ROLE OF NITRIC OXIDE IN THE CONTROL OF THE GASTRIC MOTILITY WITHIN THE NUCLEUS AMBIGUUS OF RATS

1Biological Department, Qi Lu Normal University, Jinan, China; 2High-Tech Research Center, Shandong Academy of Agricultural Sciences, Jinan, China; 3Key Laboratory of Animal Resistance, College of Life Science, Shandong Normal University, Jinan, China

This study aims to investigate whether exogenous nitric oxide (NO) plays a role in controlling gastric motility within the nucleus ambiguus (NA). Experiments were performed on male Wistar rats anaesthetized with chloral hydrate. A latex balloon, connected to a pressure transducer, was inserted into the pylorus through the fundus for continuous recording of the change of gastric smooth muscle contractile curves. Microinjection of the NO-donor sodium nitroprusside (SNP; 5 nmol) or L-arginine (L-Arg; 5 nmol) into the NA significantly inhibited gastric motility, whereas the treatment of NO-synthase inhibitor N-nitro-L-arginine methylester (L-NAME) increased gastric motility remarkably. The negative effect of SNP or L-Arg on gastric motility was abolished by bilateral subdiaphragmatic vagotomy as well as by intravenous injection of ganglionic blocker, hexamethonium bromide (Hb). These results demonstrated that NO inhibited gastric motility by activating the cholinergic preganglionic neurons in the NA and through the mediation of vagus nerves.

Key words: gastric motility, nitric oxide, hexamethonium bromide, nucleus ambiguus, vagotomy, sodium nitroprusside

INTRODUCTION

Nitric oxide (NO) functions as a nonadrenergic-noncholinergic (NANC) neurotransmitter in the vagus nerve mediating relaxation of the gastrointestinal tract (1-3). The importance of neuronal NO in control of gastric function is underscored by the fact that mice which lack the neuronal NO synthase gene have altered gastric motor function resulting in a grossly enlarged stomach (4). Yet most of the research to date focuses on peripheral nervous system (PNS), a lot remains to be explored in terms of the innervations of central nervous system (CNS) on gastrointestinal tract.

It has been reported that nitric oxide (NO) produced in the central nervous system from conversion of L-arginine (L-Arg) by the NO synthase (NOS) may be involved in gastroenteric control. In the brain stem, NOS has been found in the nucleus tractus solitarii (5-9), in the rostral and caudal ventrolateral medulla of various animal species including rat, rabbit, mouse and cat (5, 7, 9), in the dorsal motor nucleus of the vagus (DMV) of the rat (5, 10) and in the nucleus ambiguus (NA) of rat (6, 7) and cat (8). The presence of NOS containing neurons in the NA suggests that NO may serve as a physiological regulator of the parasympathetic nervous system.

Histochemical studies of NOS indicated that it is distributed in the neurons and fibers within the DMV, which regulate gastric relaxation that is independent of nicotinic receptors (11-12). L-Arg, microinjected into the DMV, significantly decreases intragastric pressure, and this effect is abolished by vagotomy. Microinjection of a NOS inhibitor, NG-nitro-L-arginine methylester, increases intragastric pressure, with the greatest impact on the DMV rostral to the obex. Overall, it was concluded that tonic release of NO in the DMV mediates gastric relaxation, at least in anesthetized animals (13). As the centrum of parasympathetic preganglionic nerve, NA as well as DMV is acting on gastric motility (14-17), but there have been no reports on the role of NO in controlling gastric motility through the NA. This nucleus is known to be a source of vagal innervation of the gut and the importance of the activity of this autonomic output in the regulation of the gastric motility is well established (18).

The present study was therefore to determine whether NO plays a role in the control of gastric motility exerted by neurons in the NA. For this purpose, the effect of microinjections of L-Arg, NO donors and NOS inhibitors into functional gastroinhibitory sites within the NA were examined in anaesthetized rats.

MATERIALS AND METHODS

Animal preparation and microinjection studies

Experiments were performed on 52 male Wistar rats (260–320 g) purchased from the Experimental Animal Center of Shandong University. Animals were maintained in a temperature-controlled environment with a 12-h light/dark cycle. They were allowed free access to food and water for one week. Prior to the experiments, animals were fasted for 24 hours, but allowed free access to water. All procedures performed were...
into the balloon to achieve a baseline pressure of 5–10 cm H\(_2\)O.

The stomach was inflated by introducing warm saline (0.5–1.0 ml) and a latex balloon attached to a thin polyethylene tube was inserted into the pylorus through the fundus and the other end was connected to a pressure transducer. The other end of the polyethylene tube was inserted into the fundus through the pylorus and a latex balloon attached to a thin polyethylene tube was inserted into the pylorus through the fundus and the other end was connected to a pressure transducer. The stomach was inflated by introducing warm saline (0.5–1.0 ml) into the balloon to achieve a baseline pressure of 5–10 cm H\(_2\)O. The normal gastric motility was recorded for 30 min before injection, and then SNP, L-Arg, or L-NAME was microinjected into the right NA, or L-NAME was microinjected into the right NA within 1 min followed by recording gastric motility for another 30 min. Hb was given i.v.10 min prior to microinjection of SNP.

Animals were anesthetized with an intraperitoneal injection of chloral hydrate (400 mg/kg body weight, i.p.). The adequacy of anesthesia was assessed by monitoring eyelid and paw pinch reflexes. Body temperature was maintained at 37±1°C with radiant heat lamp. A midline laparotomy was performed, and a latex balloon attached to a thin polyethylene tube was inserted into the pylorus through the fundus and the polyethylene tube was connected to a pressure transducer. The stomach was inflated by introducing warm saline (0.5–1.0 ml) into the balloon to achieve a baseline pressure of 5–10 cm H\(_2\)O. Gastric motility curves were recorded by a two-lead physiological recording instrument (LMS-2B, Chengdu Instrument Factory, China). The animals were placed in a stereotaxic apparatus (Stoelting 51600, USA), and a portion of the cerebellum was exposed through an occipital craniotomy. After removal of the dura, a glass micropipettes (30–50 µm external tip diameter) connected to pneumatic pumps was stereotaxically introduced into the right NA, according to the atlas of Paxinos and Watson (20). Anteroposterior coordinates ranged between –4.24 and –4.68 mm from the interaural line(1.7±0.22 mm rostra1 to the obex), mediolateral coordinates from 1.7 to 2.1 mm lateral to the midline, and vertical coordinates between 0.0 and +0.6 mm from the horizontal plane passage through the interaural line.

A series of experiments were conducted to testify the hypothesis about the effect of NO in NA on gastric motility. (1) Effects of SNP (5 nmol in 50 nL, n=7) microinjected into the NA on gastric motility. Respiratory movements and electrocardiogram were monitored continuously with BL-420 BFES (Biological Function Experimental System; Chengdu Taimeng Company, China). (2) Effects of L-Arg (5 nmol in 50 nL, n=7) on gastric motility. (3) Effects of L-NAM (5 nmol in 50 nL, n=7) on gastric motility. (4) Control experiment. Microinjection of physiological saline (PS, 50 nL, n=10) into the NA. (5) After bilateral subdiaphragmatic vagotomy, microinjection of SNP or L-Arg (5 nmol in 50 nL, n=7) into the NA. (6) To investigate whether the effect of NO on gastric motility was also via activating cholinergic preganglionic neurons within NA, the effects of SNP (5 nmol in 50 nL, n=7) microinjected into the NA on gastric motility was observed 10 min after administration of a ganglionic blocker, Hb (0.5 ml mg/100 g body weight, 15 mg/kg body weight i.v.).

Histological identification for the microinjection site

At the end of the experiments, 2% potamine sky blue (50 nL) was injected into the same microinjection site. All of the experiments were terminated by a bolus intravenous injection of pentobarbital sodium (80 mg/kg). Then, the animals were perfused transcardially with PS with subsequent 4% paraformaldehyde, and their brains were removed and fixed in 4% paraformaldehyde with 20% sucrose for at least 2–3 days. Frozen sections of the brain stem (40 µm) were cut and stained with neutral red to determine placement of the micropipette tip in the right NA (Fig. 1A and 1B). Photomicrograph images were taken using a microscope (Nikon Optiphot; Nikon) with a digital camera (Magnafire; Optronics, Goleta, CA, USA) attached to a Dell Computer. These were then exported into Adobe PhotoShop where they were untouched except for minor adjustments to brightness and contrast.

Data analysis

The total amplitude, total duration, and motility index of gastric contraction waves within 5 min before microinjection and after microinjection were measured. The motility index was defined as the product of amplitude and duration of every contraction waves in this paper. At the same time, inhibitory rate was applied to estimate the changing degree of gastric motility before and after microinjection, namely inhibitory rate = (the value before microinjection – the value after microinjection)/the value before microinjection. All values were analyzed using SPSS13.0 software (SPSS Inc. Chicago, IL, USA) and presented as mean ±S.E. Statistical analysis was performed by either one-way ANOVA followed by Student-Newman-Keuls multiple-comparisons test or Student’s t-test. Significance was accepted at the level of \(P<0.05\).

RESULTS

Effects of sodium nitroprusside

SNP (5 nmol, n=7) microinjected into the right NA evoked significant inhibition on gastric motility (Fig. 2A). Total amplitude of contraction waves decreased from 41.80±1.16 to 28.00±2.00 (Fig. 2B). Effects of L-Arg (5 nmol, n=7) microinjected into the right NA evoked significant inhibition on gastric motility (Fig. 2C). Total amplitude of contraction waves decreased from 41.80±1.16 to 28.00±2.00 (Fig. 2D).
mm/5 min (before microinjection) to 27.00±0.71 mm/5 min (P<0.01), and gastric motility index decreased from 865.44±17.92 (before microinjection) to 502.08±14.55 (P<0.01) after SNP was microinjected into NA (Fig. 2B and 2C).

**Effects of L-arginine**

L-Arg (5 nmol, n=7) microinjected into the right NA inhibited gastric motility markedly (Fig. 3A). Total amplitude of contraction waves decreased from 625.6 mm/5 min (before microinjection) to 237.7 mm/5 min (P<0.01).

**Fig. 2.** Effects of SNP microinjected into the right NA on gastric motility. Representative effects of SNP microinjected into the right NA on gastric motility (representing curve from a rat) (A). T.A.C.W. before and after microinjection of SNP into NA (B). Gastric motility index before and after microinjection of SNP into NA (C). T.A.C.W. – total amplitude of contraction waves; Micro – microinjection; **P<0.01, vs. before microinjection.

**Fig. 3.** Effects of L-Arg microinjected into the right NA on gastric motility. Representative effects of L-Arg microinjected into the right NA on gastric motility (representing curve from a rat) (A). T.A.C.W. before and after microinjection of L-Arg into NA (B). Gastric motility index before and after microinjection of L-Arg into NA (C). T.A.C.W. – total amplitude of contraction waves; Micro – microinjection; **P<0.01, vs. before microinjection.

**Fig. 4.** Effects of L-NAME microinjected into the right NA on gastric motility. Representative effects of L-NAME microinjected into the right NA on gastric motility (representing curve from a rat) (A). T.A.C.W. before and after microinjection of L-NAME into NA (B). Gastric motility index before and after microinjection of L-NAME into NA (C). T.A.C.W. – total amplitude of contraction waves; Micro – microinjection; **P<0.01, vs. before microinjection.
Fig. 5. Effects of PS microinjected into the right NA on gastric motility.

Fig. 6. Effects of SNP microinjected into the right NA on gastric motility with bilateral subdiaphragmatic vagotomy in advance. Representative effects of bilateral subdiaphragmatic vagotomy on gastric motility (A). Representative effects of SNP (5 nmol) microinjected into the right NA on gastric motility with bilateral subdiaphragmatic vagotomy in advance (B). T.A.C.W. before and after microinjection of SNP (5 nmol) into NA with and without bilateral subdiaphragmatic vagotomy in advance; ** P<0.01, vs. before microinjection (C). Gastric motility index before and after microinjection of SNP (5 nmol) into NA with and without bilateral subdiaphragmatic vagotomy in advance; ** P<0.01, vs. before microinjection (D). Average inhibitory rate of T.A.C.W. and gastric motility index in the SNP group and the vagotomy + SNP group; ** P<0.01, vs. the SNP group (E). T.A.C.W. – total amplitude of contraction waves; Micro – microinjection.

Fig. 7. Effects of L-Arg microinjected into the right NA on gastric motility with bilateral subdiaphragmatic vagotomy in advance. Representative effects of bilateral subdiaphragmatic vagotomy on gastric motility (A). Representative effects of L-Arg (5 nmol) microinjected into the right NA on gastric motility with bilateral subdiaphragmatic vagotomy in advance (B). T.A.C.W. before and after microinjection of L-Arg (5 nmol) into NA with and without bilateral subdiaphragmatic vagotomy in advance; ** P<0.01, vs. before microinjection (C). Gastric motility index before and after microinjection of L-Arg (5 nmol) into NA with and without bilateral subdiaphragmatic vagotomy in advance; ** P<0.01, vs. before microinjection (D). Average inhibitory rate of T.A.C.W. and gastric motility index in the Arg group and the vagotomy + Arg group; ** P<0.01, vs. the Arg group (E). T.A.C.W. – total amplitude of contraction waves; Micro – microinjection.
contraction waves and gastric motility index dropped to 31.20±1.85 mm/5 min (P<0.01) and 517.44±34.17 (P<0.01) respectively after microinjection, in contrast to 46.20±3.83 mm/5 min and 848.64±56.17 before injection (Fig. 3B and 3C).

Effects of L-NAME

Microinjection of the NO-synthase inhibitor L-NAME (5 nmol, n=7) induced a remarkable increase in gastric motility (Fig. 4A). Total amplitude of contraction waves increased from 43.83±3.97 mm/5 min to 83.00±8.25 mm/5 min (P<0.01), gastric motility index increased from 1003.80±88.8 to 1724.80±97.49 (P<0.01) after L-NAME application (Fig. 4B and 4C).

Effects of PS

The before and after data of PS (50 nL, n=10) treatment were 45.40±1.97 mm/5 min and 45.80±1.59 mm/5 min (P>0.05) for total amplitude of contraction waves, 811.38±11.20 and 817.48±12.60 (P>0.05) for gastric motility index (Fig. 5A), suggesting unaltered gastric motility.

Effects of sodium nitroprusside with bilateral subdiaphragmatic vagotomy pretreatment

Bilateral subdiaphragmatic vagotomy abolished the inhibitory effect that SNP imposed on gastric motility (Fig. 6A and 6B). No obvious inhibitory effect on gastric motility was observed in SNP microinjected samples preprocessed with bilateral subdiaphragmatic vagotomy (Fig. 6C and 6D). The inhibitory rate for total amplitude of contraction waves and gastric motility index was 35.20% and 41.84% respectively in the SNP group, but was 4.44% and 6.79% (Fig. 6E) respectively, in the vagotomy + SNP group (compared with the SNP group, P<0.01; n=7).

Effects of L-Arginine with bilateral subdiaphragmatic vagotomy pretreatment

Bilateral subdiaphragmatic vagotomy abolished the inhibitory effect of microinjection of L-Arg into NA on gastric motility (Fig. 7A and 7B). Total amplitude of contraction waves changed from 14.80±0.86 mm/5 min to 14.30±0.77 mm/5 min (P>0.05), gastric motility index changed from 166.08±4.82 to 157.92±3.97 (P>0.05) after L-Arg (5 nmol, n=7) was microinjected into NA with bilateral subdiaphragmatic vagotomy in advance (Fig. 7C and 7D). The inhibitory rate of total amplitude of contraction waves and gastric motility index was 31.76% and 38.67% respectively, in the Arg group, but was only 3.19% and 4.78% (Fig. 7E) respectively, in the vagotomy + Arg group (compared with the Arg group, P<0.01; n=7).

Effects of sodium nitroprusside with intravenous injection hexamethonium bromide in advance

Hb treatment alone induced significant inhibition on gastric motility (Fig. 8A and Table 1), but abolished the inhibitory effect caused by SNP (Fig. 8B). Total amplitude of contraction waves, total duration of contraction waves, frequency, gastric motility

Table 1. Representative effects of intravenous injection hexamethonium bromide on gastric motility.

<table>
<thead>
<tr>
<th>n=7</th>
<th>Before injection</th>
<th>After injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.C.W (mm/5min)</td>
<td>56.14 ± 2.68</td>
<td>8.14 ± 0.68 **</td>
</tr>
<tr>
<td>T.D.C.W (s/5min)</td>
<td>33.29 ± 2.39</td>
<td>9.86 ± 0.93 **</td>
</tr>
<tr>
<td>Frequency(time/5min)</td>
<td>4.57 ± 0.52</td>
<td>1.71 ± 0.39 **</td>
</tr>
<tr>
<td>Motility index</td>
<td>937.37 ± 59.86</td>
<td>86.06 ± 6.49 **</td>
</tr>
</tbody>
</table>

T.A.C.W. – total amplitude of contraction waves; T.D.C.W. – total duration of contraction waves; **Compared with before injection, P<0.01.

Table 2. Representative effects of SNP microinjected into the NA on gastric motility with intravenous injection hexamethonium bromide in advance.

<table>
<thead>
<tr>
<th>n=7</th>
<th>Before Micro</th>
<th>After Micro</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.A.C.W (mm/5min)</td>
<td>12.29 ± 1.05</td>
<td>12.14 ± 2.45</td>
</tr>
<tr>
<td>T.D.C.W (s/5min)</td>
<td>15.14 ± 1.30</td>
<td>15.43 ± 1.41</td>
</tr>
<tr>
<td>Frequency(time/5min)</td>
<td>2.43 ± 0.46</td>
<td>2.71 ± 0.45</td>
</tr>
<tr>
<td>Motility index</td>
<td>126.86 ± 9.15</td>
<td>132.34 ± 13.30</td>
</tr>
</tbody>
</table>

T.A.C.W. – total amplitude of contraction waves; T.D.C.W. – total duration of contraction waves; Micro – microinjection.
Effects of exogenous nitric oxide on electrocardiogram

Heart rate were compared before and after microinjections. We found that SNP (5 nmol, n=7) significantly decreased heart rate (from 378.56±16.36 times/min to 253.12±11.88 times/min, P<0.01). And L-Arg (5 nmol, n=7) has similar effect as SNP (from 376.28±15.82 times/min to 258.31±12.09 times/min, P<0.01). However, heart rate rose sharply (from 364.61±10.85 times/min to 447.36±17.58 times/min, P<0.01) after the addition of L-NAME.

DISCUSSION

The present results for the first time provided evidence that NO within the NA are involved in the regulation of gastric motility in anaesthetized rats. It was shown in functional gastroenteric sites within NA that inhibition of NOS by L-NAME enhanced gastric motility. Moreover, increasing exogenous NO, through microinjeting L-Arg, or NO donor SNP inhibited gastric motility and this inhibitory effect could be abolished by bilateral subdiaphragmatic vagotomy and Hb treatment. Thus the negative influence of SNP or L-Arg relies on the activation of cholinergic preganglionic neurons and through the vagal neural pathway.

The responses of gastric motility were specific to L-NAME, L-Arg and SNP microinjections in this study which is supported by the following observations. Firstly, 50 nl of PS did not result in any changes to gastric motility. Secondly, the effect caused by inhibiting NOS on gastric motility was opposite to that triggered by increasing NO locally. Lastly, microinjection of SNP or L-Arg (5 nmol) significantly decreased heart rate, while L-NAME displayed contrary impact. Ruggeri et al. found that within NA the inhibition of NOS by L-NAME enhanced the HR, but increasing endogenous NO, through microinjecting L-Arg, or donating NO by SNP suppressed the HR (21). Our results were the same as these results, further implying that elevation of NO level via L-Arg, or SNP in the right NA inhibited the gastric motility is indeed credible.

Fig. 9. Effects of microinjection of SNP, L-Arg and L-NAME into the right NA on electrocardiogram (A). Representative effects of L-Arg (5 nmol) microinjected into the right NA on electrocardiogram (B). Representative effects of L-NAME (5 nmol) microinjected into the right NA on electrocardiogram (C). Micro – microinjection; **Compared with before microinjection, P<0.01.
activate protein kinase, which regulates the activity of proteins through phosphorylation (28).

In conclusion, this study suggests that the exogenous NO within the NA is involved in the regulation of gastric motility. It inhibited gastric motility by activating the cholinergic preganglionic neurons in the NA and the inhibitory effect was mediated by vagus nerves.

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Author’s address: Sun Hong-Zhao, Biological Department, Qi Lu Normal University, No.36, Lishan Road, Jinan 250013, China.
E-mail: send to: sunhongzhao18@126.com