glucose, and 10 mM citrate). This procedure ensures clearing the stomach of residues while maintaining euglycemia and normovolemia (Souza et al., 2009).

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Under ether anesthesia, the rats underwent laparotomy, and a midgastric incision was made for the insertion of a plastic tube (6 mm outer diameter) whose distal end was positioned at the gastric fundus or at the first 1.0 cm of the duodenal bulb, respectively, referred to as the *gastric cannula* or *duodenal cannula*. They were fixed at the gastric wall, and the proximal end was externalized at the interscapular region. A polyethylene cannula (PE-50, Intramedic Clay Adams, Franklin Lakes, NJ, USA) with a thermocouple sensor was inserted into the carotid artery to determine cardiac output (CO; in ml min^{-1}) by thermodilution (Palheta et al., 2010). The right jugular vein received two catheters united by cyanoacrylate glue. The first one consisted of a balloon catheter (0.51 mm inner diameter, 0.94 mm outer diameter; Silastic, Dow Corning, Midland, MI, USA), manufactured as described previously (Palheta et al., 2010). The length of the second catheter (PE-10) was 1.0 cm shorter than the former. The balloon catheter was passed into the right jugular vein and advanced down to the superior vena cava so that its tip laid at the right atrium to stretch it (Palheta et al., 2010). Once externalized, the vascular catheters were connected to pressure transducers coupled to a digital acquisition system (PowerLab/8SP, AD Instruments, Bella Vista, NSW, Australia) for the continuous recording of mean arterial pressure (MAP; in mmHg) and central venous pressure (CVP; in cmH_2O). This technique allowed simultaneous mechanical stretching of the right atrium and CVP monitoring. Three stainless steel wires (0.203 mm Teflon-coated; A.M. Systems, Carlsborg, WA, USA) were fixed bilaterally onto the chest muscles and hip muscle of the left paw, externalized at the interscapular region. After connecting them to a bioamplifier (ML132 BioAmp) coupled to the digital system, an electrocardiogram signal was derived to continuously record heart rate (HR; in beats per minute [bpm]). A 72 h interval was allowed for recovery after surgery.

Experimental design for upper gastrointestinal transit assessment

Gastric emptying of liquid test meal

To test the effect of AS on GE, the rats were continuously monitored for up to 70 min. During the initial 20 min (termed the basal interval; Fig. 2), the atrial balloon was left untouched. The rats were then randomly assigned to either the control or AS protocol as previously reported (Palheta et al., 2010). For the rats subjected to AS, the balloon was distended once for 5 min (i.e., 20–25 min interval; Fig. 2; *AS*) with 50 μl saline. For the rats in the control group, the balloon remained empty during the 20–25 min interval (Fig. 2; *Sham*, Sh) as well as up to the end of the study. After balloon deflation or the sham-procedure, the rats were given a 5 min rest interval (Fig. 2, *R*). Ten minutes later, the rats received 1.5 ml of a test meal (0.5 mg ml^{-1} of phenol red in 5% glucose solution; Fig. 2, *Gavage*) via the gastric cannula. After a 10, 20, or 30 min postprandial interval, the animals were sacrificed (Fig. 2, *PP-10*, *PP-20*, and *PP-30*, respectively) by an overdose of thiopental for the determination of fractional gastric dye recovery as previously described (Souza et al., 2009). The other rats were subjected to AS for 5 min with 30 or 70 μl after the basal interval. Fifteen minutes later, the rats received the test meal and were sacrificed 10 min postprandially for the determination of gastric dye recovery as described below.

After laparotomy, the gut was divided into consecutive segments: the stomach and small intestine. The volume of each individual segment was calculated by submerging it in a graduated cylinder that contained 100 ml of 0.1 N NaOH. After segment homogenization, the proteins were precipitated with 0.5 ml of 20% trichloroacetic acid. After centrifugation, 3 ml of the supernatant was added to 4 ml of 0.5 N NaOH, and the samples were read by a spectrophotometer at 560 nm to construct dilution curves by plotting the dye concentrations against optical densities. The value of fractional (%) gastric dye recovery is expressed according to the following equation:

$$\text{Gastric dye retention}(\%) = 1 - \left[\frac{\text{amount of phenol red recovered in stomach}}{\text{total amount of phenol red recovered from all segments}}\right] \times 100$$

Surgical and pharmacological protocols

To determine the effect of hypovolemia on the present phenomenon, a separate group of rats was pretreated (4 h) subcutaneously with 5 ml of polyethylene glycol (PEG; 20 M carbowax; Union Carbide; 30% w/w; Stricker and MacArthur, 1974). After blood sample collection for hematocrit analysis, the animals were randomly subjected to control or 50 μl AS protocols, followed by test meal administration and sacrifice 10 min later for GE assessment as described above.

To verify the role of cardiac afferent C-fibers on the present phenomenon, another set of rats was anesthetized and treated with capsaicin (0.1 ml, 1 mg ml^{-1}) according to Kaufman and Deng (2004). After 15 min, they received a second dose of capsaicin, also instilled into the pericardial space, which was rinsed 30 min later with isotonic saline (5 ml), followed by suture of the incision. The respective controls were subjected to the same procedure, with the exception that they were instilled with vehicle (10% Tween) instead of capsaicin. Three days later, both capsaicin- and vehicle-treated rats were subjected to 50 μl AS, fed the test meal, and sacrificed 10 min later for GE assessment as described above.

To evaluate the neural pathways involved in the present phenomenon, other rats were subjected to bilateral subdiaphragmatic vagotomy via circular seromuscular myotomy of the esophagus 2 cm from the gastroesophageal junction (Hansen and Krueger, 1997). In another group, the rats were subjected to celiac ganglionectomy and splanchnic nerve sectioning (Fujita and Donovan, 2005). Three days later, the denervated and respective sham-operated rats were subjected to the 50 μl AS protocol, fed the test meal, and sacrificed 10 min later for GE assessment as described above.

To assess neurotransmission involved in the present phenomenon, other rats received an intravenous (1 ml·kg^{-1}) injection of one of the following agents: saline (control), hexamethonium (10 mg·kg^{-1}), atropine (0.5 mg·kg^{-1}), guanethidine sulfate (10 mg·kg^{-1}), or ondansetron (20 μg·kg^{-1}). The nitrergic contribution was assessed by pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME; 3 mg·kg^{-1}), L-arginine (100 mg·kg^{-1}) + L-NAME (3 mg·kg^{-1}), or methylene blue (3 mg·kg^{-1}). The role of K^+-ATP channels was assessed by pretreatment with glibenclamide (1 mg·kg^{-1}) either alone or combined with diazoxide (3 mg·kg^{-1}). All of the doses were selected based on previous studies (Gondim et al., 1999; Medeiros et al., 2008). After 10 or 60 min (guanethidine subset) pharmacological pretreatment, the rats were randomly subjected to the control or 50 μl AS protocol, fed the test meal, and sacrificed 10 min later for gut motility assessment as described above.

Assessment of small intestinal transit

A separate set of rats was subjected to either 50 μl AS or the sham protocol and received 1 ml of the liquid test meal 15 min later injected directly into the duodenum. After 20 min, the rats were sacrificed to determine fractional dye recovery. After laparotomy, the stomach and first 1 cm segment of the duodenum (with the cannula tip) were removed, comprising segment 1. The remaining gut was carefully removed and stretched. Obstructive ligatures were performed to obtain five consecutive small intestine segments (~20 cm long). Each segment was homogenized, and the dye content was determined by spectrophotometry as described above. Fractional marker recovery was calculated for each gut segment as the ratio

between the amount obtained in it and the sum of the amounts of all of the gastrointestinal segments, including the gastroduodenal segment. The data obtained for each individual segment was multiplied by the number of respective segments and summed to calculate the mass geometric center of the marker distribution throughout the gut as previously reported (Graça et al., 2008).

Plasma nitric oxide analysis

A separate group of instrumented rats was decapitated immediately after the 50 μl AS or sham protocol. Blood samples were collected, and the plasma was stored at 20 °C. The plasma levels of nitric oxide (NO) were measured by the light emission produced by the reaction of NO with ozone (O_3) detected by chemiluminescence as previously reported (Grau et al., 2007).

Statistical analysis

At the end of the experiments, the locations of the gastric and duodenal cannulas and balloon catheter were determined. Data from rats with balloon misplacements or balloons that were not inflated were discarded, yielding subgroups with 6–7 rats each. The hemodynamic data recorded throughout the studies were pooled as mean MAP, CVP, and HR values into consecutive intervals: Basal, during AS or Sham, and after AS or Sham. Each subgroup consisted of 6–7 rats. The data are expressed as mean ± SEM or median within interquartile intervals (for the intestinal transit index). Differences in the gastric recovery values between groups was assessed by one-way analysis of variance (ANOVA) followed by the Student Newman–Keuls post hoc test. Differences in the intra-group hemodynamic data between the basal period and successive intervals were compared using repeated-measures ANOVA followed by Bonferroni's test. Differences in small intestinal transit index values were compared using the unpaired Student's *t*-test. Values of $p<0.05$ were considered statistically significant.

Results

In control rats, MAP, HR, and CVP remained stable throughout the study (Table 1). Stretching the atrium by balloon inflation with 50 or 70 μl but not 30 μl was contemporaneously associated with sharp increases in HR and CVP but did not modify MAP or CO values. Considering the fractional gastric recovery data obtained from control and 30, 50, and 70 μl AS rats, AS induced a delay in GE, with a positive linear pattern according to the regression equation $y=0.18x+53.3$, $r=0.67$ ($p<0.05$; Fig. 1). To further investigate the underlying mechanisms involved in the present phenomenon, we employed the same volume of intra-atrial balloon distension (50 μl) that inhibits water intake and increases renal water and electrolyte output in awake rats as previously reported (Kaufman, 1984; Kaufman and Stelfox, 1987).

In addition to transient increases in HR (Fig. 2B) and CVP (Fig. 2C), 50 μl AS also delayed the rate of GE at all postprandial times evaluated (i.e., 10, 20, and 30 min after the test meal gavage feeding; Fig. 2D) compared with the respective values in sham rats.

Effects of hypovolemia

Compared with control rats, PEG pretreatment increased ($p<0.05$) hematocrit values in both sham and 50 μl AS rats (41.3 ± 1.6% vs. 50.6 ± 2.5% and 52.8 ± 1.5%, respectively), without altering MAP (122.4 ± 9.6 mmHg vs. 119.1 ± 4.0 mmHg and 115.5 ± 2.9 mmHg, respectively), moreover the cardiovascular parameters analyzed one can see in Supplementary Table II. In PEG-pretreated rats, 50 μl AS also increased ($p<0.05$) gastric dye recovery values (Fig. 3C). However, the magnitude of gastric retention (i.e. the difference between the mean values of gastric residual content of atrial stretched and its respective sham groups) in PEG-treated rats was lower ($p<0.05$) than in the respective vehicle-treated rats (Δ = 13.7 ± 1.4% vs. 22.7 ± 2.7%, respectively).

Table 1
Comparison of hemodynamic indices and gastric dye recovery (%) of a liquid test meal in awake rats subjected to atrial stretch (AS) and control (sham stretch [Sh]) protocols.

Group	Hemodynamic index	Basal	During AS or Sh	After AS or Sh	Gastric dye recovery (%)
Control	MAP	115.4 ± 3.2	113.9 ± 5.0	112.3 ± 6.0	44.0 ± 4.5
	CVP	2.2 ± 0.8	1.2 ± 0.5	1.6 ± 0.5	
	HR	364 ± 12	372 ± 9	373 ± 1	
	CO	132.2 ± 8.6	134.4 ± 12.3	130.2 ± 17.1	
AS 30 μl	MAP	116.6 ± 2.2	115.8 ± 2.4	114.3 ± 3.2	58.2 ± 3.1#
	CVP	1.0 ± 0.4	1.5 ± 0.4	0.3 ± 0.3	
	HR	326 ± 11	339 ± 20	339 ± 13	
	CO	123.5 ± 9.4	122.5 ± 9	122.5 ± 12.4	
AS 50 μl	MAP	118.3 ± 3.0	119.9 ± 4.0	117.9 ± 3.8	69.8 ± 3.5#
	CVP	1.2 ± 0.5	5.0 ± 1.0*	1.4 ± 0.6	
	HR	371 ± 7	421 ± 22*	368 ± 10	
	CO	136 ± 6.8	151.6 ± 16.4	140 ± 7.2	
AS 70 μl	MAP	117.7 ± 4.1	117.9 ± 3.6	116.7 ± 4.4	66.1 ± 1.7#
	CVP	1.8 ± 1.0	5.8 ± 1.5*	1.7 ± 1.1	
	HR	341 ± 14	392 ± 17*	339 ± 17	
	CO	125.3 ± 11	111.1 ± 17	130 ± 9.4	

Mean arterial pressure (MAP; in mmHg), central venous pressure (CVP; in cmH_2O), and heart rate (HR; in beats·min^{-1}) were continuously recorded for 45 min. Cardiac output (CO; in mL·min^{-1}) was determined by thermodilution. After a basal interval (the first 20 min of monitoring), the rats were subjected or not (sham stretch [Sh]) to the AS protocol by distending an intra-atrial balloon catheter with 30, 50, or 70 μl saline. Hemodynamic data obtained during the last 25 min of monitoring were pooled into after AS or Sh interval. Fractional gastric dye recovery of a liquid test meal was determined by spectrophotometry.

* $p<0.05$, vs. CVP and HR basal values.

\# $p<0.05$, vs. Sham AS (ANOVA followed by Student–Newman–Keuls or Bonferroni's test).

Effects of capsaicin administration

Capsaicin treatment prevented the AS-induced GE delay. Notably, intrapericardiac pretreatment with capsaicin abolished the tachycardiac response (see Supplementary Table I.) and the gastric retention elicited by AS (Fig. 3A).

Effects of gut autonomic denervation

Compared with the GE delay induced by 50 μl AS in sham-operated rats, the subdiaphragmatic-vagotomy or splanchnicotomy + ganglionectomy procedures prevented the gastric retention elicited by 50 μl AS

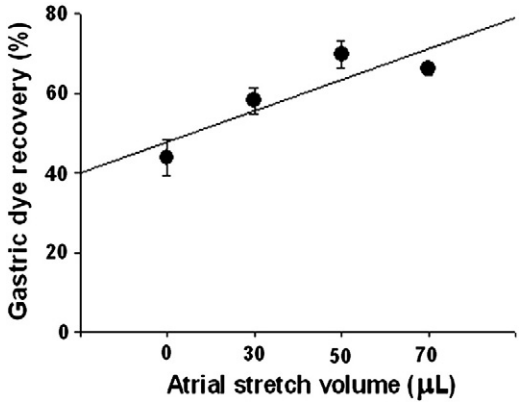

Fig. 1. Relationship between the volume of stretch of an intra-atrial balloon and the respective fractional gastric dye recovery values in awake rats. After a basal period, the rats were randomly subjected to right atrial stretch with 30, 50 or 70 μl saline for 5 min or not (0 μl; sham stretch protocol). They were then gavage-fed a liquid test meal and sacrificed 10 min later for gastric recovery (GR) analysis. Each subgroup consisted of 6 rats. The dots indicate mean GR values, and their respective vertical lines denote the SEM.

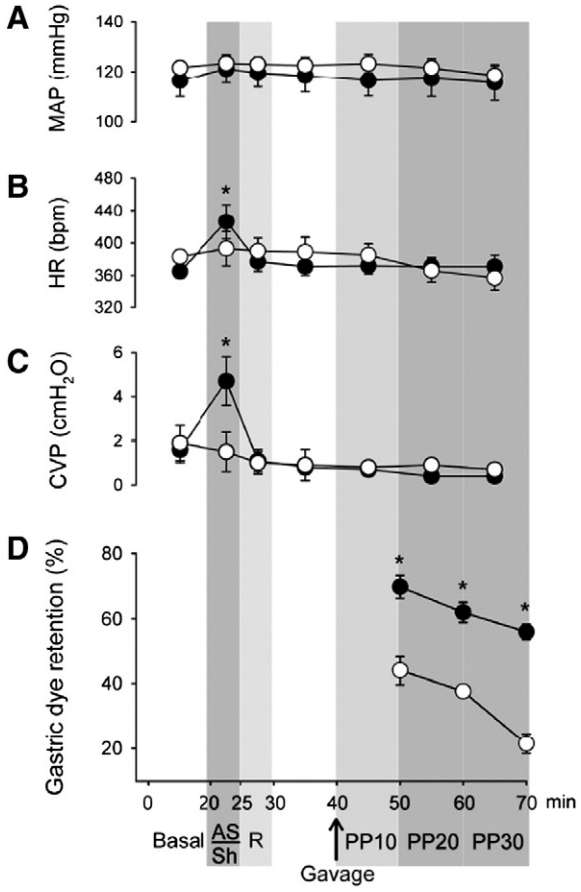

Fig. 2. Effects of 50 μl atrial stretch (●, AS) and sham stretch (○, Sh) on mean arterial pressure (MAP; panel A), heart rate (HR; panel B), central venous pressure (CVP; panel C), and fractional gastric dye recovery (panel D). After the first 20 min (Basal), the rats were randomly subjected to right atrial stretch with 50 μl saline for 5 min or a sham stretch protocol (20–25 min). A 5 min rest (R) interval occurred after balloon deflation. Ten minutes later (40 min), the rats were gavage-fed (1.5 ml) a liquid test meal (phenol red in glucose solution) and sacrificed 10, 20, or 30 min later (PP-10, PP-20, and PP-30, respectively). Gastric dye recovery was determined by spectrophotometry. Each subgroup consisted of 6–7 rats. Dots indicate mean MAP, HR, CVP, and GR values, and respective vertical lines denote the SEM. * $p<0.05$, vs. basal period or sham stretch (ANOVA followed by Student–Newman–Keuls or Bonferroni's test).

(Fig. 3B). The hemodynamic changes induced by AS in extrinsic denervated and their respective sham-operated groups are shown in Supplementary Table I.

Effects of pharmacological agents

Hexamethonium treatment caused significant ($p<0.05$) arterial hypotension (Supplementary Table II), and prevented the GE delay induced by 50 μl AS (Fig. 3C). Atropine treatment induced significant ($p<0.05$) tachycardia (Supplementary Table II), but neither guanethidine nor ondansetron treatment significantly modified the hemodynamic levels, as one can see in Supplementary Table II. Neither atropine nor guanethidine impaired the AS-induced GE delay, whereas it was significantly prevented by ondansetron treatment (Fig. 3C).

Effects of nitrergic intervention

L-NAME treatment induced significant ($p<0.05$) arterial hypertension (from 109.7 ± 11.7 to 134.0 ± 13.9 mmHg and from 110.7 ± 2.4 to 135.2 ± 0.9 mmHg in sham- and AS-treated rats, respectively) and prevented the AS-induced GE delay, changes that did not occur in rats pretreated with L-arginine + L-NAME (Fig. 4A and Supplementary Table III). Fig. 4B shows that 50 μl AS also significantly increased ($p<0.05$) fractional gastric recovery values in methylene blue-pretreated rats. The 50 μl AS-induced GE delay was prevented ($p<0.05$) in rats pretreated with glybenclamide but not in rats pretreated with diazoxide + glibenclamide (Fig. 4C).

Measurements of intestinal transit and plasma nitric oxide levels

Finally, as shown in Fig. 5, compared with the respective values in sham rats, 50 μl AS also increased plasma NO levels (7.5 ± 1.1 vs. 14.5 ± 2.0 nmol·μg protein^{-1}, respectively) and delayed ($p<0.05$) the progression along the small intestine of the dye mass center of the test meal injected directly into the duodenum (median and range: 3.4 and 3.0–3.5 vs. 2.4 and 2.1–2.7, respectively).

Discussion

The main result of this work was that transient (5 min) mechanical stretching of the right atrium delayed the GE rate of a liquid test meal in awake rats. The magnitude of the GE delay depended on the intensity of AS and was attenuated by prior hypovolemia. The mechanism of this effect appears to involve cardiac afferent C-fibers and serotonin 5-hydroxytryptamine-3 (5-HT_3) receptors because capsaicin and ondansetron treatments prevented it. The GE delay was also prevented by autonomic denervation caused by sub-diaphragmatic vagotomy and splanchnicectomy + ganglionectomy and pharmacological treatment with hexamethonium, L-NAME, and glibenclamide. Nicotinic non-adrenergic, non-cholinergic (NANC) pathways related to NO/K^+_{ATP} channel transduction likely participated in the onset of such a phenomenon.

The double cannulation of the right atrium is a useful technique to simultaneously induce AS and monitor CVP (Palheta et al., 2010). Central venous pressure is considered equivalent to right atrial pressure and indicates right heart preload, and it is influenced by intravascular volume, venous tone, and right ventricular function (Pittman et al., 2004). Putative impairment in cardiovascular function appears unlikely because the present basal hemodynamic values were similar to those previously reported (Benedetti et al., 2008) and remained at stable levels throughout the experiments. According to Kaufman (1984), 50 μl distension of an intra-atrial balloon does not obstruct venous return in adult rats. In fact, no difference was found between CO values recorded before and immediately after 30, 50, or 70 μl AS.

Gut transit was assessed using the dye dilution technique, a simple and reliable method (Souza et al., 2009). Putatively, AS increases gastric acid secretion, which alters the phenolphthalein analysis and inhibits GE via chemical stimulation of the duodenum (Tack, 2006). However, such a bias in the present gastric retention is unlikely because 50 μl AS also increased gastric recovery values in a separate set of omeprazole (20 mg·kg^{-1}, 1 mL·kg^{-1} i.v.)-pretreated rats (36.0 ± 1.8% in sham rats vs. 50.0 ± 3.2% in AS rats).

The present results support a functional relationship between the cardiovascular and gastrointestinal systems (Sjövall et al., 1986; Addisu et al., 2008; Michell et al., 2008). The fact that mechanical stretch of the right atrium volume-dependently increased the gastric retention of liquid in awake rats confirms the notion of a hemodynamic influence on gut motor behavior. Such an idea is reinforced by the attenuation of AS-induced GE delay caused by PEG pretreatment. We previously showed that bleeding accelerates the GE of a liquid test meal in awake rats, whereas saline infusion delays it (Gondim et al., 1999).

Changes in venous return are well known to activate myelinated and unmyelinated afferent fibers, triggering modulatory neurohormonal inputs to several brain areas involved in blood volume balance (Coleridge et al., 1964; Antunes-Rodrigues et al., 2004; Benedetti et al., 2008). In the present work, we considered that the reflex activation of such mechanoreceptors, manifested by a tachycardia response (Kappagoda et al., 1972; Kaufman et al., 1981; Benedetti et al., 2008) during 50 and 70 μl AS, is involved in the GE delay. The role of afferent

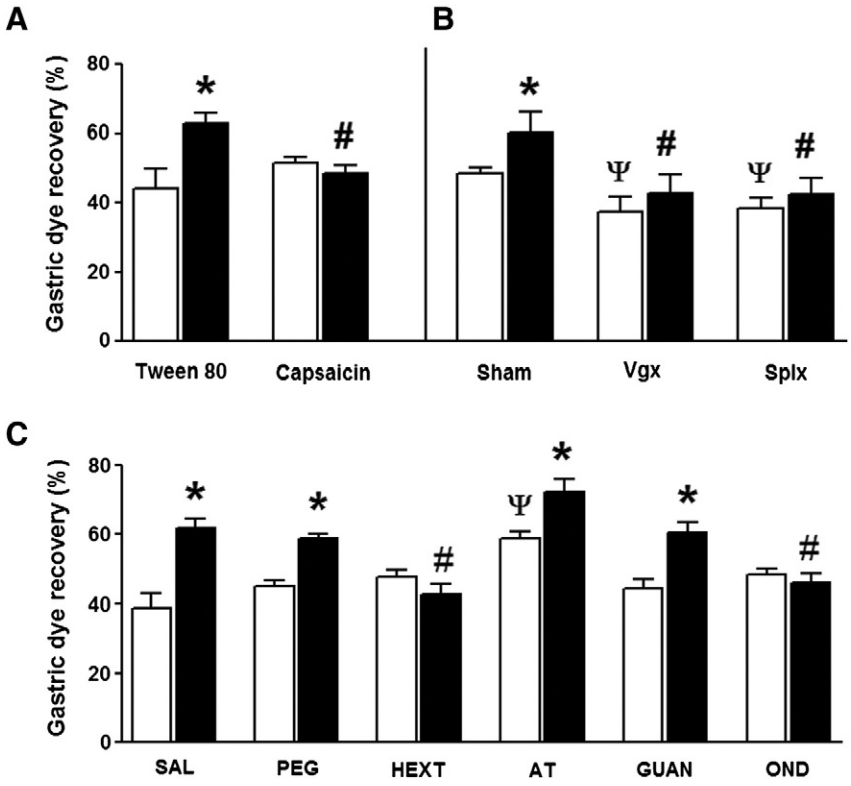

Fig. 3. Effects of cardiac afferent denervation, gut extrinsic denervation, and autonomic antagonist pretreatment on gastric emptying delay in awake rats subjected to distention of an intra-atrial balloon (■, 50 μl AS) or a sham stretch protocol (□, control). A, Comparison of pericardiac instillation (0.2 ml) pretreatment with capsaicin (1.0 mg·kg^{-1}) or its vehicle (Tween 10%). B, Comparison of subdiaphragmatic vagotomy (Vgx) and splanchnicectomy + ganglionectomy (Splx) pretreatment with respective sham surgery. C, Comparison of effects of pretreatment with vehicle (saline [SAL]; 1.0 ml·kg^{-1}, i.v.), polyethylene glycol (PEG; 5 ml, 30% w/w, s.c.), atropine (AT; 0.5 mg·kg^{-1}, i.v.), guanethidine (GUAN; 10 mg·kg^{-1}, i.p.), or ondansetron (OND; 20 μg·kg^{-1}, i.v.). $p<0.05$, vs. respective sham stretch and 50 μl AS rats; $^{\#}p<0.05$, vs. respective vehicle or sham operation rats subjected to 50 μl AS; $^{\Psi}p<0.05$, vs. respective vehicle or sham operation in sham stretch rats (ANOVA followed by Student–Newman–Keuls test).

pathways in such a phenomenon is indicated by the pharmacological prevention of both the GE delay (Supplementary Figure I) and tachycardia response by cardiac afferent-C fibers denervation with capsaicin or blockade of 5-HT$_3$ receptors with ondansetron (Supplementary Tables I and II, respectively). According Lupiński et al. (2011), the afferent C-fibers and 5-HT$_3$ receptors from the heart have cardioprotective properties against acute myocardial infarction in rats.

Patients with acute myocardial infarction can present gastric dysmotility (Krack et al., 2005). Electrical, mechanical, or chemical cardiac stimuli increase afferent traffic at vagal heart receptors, eliciting gastric relaxation and vomiting via a vago-vagal reflex (Abrahamsson and Thorén, 1972, 1973). For example, intravenous serotonin administration may trigger efferent vagus nerve activity, altering gastric motility in rats (Yoshioka et al., 1990) and eliciting nausea and vomiting (McLean et al., 2006). The abolition of the AS-induced GE delay by bilateral subdiaphragmatic vagotomy suggests that the present phenomenon is mediated by descending vagal pathways. Such a hypothesis is further supported by our previous data, in which the hypervolemia-induced GE delay was prevented by identical parasympathetic denervation (Gondim et al., 1999).

The present finding that the AS-induced GE delay was prevented by splanchnicectomy + celiac ganglionectomy suggests a role of sympathetic pathways. However, identical sympathetic deafferentation did not interfere with the GE delay elicited by saline infusion (Gondim et al., 1999). Curiously, the AS-induced GE delay was not affected by pretreatment with guanethidine, a sympathoplegic-adrenergic agent (Fukuda et al., 2005). The recruitment of sympathetic fibers that modulate gut motor behavior may release several biologically active agents, in addition to catecholamines, at the synaptic cleft (Hansen, 2003), and further research is necessary to establish the substances involved in such efferent signals.

In the present study, hexamethonium treatment prevented the AS-induced GE delay, indicating the involvement of nicotinic ganglionic nerves. The inhibition of GE caused by saline infusion was also blocked by hexamethonium (Gondim et al., 1999), suggesting a similar nicotinic ganglionic pathway in these phenomena. However, atropine did not alter the AS-induced GE delay, but it increased fractional gastric recovery in sham rats. Thus, we hypothesize that such a phenomenon involves NANC pathways. A previous work found gastric relaxant responses due to stimulation of cardiac vagal fibers via NANC efferent mediators (Abrahamsson and Thorén, 1973).

Vagal efferent nerves may release NO from the gastric myoenteric plexus via relaxation of the stomach induced by nicotinic receptor stimulation (Nakamura et al., 1998). The involvement of NOergic pathways in the present phenomenon is supported by three findings: (*i*) 50 μl AS increased plasma NO levels, (*ii*) L-NAME treatment prevented the AS-induced GE delay, and (*iii*) L-arginine pretreatment impaired the blunting effect of L-NAME on gastric retention caused by AS. Nitric oxide is the natural activator of the guanylyl cyclase enzyme. Notably, the magnitude of AS-induced gastric retention was significantly lower in methylene blue-pretreated rats than in vehicle-pretreated rats ($\Delta = 12.4 \pm 4.5\%$ vs. $22.7 \pm 2.7\%$, respectively), supporting the hypothesis of mediation by a NO-guanylyl cyclase pathway. Additionally, NO inhibits the pacemaker activity of interstitial cells of Cajal through a cyclic guanosine monophosphate (cGMP) dependent activation of ATP-sensitive K^+ channels (Koh et al., 2000; Park et al., 2007). In fact, the K^+_{ATP} channels regulate the smooth muscle excitability by interfering with the establishment of the transmembrane electrical difference (Koh et al, 1998; Rodrigo and Standen, 2005). A role for such K^+_{ATP} channels in the AS-induced GE delay is suggested by the glybenclamide-induced prevention of this phenomenon, which persisted in diazoxide + glybenclamide-pretreated rats.

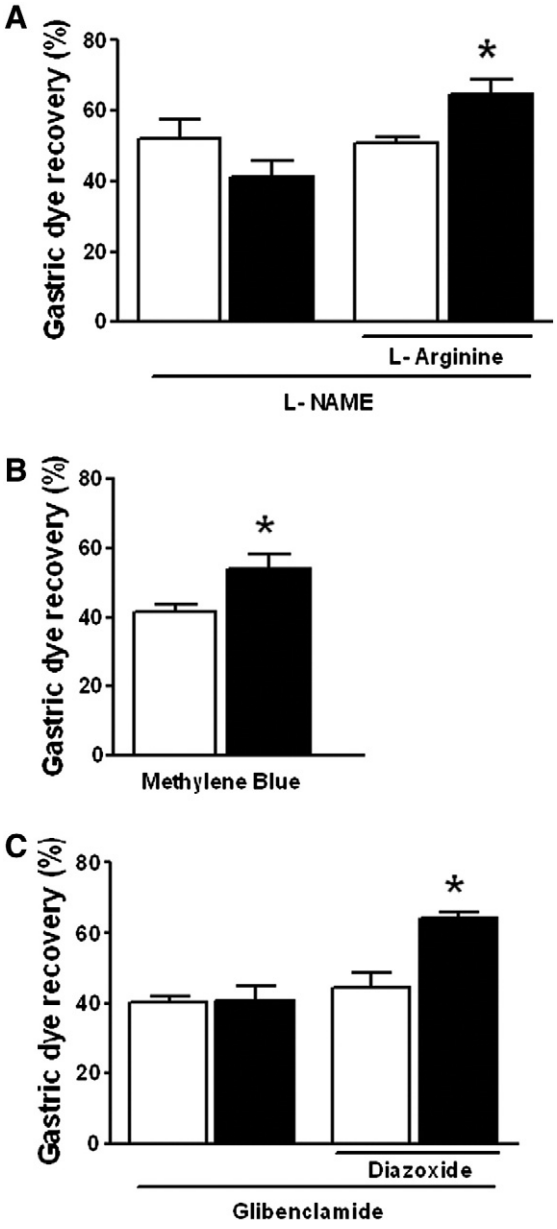

Fig. 4. Effects of different pharmacological pretreatments on fractional gastric dye recovery in awake rats subjected to distention of an intra-atrial balloon (■, 50 μl AS) or a sham protocol (□, control). A, Comparison of L-NAME (3 mg·kg^{-1}) and L-ARG (100 mg·kg^{-1}) + L-NAME (3 mg·kg^{-1}) pretreatments. B, Comparison between control and AS protocols after methylene blue (3 mg·kg^{-1}) pretreatments. C, Comparison of vehicle, glibenclamide (1 mg·kg^{-1}, i.p.), and glibenclamide (1 mg·kg^{-1}, i.p.) + diazoxide (3 mg·kg^{-1}, i.v.) pretreatments on gastric emptying delay induced by AS. Each subgroup consisted of 6–7 rats. *$p<0.05$, comparison between respective vehicle-pretreated and drug-pretreated 50 μl AS rats (ANOVA followed by Student–Newman–Keuls test).

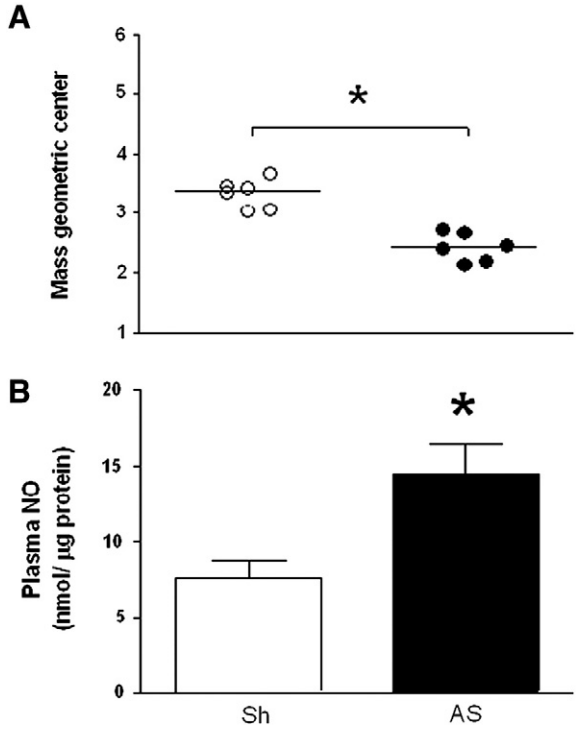

Fig. 5. Effects of 50 μl atrial stretch (●, AS) or sham stretch (○, Sh) protocols on small intestinal transit index and plasma nitric oxide (NO) values. A, Values of mass geometric center distribution from the stomach and proximal duodenum (segment number 1) to the five consecutive small intestine segments (segment number 6) in awake rats subjected to AS (●) or Sham protocols (○). B, Effects of AS or Sh protocols on plasma NO levels determined by spectrophotometry. Horizontal bars are medians of mass geometric center. Bars indicate mean plasma NO values, and respective vertical lines denote the SEM. *$p<0.05$, comparisons between Sh and AS rats (Student's t-test for unpaired means).

An important aspect of the present work is that the GE delay was observed 20 min after the end of intra-atrial balloon distension, which outlasted the increases in CVP and HR that paralleled AS. A similar outlast also occurred with an increase in gastric tonus elicited by AS in anesthetized rats (Palheta et al., 2010). Atrial stretching is well known to release prostaglandins, oxytocin, and atrial natriuretic peptide (ANP), which help mammals excrete blood volume excess (Ruskoaho et al., 1997; Skvorak and Dietz, 1997). Some of these agents (e.g., oxytocin) may also interfere with gut motility (Wu et al., 2003; Qin et al., 2009). We previously showed that 50 μl AS increased ANP blood levels, in addition to increasing gastric tonus (Palheta et al., 2010). Thus, the involvement of a putative humoral pathway in the present phenomenon should be considered.

Nevertheless, because AS increased gastric tone in anesthetized rats (Palheta et al., 2010), one should expect an increased flow of a liquid test meal to the small intestine instead of the present GE delay. Although these findings may appear discrepant, such a phenomenon also occurs under other conditions. For example, acute hypervolemia increases gastric tonus in anesthetized rats (Graça et al., 2002) but delays the GE of liquids in awake rats (Gondim et al., 1999). In addition, oxytocin increases intragastric pressure in anesthetized rats (Qin et al., 2009) but decreases both the GE and intestinal transit of liquids in awake rats (Wu et al., 2003). Besides the obvious influence of anesthesia on the neuro-humoral control of gut motor function (Fukuda et al., 2005), this phenomenon can be understood when one considers the inhibitory influences of both the pylorus and small intestine on the rate of GE, collectively known as the "breaking effect" (Hansen, 2003). We found that hypervolemia caused by saline infusion enhanced duodenal motility in anesthetized dogs, whereas it decreased the gastroduodenal flow of liquid (Santos and Oliveira, 1998). Atrial stretch also delays the progression of a test meal injected directly into the duodenum, indicating an increased resistance by the upper small intestine to the gut transit of liquid. We speculate that the present GE delay of a liquid induced by AS may be involved in gut dysmotility complaints (i.e., bloating and dyspepsia) of heart failure patients (Krack et al., 2005).

In summary, we found that mechanical stretching of the right atrium elicited a GE and intestinal transit delay of a liquid test meal in awake rats. The magnitude of the GE delay depended on the volume of intra-atrial balloon distension. Such a phenomenon appears to be elicited by capsaicin-sensitive afferent cardiac neurons and mediated

by both sympathetic and parasympathetic nerves and involve a NANC mechanism related to NO/K^+-ATP channel pathways.

Conflict of interest statement

This work has no competing interests.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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Author contribution

RCP, MTBS, HLGB, ADNP, and KVCB performed experiments. RCP, JRVG, PJCM, and RBO discussed the results and revised the paper. AAS and RCP designed the experiments, discussed the results, and wrote the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2013.01.016.

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