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Effect of nitric oxide on electric and mechanical activities of gastric antral circular muscles in guinea pigs[1]

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KEY WORDS nitric oxide; nitroprusside; electromyography; gastrointestinal motility

ABSTRACT

AIM: To study the effect of exogenous nitric oxide (NO) on electric and mechanical activities of gastric antral circular muscle in guinea pigs *in vitro*. **METHODS**: Mechanical and electric activities of gastric antral circular muscle in guinea pigs were recorded simultaneously. **RESULTS**: Sodium nitroprusside (SNP, 0.5 $\mu mol \cdot L^{-1}$), an NO donor, inhibited the frequency and amplitude of fast wave and spontaneous contraction of the strips ($P < 0.01$). SNP-induced inhibition was not blocked by tetrodotoxin, atropine, phentolamine, and propranolol ($P > 0.05$), but diminished by methylene blue ($P < 0.01$) and oxyhemoglobin ($P < 0.01$). **CONCLUSION**: Exogenous NO inhibits gastric antral myoelectric and mechanical activities in guinea pigs. The inhibitions are produced by NO acting on extracellular membrane and enhancing the level of cGMP.

INTRODUCTION

Nitric oxide (NO) is the major endothelium-derived relaxing factor (EDRF), and it is thought to relax smooth muscle cells[1]. NO is synthesized by a variety of cells, including gastrointestinal smooth muscle cells[2]. NO is a non-adrenergic non-cholinergic (NANC) inhibitory neurotransmitter that inhibits gastrointestinal motility[3]. Effects of NO and its donors, such as sodium nitroprusside (SNP), on motility of esophagus, jejunum, ileum, and colon have been reported[4-7]. However, nobody reported the effect of NO on gastric antral circular muscle in guinea-pigs. The aim of the present study was to investigate the effect of SNP on electric and mechanical activities of antral circular smooth muscle in guinea pig stomach and analyze its mechanism.

MATERIALS AND METHODS

EWG/B guinea pigs of either sex, bred by Experimental Animal Center, Norman Bethune University, Certificate № 10-6004, weighing (300 ± 50) g, were stunned and bled. The abdomen of each guinea pig was opened along the midline and stomach was removed and placed in pre-oxygenated Tyrode's solution at room temperature. The mucous layer was removed and strips (about 2.0 mm × 20.0 mm) of gastric antral circular muscle were prepared. The longer axis of the stomach was cut parallel to the circular muscle fibres. Muscle strips were placed in a chamber and pinned in silica gel floor. One end of the strip was pinned to the floor of the chamber to record extra cellular electric activity with an Ag-AgCl electrode. The other end was attached to an isometric force transducer (CJY100, Beijing, China) to record contraction. The chamber (8 mL volume) was constantly perfused with pre-oxygenated Tyrode's solution at 3 mL/min. Temperature was maintained at (37.0 ± 0.5) ℃ by a water bath thermostat (*WC*/09-05, Chongqing, China). The Ag-AgCl electrode and isometric force transducer were connected to a polygraph (RM6200, Nihon Kohden, Tokyo, Japan). The muscle strips were allowed to incubate for at least 2 h before experiments were started.

The Tyrode's solution used in this study contained ($mmol \cdot L^{-1}$) NaCl 147, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2, NaH_2PO_4 0.42, Na_2HPO_4 1.81, glucose 5.5, the pH was 7.36 ± 0.01.

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Drugs Sodium nitroprusside (Nakarai Chemical, Ltd, Tokyo, Japan), atropine, phentolamine, and propranolol (Beijing, Chemical Reagent Plant, China), methylene blue (Shenyang, No 3 Chemical Reagent Plant, China), tetrodotoxin and oxyhemoglobin (Sigma Chemical Co, USA).

Statistics Results were expressed as $\bar{x} \pm s$. Significance was tested by t-test and values of $P < 0.05$ were considered significant.

RESULTS

Effect of SNP on electric and mechanical activities of gastric antral circular muscle The spontaneous contraction and fast wave usually appeared after incubating the muscle strips in Tyrode's solution for about 2 h. SNP ($0.5\ \mu mol \cdot L^{-1}$, $n=5$) markedly decreased the frequency and amplitude of fast waves and completely inhibited the contractile responses. The amplitude of slow waves was slightly diminished while the frequency was not affected by SNP (Fig 1A). These SNP-induced inhibitions returned progressively to control level after washing out.

Effects of tetrodotoxin (TTX) and receptor blockers on the SNP-induced inhibition To determine the relationship between SNP-induced inhibition and NANC inhibitory nerve in Auerbach's plexus, we observed the effects of TTX, adrenergic and cholinergic receptor blockers on the SNP-induced inhibition, respectively. TTX ($1\ \mu mol \cdot L^{-1}$, $n=4$) slightly increased basic tone, but did not affect electric activity and phasic contraction of the muscle strips. The SNP-induced inhibitions of gastric antral circular muscle were not affected by pretreatment with TTX (Tab 1), atropine ($1\ \mu mol \cdot L^{-1}$, $n=6$) (Fig 1B, Tab 1), phentolamine ($1\ \mu mol \cdot L^{-1}$, $n=5$) (Tab 1) and propranolol ($1\ \mu mol \cdot L^{-1}$, $n=4$) (Tab 1).

Effects of oxyhemoglobin and methylene blue on the SNP-induced inhibition To further analyze the mechanism by which NO inhibits the gastric electric and mechanical activities, we tested the action of oxyhemoglobin (Oxy-Hb), an extra cellular NO scavenger[5], and methylene blue (MB) which inhibits soluble guanylate cyclase[5], on the SNP-induced inhibitions. Oxy-Hb ($5\ \mu mol \cdot L^{-1}$, $n=4$), markedly diminished the inhibitory effect of SNP on fast wave and motility of the strips (Tab 1). MB ($5\ \mu mol \cdot L^{-1}$, $n=7$) also significantly diminished the inhibitory effect of SNP on fast wave and motility (Fig 1C, Tab 1), but could not completely abolish the inhibitory effect. We also observed that higher concentration of MB ($10\ \mu mol \cdot L^{-1}$) did not completely block SNP-induced inhibitions (data unshown).

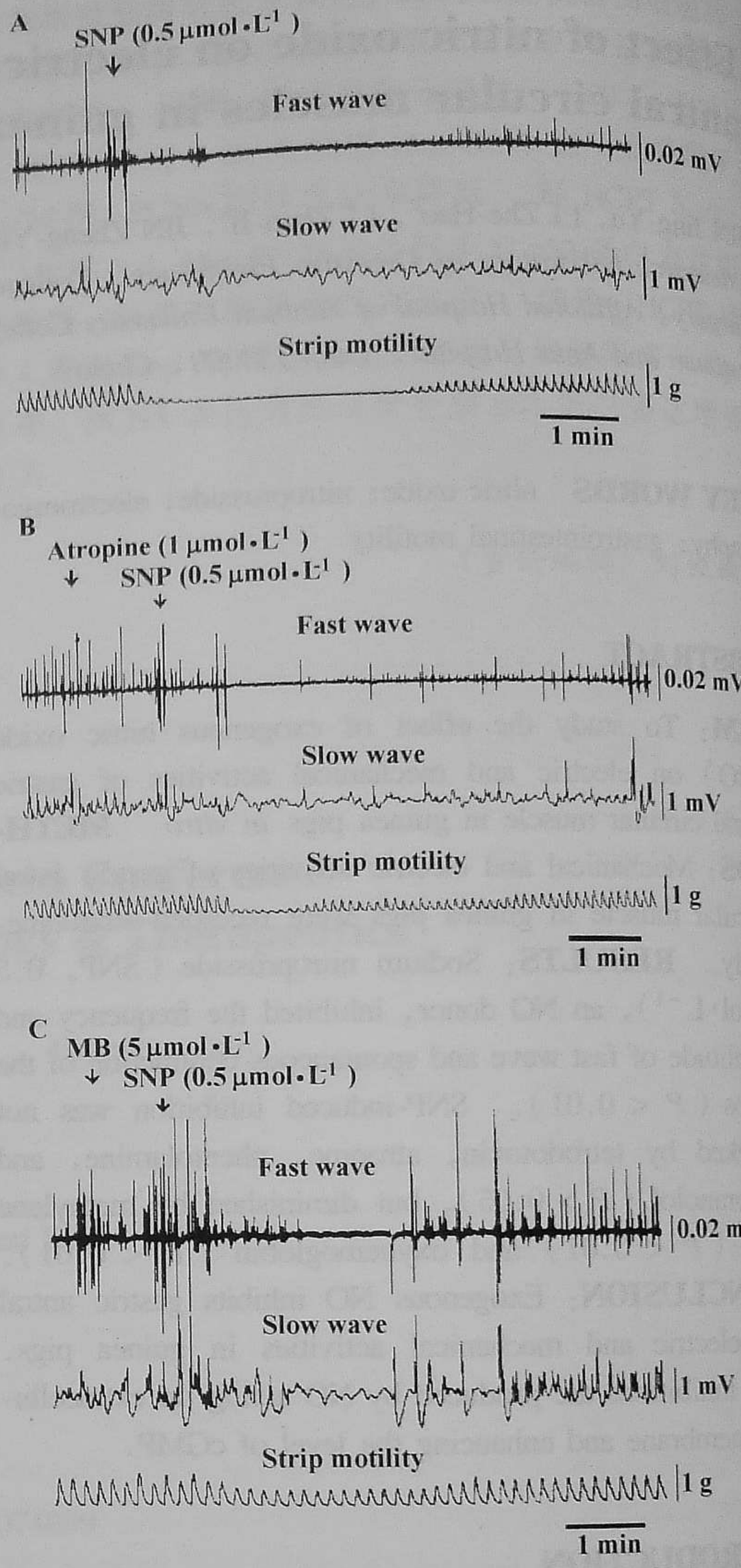

Fig 1. Effects of different drugs on electric and mechanical activities. A: SNP ($0.5\ \mu mol \cdot L^{-1}$, $n=5$) inhibits electric and mechanical activities. B: SNP-induced inhibition was not affected by atropine ($1\ \mu mol \cdot L^{-1}$, $n=6$). C: Methylene blue ($5\ \mu mol \cdot L^{-1}$) markedly diminished SNP-induced inhibition ($n=7$).

DISCUSSION

The present data shows that SNP inhibits spontaneous

Tab 1. Effects of drugs on electric and mechanical activities. $x \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **SNP group.**

Drugs	*n*	Change of fast wave		Change of strip motility	
		Frequency/%	Amplitude/%	Frequency/%	Amplitude/%
SNP	5	−89.9 ± 20.1	−94.9 ± 10.2	−92.3 ± 15.3	−96.5 ± 7.8
TTX + SNP	4	−82.9 ± 16.4[a]	−85.4 ± 13.1[a]	−84.2 ± 18.6[a]	−78.6 ± 17.9[a]
Atropine + SNP	6	−89.3 ± 7.4[a]	−85.7 ± 12.1[a]	−84.5 ± 13.4[a]	−82.0 ± 18.0[a]
Propranolol + SNP	5	−79.5 ± 28.6[a]	−88.2 ± 14.1[a]	−72.8 ± 31.4[a]	−68.8 ± 22.5[a]
Phentolamine + SNP	4	−79.8 ± 13.4[a]	−82.5 ± 13.1[a]	−85.4 ± 11.0[a]	−85.1 ± 13.2[a]
Oxy-Hb + SNP	4	−8.5 ± 2.5[c]	−9.1 ± 5.3[c]	−11.5 ± 10.9[c]	−6.2 ± 2.7[c]
MB + SNP	7	−19.2 ± 22.9[b]	−20.1 ± 22.6[b]	−22.1 ± 16.0[c]	−34.8 ± 18.8[c]

electric and mechanical activities of antral circular muscle in the guinea pig stomach. Our results demonstrate that the response of gastric antral smooth muscle to SNP is the same as that of other smooth muscles of the gastrointestinal tract, for example, jejunum and colon[5-7].

The mechanism by which SNP inhibits electric and mechanical activities of gastric antral circular muscle still remains to be elucidated. SNP is an established and commonly used NO-donor[3-8]. Therefore, it can be considered that the SNP-induced inhibition appears to be due to NO released from SNP. The mechanism by which NO inhibits smooth muscle motility is not fully clear. According to the experimental conditions, we can presume two possible routes for the exogenous NO action: nervous and direct routes. A number of studies have shown that some of the nerves distributed in the stomach are NANC inhibitory nerve, but the identification of the NANC inhibitory neurotransmitter remains unclear. Recent studies have strongly suggested that NO is an NANC inhibitory neurotransmitter[7-9]. In the present experiment, SNP may stimulate intermediary neuron releasing another NANC inhibitory transmitter in Auerbach's plexus, example VIP, ATP, and so on, to inhibit target smooth muscle cells. However, the present results show that SNP-induced inhibitions were not affected by TTX and receptor blockers. There is thus little possibility of nervous route to be involved. Another possible route is that NO directly acts on the smooth muscle. Oxy-Hb, an NO scavenger, markedly diminished the SNP-induced inhibition. Oxy-Hb acts only at the extracellular sites, because it is a big molecule impermeable to cell membrane. There is evidence that exogenous NO may directly act on calcium-dependent potassium channels[1]. No may also be having an intracellular action via cGMP pathway. NO has been shown to activate guanylate cyclase and enhance production of cGMP[10]. The present result indicates that methylene blue, which inhibits soluble guanylate cyclase, obviously diminished SNP-induced inhibitions. Therefore, we may suggest that SNP-induced inhibition appears partially through enhancing cGMP.

In summary, the present study shows that exogenous NO inhibits electric and mechanical activities of the gastric antral circular muscle in the guinea pigs. The effects are produced by NO action on the extracellular membrane and by enhancing intracellular cGMP.

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一氧化氮对豚鼠胃窦环行肌电活动和收缩运动的影响[1]

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关键词 一氧化氮；硝普盐；肌电描记术；胃肠活动

目的：在体外研究一氧化氮对豚鼠胃窦环行肌电活动和收缩运动的影响. **方法**：用常规方法同时记录胃窦肌条的电活动和收缩运动. **结果**：在豚鼠胃窦环行肌条上，一氧化氮供体硝普钠(0.5 μmol·L^{-1})能显著抑制电活动快波和运动. 这种抑制作用不受河鲀毒、阿托品、酚妥拉明和普萘洛尔(各1 μmol·L^{-1})的影响($P>0.05$)，但可被亚甲基蓝(5 μmol·L^{-1})和氧合血红蛋白 (5 μmol·L^{-1})明显减弱($P<0.01$). **结论**：一氧化氮抑制豚鼠胃窦环行肌电活动和收缩运动. 这种抑制效应是通过一氧化氮对平滑肌细胞膜的直接作用和增加细胞内环磷酸鸟苷来实现的.

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