

## Neural Mechanisms and Delayed Gastric Emptying of Liquid Induced Through Acute Myocardial Infarction in Rats

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### Abstract

**Background:** In pathological situations, such as acute myocardial infarction, disorders of motility of the proximal gut can trigger symptoms like nausea and vomiting. Acute myocardial infarction delays gastric emptying (GE) of liquid in rats.

**Objective:** Investigate the involvement of the vagus nerve,  $\alpha$  1-adrenoceptors, central nervous system GABA<sub>B</sub> receptors and also participation of paraventricular nucleus (PVN) of the hypothalamus in GE and gastric compliance (GC) in infarcted rats.

**Methods:** Wistar rats, N = 8-15 in each group, were divided as INF group and sham (SH) group and subdivided. The infarction was performed through ligation of the left anterior descending coronary artery. GC was estimated with pressure-volume curves. Vagotomy was performed by sectioning the dorsal and ventral branches. To verify the action of GABA<sub>B</sub> receptors, baclofen was injected via icv (intracerebroventricular). Intravenous prazosin was used to produce chemical sympathectomy. The lesion in the PVN of the hypothalamus was performed using a 1mA/10s electrical current and GE was determined by measuring the percentage of gastric retention (% GR) of a saline meal.

**Results:** No significant differences were observed regarding GC between groups; vagotomy significantly reduced % GR in INF group; icv treatment with baclofen significantly reduced %GR. GABA<sub>B</sub> receptors were not conclusively involved in delaying GE; intravenous treatment with prazosin significantly reduced GR% in INF group. PVN lesion abolished the effect of myocardial infarction on GE.

**Conclusion:** Gastric emptying of liquids induced through acute myocardial infarction in rats showed the involvement of the vagus nerve, alpha1- adrenergic receptors and PVN. (Arq Bras Cardiol. 2015; 104(2):144-151)

**Keywords:** Rats; Gastric Emptying; Myocardial Infarction; Midline Thalamic Nuclei Receptors, GABA; Gastrointestinal Motility.

### Introduction

The gastric emptying (GE) process results from mechanism actions that inhibit or stimulate the motor activity of the stomach, pylorus and duodenum. The central nervous system connects itself with the enteric nervous system through the vagus nerve and sympathetic nervous system, participating in GE control<sup>1</sup>. The vagus nerve controls food movement throughout the digestive tract. If this mechanism is impaired, the stomach muscles and intestines do not function normally and food transportation slows down or stops completely. The dorsal vagal complex consists of the solitary tract nucleus with neurons that receive afferent information, area postrema and the dorsal nucleus of the vagus, where stimulatory and inhibitory motoneurons are located, with the axons being efferent pathways of the vagus nerve<sup>2</sup>. The dorsal vagal complex is more influenced by higher structures, such as the paraventricular nucleus of

hypothalamus, which under certain conditions can modify gastric motility and GE<sup>2-4</sup>. In rats, GABA<sub>B</sub> receptors are located in the presynaptic afferent endings of the vagus that project into the solitary tract nucleus<sup>5</sup>. Presynaptic GABA<sub>B</sub> receptors are involved in regulation of neurotransmitter release, as the effect of (an agonist for these receptors) is to reduce the release of stimulatory and inhibitory synaptic transmitters<sup>6,7</sup>. In pathological situations, such as acute myocardial infarction, disorders of motility of the proximal gut can trigger symptoms like nausea and vomiting<sup>8</sup>.

Experimental studies on the association of myocardial infarction and gastric emptying<sup>9,10</sup> are very rare, and one study<sup>10</sup> considered that delayed gastric emptying may be due to stress caused by ischemia. However, the underlying mechanisms of delayed gastric emptying were not addressed. Therefore, the present study aimed to determine the involvement of neural mechanisms related to delayed gastric emptying.

### Methods

Male Wistar rats (n = 8-15) were used to perform this experiment, weighing 220 to 300 g, supplied by the Central Animal Facility of Universidade Estadual de Campinas.

The study protocol was approved by SBCAL (Brazilian Society of Laboratory Animal Sciences) ([www.ib.unicamp.br/ceea/principios](http://www.ib.unicamp.br/ceea/principios)) (Protocol N<sup>o</sup>. 1021-2).

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Rats had an adjustment period of four weeks to laboratory conditions with controlled temperature (22 - 26°C) and artificial light cycle of 12 hours and were given water *ad libitum*. In surgical procedures such as vagotomy or implantation of cannula into the lateral brain ventricle or electrolytic lesion of the paraventricular nucleus, the rats were previously sedated with intra-peritoneal (*ip*) injection of thiopental 75mg/Kg. After the procedures or the study, the animals were kept in individual cages, receiving water and food *ad libitum*. In order to study GC, the animals were anesthetized with *ip* administration of ketamine (85mg/kg) + xylazine (10mg/kg).

The drugs prazosin (PRA) and baclofen (BAC) (both from Sigma, USA) were diluted at the time of the study, using sterile saline as vehicle (V).

### Myocardial infarction

Myocardial infarction was induced by ligation of the left anterior descending coronary artery, according to the technique recommended by Johns & Olson<sup>11</sup>. The rats were anesthetized with ether and thoracotomy was performed. Through gentle pressure applied to the right hemithorax, the heart was exposed and a ligature was performed around the proximal left coronary artery in its proximal segment, between the pulmonary artery cone and the left atrial apex. Only the animals with major infarction, i.e., involving 40% or more of the entire area of the left ventricle, were considered for the study. Twenty-four hours after the surgical procedure, studies were performed by measuring gastric compliance (GC), vagotomy, intracerebroventricular injection with GABA<sub>B</sub>, intravenous treatment with prazosin and electrolytic paraventricular nucleus lesions.

In order to measure GC, rats were divided into three groups twenty-four hours before: rats were submitted to myocardial infarction (INF), and also to simulated infarction (SH) and naive (NA). Only the INF and SH groups were used to determine GE. Rats in all groups were fasted and GE or GC were evaluated twenty-four hours after these groups were formed, between 2:00 pm and 5:00 pm, and access to water was cancelled an hour before the test.

### Gastric Complacence

The technique described by Bustorff-Silva et al<sup>12</sup> was used to measure GC. In brief, anesthetized rats were submitted to the following procedures: tracheotomy, abdominal incision, pylorus ligation, fixation of distal esophagus with an orogastric polyethylene tube filled with saline solution and connected through a three-way stopcock to an infusion pump (model LF 2001 Lifemed, Brazil) and a pressure monitor (Biomotor 7.0, BESE, Belo Horizonte - Brazil). Thirty minutes after these procedures, saline solution at 37 °C was infused into the stomach of each animal, at a rate of 1.5 mL/100g weight/min intermittently, every 20 seconds (s) at 1-minute intervals. Every 20s (1/3 the volume) the infusion was stopped and the system was balanced for 50s, while recording intragastric pressure (IGP). Intragastric pressure corresponding to 1/3, 2/3 and the total volume were recorded. The procedure was repeated twice with 30-min intervals. The results of each

animal corresponded to the mean of three measurements at each point of IGP. To estimate the GC, curves of volume/pressure were constructed and calculated through the following formula:

$$\text{Compliance (mL/mmHg)} = V_1 - V_0 / P_1 - P_0,$$

Where  $V_0$  = initial volume and  $V_1$  = final volume;

$P_0$  = initial IGP and  $P_1$  = final IGP.

### Gastric emptying

A saline solution containing phenol red (6mg/dl) with a volume of 1.5 mL/100g bodyweight was used as a test meal in the GE study. Gastric emptying was assessed indirectly in awaken rats, to determine the percentage of gastric retention (% GR) of test meal, after ten minutes of the orogastric administration, using a standardized technique<sup>13</sup>.

The rats were placed vertically and the test meal infused by gavage to reach the stomach. After the administration, the animals remained in the cage for 8 min and 30 sec. After being anesthetized with ether, the orogastric tube was introduced, keeping the animals anesthetized by ether inhalation. The abdomen was opened longitudinally and the pylorus clamped, exactly 10 minutes after orogastric infusion. All steps were timed. The gastric residue was aspirated and then five washes were performed with 2 mL of distilled water at each time, taking care to always aspirate using the same syringe. Complete emptying was confirmed by direct visualization of the viscera. The probe was then removed and negative pressure was applied to the euthanized animal. The gastric residue obtained plus the washings were transferred to a 25 mL graduated cylinder, and the aspiration tools (probe and syringe) were washed three times with one mL of water each time, and the volume was added to the beaker.

To determine the percentage of gastric retention (GR%) 2.0 mL were taken from the total volume recovered, and transferred in duplicate to 10 mL volumetric flasks, to which 5.0 mL of a trisodium phosphate solution were added at a concentration of 27.5 g/L. The same procedure was performed with one mL of the test meal. The final volume was completed to 10 mL with distilled water. The readings were made in a spectrophotometer (Spectrophotometer B 382, Micronal) at a wavelength of 560 nM. GR was calculated using the following formula:

$GR = \text{vrg} \times \text{arg} \div \text{vrp} \times \text{arp}$  and the result was multiplied by 100.

Where: vrg = volume of gastric residue; arg = gastric residue absorbance; vrp = volume of the test meal; arp = absorbance of the test meal.

### Vagotomy

Two weeks before the vagotomy study, rats underwent subdiaphragmatic vagotomy (VGX), in which the dorsal and ventral branches of vagus nerve were sectioned, while other animals were submitted to sham procedure (VGS), constituting the controls. Twenty-four hours before the procedure of GE, animals VGX and VGS, were submitted to surgical infarction, while others were submitted to the same simulated surgery.

### Intracerebroventricular baclofen injection

To study the involvement of GABA<sub>B</sub> receptors in the central nervous system eight days before the GE study, each animal, underwent implantation of a cannula (21G) in the right lateral ventricle, using techniques and coordinates related to bregma<sup>14</sup>, as previously described<sup>15</sup>. Twenty-four hours prior to the GE study, rats were submitted to surgical infarction (INF), while others were submitted to the same simulated surgery (SH). Ten minutes before GE assessment, the two groups were treated with an intracerebroventricular (*icv*) injection of 10 $\mu$ L of saline solution (V) or an equal volume of solution containing 1 $\mu$ g of BAC. The dose of BAC used was based on the literature<sup>16</sup>. GE was evaluated ten minutes after the end of the injection.

### Involvement of alpha1-adrenergic receptors

To study the involvement of alpha-1adrenergic receptors, both INF and SH animals, were treated intravenously (*iv*) through a tail vein, with saline as vehicle (V) or prazosin at a dose of 1mg/kg, 24 hours after the surgery, and dose given was based on the literature<sup>17</sup>. GE was evaluated fifteen minutes after the injection.

### Paraventricular nucleus of the hypothalamus lesion

To evaluate the involvement of the paraventricular nucleus, ten days prior to the GE study, the animals were submitted to a restricted PVN lesion (group PVNX) at two points, bilaterally, with passage of a 1 mA/10 s electrical current injury, using nickel and chromium electrodes with 0.25 mm in diameter. The coordinates in relation to the bregma, were the following: anterior-posterior (AP) -1.2 and -1.5 mm, lateral  $\pm$  0.5 mm, vertical 7.8 mm and 8.0 points corresponding to AP, as shown in another study<sup>15</sup>. In rats with sham lesion (PVNS), the same coordinates were used, except the vertical one, in which the depth was 7.5 mm, without the passage of an electrical current.

Twenty-four hours before the GE procedure, PVNX and PVNS animals were divided into INF and SH groups. After the experiments, all rats were euthanized. In the INF group, the hearts were removed, sectioned in the sagittal plane, using the left auricle, the interventricular sulcus and left ventricular outflow tract as reference points. Then, the two halves were fixed in 10% formalin and embedded in paraffin. The histological sections stained with hematoxylin-eosin were used to determine % of infarction area in relation to the entire area of the left ventricle, using a standard technique<sup>18</sup>.

In the PVNX group, the brains were removed, fixed, embedded in paraffin and histological sections were stained with toluidine blue. To confirm the site of injury, the sections, under microscopic view, were compared to the Paxinos & Watson<sup>19</sup> atlas in another study performed in the same laboratory<sup>15</sup>.

In animals with implantation of a cannula into the lateral ventricle, the assessment was made with an *icv* injection of 10 $\mu$ L of an Evans Blue solution at 1% at the end of the GE study. In this group, after euthanization, the brains were removed and fixed in 10% formalin, and coronal sections were obtained and confirmed when the dye injected *icv* was found in the fourth ventricle.

### Statistical Analysis

The SAS (Statistical Analysis System) for Windows software, version 9.2 (SAS Institute Inc., 2002-2008, Cary, NC, USA) was used in the statistical analysis.

The results of GC and GR are presented as mean  $\pm$  SEM. Statistical analysis was performed using ANOVA, followed by Tukey test when necessary. It was established the value of  $\alpha = 0.05$  for both tests.

### Results

There were no significant differences in GC in the comparison between groups (mean  $\pm$  SEM of group INF = 0.16  $\pm$  0.03 mL / mmHg, N = 9, group SH = 0.17  $\pm$  0.03 mL/mmHg N = 9, group NA = 0.16  $\pm$  0.01 mL / mmHg, N = 8). The percentage of infarcted area of left ventricle, mean  $\pm$  SEM, in rats of INF group was 47.8  $\pm$  2.7%.

The results of the study prior to the vagotomy are shown in Figure 1. There were significant differences between GR% of the animals in the VGS+SH vs. VGS+INF groups (mean  $\pm$  SEM = 36.6  $\pm$  2.0%, N = 10; and 48.0  $\pm$  2.3%, N = 15, respectively), which indicated that infarction determined delayed GE in animals with sham vagotomy when compared to their controls. Moreover, the VGX+INF subgroup presented significantly lower GR% (28.7  $\pm$  2.8%, N = 11) than the VGS + INF group and it did not differ from the VGX + SH group (25.9  $\pm$  1.5%, N = 10). However, the previous vagotomy also significantly reduced GR% in SH group (VGX + SH) when compared to the VGS group (VGS + SH). Therefore, the reduction in GR% caused by vagotomy in INF group was 40% higher. In this study, the infarcted area of left ventricle, mean  $\pm$  SEM, in VGS + INF animals was 51.7  $\pm$  2.3% and in VGX + INF animals was 50.2  $\pm$  2%.

Intracerebroventricular treatment with BAC (Figure 2) significantly reduced GR% in the control group (SH+BAC = 17,8  $\pm$  2,6%, N = 11) when compared to treatment with V (SH + V = 32,0  $\pm$  2,9%, N = 9), as well as in the INF +BAC group when compared to INF+V (26.0  $\pm$  3.3%, N = 11 and 40.2  $\pm$  2 1%, N = 10, respectively). Although myocardial infarction increased the GR% in the group of rats treated with vehicle (INF + V), this result did not significantly differ from its control group (SH + V). Additionally, it was found that the mean reduction in GR% determined by BAC was 25% higher in the SH groups than that observed in the INF groups. The infarcted area of the left ventricle, mean  $\pm$  SEM, in INF + V animals was 51.2  $\pm$  2.7% and 52,2  $\pm$  3 % in INF + BAC animals.

Figure 3 shows results from the *iv* treatment with PRA, which significantly reduced GR% in infarcted group (INF + PRA = 22.0  $\pm$  1.5%, 10) when compared to the vehicle-treated group (INF + V = 42.1  $\pm$  2.4%, 10). The same phenomenon occurred among animals from the SH group (SH + PRA = 22.3  $\pm$  1.8%, N = 12 vs SH + V = 30.5  $\pm$  1.3%, N = 11); however, GR% reduction was 77% higher in the INF group. The infarcted area of the left ventricle, in mean  $\pm$  SEM, in INF + V animals was 55.1  $\pm$  1.2% and 55.2  $\pm$  1.2% in INF + PRA animals.

Figure 4 shows the results of the lesion seen in the paraventricular nucleus in a prior study. Rats with electrolytic

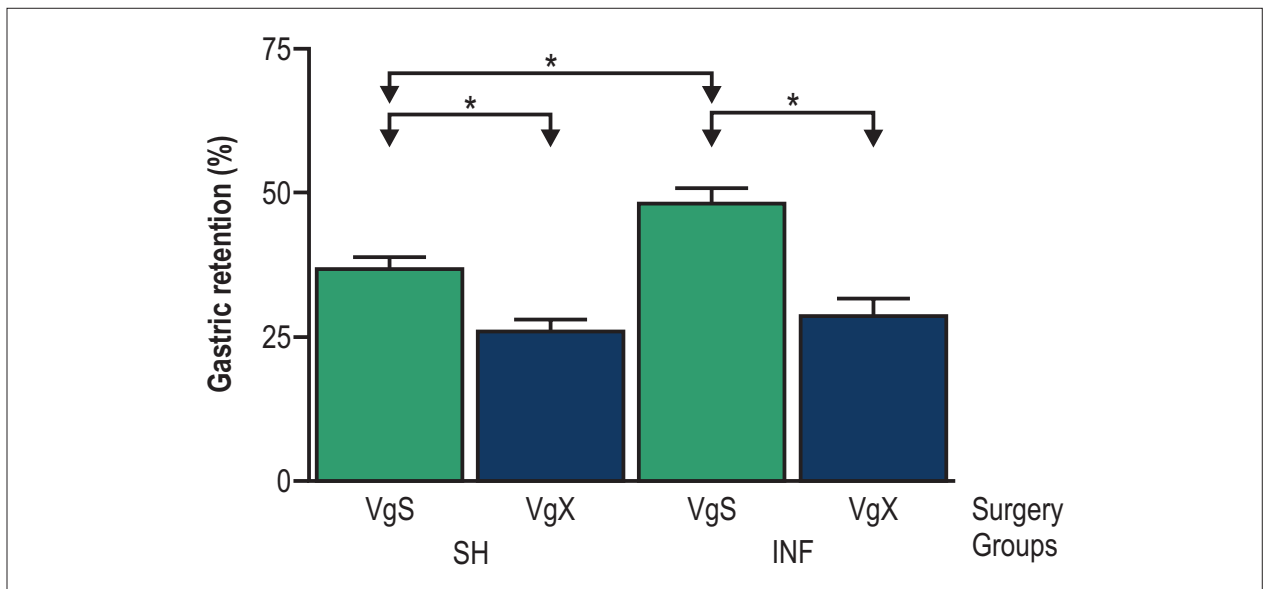


Figure 1 – Results of gastric retention (%) in groups of rats with myocardial infarction (INF) and vagotomy (Vg) \*  $p < 0.05$ .

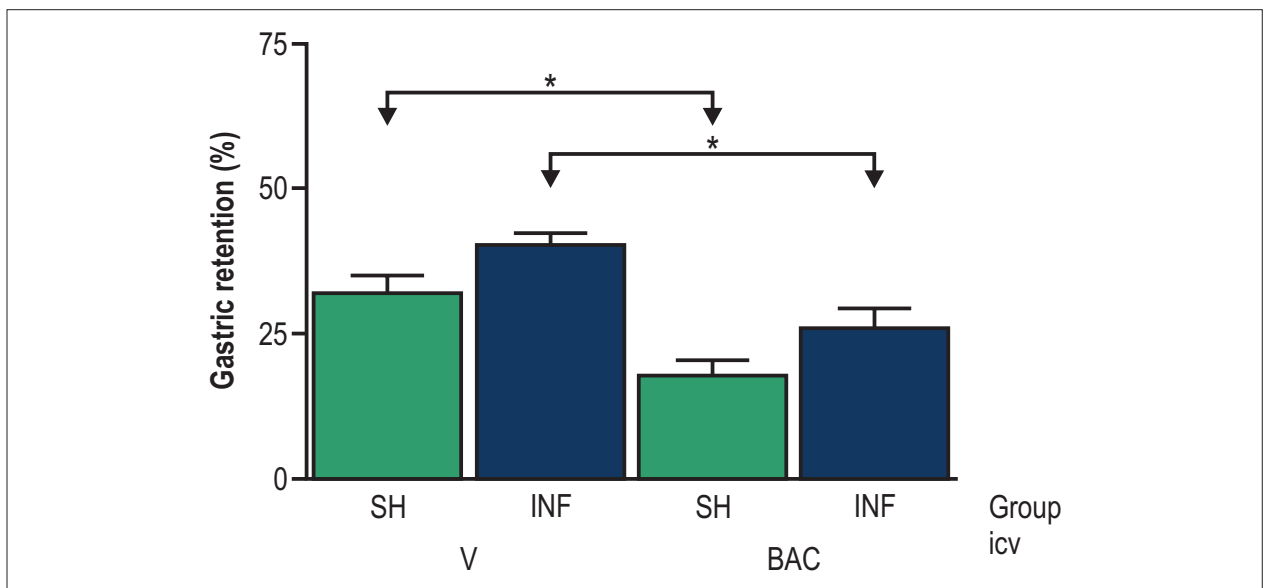


Figure 2 – Results of gastric retention (%) in groups of rats with myocardial infarction and inserted metal probe, with baclofen (BAC) or saline (V) injected into the lateral ventricle of the brain \*  $p < 0.05$ .

PVN lesion and infarction showed a significantly lower GR% (PVNX + INF =  $25.0 \pm 3.0\%$ , N = 8) compared to animals with sham lesion (PVNS) and infarction (PVNS+INF =  $41.2 \pm 1.7\%$ , N=8). The same lesion did not reduce GR% in control animals (PVNX + SH =  $28.5 \pm 2.9\%$ , N = 8 vs. PVNS + SH =  $32.1 \pm 1.9\%$ , N = 10). The infarcted area of the left ventricle, in mean  $\pm$  SEM, in PVNS + INF animals was  $52.2 \pm 0.4\%$  and  $55.4 \pm 0.8\%$  in PVNX + INF animals. As for the histological assessment of the brains after the PVN lesion, only lesions involving 100% of the paraventricular region were considered for this study.

## Discussion

Decrease in gastric tone determines slower GE of liquid<sup>20</sup>. Therefore, under the experimental conditions of this study, acute myocardial infarction did not induce changes in gastric tone in rats. However, as GE is a coordinated action of the stomach, pylorus and duodenum<sup>21</sup>, it is possible that the determinant factor of delayed GE in myocardial infarction did not act directly on the stomach. Camurça et al<sup>9</sup> detected that the transit of liquid in the small intestine is slower in acute myocardial infarction, in addition to delayed GE. It was unclear whether the two disorders are

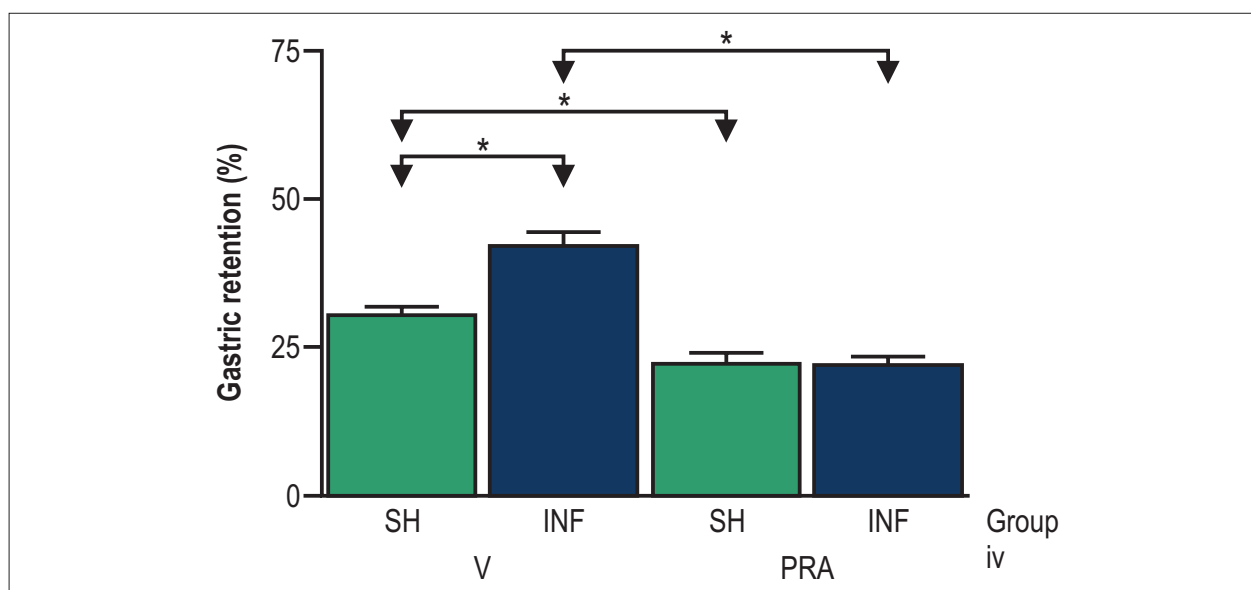


Figure 3 – Results of gastric retention (%) in groups of rats with myocardial infarction that received prazosin (PRA) or Saline (V) injection \*  $p < 0.05$ .

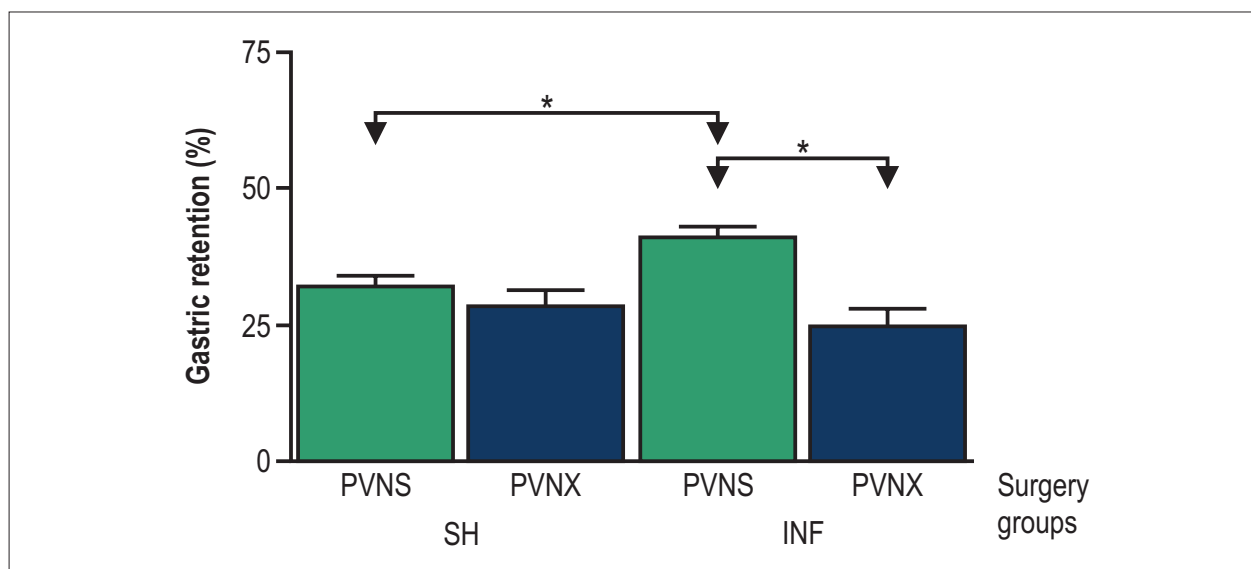


Figure 4 – Results of gastric retention (%) in groups of rats with myocardial infarction and lesions of the paraventricular nucleus \*  $p < 0.05$ .

dependent on the same phenomenon or if the effect on GE is a consequence of what occurs after the stomach. As no changes were observed in GC, an alternative speculation to explain delayed GE of liquid in myocardial infarction would be an increase the resistance, of unknown nature, to the meal flow into the small intestine.

Intracerebroventricular treatment with Baclofen significantly reduced GR% in control group (SH) when compared to controls treated with saline and, similarly, in the infarcted group (INF) (Figure 1). In comparative terms, it was surprising that the largest reduction occurred in the control groups. As a result, it was not

verified the involvement of GABA<sub>B</sub> receptors in delayed GE through INF. Baclofen in the central nervous system increases GE of liquids through its capacity to block the dorsal vagal complex, mechanical inhibitory stimuli that act on proximal stomach, conveyed through the afferent vagus nerve fibers<sup>22-25</sup>. As a result, there is an increased tone in this segment of functional stomach, leading to faster GE of a saline meal. What was expected, considering that the infarction did not modify gastric compliance and may have resulted in an increased tone with the same intensity in controls and infarcted groups, overcoming the condition that determined the delayed GE in infarction.

The previous sub-diaphragmatic section of the ventral and dorsal branches of the vagus nerve significantly reduced GR in infarcted animals and also in SH animals (Figure 3). The result observed between sham animals was expected, as this type of vagotomy can increase gastric emptying of liquid increasing the tone of the proximal stomach<sup>26</sup>. However, GR% reduction through vagotomy in infarcted animals was higher (approximately 40%), when compared to the mean reductions of GR% between the two groups.

Vagotomy modifies the motor activity of the stomach by blocking the arrival of inhibitory afferent stimuli to the solitary tract and abolishes the efferent stimuli, which originate in the dorsal vagal nucleus<sup>1,27,28</sup>. Afferent fibers of the vagus nerve carry sensory information from other regions of the gastrointestinal tract and the efferent pathways innervate from the gastric fundus to the descending colon<sup>29-30</sup>.

Thus, regarding the procedure employed in this study, it is unknown whether we are blocking afferent or efferent stimuli and in which part of the gastrointestinal tract. However, results suggest the participation, at least partially, of the vagus nerve in delayed GE induced by myocardial infarction.

The role of alpha1-adrenoceptors as stimulatory receptors particularly involves smooth muscle contraction, especially the contraction of vascular smooth muscle fibers, determining local vasoconstriction and acting on blood pressure control<sup>31</sup>. Prazosin is a peripheral antagonist that binds to these receptors in vessels<sup>32,33</sup> and has no significant influence on gastric tone and phasic contractions in the stomach<sup>34</sup>.

Intravenous treatment with Prazosin significantly reduced the effect of myocardial infarction on GE (Figure 3). This fact is related to the one proposed by Camurça et al<sup>9</sup>, that delayed GE in myocardial infarction would result of increased sympathetic activity as it was observed in the present study, with a possible involvement of the vascular system.

In addition, it was also found that there was a significant GR reduction in SH group, although the reason for this effect remains unknown. In a previous study, carried out in the same laboratory, on the effect of myocardial infarction on GE<sup>10</sup>, it was found that sham animals showed a non-significant GR increase, when compared with the naive group. This fact was attributed to the combined effect of anesthesia + surgery; these procedures were performed twenty-four hours before the sham group was created. This combined effect, if confirmed in this study, might be less intense at vascular level in the SH group than in the INF group and it can also explain the results of treatment with prazosin. Nevertheless, GR reduction induced through Prazosin was 77% higher in infarcted animals, suggesting involvement of alpha1-adrenoceptors in this condition.

The paraventricular nucleus (PVN) is a major integrative region of the hypothalamus that maintains homeostasis.

Practically, PVN is involved in food intake, response to stress and it also modulates metabolic rate and thermoregulation, and participates in regulation of cardiovascular function and the autonomic nervous system<sup>35</sup>. This hypothalamic structure participates in cardiovascular autonomic regulation<sup>36,37</sup>. Heart failure has been associated with changes in specific brain areas, as well as the activation of neurons in PVN, which are related to abnormalities in the production of vasopressin, blood volume regulation and sympathetic stimulation (excitation)<sup>38</sup>.

In this study, infarcted animals with electrolytic lesion of the PVN showed significantly lower GR when compared to infarcted animals with sham lesion. This type of lesion does not reduce GR% in control animals (Figure 4). These results indicate that the PVN lesion abolished the effect of recent myocardial infarction on GE in rats. This finding is an evidence of the important participation of PVN in GE delay in myocardial infarction. It is possible that the partial results obtained with vagotomy and alpha1-adrenergic blockade occurred due to the fact that each one of these procedures affected only one part of the set of changes under PVN effect, determining delayed GE in myocardial infarction.

## Conclusion

The results of this study suggest the involvement of the vagus nerve, the alpha1-adrenergic receptors and the PVN in the delayed GE induced by recent myocardial infarction in rats.

## Author contributions

Conception and design of the research: Nunes WRR, Collares EF, Almeida EA; Acquisition of data: Nunes WRR, Ozaki MR, Vinagre AM, Collares EF; Analysis and interpretation of the data: Nunes WRR, Ozaki MR, Collares EF, Almeida EA; Statistical analysis: Nunes WRR, Vinagre AM; Writing of the manuscript: Nunes WRR; Critical revision of the manuscript for intellectual content: Ozaki MR, Collares EF, Almeida EA.

## Potential Conflict of Interest

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## Study Association

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