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

Xue-Ying Zhang, Hong-Bin Ai, Xi-Yun Cui

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 quantums 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.

© 2006 The WJG Press. All rights reserved.

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

Zhang XY, Ai HB, Cui XY. Effects of nucleus ambiguus and dorsal motor nuclei of vagus on gastric $H^+$ and $HCO_3^-$ secretion in rats. World J Gastroenterol 2006; 12(20): 3271-3274

http://www.wjgnet.com/1007-9327/12/3271.asp

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. However, Kerr 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 narcine or pentobarbital sodium. 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. 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. 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.

It was reported that the medulla is the essential center of respiratory rhythm in mammals. 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. A group of respiratory neurons in the rostral NA complex is involved in the generation of inspiratory and expiratory drives which enables spontaneous respiration. 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\(^+\) and HCO\(_3\)\(^-\) secretion after pylorus ligation (mean ± SD)

|        | Gastric juice | Titratable H\(^+\) quantum | H\(^+\) concentration | HCO\(_3\)\(^-\) quantum |
|--------|---------------|-----------------------------|------------------------|------------------------|
|        | (mL/3 h)      | (μmol/3 h)                  | (mol/L)                | (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           |
| 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            |

Table 2 Effects of stimulation of DMV on gastric H\(^+\) and HCO\(_3\)\(^-\) secretion after pylorus ligation (mean ± SD)

|        | Gastric juice | Titratable H\(^+\) quantum | H\(^+\) concentration | HCO\(_3\)\(^-\) quantum |
|--------|---------------|-----------------------------|------------------------|------------------------|
|        | (mL/3 h)      | (μmol/3 h)                  | (mol/L)                | (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 ± 0.25                 | 109.0 ± 21.7           | 0.11 ± 0.01            | 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                 | 115.8 ± 16.5           | 0.13 ± 0.03            | 11.5 ± 3.1            |

*P < 0.05, *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\(^{19}\) 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\(^{20}\), 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). Stimulation 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 volume of gastric juice, titratable H\(^+\) quantum, H\(^+\) concentration, HCO\(_3\)\(^-\) quantum. Values for titratable H\(^+\) quantum were determined by back titration to pH 7.0 using 0.01 N NaOH. Values for titratable H\(^+\) 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\(^+\) and HCO\(_3\)\(^-\) 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\(^+\) quantum after oesophagus perfusion (Figure 2).
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 $\text{H}^+$ and $\text{HCO}_3^-$ secretion have not been examined up to now. In the present study, electrical stimulation of NA produced pronounced changes in $\text{HCO}_3^-$ secretion but no changes in $\text{H}^+$ secretion. However, electrical stimulation of DMV produced pronounced changes in $\text{H}^+$ secretion but no changes in $\text{HCO}_3^-$ secretion. The results of gastric $\text{H}^+$ secretion after stimulation of NA were consistent with those after pylorus ligation and esophagus perfusion. Pagani et al.[22] 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[7,11]. 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.[22], 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[23].

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

### Table 3 Effects of stimulation of NA and DMV on respiratory frequency and heart rate (mean ± SD)

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

*$P < 0.01$.*
induces bradycardic responses, and the inhibitory rate of NA to the heart rate is not significantly different from that of DMV. Machado and Brody\textsuperscript{[15]} 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 cardiacplexuses\textsuperscript{[12]}. 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 \textit{et al.}\textsuperscript{[13]} 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 motoneuraxis and located in the medullary visceral zone (MVZ), named “life center” which modulates the activities such as circulation, respiration, and digestion\textsuperscript{[17]}. In our study, electrical stimulation of NA significantly increased HCO\textsubscript{3} secretion, but induced no changes in H\textsuperscript{+} secretion. In contrast, electrical stimulation of DMV significantly increased H\textsuperscript{+} secretion, but induced no changes in HCO\textsubscript{3} 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 vagothymus.

REFERENCES

1. ELIASSON S. Activation of gastric motility from the brainstem of the cat. Acta Physiol Scand 1954; 30: 199-214
2. Kerr FW. Preserved vagal visceromotor function following destruction of the dorsal motor nucleus. J Physiol 1969; 202: 755-769
3. Kerr FW, Preshaw RM. Secretomotor function of the dorsal motor nucleus of the vagus. J Physiol 1969; 205: 405-415
4. Chan YS, Ko JK, Cho CH. Role of dorsal motor nucleus of vagus in gastric function and mucosal damage induced by ethanol in rats. Dig Dis Sci 1995; 40: 2312-2316
5. Garrick T, Buack S, Bass P. Gastric motility is a major factor in cold restraint-induced lesion formation in rats. Am J Physiol 1986; 250: G191-G199
6. Ai HB, Zhang ZD. [Studies on the mechanism of gastric mucosal injury induced by water-immersion stress in rats]. Sheng Li Xue Bao 1990; 42: 496-502
7. Grabauskas G, Moises HC. Gastrointestinal-projecting neurones in the dorsal motor nucleus of the vagus exhibit direct and viscerotopically organized sensitivity to orenix. J Physiol 2003; 549: 37-56
8. Lee CH, Jung HS, Lee TY, Lee SR, Yuk SW, Lee KG, Lee BH. Studies of the central neural pathways to the stomach and Zusanli (ST36). Am J Chin Med 2001; 29: 211-220
9. Hayakawa T, Takanaga A, Tanaka K, Maeda S, Seki M. Cells of origin of vagal motor neurons projecting to different parts of the stomach in the rat: confocal laser scanning and electron microscopic study. Anat Embryol (Berl) 2003; 207: 289-297
10. Hopkings DA, Armour JA. Brainstem cells of origin of physiologically identified cardiopulmonary nerves in the rhesus monkey (Macaca mulatta). J Auton Nerv Syst 1998; 68: 21-32
11. Hsieh JH, Chen RF, Wu JJ, Yen CT, Chai CY. Vagal innervation of the gastrointestinal tract arises from dorsal motor nucleus while that of the heart largely from nucleus ambiguus in the cat. J Auton Nerv Syst 1998; 70: 38-50
12. Cheng Z, Powley TL. Nucleus ambiguous projections to cardiac ganglia of rat atria: an anterograde tracing study. J Comp Neurol 2000; 424: 588-606
13. Takanaga A, Hayakawa T, Tanaka K, Kawabata K, Maeda S, Seki M. Immunohistochemical characterization of cardiac vagal preganglionic neurons in the rat. Auton Neurosci 2003; 106: 132-137
14. Kopylov EV, Smirnov SI. [Changes in the electrical activity of the gastric pyloric area during stimulation of the nucleus ambiguus]. Fiziol Zh SSSR Im I M Sechenova 1991; 77: 91-99
15. Machado BH, Brody MJ. The nucleus ambiguus region participates in arterial pressure regulation. Neurosci Lett 1992; 135: 91-94
16. Merrill EG. Where are the real respiratory neurons? Fed Proc 1981; 40: 2389-2394
17. Ezure K. Synchronization of respiratory neurons in the brainstem of the cat. Prog Neurobiol 1990; 35: 429-450
18. Nakazawa K, Umezaki T, Zheng Y, Miller AD. Behaviors of bulbar respiratory interneurons during fictive swallowing and vomiting. Otolaryngol Head Neck Surg 1999; 120: 412-418
19. Zimmermann M. Ethical considerations in relation to pain in animal experimentation. Acta Physiol Scand Suppl 1986; 554: 221-233
20. Paninos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 4 th ed. Sydney: Academic Press, 1998
21. Mei MH, Chen Q. [Interaction of vagal stimulation and duodenal acidification in the regulation of pancreatic secretion]. Sheng Li Xue Bao 1985; 37: 410-415
22. Patani FD, Norman WP, Kasbekar DK, Gilliss RA. Effects of stimulation of nucleus ambiguous complex on gastroduodenal function. Am J Physiol 1984; 246: G253-G262
23. Hsieh JH, Chang YC, Chung JL, Hsiao MC, Chen SC, Yen CT, Chai CY. The relationship between FTL and NA, DMV or CVM in central cardiovascular control. Chin J Physiol 2001; 44: 169-179
24. Rentero N, Cividjian A, Trevaks D, Peggignot JM, Quintin L, McAlmon RM. Activity patterns of cardiac vagal motoneurons in rat nucleus ambiguus. Am J Physiol Regul Integr Comp Physiol 2002; 283: R1327-R1334
25. Yang ZJ, Rao ZR, Ju G. Evidence for the medullary visceral zone as a neural station of neuroimmunomodulation. Neurosci Res 2000; 38: 237-247