Acute blood volume expansion delays the gastrointestinal transit of a charcoal meal in awake rats

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Abstract

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The present study evaluates the effect of blood volume expansion on the gastrointestinal transit of a charchoal meal (2.5 ml of an aqueous suspension consisting of 5% charcoal and 5% gum arabic) in awake male Wistar rats (200-270 g). On the day before the experiments, the rats were anesthetized with ether, submitted to left jugular vein cannulation and fasted with water *ad libitum* until 2 h before the gastrointestinal transit measurement. Blood volume expansion by *iv* infusion of 1 ml/min Ringer bicarbonate in volumes of 3, 4 or 5% body weight delayed gastrointestinal transit at 10 min after test meal administration by 21.3-26.7% ($P<0.05$), but no effect was observed after 1 or 2% body weight expansion. The effect of blood volume expansion (up to 5% body weight) on gastrointestinal transit lasted for at least 60 min ($P<0.05$). Mean arterial pressure increased transiently and central venous pressure increased and hematocrit decreased ($P<0.05$). Subdiaphragmatic vagotomy and yohimbine (3 mg/kg) prevented the delay caused by expansion on gastrointestinal transit, while atropine (0.5 mg/kg), L-NAME (2 mg/kg), hexamethonium (10 mg/kg), prazosin (1 mg/kg) or propranolol (2 mg/kg) were ineffective. These data show that blood volume expansion delays the gastrointestinal transit of a charcoal meal and that vagal and yohimbine-sensitive pathways appear to be involved in this phenomenon. The delay in gastrointestinal transit observed here, taken together with the modifications of gastrointestinal permeability to salt and water reported by others, may be part of the mechanisms involved in liquid excess management.

Introduction

The gastrointestinal (GI) tract, besides carrying out its function of nutrient digestion and absorption/secretion, is vitally important in maintaining water, electrolyte and acid-base regulation (1,2). Small intestine fluid absorption capacity in humans is about 8-9 liters per day (3) and the intestinal lumen fluid is a potential liquid reservoir readily accessible to body needs (4). However, its functional coupling with the other body systems involved in liquid homeostasis and its role in acute volume homeostasis are not well understood.

Blood volume (BV) expansion decreases

intestinal fluid/electrolyte absorption and increases secretion (5) while intestinal water and sodium absorption increases during BV retraction (6). Hypervolemia may also increase uretero-pelvic pressure, which has been shown to decrease jejunal absorption (7). This reflex pattern has been considered to be important for the management of increased body fluid (7). Since GI motility is coupled to absorption and secretion (8), GI motility changes may be important for the management of liquid volume imbalances.

We have shown that BV changes modify the gastrointestinal tonus in anesthetized dogs: volume overload is followed by decreased gastric and jejunal compliances while bleeding increases them (9,10). In a barostatically perfused system we have also observed, in anesthetized dogs and rats, that the resistance offered by the gastroduodenal (GD) segment to the flow of liquid is modulated by BV levels: BV expansion decreased the GD flow while BV retraction increased it (11,12).

This evidence comes from experiments carried out on anesthetized animals after rather invasive surgical procedures. Searching for an experimental model that avoids the influence of anesthesia on the autonomic nervous system, in the present study we evaluated the effect of BV expansion on the GI transit of a charcoal meal in awake rats and the neural mechanisms involved in this process.

Material and Methods

Surgical procedures

On the day before the experiment, male Wistar rats (N = 181, 200-270 g) were anesthetized with ether and a polyethylene catheter (PE 50) was inserted into the left external jugular vein. The catheter was passed under the skin and its free end fixed outside through a dorsal skin incision between the shoulders. The rats were fasted for 24 h but received water *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", NIH, Bethesda, MD.

GI transit measurement

For GI transit measurements, we used a modification of the method reported by Green (13). First, 2.5 ml of an aqueous suspension of 5% charcoal and 5% gum arabic was administered by gavage. After 10, 15, 30, 45 and 60 min, the animals were sacrificed with an *iv* thiopental overdose. The small intestine was removed after laparotomy, its length measured (cm) from the pyloric sphincter to the ileocecal junction and the distance traveled by the front of the meal was recorded as percent of the small intestine length. Since the intestines of all animals were of quite similar length (mean 110.3 ± 4.1 cm), the GI transit index is defined as distance the marker traveled/total length of intestine x 100 and is reported as % at a specified time after receiving the test meal by gavage. Unless otherwise identified 10 min was used for GI transit measurement.

Treatments and experimental design

The animals were randomly assigned to one of the two following protocols: normovolemic control or BV expansion. Groups consisting of 5-8 animals were used for each subset of experiments. GI transit index was determined 10 min after receiving the test meal in normovolemic control animals and in animals previously submitted to an *iv* infusion of Ringer bicarbonate (140 mEq/l Na^+, 4 mEq/l K^+, 124 mEq/l Cl^-, 20 mEq/l HCO_3^-), at the rate of 1 ml/min in volumes of 1, 2, 3, 4 or 5% body weight. GI transit was also determined 15, 30, 45 and 60 min after the test meal in normovolemic controls and in animals previously submitted to 5% BV expansion.

Investigation of neural mechanisms

Atropine sulfate (0.5 mg/kg; Sigma Chemical Co., St. Louis, MO), Nω-nitro-L-arginine methyl ester (2 mg/kg; L-NAME, Sigma), prazosin chloride (1 mg/kg; Pfizer, Guarulhos, SP, Brazil), yohimbine hydrochloride (3 mg/kg; Sigma), propranolol hydrochloride (2 mg/kg; Sigma) or hexamethonium bromide (10 mg/kg; Sigma), were injected *iv* for investigation of neural mechanisms. GI transit measurements were performed in drug controls or in animals submitted to 5% BV expansion, after drug pretreatment. The time elapsed between drug pretreatment and test meal administration was 10 (prazosin, yohimbine, L-NAME) or 30 min (atropine, propranolol or hexamethonium). All animals were sacrificed 10 min after administration of the test meal.

In another group, submitted to a 6-h fasting period, a subdiaphragmatic vagotomy was performed 24 h prior to the experiments. A complete esophageal transection was performed 1 cm above the gastroesophageal junction and the esophageal lumen was reconstituted by inserting and fixing a 0.7-cm plastic tube (0.4 cm ID) (14). Sham operation for this group consisted of laparotomy.

Hemodynamic data

In separate groups of 4-5 awake rats, mean arterial pressure (MAP) was monitored for 45 min before and after the submission to the different experimental treatments, including drug administration. For this purpose, a catheter was placed into the carotid artery and connected to a mercury (Hg) manometer. Central venous pressure (CVP) was monitored in awake animals before and after 5% BV expansion (N = 5). A catheter was inserted into the right external jugular vein, positioned in the right atrium and connected to a low pressure transducer (Statham P23) which was connected to a Mark IV Physiograph (Narco Byo-Systems, Houston, TX).

Intracardiac blood samples from controls (N = 5) and animals submitted to 5% BV expansion (N = 5) were also collected for hematocrit (Ht) determination after sacrifice.

Statistical analysis

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

Results

Figure 1 shows the effect of BV expansion on the GI transit of a charcoal meal when measured 10 min after test meal administration. BV expansion up to 1 and 2% body weight caused a slight but nonsignificant decrease in GI transit rates while BV expansion of 3, 4 and 5% body weight significantly decreased GI transit ($P<0.05$). Figure 2 shows that GI transit rates were decreased for at least 60 min after 5% BV expansion ($P<0.05$).

Table 1 shows GI transit rates in drug-pretreated or vagotomized animals submitted or not (drug controls) to 5% BV expansion. As can be seen, either subdiaphragmatic vagotomy or yohimbine prevented the effect of BV expansion on GI transit (Figure
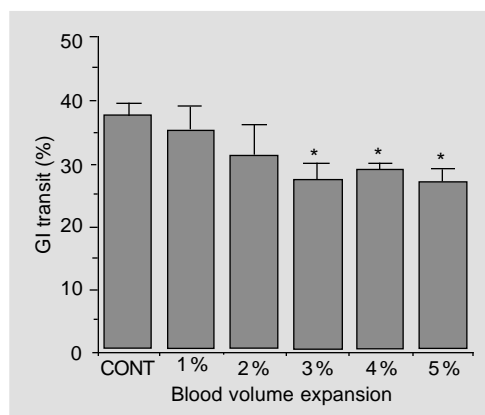

Figure 1 - Effect of acute blood volume expansion on gastrointestinal (GI) transit rates, 10 min after intragastric administration of a charcoal meal (2.5 ml of an aqueous suspension of 5% charcoal and 5% gum arabic) to controls (CONT; N = 6) and animals submitted to blood volume expansion by *iv* infusion of Ringer-bicarbonate, 1 ml/min, in volumes of 1, 2, 3, 4 and 5% body weight (N = 5, 5, 5, 5 and 6, respectively).*P<0.05 compared to control (Student-Newman-Keuls test).

Figure 2 - Comparison of gastrointestinal (GI) transit rates of an aqueous charcoal meal (2.5 ml of an aqueous suspension of 5% charcoal and 5% gum arabic) in controls (CONT; N = 6, 5, 7, 6 and 6, respectively) and in animals submitted to 5% blood volume expansion (EXP; N = 5, 5, 5, 8, 7, and 6, respectively) by *iv* infusion of Ringer-bicarbonate, 1 ml/min, 10, 15, 30, 45 and 60 min after the intragastric test meal administration. *P<0.05 compared to control (Student-Newman-Keuls test).
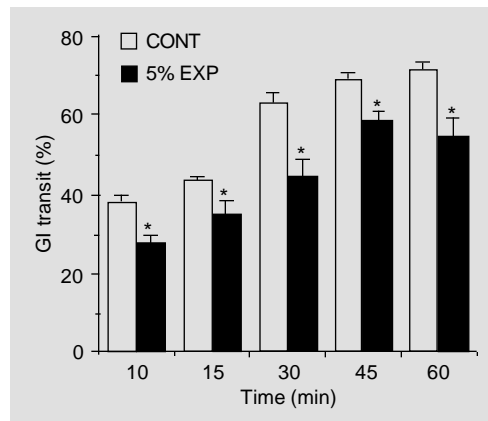
Figure 3 - Effect of subdiaphragmatic vagotomy (VAG) and yohimbine (YOH) administration (3 mg/kg) on gastrointestinal (GI) transit delay of an aqueous charcoal meal due to 5% blood volume (BV) expansion (EXP) by *iv* infusion of Ringer-bicarbonate, 1 ml/min in awake rats. VAG and VAG + EXP refer to vagotomized animals submitted or not to BV expansion (N = 5 in each group) and YOH and YOH + EXP to yohimbine-pretreated animals submitted or not to BV expansion (N = 5 in each group). NS, Not significant (Student-Newman-Keuls test).
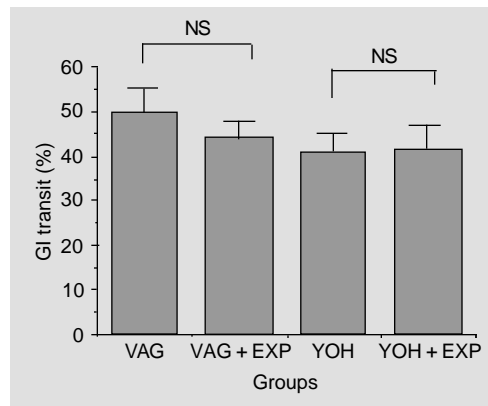
Table 1 - GI transit indexes 10 min after test meal administration in drug-pretreated or vagotomized controls (Control) and in pretreated or vagotomized animals submitted to 5% blood volume expansion (Expansion) with *iv* infusion of Ringer-bicarbonate, 1 ml/min.

The number of animals is given within parentheses. *P<0.05 compared to None-Control (Student-Newman-Keuls test); +P<0.05 compared to the respective treatment control (Student-Newman-Keuls test).

Treatment	Control	Expansion
None	38.1 ± 1.6 (6)	27.5 ± 1.8*+ (6)
Vagotomy	50.2 ± 4.6* (5)	44.6 ± 3.3* (5)
Hexamethonium (10 mg/kg)	45.9 ± 3.1 (6)	30.1 ± 3.4+ (5)
Atropine (0.5 mg/kg)	30.1 ± 2.3* (5)	22.2 ± 2.1*+ (5)
L-NAME (2 mg/kg)	31.1 ± 1.8* (5)	21.7 ± 2.6*+ (5)
Prazosin (1 mg/kg)	35.7 ± 3.1 (5)	26.4 ± 2.4*+ (5)
Yohimbine (3 mg/kg)	41.3 ± 3.5 (5)	42.2 ± 4.9 (5)
Propranolol (2 mg/kg)	44.1 ± 2.5 (5)	25.7 ± 2.6*+ (5)

3). Despite preventing the BV expansion effect on GI transit, we still observed a trend for a decrease in GI transit after BV expansion in vagotomized animals, which however was not statistically significant. Hexamethonium, atropine, L-NAME, prazosin or propranolol pretreatments, however, were ineffective (Table 1).

BV expansion (5%) transiently increased MAP during the expansion period (from 108.2 ± 3.2 to 119.7 ± 4.1, P<0.05, N = 5). However, MAP levels were not significantly modified after expansion was completed (expanded period, 116.1 ± 3.1). Table 2 shows that atropine and yohimbine decreased MAP. L-NAME increased while hexamethonium, propranolol and prazosin significantly decreased MAP values (P<0.05). BV expansion also increased CVP values (from 3.6 ± 1.6 to 9.6 ± 3.2 cmH$_2$O, P<0.05, N = 5) while decreasing the mean hematocrit (from 49.6 ± 1.6 to 34 ± 1.1, P<0.05, N = 5).

Discussion

We have observed that acute BV changes modify the GI motility in anesthetized rats and dogs: gastric and jejunal compliance (9,10), as well as the resistance offered by the gastroduodenal segment to the flow of liquid (11,12). We have also recently extended these observations to the ileocolonic region (15). The present study, which has been reported in abstract form (16), extend these observations from anesthetized to awake rats, avoiding the interference of anesthesia on the autonomic nervous activity. Furthermore, we evaluated the relationship between the volume infused and the GI transit delay and studied the neural mechanisms involved in this phenomenon.

The results show that BV expansion delays the GI transit of an aqueous charcoal meal in awake rats. BV expansion of 1 and 2% body weight had no significant effect on GI transit but BV expansion of 3, 4 and 5% body weight significantly decreased GI tran-

sit rates. This effect persisted for at least 60 min after expansion of 5% body weight.

The factors that influence the rate of propulsion of a meal through the small intestine are not completely understood. It has been demonstrated that a delay in gastric emptying may determine a delay in GI transit (17). In fact, we have reported that BV expansion delays the gastric emptying of liquid in awake rats (14,16). However, changes in small intestine transit time can occur independently of changes in gastric emptying (17) and the rate of gastric emptying can influence the transit of food down the first 70 cm of the human intestine, but have little or no influence down the entire small intestine in normal subjects (17).

In this respect, GI transit delay due to BV expansion may not be essentially determined by a gastric emptying delay, since BV expansion modifies jejunal compliance (10) and in animals submitted to fundectomy, BV expansion also did not delay gastric emptying, while the final GI transit was markedly delayed, thus indicating the activation of small bowel resistance by BV expansion (14).

Subdiaphragmatic vagotomy prevented the effect of BV expansion on GI transit. These findings indicate that vagal pathways are necessary for the full expression of the phenomenon. However, the effect of vagotomy may be incomplete since we still observed a tendency to a delay in GI transit after BV expansion in animals submitted to subdiaphragmatic vagotomy. Cholinergic pathways appear not to be involved since atropine did not block the effect of BV expansion on GI transit. L-NAME was also unable to prevent the effect of BV expansion on GI transit.

Yohimbine (an α_2 antagonist) prevented the effect of BV expansion on GI transit, while hexamethonium (a ganglion blocker), propranolol (a ß blocker) and prazosin (an α_1 antagonist) were ineffective. Alpha-2 receptors are located both in the central nervous system and peripherally (18). Our find-

Table 2 - Effect of drug pretreatment on mean arterial pressure (MAP) before and after 5% blood volume expansion (EXP) with *iv* infusion of Ringer-bicarbonate, 1 ml/min.

The number of animals is given within parentheses. *P<0.05 compared to basal MAP levels (Student-Newman-Keuls test).

Treatment	MAP	
	Before EXP	After EXP
Atropine (0.5 mg/kg)	100.1 ± 3.6	102.2 ± 3.9 (5)
L-NAME (2 mg/kg)	142.6 ± 5.6*	148.4 ± 3.8* (5)
Hexamethonium (10 mg/kg)	72.1 ± 3.4*	80.1 ± 4.2* (5)
Yohimbine (3 mg/kg)	98.1 ± 4.4	96.3 ± 4.2 (4)
Prazosin (1 mg/kg)	70.2 ± 3.4*	82.1 ± 4.2* (4)
Propranolol (2 mg/kg)	82.4 ± 3.4*	87.1 ± 4.2* (4)

ings suggest that central rather than peripheral α_2 receptors are activated by BV expansion, since peripheral activation would be associated with an increased sympathetic activity and thus would be blocked by hexamethonium and/or by prazosin and propranolol. Central α_2 activation causes a significant decrease in sympathetic activity (19), similar to that observed after BV expansion (20), and mediates GI motility inhibition (18). However, it has been demonstrated that yohimbine can also modulate vagal activity and act upon non-adrenergic receptors (21).

Our findings point to an involvement of neural pathways. However, increased CVP also leads to a release of several hormones and autacoids, such as the atrial natriuretic peptide (ANP), which could interfere with GI motility, absorption and secretion. In fact, it has been demonstrated that ANP can increase the magnitude of spontaneous duodenal phasic contractions (22) and reduces fluid and electrolyte intestinal absorption (23).

Hepatorenal and hepatointestinal reflex systems are of paramount importance for Na^+ and ultimately liquid homeostasis (24). BV expansion causes important homeostatic modifications to manage increased body liquid volume and attain a new steady state. Diuretic and natriuretic responses following BV expansion have been extensively studied (25), as well as decreased intestinal sodium/

water absorption and increased secretory rates (6). Even subtle postural changes (tilting and standing up) simulating BV changes might modify intestinal salt and water permeability rates in man (26).

Since GI motility is related to absorption and secretion (8), the delay in GI transit of liquid observed here, taken together with the absorptive and secretory modifications described by others (5,6), may be part of the body strategies involved in the management of liquid excess.

Acknowledgments

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