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Research Article

The impact of the vagus nerve over gastric motility in spinal cord transection – an experimental model

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Abstract. Introduction. Cervical Spinal Cord Transection (SCT) determines is responsible with a delay in large bowel transit, but also determines an increase of the rate of functional gastric outlet syndrome (for both liquids and solids). The primary factor related to these outcomes is the loss of supra-spinal sympathetic inhibition. The aim of this study is to determinate the involvement of vagal pathways in GI alterations in SCT. Material and methods. We have studied 24 adult male Wistar rats, which had access to food and water ad libitum before the first phase of surgery. The animals were equally divided into three groups: SCT-group, SCT + cervical bilateral vagotomy (CBV) group control group. Results. A decreased parasympathetic control over the stomach (via CBV), causes an increase of the fundic region distention (intra-gastric volume growth), which, in our study, has proven to be responsible to an increase of the gastric volume and a delayed emptying in SCT+CBV model. Conclusions. In summary, the experiment itself is a preliminary phenomenon study of SCT and concomitant parasympathetic inhibition (via CBV) in which the results registered show that the increase of GV determines the increase of the gastric compliance in complete cervical SCT.

Key Words: spinal cord injury, gastric volume, vague nerve, laxatives, Wistar rats

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Introduction

The stomach's physiological function is that of a temporary reservoir for solid food and liquids; after food homogenization, the organ acts as a pump, sending its contents via the pylorus into the small intestine for subsequent absorption and digestion. The stomach's motility varies from one segment of the stomach to the other. Thus, the walls of the fundus and of the proximal part of the gastric body are involved in tonic contractions, while the distal part of the gastric body, together with the pyloric antrum and the pylorus itself, are responsible for implementing phasic contractions (Kelly 1981; Holmes 2012).

Gastric compliance phenomenon (GC) is best described as the ratio between intra-gastric volume (IV) and intra-gastric pressure (IP). The region of the stomach which is mainly involved in altering IV/IP ratio is the fundus of the stomach (Kelly 1981; Browning et al 2006; Tong&Holmes 2009), which has the highest capacity of adapting its' fibers to the intraluminal content of the stomach.

In physiologic conditions a decrease of the gastric tonus, determines an increase of the IV, which leads to a constant IP. It seems that the supra-spinal sympathetic inhibition is the main factor involved in the majority of the phenomena that appear related to gastric motility variations and alterations (Menardo et al 1987; Longo et al 1989).

Spinal cord transection (SCT) is associated to several imbalances at the level of the gastrointestinal (GI) system: a delayed large bowel evacuation (Menardo et al 1987), colon and anorectal functional alterations (Longo et al 1989). In experimental rat-models, several studies have reported a decrease of the distal colon's motility (Meshkinpour et al 1985). Few and controversial reports have addressed the alterations of the upper-GI's tract reservoir and pump functionality in SCT scenarios (both clinical and experimental) (Bueno et al 1978; Yamada 1987)

The aim of the current study was to determine the role that vagal pathways play in the gastrointestinal alterations for patients with SCT.

Material and methods

1. SCT protocol

All animals used for this study were treated according to the Helsinki protocols and in accordance with the "Guide for the Care and Use of Laboratory Animals" [DHEW Publication no. (NIH) 85-23, Bethesda, Maryland, USA]. An institutional Ethics' Committee approval was granted prior to any experimental steps being performed.

For this study, we have used 24 adult male Wistar rats (body weight ranging from 295-366 g) with unattended access to food and water before the first phase of surgery. Animals were equally divided into three groups: Spinal Cord Transection (SCT) group 1, SCT + Cervical Bilateral Vagotomy (CBV) group 2, and Sham/control group 3.

For the first phase of the surgical experimental model, ketamine and xilasine 2% (0.006 mg/g of body weight) were chosen to be administrated intramuscular (im.) in order to achieve animal sedation without myorelaxation. Experimental simulation of SCT – used for groups 1 and 2 – was initially designed to pe performed via a dorsal cervical laminectomy followed by an inter-spinous ligament dissection, exposing the seventh cervical (C7) and first thoracic (T1) vertebrae. At this level, SCT was completed using a fine cut scissor (SCT-group 1 – 8 animals). Same procedures + Cervical Bilateral Vagotomy (CBV) were performed 24 hours later for group 2 (SCT+CBV group – 8 animals).

Cervical laminectomy, without subsequent inter-spinous ligament dissection and SCT was done for the third group (Sham/control group – 8 animals).

After recovery from the anesthesia phase, successful SCT was assessed by careful inspection of the lesion with the aid of a 10x surgical loupe magnification system coupled to an optic light. A complete transection was confirmed in all cases and with concomitant clinical parameters' evaluation (i.e. presence of paraplegia, lack of nociception and somatic reflexes below the lesion, hyperreflexia/hyperextension of the tail, hematuria or sub-corneal hemorrhage, as well as urinary retention).

After initial surgery the animals were placed on separate warming pads in isolation cages, with water ad libitum, but without access to dry food, for postoperative day 0. Urinary bladder emptying in all SCT-animals was accomplished successfully by manual hypogastric compressions three to four times a day, performed by an assistant.

The second phase of the surgical procedure implied the following steps, for all 3 groups of experimental animals:

After the initial 24 hours under complete anesthesia with myorelaxation, using Urethrane 20% (1.2 g/kg body weight), injected intraperitoneal (ip.) we have dissected the right common carotid artery, the jugular vein and of the vagus nerve; the artery and nerve were surgically separated, inside the carotid sheath. Both artery and vein were distally ligatured without further transection and had a polyethylene catheter, filled with saline solution and heparin (500 U/ml) introduced downwards (towards the heart) for measurement of the mean arterial pressure (MAP) and heart rate (HR) of the animal, and to obtain a central venous access (in the event of intravascular medication administration needed) (Figure 1). A tracheostomy was also undertaken, at this stage, in order to facilitate spontaneous breathing (Figure 1).

2. Gastric volume (GV) measurement

Measurements of the gastric volume (GV) was obtained using a latex balloon catheter (3 ml total useful capacity, created from latex gloves fingertips - [outside diameter (OD): 1,5 mm; inside diameter (ID): 2 mm; 20 cm in length]) which was introduced per os and positioned in the proximal stomach (fundic area). The opposite end of the catheter was connected to a three-way valve and then to the lower end of an U-shaped glass reservoir (non-modifiable volume: 30 ml; ID: 2.5 cm), equipped with an electronic volume sensor coupled to a plethysmometer (model 7140, Ugo Basille®, Comerio, Italy) (Figures 2 and 3). A syringe was connected to the three-way valve to fill the balloon, the catheter and the reservoir with a pre-warmed (37°C) standard ionic solution (0,3 ml Imbebiente BBC ® 97, – and 99.7 ml of 45 mg% NaCl).

The reservoir's liquid level baseline was that of 4 cm above the animal's sternal xyphoid appendix, in order to simulate the basal intra-gastric pressure value of 4 cm H_2O.

The gastric balloon (volume) changes were transmitted through the vessel system to the reservoir which displayed the real-time values using the plethysmomether (Figure 3). The gastric volume (GV) values (in ml) were recorded every 1 out of 10 min of monitoring. The GV variations were studied by modifying the height of the reservoir (from 4 cm above the xyphoid process, to 8 cm, 12 cm), thus artificially augmenting the IP.

As a general principle, due to its large volumetric capacity, the reservoir functions as a barostat – distending the stomach under

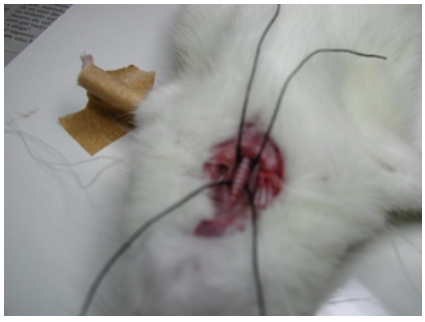

Fig. 1. Trachea exposed prior to tracheostomy; carotid sheath also exposed

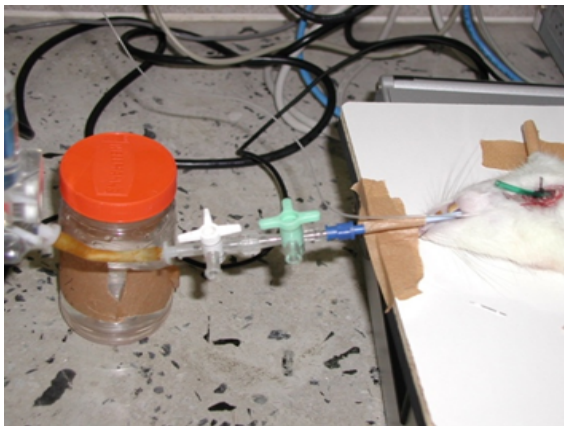

Fig. 2. The GV measuring system (note the three-way valve system)

Fig. 3. Example of GV displayed by the plethysmomether (note the close proximity of the experimental rat model)

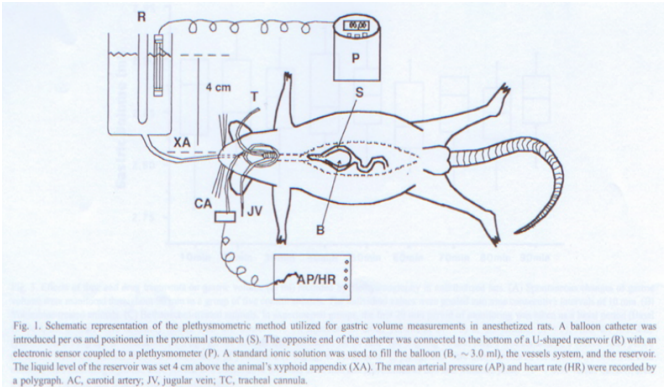

Fig. 1. Schematic representation of the plethysmometric method utilized for gastric volume measurements in anesthetized rats. A balloon catheter was introduced per os and positioned in the proximal stomach (S). The opposite end of the catheter was connected to the bottom of a U-shaped reservoir (R) with an electronic sensor coupled to a plethysmometer (P). A standard ionic solution was used to fill the balloon (B, ~ 3.0 ml), the vessels system, and the reservoir. The liquid level of the reservoir was set 4 cm above the animal's xyphoid appendix (XA). The mean arterial pressure (AP) and heart rate (HR) were recorded by a polygraph. AC, carotid artery; JV, jugular vein; TC, tracheal cannula.

Fig. 4. Schematic representation of the plethysmometric method utilized for gastric volume measurements (Graca et al 2000)

constant pressure. In this manner, real-time measurements of GV indicate gastric compliance (as per ratio described above). For the statistical analysis of GV variation data, the information was obtained from monitoring each animal (10 min at every pressure increase) and registered in distinct experimental groups Microsoft Excel® Databases. Furthermore, the overall global results for each separate group of experimental animals were calculated as arithmetical mean of the results obtain from each individual animal.

3. Cardiovascular parameters

Real-time continuous mean arterial pressure (MAP) was determined by connecting the common carotid catheter to a pressure transducer (P 100B, Narco-Biosystems®, Houston TX, USA), which was furthermore coupled to a polygraph (Mark IV, Narco-Biosystems®, Houston TX, USA). MAP values (mm Hg), were recorded every minute. At every 10-min interval, the chart speed was increased from 0.025 to 1 cm/s during 1 min thus accurately calculating the heart rate (HR) values (measured in beats per minute – BPM).

The entire experimental model's design is best described by the schematic representation in Figure 4.

4. Statistical analysis

Cardiovascular and GV data were assessed after monitoring each animal for 10 min at every pressure increase. The overall global results for each separate group (1 to 3) were calculated as arithmetical mean of the results obtained from each individual animal separately.

Further on, the results were expressed as Mean ± SEM and graphically represented as columns, using the same descriptive statistic software, as mentioned above. The Unpaired t-Student test was used to compare the differences in GV between Sham/control group 3 and SCT-group 1, SCT+CBV group 2. The comparison was made for every pressure value variation (4, 8 and 12 cm H2O respectively) recorded at every 10 minutes.

The differences between MAP and HR in all experimental groups were interpreted using the sum between the arithmetical mean and the standard deviation (SD) of the values measured after pressure alteration (after 4, 8 and 12 cm H_2O). A p value < 0.005, was considered statistically significant.

All statistical data resulted from using the Graph Pad ® software.

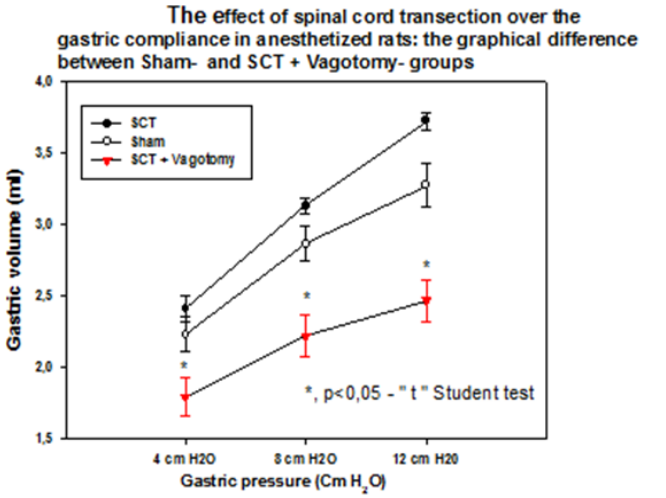

Fig. 5. The effect of SCT over gastric compliance (IP/IV) and different IP for the three studied groups

Results

Figure 5 demonstrates that CBV (SCT+CBV group 2) is involved in a decrease of the GV at each gastric pressure (IP) augmentation (1.79±0.13; 2.22±0.14; 2.47±0.14 ml; $p<0.05$) when compared to Sham/control group 3 (2.4±0.1; 2.87±0.1; 3.27±0.12 ml). Table 1 schematically describes that MAP values, measured at a 4 cm H_2O pressure, increase after CBV (97.1±24.9 mm Hg) in comparison to plain SCT-animals from group 1 (65.8±22 mm Hg); however, the reported HR values do not vary after CBV.

In Figure 6 one can observe the same statistical relevant data concerning the addition of CBV to SCT in the decrease of the GV values (2.44±0.07; 2.78±0.08; 2.96±0.16 ml; $p<0.05$). This statement can be done, only when comparing the results to those of the control group 3.

Discussion

As reported by several other studies (Graca et al 2000; Bueno et al 1978; Yamada 1987; Osborn et al 1990; Atkinson & Atkinson 1996; Maiorov et al 1997; Gondim et al 1998; Tong et al 2011), in the event of a traumatic or artificial SCT, the basal adrenergic activity decreases in both humans and experimental animals, during the spinal shock and hyper-reflexia phase. This indicates that a potential baseline or hyperactive parasympathetic response, involving the remnant vagal pathways are responsible with the inhibition of GI motility is one of the possible players affecting the gastric evacuation GE alterations after SCT (Graca et al 2000; Gondim et al 1999; Gondim et al 2001; Beyak et al 2006). Lower limb paraplegia, leading to a decrease in physical activity, a general phenomenon after SCT, could also contribute to enhancing the decrease of the GI motility, thus leading to an increase of the GV (Fealey et al 1984; Osborn et al 1990; Maiorov et al 1997; Gondim et al 1999; Gondim et al 2001). The complete absence of supra-spinal control over the paravertebral sympathetic centers, after SCT, could also be a true or possible factor leading to inappropriate sympathetic misfires, after visceral stimulation, such as cavitary organs volumetric distension, in relations to their contents (Segal et al 1987, 1995; Kao et al 1998; Gondim et al 1999; Gondim et al 2001; Zheng et al 2005; Beyak et al 2006; Weaver et al 2006).

Table 1. HR and MAP variations between the three groups of experimental rats

	MAP (mm Hg)				HR (BPM)			
	Basal	**4 cm H_2O**	**8 cm H_2O**	**12 cm H_2O**	**Basal**	**4 cm H_2O**	**8 cm H_2O**	**12 cm H_2O**
SCT group 1	63.5±21.1	65.8±22	70.8±21.2	71.5±21.5	227.1±25.2	237.7±33.0	222.4± 25.4	214.7±28.2
SCT + CBV group 2	74.1±19.9	97.1±24.9	105.5±23.5	100±21	253.3±70.1	236.8±56.3	225.1±45.8	217.3±43.9
Sham/control group 3	88.2±14.4	86.4±17.6	85.2±14.2	92.5±20	427.0±19.2	422.3±23.3	398.5±12.3	380.2±20.2

In our experimental model we have tried to completely reduce the parasympathetic control over the stomach (via the vagus nerve), by performing CBV, thus trying to annihilate the increase of the distention of the proximal fundical region (intra-gastric volume growth). Since normal basal values of the fundical intraluminal pressure is of 4 cm H_2O, and the values of 8 and 12 cm H2O can be reached only in experimental situations (such as ours) or in isolated physiological or artificial states (i.e. ingestion of effervescent liquids, pylorus stenosis etc.), our preliminary studies are relevant mainly for an observational study of the phenomenon of SCT.

The catastrophic effects seen in a collapse of the MAP, observed in group 2, where CBV was associated to SCT, is one other drawback of our experimental model, which cannot extrapolate our results in the clinical setting (CBV is rarely or never seen as a concomitant post-traumatic state in SCT patients).

Conclusions

To summarize, our preliminary experimental model is purely an observational phenomenon study of a hypothetical association of SCT to CBV. Our results, reveal that an increased IV in SCT animals, is a prerequisite of an increase in gastric compliance, and that one of the potential influential factors could be the influence of a baseline or hyper-active isolated remnant parasympathetic system (manifested at the GI level, via the vagus nerve). If CBV is performed, thus interrupting vagal pathways, this influence is lost.

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