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Effects of Nitric Oxide on Slow Waves and Spontaneous Contraction of Guinea Pig Gastric Antral Circular Muscle

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Abstract. We examined the effects of nitric oxide (NO) donors, S-nitroso-L-cysteine (Cys-NO) and 3-morpholinosydnonimine hydrochloride (SIN-1), on slow waves and contractile activity in the circular muscle of guinea pig gastric antrum. In the presence of atropine and guanethidine, electrical field stimulation (EFS) reduced the amplitude of phasic contraction. The effect of EFS was significantly inhibited by both the NO synthase inhibitor Nω-nitro-L-arginine methyl ester and a soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). Cys-NO and SIN-1 mimicked the effect of EFS on phasic contraction and reduced the amplitude of slow waves in a concentration-dependent manner, with no effect on frequency and resting membrane potential. Phasic contraction was more sensitive to NO donors than slow waves. The inhibitory effects of NO donors were antagonized by ODQ and mimicked by a membrane permeable cGMP analogue 8-bromo-cGMP. Several K⁺ channel blockers such as apamin, iberiotoxin, and glibenclamide had no effect on the inhibitory action of SIN-1. These results suggest that NO inhibits the phasic contraction and slow waves through cGMP-dependent mechanisms in guinea pig gastric antrum. The effect of NO is unlikely to be mediated by the activation of Ca²⁺-activated or ATP-sensitive K⁺ channels.

Keywords: nitric oxide, slow wave, gastric antrum, circular muscle, cGMP

Introduction

Gastric antrum, like other gastrointestinal tissues, exhibits spontaneous motility in the absence of nerve input. This activity is known to be regulated by spontaneous rhythmic depolarization and repolarization of membrane potential termed slow waves (1).

Nitric oxide (NO) or a NO-related compound is now widely recognized as an important physiological mediator of nonadrenergic-noncholinergic (NANC) relaxation of gastrointestinal smooth muscle (2). However, the effect of NO on spontaneous motility and slow wave has rarely been reported. In the canine ileum, the NO donor 3-morpholinosydnonimine hydrochloride (SIN-1) abolished circular muscle contraction and induced hyperpolarization. In addition, SIN-1 slightly increased the slow wave frequency and reduced the slow wave duration and amplitude (3). In guinea pig proximal colon, NO and NO donors hyperpolarized membrane potential by activating a tetraethylammonium (TEA)-resistant membrane K⁺ conductance (4). In canine gastric antrum, sodium nitroprusside (SNP) induced hyperpolarization and dramatically inhibited the slow wave plateau and duration with little effect on the slow wave upstroke (5).

It is generally accepted that NO increases the intracellular cGMP concentration through activation of soluble guanylate cyclase (sGC), and cGMP induces various cellular responses via protein kinase G-dependent phosphorylation of intracellular target proteins including ion channels (6). However, the mechanism of action of NO varies by species, strains, regions, and also aging. For example, NO hyperpolarizes dog proximal colon, but not rat proximal colon or guinea pig gastric fundus (7). It was reported that NO increased the synthesis of cGMP, but the increase of cGMP did not correlate with relaxation in rat duodenum (8), rat proximal colon (9), and monkey myometrial smooth muscle (10).
NO can induce relaxation by direct binding to target proteins in vascular smooth muscle (11) and guinea pig proximal colon (12). It is also known that cGMP induces a response by its direct action on ion channels or phosphodiesterases (PDEs) (13). Moreover, it was even suggested that NO induces responses by an unknown mechanism (14).

In the present study, we investigated whether the effect of NO on the spontaneous motility and electrical activity in gastric smooth muscle is mediated mainly by cGMP and hyperpolarization.

Materials and Methods

Tissue preparation

Male guinea pigs weighing 200 – 350 g were exsanguinated after stunning. The antral region of the stomach was dissected out, and contents were carefully removed in a beaker containing oxygenated Krebs solution. The composition of the Krebs solution was 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 2.5 mM CaCl2, and 11 mM glucose. The cleaned segment was then pinned flat to the Sylgard bottom of a dissecting dish filled with continuously oxygenated (95% O2 – 5% CO2) Krebs solution. After removal of the mucosal layer, muscle strips (2 × 8 mm) were cut parallel to the circular muscle fiber.

Organ bath recording of muscle tension

The strips were suspended to the tissue holder and placed between two platinum plates, which were located in 20-ml chambers filled with warmed (37°C) and oxygenated (95% O2 – 5% CO2) Krebs solution. The upper end of the strip was connected to the isometric force transducer (FT-03; Grass-Telefactor, West Warwick, RI, USA). The output of the transducer was processed through MacLab/2e (AD Instruments, Castlehill, Australia) and recorded on a Macintosh LC III computer. Two platinum plates were connected to the electrical stimulator (S88, Grass-Telefactor). An initial 0.5 g of tension was loaded onto each muscle strip during a 60-min equilibration period with rinsing every 15 min during this period. The muscarinic antagonist atropine (1 μM) and adrenergic transmission blocker guanethidine (50 μM) were applied to induce a NANC condition. Electrical field stimulations (EFS) (1-ms pulse duration, 80-V, 1-min train duration) were applied via paired platinum plates in a stepwise-increment of frequency (1 – 20 Hz).

Intracellular recordings

The contraction of circular muscle and membrane potential were recorded simultaneously by using the intracellular microelectrode technique. One half of the length of the strip was carefully pinned onto the Sylgard plate of a 2-ml organ bath with the submucosal surface facing up. The free end of the strip was tied to an isometric force transducer (MLT-100, AD Instruments) to measure mechanical activity. Strips were stretched to a resting tension of 0.5 g.

Muscle cells were impaled with glass microelectrodes (40 – 80 MΩ) filled with 3 M KCl. Microelectrodes were prepared from glass capillaries (1BBL, 1B100F-4; WPI, Inc., Sarasota, FL, USA) with inner and outer diameters of 0.5 and 1.0 mm, respectively. The micro-electrode was connected to a high-impedance electrometer (Duo 773, WPI, Inc.). The electrical activities of the muscle cells were recorded from the microelectrode and mechanical signals were recorded simultaneously from the force transducer. The recordings, storage, and analysis of these data were performed by using the software Chart 3.51 (AD Instruments). The muscle strips were allowed to equilibrate for approximately 2 h before experiments were begun. During experiments, oxygenated (95% O2 – 5% CO2) and pre-warmed Krebs solution (pH 7.4) were continuously pumped into the bath at a rate of 1.6 ml/min. The temperature of the bath solution was maintained at 37.0 ± 0.5°C.

Drugs and solutions

SIN-1, iberiotoxin (IbTX), and 1H-[1,2,4]oxadiazolo[4,3-c]quinoxalin-1-one (ODQ) were purchased from RBI (Natick, MA, USA). The following drugs were from Sigma (St. Louis, MO, USA): sodium nitrite, L-cysteine, Nω-nitro-L-arginine methyl ester (L-NAME), 8-bromo-cyclic GMP (8-br-cGMP), apamin, glibenclamide, dimethyl sulfoxide (DMSO). All drugs were dissolved in distilled water except ODQ and glibenclamide that were dissolved in DMSO.

S-nitroso-L-cysteine (Cys-NO) was prepared according to the method of Barbier and Lefebvre (15). Briefly, acidified NaNO2 (200 mM, pH <2) and L-cysteine (200 mM) were mixed together at 4°C. The solution turned bright red as Cys-NO was formed. Cys-NO solution was prepared immediately before use, kept on ice, and protected from light.

Data and statistical analyses

For the fitting of the concentration-response curves of NO donors and 8-br-cGMP, the following logistic function was used: \( R/R_{\text{cont}} = \frac{1}{1 + \left(\frac{[C]}{IC_{50}}\right)^h} \), where R is the response at a given concentration ([C]) of each drug, \( R_{\text{cont}} \) is the control response in the absence of the drug, and h is the slope factor of the curve. IC_{50} was defined as the concentration of the drug at which R was inhibited to 50% of \( R_{\text{cont}} \), and it was determined from
the above function. Data are presented as means ± S.E.M., with n, the number of strips.

Differences in the data were evaluated using one way or two way repeated measures ANOVA followed by the Newman-Keuls multiple comparison test. P values less than 0.05 were considered statistically significant.

Results

Effects of EFS on spontaneous motility

Circular muscle strips of the guinea pig gastric antrum exhibited rhythmic spontaneous contraction. In the presence of atropine and guanethidine, EFS (1 – 20 Hz, 1-ms pulse duration, 1-min train duration) reduced the amplitude of spontaneous phasic contraction in a frequency-dependent fashion (Fig. 1A). EFS also reduced tonic contraction and induced rebound excitation following cessation. However, tonic relaxation and rebound excitation varied among strips, and they were changed relatively little by EFS. EFS had no effect on the frequency of phasic contraction. The NO synthase inhibitor L-NAME (100 and 300 μM) significantly inhibited the EFS-induced reduction of spontaneous phasic contraction (two-way repeated measures ANOVA, F\text{ratio} = 12.2, P = 5.11 \times 10^{-5}) (Fig. 1B). However, the response in the presence of L-NAME at 100 μM did not differ from that at 300 μM L-NAME. An sGC inhibitor ODQ (1 and 10 μM) significantly attenuated the EFS-induced reduction in contraction (two-way repeated measures ANOVA, F\text{ratio} = 26.3, P = 1.94 \times 10^{-8}) (Fig. 1C), but the responses between ODQ 1 and 10 μM were not different. There was no significant difference between the effect of L-NAME (100 μM) and that of ODQ (10 μM) on the response observed at 20-Hz of NANC nerve stimulation (P = 0.426) (Fig. 1D).

Effects of NO donors on the mechanical and electrical activity

Gastric antral circular muscle showed spontaneous phasic contraction and slow waves during microelectrode recordings. The upstroke and the plateau amplitudes of slow waves were 31.6 ± 1.1 and 31.4 ± 1.2 mV, respectively, under the control condition. The resting membrane potential (most negative potential

Fig. 1. Responses to NANC nerve stimulation in the circular muscle of guinea pig gastric antrum. Electrical field stimulation (EFS) (1 – 20 Hz, 1-ms, 1-min trains) reduced phasic contraction in a frequency-dependent manner (A). EFS also reduced tonic contraction and induced rebound excitation following cessation; however, these are changed relatively little by EFS. The EFS-induced reduction of phasic contraction was significantly inhibited by L-NAME (100 and 300 μM) (two-way repeated measures ANOVA, F\text{ratio} = 12.2, P = 5.11 \times 10^{-5}) and ODQ (1 and 10 μM) (F\text{ratio} = 26.3, P = 1.94 \times 10^{-8}), suggesting that NO plays an important role in inhibitory neurotransmission (B, C) (n = 5). There was no significant difference between the inhibitory effects of L-NAME (100 μM) and ODQ (10 μM) on NANC stimulation at 20 Hz (P = 0.426) (D). (*P<0.05, **P<0.01: taken by two way repeated measures ANOVA test followed by Newman-Keuls multiple comparison test)
between slow waves) was $-61.9 \pm 0.7$ mV, while the
duration of slow waves was $6.6 \pm 0.3$ s, and the fre-
cquency of slow waves was $5.5 \pm 0.2$ cycles per minute
($n = 19$).

We used two NO donors from different classes (16).
Application of Cys-NO ($0.001 - 10$ $\mu$M), an $S$-nitro-
sothiol, for 3 min reversibly inhibited phasic contraction
and slow waves in a concentration-dependent manner
(Fig. 2: A – D). Among the slow wave parameters, up-
stroke amplitude (two-way repeated measures ANOVA,
$F_{\text{ratio}} = 150.6, P = 3.01 \times 10^{-17}$), plateau amplitude ($F_{\text{ratio}} = 167.1, P = 3.64 \times 10^{-18}$), and duration ($F_{\text{ratio}} = 25.7, P = 4.94 \times 10^{-6}$) were significantly reduced in a concentra-
tion-dependent manner (Fig. 2: E and F). At higher con-
centrations (Cys-NO $\geq 1$ $\mu$M), the upstroke was barely
distinguishable from the plateau (Fig. 2: C and D).

![Figure 2](image_url)

**Fig. 2.** Response to Cys-NO in the circular muscle of guinea pig gastric antrum. Cys-NO reversibly reduced both phasic
contraction (upper trace) and slow waves (lower trace) in a dose-dependent manner (A – D). All tracings are from the same
cell. Sampling sites are indicated by arrows. Phasic contraction (two-way repeated measures ANOVA, $F_{\text{ratio}} = 3682.3,$
$P = 2.27 \times 10^{-58}$), upstroke ($F_{\text{ratio}} = 150.6, P = 3.01 \times 10^{-17}$), and plateau amplitudes ($F_{\text{ratio}} = 167.1, P = 3.64 \times 10^{-18}$) of slow
waves were dramatically reduced by Cys-NO in a dose-dependent manner. Spontaneous phasic contraction showed the most
sensitivity to NO donors. Plateau amplitude was more sensitive to NO than upstroke amplitude (D, E). The duration of slow
wave was also reduced slightly but significantly by Cys-NO (two-way repeated measures ANOVA, $F_{\text{ratio}} = 25.7, P = 1.94 \times 10^{-6}$).
However, frequency ($F_{\text{ratio}} = 1.99, P = 0.165$) and resting membrane potential ($F_{\text{ratio}} = 0.048, P = 0.827$) were not significantly
affected by Cys-NO (F, G) ($n = 4$).
Phasic contraction was more sensitive to Cys-NO than slow waves. The phasic contraction was more rapidly affected by Cys-NO and recovered more slowly after washout than slow waves. The IC50 of Cys-NO for phasic contraction, upstroke amplitude, and plateau amplitude were 0.03 ± 0.003, 0.95 ± 0.13, and 0.68 ± 0.09 μM, respectively (n = 4). Among the slow wave parameters, the amplitude of the plateau was most sensitive. However, frequency (two-way, repeated measures ANOVA, F ratio = 1.99, P = 0.165) and resting membrane potential (F ratio = 0.048, P = 0.827) were not significantly affected by Cys-NO (Fig. 2: F and G).

Another NO donor, SIN-1 (0.01 – 100 μM) showed similar effects to that of Cys-NO (Fig. 3: A – D). The phasic contraction (two-way repeated measures ANOVA, F ratio = 381.8, P = 3.03 × 10^-13), upstroke (F ratio = 98.3, P = 7.24 × 10^-17), and plateau (F ratio = 219.3, P = 9.2 × 10^-28) were inhibited by SIN-1 in a concentration-depen-

![Figure 3](image.png)

Fig. 3. Response to SIN-1 in the circular muscle of guinea pig gastric antrum. SIN-1 reduced both phasic contraction (upper trace) and slow waves (lower trace) in a dose-dependent manner (A – D). Tracings are from the same cell. Sampling sites are indicated by arrows. Phasic contraction (two-way repeated measures ANOVA, F ratio = 381.8, P = 3.03 × 10^-13), upstroke (F ratio = 98.3, P = 7.24 × 10^-17), and plateau amplitudes (F ratio = 219.3, P = 9.2 × 10^-28) of slow waves were dramatically reduced by SIN-1 in a dose-dependent manner. Spontaneous phasic contraction was most sensitive to NO donors, while plateau amplitude was more sensitive than upstroke amplitude (D, E). The duration of slow waves was also reduced slightly but significantly by SIN-1 (two-way repeated measures ANOVA, F ratio = 22.5, P = 6.34 × 10^-4). The frequency was unaffected by SIN-1 (F ratio = 2.60, P = 0.109) and did not show dose-dependence (F). Changes in RMP were not significant (F ratio = 1.08, P = 0.302) and only hyperpolarized about 2 mV at higher concentrations of SIN-1 (G) (n = 7).
dent manner. The IC_{50} of SIN-1 for phasic contraction,
upstroke amplitude, and plateau amplitude were
0.69±0.12, 38.6±2.4, and 24.7±2.9 M, respectively
(n=7, Fig. 3E). At the higher concentration of SIN-1
(100 M), upstroke and plateau were barely discernible
from each other (Fig. 3D). Slow wave duration was
significantly reduced by SIN-1 (10 M) (two-way
repeated measures ANOVA, F_{ratio}=22.5, P=6.34×10^{-4}).
The change of slow wave frequency by SIN-1 was
variable and statistical significance was not detected
(F_{ratio}=2.60, P=0.109) (Fig. 3F). The resting membrane
potential was hyperpolarized slightly by higher concen-
trations of SIN-1 (3 M). However, this effect was
neither concentration-dependent nor significant (F_{ratio}=
1.08, P=0.302) (Fig. 3G).

Involvement of cGMP-dependent pathway in the action
of NO on mechanical and electrical activity
The inhibitory actions of Cys-NO (1 M) and SIN-1
(30 M) on the mechanical and electrical activity were
antagonized by ODQ (10 M) (Fig. 4). A membrane
permeable cGMP analogue, 8-br-cGMP (0.1 – 300 M)
mimicked the inhibitory action of NO donors on sponta-
neous contraction and slow waves (Fig. 5: A – D). The
phasic contraction (two-way repeated measures ANOVA,
F_{ratio}=284.0, P=3.21×10^{-5}), upstroke (F_{ratio}=55.9,
P=2.69×10^{-10}), and plateau (F_{ratio}=154.1, P=1.07×10^{-14}) were inhibited by 8-br-cGMP in a concentration-
dependent manner. The IC_{50} of 8-br-cGMP for phasic
contraction, upstroke amplitude, and plateau amplitude were
2.36±0.72, 121±17, and 67.5±12 M, respectively
(n=5, Fig. 5E). At the higher concentration of 8-br-cGMP (300 M), the upstroke was barely distinguishable from the plateau (Fig. 5D). The duration of slow waves was reduced by 8-br-cGMP (10 M) (two-
way, repeated measures ANOVA, F_{ratio}=91.3, P=6.12×10^{-14}). However, neither frequency (F_{ratio}=2.72, P=
0.103) nor resting membrane potential (F_{ratio}=0.0061,
P=0.938) was significantly affected. (Fig. 5: F and G).

Effects of NO and 8-bromo-cGMP on the action potentials of slow wave
Some circular muscle strips of the guinea pig gastric
antrum showed spikes superimposed on the plateau of the slow wave. Cys-NO (1 M) and 8-br-cGMP abol-
ished these spikes without changing resting membrane potential (Fig. 6).

Effects of several K+ channel blockers on the action of
SIN-1
Pretreatment with apamin (0.1 M), a blocker of
small-conductance Ca^{2+}-activated K+ channels, insignifi-
cantly increased the amplitude of slow waves from
32.6±1.73 to 34.5±1.71 mV. Moreover, in the pres-
ence of apamin, SIN-1 (30 M) still inhibited the slow
waves in guinea pig gastric antrum (Fig. 7: A – C).
There was no significant difference between the SIN-1-
induced inhibition ratios in the presence (49.1±15.8%)
and that in the absence (49.3 ± 16.3%) of apamin (n = 5, Fig. 7D). The resting membrane potential was insignificantly depolarized by apamin from −66.9 ± 4.82 to −65.1 ± 3.82 mV. Either in the presence or in the absence of apamin, SIN-1 did not hyperpolarize the resting membrane potential (one-way ANOVA, \( F_{\text{ratio}} = 0.139, P = 0.497 \)) (n = 5, Fig. 7E).

Application of IbTX (0.1 \( \mu \)M), a blocker of large-conductance \( \text{Ca}^{2+} \)-activated \( \text{K}^{+} \) channels, slightly but not significantly increased slow wave amplitude from 27.5 ± 1.97 to 30.8 ± 2.95 mV. In addition, IbTX did not inhibit the action of SIN-1 (30 \( \mu \)M). Slow wave amplitude was still significantly decreased by SIN-1 to 16.2 ± 3.84 mV in the presence of IbTX (Fig. 8: A – C). The inhibition ratios by SIN-1 (30 \( \mu \)M) in the presence and in the absence of IbTX (0.1 \( \mu \)M) were 48.4 ± 13.4%
and 45.1 ± 14.8%, respectively (n = 7, Fig. 8D). There was no significant difference between the two values. IbTX insignificantly depolarized the resting membrane potential from −63.7 ± 1.71 to −60.5 ± 2.43 mV. SIN-1 did not significantly hyperpolarize the resting membrane potential either in the presence or in the absence of IbTX (one-way ANOVA, F ratio = 0.485, P = 0.506) (Fig. 8E).

Preincubation of glibenclamide (10 μM), a blocker of ATP-sensitive K+ channels, did not significantly change the amplitude of slow waves. SIN-1 significantly inhibited the amplitude of slow waves even after the preincubation of glibenclamide (Fig. 9: A – C). Inhibition ratios by SIN-1 in the presence and in the absence of glibenclamide were 45.5 ± 9.37 and 47.9 ± 9.81%, respectively.

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**Fig. 6.** Effects of NO and cGMP on action potentials. Some strips of guinea pig gastric antrum exhibited spikes on the plateau of slow waves. Cys-NO (1 μM) and 8-br-cGMP abolished these spikes without hyperpolarization. A and B were recorded from different muscle strips.

**Fig. 7.** Effect of apamin on the action of SIN-1 on slow waves of the circular muscle of guinea pig gastric antrum. Apamin (APA, 0.1 μM), a blocker of small-conductance Ca2+-activated K+ channels, insignificantly increased the amplitude of slow waves. Moreover, in the presence of apamin, SIN-1 (30 μM) still inhibited the slow waves in guinea pig gastric antrum (A – C). There was no significant difference between the SIN-1-induced inhibition ratios in the presence and that in the absence of apamin (D). The resting membrane potential was not affected by apamin. Either in the presence or in the absence of apamin, SIN-1 did not hyperpolarize the resting membrane potential (one-way ANOVA, F ratio = 0.139, P = 0.497) (E). (n = 5, *P<0.05 taken by the one way ANOVA test followed by the Newman-Keuls multiple comparison test)
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(n = 4, Fig. 9D). There was no significant difference between the two values. The resting membrane potential was not affected by glibenclamide alone or by SIN-1 in the presence of glibenclamide (one-way ANOVA, F ratio = 2.72 × 10^-4, P = 0.987) (Fig. 9E).

Discussion

In the present study, we observed that NANC nerve stimulation reduced the amplitude of phasic contraction in circular muscle of the guinea pig gastric antrum. L-NAME remarkably inhibited the effect of EFS, indicating that a significant portion of the inhibitory response depends upon synthesis of NO, as in other regions of the gastrointestinal tract (2). On the other hand, L-NAME increased neither the resting tone nor the phasic contraction in our experiments, suggesting that NO is not tonically released in this tissue.

The NO donors Cys-NO and SIN-1 mimicked the effect of EFS. In addition, they also inhibited spontaneous electrical activity; i.e., the slow wave. NO donors reduced the upstroke, plateau, and duration of slow waves, while leaving slow wave frequency unaffected. The reduction of plateau amplitude and the duration of slow waves was consistent with results from canine gastric antrum (17) and ileum (3). However, reduction of upstroke amplitude and lack of effect on frequency by NO conflicts with observations in the canine gastric antrum (17), canine ileum (3), and murine small intestine (18). We found that slow waves are less sensitive to NO donors than phasic contraction, which has also been reported in the above-mentioned tissues. It has been suggested that reduction of contractile protein sensitivity for Ca^{2+} is one of the mechanisms underlying the different sensitivity to NO between phasic contraction and slow waves (17, 19).

Our experiment showed that the inhibitory action of both EFS and NO donors was largely antagonized by ODQ, a sGC inhibitor. Moreover, a membrane permeable cGMP analogue, 8-br-cGMP, was found to mimic the effects of EFS or NO donors. These results suggest that NO action depends on sGC activation and the subsequent production of cGMP, which results in the reduction of the mechanical and electrical activity of...
circular muscle in guinea pig gastric antrum.

Some circular muscle strips of the guinea pig gastric antrum showed spikes superimposed on the plateau of the slow wave. These spikes are known to be inhibited by L-type Ca\(^{2+}\) channel blockers such as nifedipine (20) and occur when membrane potential reaches the threshold by slow wave plateau (21). Cys-NO and 8-br-cGMP abolished these spikes without changing resting membrane potential, which implies that NO could inhibit the activation of L-type Ca\(^{2+}\) channels by modulating the electrical activity of circular muscle in guinea pig gastric antrum. This is supported by the report that NO inhibits L-type Ca\(^{2+}\) current in canine colonic myocytes (22).

In the gastrointestinal tract, it is generally accepted that NO acts on smooth muscle cells to induce hyperpolarization and relaxation (11, 23 – 25). It was reported that NO directly activated large conductance Ca\(^{2+}\)-activated K\(^+\) channels in guinea pig proximal colon (12) or, in contrast, through a cGMP dependent pathway in guinea pig ileal longitudinal muscle (26, 27). Alternatively, NO has been reported to mediate NANC relaxation by a mechanism independent of changes in mem-

brane potential in rat proximal colon (28) and canine gastric fundus (19). In our present study, several K\(^+\) channel blockers such as apamin, IbTX, and glibenclamide did not inhibit the effect of SIN-1 on slow waves. In addition, neither NO donors nor 8-br-cGMP hyperpolarized membrane potential. These data suggest that, at least in our experimental conditions, the inhibitory effect of NO on spontaneous contraction and slow waves is not mediated by activation of Ca\(^{2+}\)-activated K\(^+\) channels or ATP-sensitive K\(^+\) channels. Similar results were reported in rat ileum, where the relaxation induced by SIN-1 or cGMP analogue were not affected by apamin, charybdotoxin, IbTX, or glibenclamide (29). However, we cannot exclude the possibility that the action of SIN-1 involves other K\(^+\) channels (30).

In summary, we found that NO donors inhibited phasic contraction and slow waves without hyperpolarization in circular muscle of the guinea pig gastric antrum. These effects are mediated largely by cGMP and unlikely to involve the activation of Ca\(^{2+}\)-activated or ATP-sensitive K\(^+\) channels.

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**Fig. 9.** Effect of glibenclamide on the action of SIN-1 on slow waves of the circular muscle of guinea pig gastric antrum. Glibenclamide (10 μM), a blocker of ATP-sensitive K\(^+\) channels, did not significantly change the amplitude of slow waves. SIN-1 significantly inhibited the amplitude of slow waves even after the preincubation of glibenclamide (A – C). There was no significant difference between the SIN-1-induced inhibition ratios in the presence and that in the absence of glibenclamide. The resting membrane potential was not affected by glibenclamide alone or by SIN-1 in the presence of glibenclamide (one-way ANOVA, F\(_{\text{rest}}\) = 2.72 × 10\(^{-4}\), P = 0.987) (E). (n = 4, *P<0.05 taken by the one way ANOVA test followed by the Newman-Keuls multiple comparison test)
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References


