



## Effects of nuclei ambiguus and dorsal motor nuclei of vagus on gastric $H^+$ and $HCO_3^-$ secretion in rats

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### Abstract

**AIM:** To determine the effects of electrical stimulation of nucleus ambiguus (NA) and dorsal motor nuclei of vagus (DMV) on gastric acid and bicarbonate secretion in rats.

**METHODS:** NA and DMV in rats were electrically stimulated. Pylorus ligation or esophagus perfusion was used to collect the gastric secretion. The titratable  $H^+$  quantum,  $H^+$  concentration,  $HCO_3^-$  secretion quantum were measured.

**RESULTS:** Electrical stimulation of NA had no effects on the volume of gastric juice, titratable acidity and acid concentration, but elicited a pronounced increase in the total bicarbonate. However, electrical stimulation of DMV significantly increased the titratable acidity, the volume of gastric juice and the acid concentration. Similarly, electrical stimulation of either NA or DMV decreased the respiratory frequency and sinus bradycardia.

**CONCLUSION:** NA in rats can not control the secretion of gastric acid but the secretion of bicarbonate in gastric juice, while DMV controls the secretion of gastric acid.

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**Key words:** Rat; Nucleus ambiguus; Dorsal motor nuclei of vagus; Gastric acid; Gastric bicarbonate

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### INTRODUCTION

The knowledge of brain stem nuclei controlling vagal parasympathetic outflow to the gastrointestinal tract has been derived principally from anatomic and physiological investigations of the dorsal motor nuclei of vagus (DMV). It was reported that electrical stimulation or lesion of DMV results in pronounced alterations in gastric motility and secretory function<sup>[1-4]</sup>. However, Kerr<sup>[2]</sup> showed that DMV is not the only origin of the vagal parasympathetic nerve innervating the stomach and vagal parasympathetic neurons might come from the nucleus ambiguus (NA). Studies indicate that the increased gastric motility in cold water-immersion stress rats is significantly inhibited, but there is no change in gastric acid secretion in rats pretreated with narceine or pentobarbital sodium<sup>[5,6]</sup>. These results suggest that the primary parasympathetic center innervating gastric motility and acid secretion consists of different nuclei. Moreover, recent morphological studies suggest that vagal parasympathetic neurons innervating the stomach are largely located in DMV and partly in NA<sup>[7-9]</sup>. So far, whether there are differences in the functions of DMV and NA in controlling gastric secretion remains unknown.

A great number of morphologic and physiological studies suggest that parasympathetic preganglionic neurons of the heart are located in DMV, NA and intermediate zone<sup>[10-13]</sup>. Previous experimental data have established that electrical stimulation of NA induces bradycardic responses, which can be abolished by injection of lidocaine into the rostral ventrolateral medulla<sup>[14,15]</sup>.

It was reported that the medulla is the essential center of respiratory rhythm in mammals<sup>[16]</sup>. Respiratory neurons can be divided into two groups according to their positions: dorsal respiratory group and ventral respiratory group. NA is a component of ventral respiratory group<sup>[17]</sup>. A group of respiratory neurons in the rostral NA complex is involved in the generation of inspiratory and expiratory drives which enables spontaneous respiration<sup>[18]</sup>. The aim of this study was to investigate the effects of electrical stimulation of NA and DMV on gastric acid and bicarbonate secretion as well as respiratory frequency and heart rate.

### MATERIALS AND METHODS

#### Animals

Male Wistar rats weighing 270-310 g were provided by

**Table 1** Effects of stimulation of NA on gastric H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> secretion after pylorus ligation (mean ± SD)

	<i>n</i> = 8	Gastric juice (mL/3 h)	Titratable H <sup>+</sup> quantum (μmol/3 h)	H <sup>+</sup> concentration (mol/L)	HCO <sub>3</sub> <sup>-</sup> quantum (μmol/3 h)
Right	Control group	0.35 ± 0.18	47.6 ± 14.1	0.17 ± 0.08	11.0 ± 1.85
Right	Stimulus group	0.45 ± 0.24	43.1 ± 13.0	0.12 ± 0.09	15.8 ± 1.46 <sup>b</sup>
Left	Control group	0.48 ± 0.23	46.6 ± 20.1	0.11 ± 0.05	10.8 ± 3.2
Left	Stimulus group	0.70 ± 0.30	58.0 ± 17.0	0.09 ± 0.03	17.2 ± 1.7 <sup>b</sup>

<sup>b</sup>*P* < 0.01.

Experimental Animal Center of Shandong University, China. The animals were individually housed in cages at 22 ± 2 °C with free access to food and water in a normal day/night cycle. All experiments were conducted according to the guidelines of the International Association for the Study of Pain<sup>[19]</sup> and every effort was made to minimize both the animal suffering and the number of animals used.

### Experimental methods

The rats were intra-abdominally anesthetized with urethane (1 g/kg) and placed in a Jiangwan type I stereotaxic apparatus (made in China). The dorsal surface of the brain stem was exposed by limited occipital craniotomy. According to the coordination of the NA as defined by the atlas<sup>[20]</sup>, an electrode (40-60 μm in diameter and 10-16 kΩ in resistance) was inserted vertically into the right or left side of the brain stem at a level 12.7 mm caudal to Bregma and 2.0 mm lateral to the midline, and to a depth of 9.6 mm below the dorsal surface. In the study on DMV, the electrode was inserted vertically into the right or left side of the brain stem at a level 13.8 mm caudal to Bregma and 0.7 mm lateral to the midline, and to a depth of 8.3 mm below the dorsal surface. Stimulation came from a BL-410 biological experimental system (Chengdu Taimeng Company, China). Stimulus parameters were 0.1 mA, 0.3 ms, 40 Hz and the stimulus duration was 10 min.

To study the effects of stimulation of NA and DMV on gastric secretion, pylorus ligation was used. A 2-mm tracheal cannula was inserted into the tracheal. The site between pylorus and duodenum was ligated. Three hours later, the site between cardia and esophagus was also ligated. The stomach was removed and gastric secretion was collected. Indices used to study gastric secretion were the volume of gastric juice, titratable H<sup>+</sup> quantum, H<sup>+</sup> concentration, HCO<sub>3</sub><sup>-</sup> quantum. Values for titratable H<sup>+</sup> quantum were determined by back titration to pH 7.0 using 0.01 N NaOH. HCO<sub>3</sub><sup>-</sup> secretion was determined as previously reported<sup>[21]</sup>.

In the study on NA, we also used the method of esophagus perfusion to collect gastric secretion. A 2.5-mm tracheal cannula was inserted into the tracheal. A polyethylene tube (2 mm in diameter) was inserted into the esophagus to perfuse warm (37 °C) normal saline at 2.0 mL/min. A 3-mm polyethylene tube was inserted into the stomach from the joint of cardia and duodenum to collect the gastric secretion. Base-line secretion values were determined from three consecutive 20-min values before stimulation. The three consecutive 20-min values were

**Table 2** Effects of stimulation of DMV on gastric H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> secretion after pylorus ligation (mean ± SD)

	<i>n</i> = 8	Gastric juice (mL/3 h)	Titratable H <sup>+</sup> quantum (μmol/3 h)	H <sup>+</sup> concentration (mol/L)	HCO <sub>3</sub> <sup>-</sup> quantum (μmol/3 h)
Right	Control group	0.62 ± 0.17	49.0 ± 12.5	0.08 ± 0.02	11.0 ± 3.4
Right	Stimulus group	1.00 ± 0.23 <sup>a</sup>	109.0 ± 21.7 <sup>b</sup>	0.11 ± 0.01 <sup>a</sup>	10.2 ± 2.6
Left	Control group	0.67 ± 0.14	60.8 ± 14.3	0.09 ± 0.02	9.0 ± 3.6
Left	Stimulus group	0.97 ± 0.21 <sup>a</sup>	115.8 ± 16.5 <sup>b</sup>	0.13 ± 0.03 <sup>a</sup>	11.5 ± 3.1

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01.

taken from beginning of the stimulation of NA to assess the change of secretion function. The index used to study gastric secretion was titratable H<sup>+</sup> quantum. Values for titratable H<sup>+</sup> quantum were determined by back titration to pH 7.0 using 0.01 N NaOH.

Respiration and electrocardiogram were continuously monitored using the BL-410 biological experimental system. Respiratory frequency and heart rate during stimulation were determined from the first 30 s of the stimulation period. Rectal temperature was monitored and maintained at 37-38 °C with a bulb.

### Histological identification of stimulated sites

At the end of the experiments, the direct current of 1 mA was given to the stimulated site in the brain stem for 15 s. Then, 0.9% sodium chloride solution and 1% potassium ferrocyanide formalin solution were injected respectively into the aorta and out flowed from the right atrium. The brain was removed and fixed in a 10% formalin solution for 2-3 d. The brains were sectioned at 40 μm with a freezing microtome. Histological sections were stained with neutral red and examined microscopically. Only the results obtained when the tips of the stimulus electrode were just within the NA were used for statistical analysis.

### Statistical analysis

Data were expressed as mean ± SD. The difference between two groups was evaluated by Student's *t* test. *P* < 0.05 was considered statistically significant.

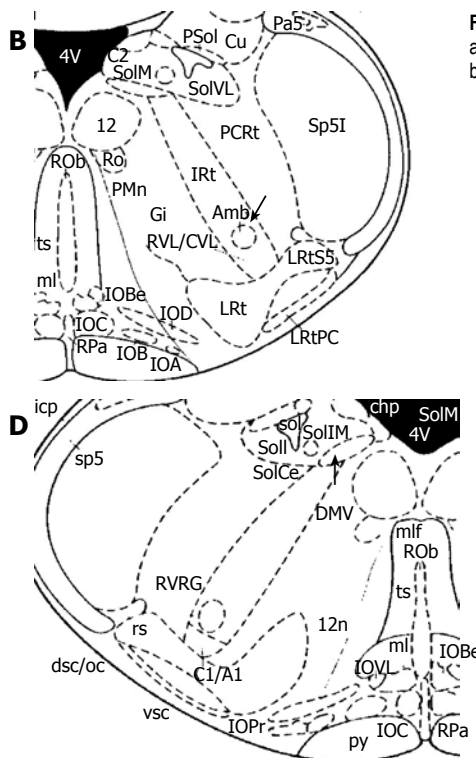
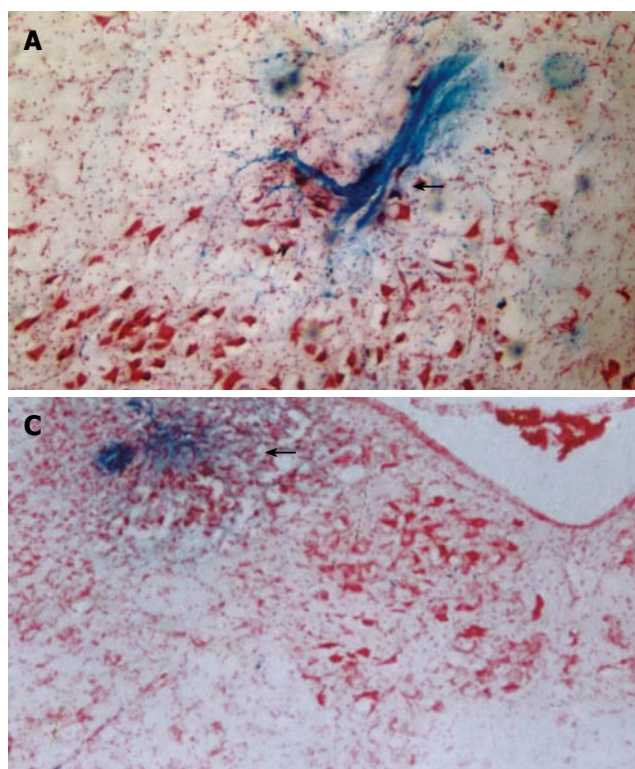
## RESULTS

### Histological identification of stimulated sites

The NA and DMV with their corresponding brain atlas are shown in Figure 1. The stimulated nuclei were identified according to the right atlas.

### Effects of stimulation of NA and DMV on gastric H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> secretion

Electrical stimulation of NA had no effects on the volume of gastric juice, titratable acidity and acid concentration, but elicited a pronounced increase in the total bicarbonate (*n* = 8, *P* < 0.01, Table 1). Electrical stimulation of DMV significantly increased the titratable acidity, the volume of gastric juice and acid concentration, with no change in bicarbonate quantum after pylorus ligation (Tables 1 and 2) and in titratable H<sup>+</sup> quantum after oesophagus perfusion (Figure 2).



**Figure 1** NA (A), DMV (C) and their corresponding brain atlas (B, D).

**Table 3** Effects of stimulation of NA and DMV on respiratory frequency and heart rate (mean  $\pm$  SD)

	<i>n</i> = 8	Respiratory frequency (times/min)	Inhibitory rate of respiratory frequency (%)	Heart rate (beats/min)	Inhibitory rate of heart rate (%)
Right NA	Control	91.2 $\pm$ 19.4		394.3 $\pm$ 70.1	
	Stimulation	34.9 $\pm$ 23.0 <sup>b</sup>	51.5 $\pm$ 17.3	261.7 $\pm$ 77.1 <sup>b</sup>	29.1 $\pm$ 8.9
Left NA	Control	101.8 $\pm$ 23.2		385.1 $\pm$ 37.6	
	Stimulation	45.6 $\pm$ 19.8 <sup>b</sup>	64.7 $\pm$ 19.7	244.5 $\pm$ 44.3 <sup>b</sup>	29.4 $\pm$ 12.3
Right DMV	Control	100.3 $\pm$ 7.6		402.8 $\pm$ 47.9	
	Stimulation	48 $\pm$ 11.5 <sup>b</sup>	51.6 $\pm$ 12.3	268.5 $\pm$ 62.4 <sup>b</sup>	34.2 $\pm$ 9.7
Left DMV	Control	102.8 $\pm$ 13.0		412.5 $\pm$ 33.3	
	Stimulation	60 $\pm$ 11.4 <sup>b</sup>	41.3 $\pm$ 11.4	295.5 $\pm$ 52.2 <sup>b</sup>	28.4 $\pm$ 10.7

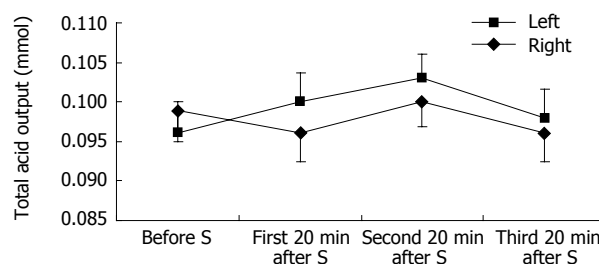
<sup>b</sup>*P* < 0.01.

### Effects of stimulation of NA and DMV on respiratory frequency and heart rate

Both respiratory frequency and heart rate were inhibited significantly after stimulation of NA and DMV (*n* = 8, *P* < 0.01). There were no significant differences in the inhibitory rate of NA and DMV (Table 3).

## DISCUSSION

To our knowledge, the effects of NA and DMV on gastric H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> secretion have not been examined up to now. In the present study, electrical stimulation of NA produced pronounced changes in HCO<sub>3</sub><sup>-</sup> secretion but no changes in H<sup>+</sup> secretion. However, electrical stimulation of DMV produced pronounced changes in H<sup>+</sup> secretion but no changes in HCO<sub>3</sub><sup>-</sup> secretion. The results of gastric H<sup>+</sup> secretion after stimulation of NA were consistent with those after pylorus ligation and



**Figure 2** Effect of stimulation of NA on gastric acid secretion after oesophagus perfusion (S: stimulation).

oesophagus perfusion. Pagani *et al*<sup>[22]</sup> found that electrical stimulation of cat NA produces no changes in gastric acid secretion. Morphological studies suggested that vagal parasympathetic neurons innervating the stomach are largely located in DMV and partly in NA<sup>[7,11]</sup>. These results indicate nervous fibers from NA and DMV innervate different cells of the stomach. NA innervates the nonacid-secreting cells and DMV innervates the acid-secreting cells. Our previous study also showed that gastric motility decreases significantly after stimulation of rat NA. Although this result is not consistent with that of Pagani *et al*<sup>[22]</sup>, the gastric motility changes when NA is stimulated. All the results suggest the nervous fibers from NA mainly innervate gastric smooth muscles while the fibers from DMV innervate both gastric glands and gastric smooth muscles, which is consistent with the report of Kerr<sup>[2]</sup>.

Morphologic and physiological studies suggest that parasympathetic preganglionic neurons of the heart are located in DMV, NA and intermediate zone<sup>[12]</sup>, especially in NA. The NA, DMV, caudal ventrolateral medulla (CVLM) and lateral tegmental field (FTL) are located in the cardio-inhibitory area<sup>[23]</sup>. Electrical stimulation of NA and DMV



induces bradycardic responses, and the inhibitory rate of NA to the heart rate is not significantly different from that of DMV. Machado and Brody<sup>[15]</sup> also reported that electrical stimulation of NA induces bradycardic responses, which can be abolished by injection of lidocaine into the rostral ventrolateral medulla. NA efferent fascicles contain more large fibers (presumably B-type), whereas the DMV contains more fine caliber fibers (presumably C-type), and vagal control of the heart involves the convergence and integration of distinct NA and DMV projections within the cardiac plexuses<sup>[12]</sup>. In our study electrical stimulation of NA and DMV significantly decreased the respiratory frequency, and the inhibitory rate of respiratory frequency in NA was not significantly different from that in DMV. Rentero *et al*<sup>[24]</sup> investigated the activity pattern of cardiac motoneurons in rat NA using extra-cellular recordings and found that cardiac vagal motoneuron firing is modulated by the central respiratory cycle, showing peak activity during inspiration, which may be the mechanisms of respiratory sinus arrhythmias.

Both NA and DMV are the visceral motonucleus and located in the medullary visceral zone (MVZ), named "life center" which modulates the activities such as circulation, respiration, and digestion<sup>[25]</sup>. In our study, electrical stimulation of NA significantly increased HCO<sub>3</sub><sup>-</sup> secretion, but induced no changes in H<sup>+</sup> secretion. In contrast, electrical stimulation of DMV significantly increased H<sup>+</sup> secretion, but induced no changes in HCO<sub>3</sub><sup>-</sup> secretion. Similarly, stimulation of NA and DMV induced respiratory sinus arrhythmias. All these findings indicate that the effects of NA and DMV on gastric secretion, respiration and heart rate are mediated by the vagus.

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