Effects of endogenous and exogenous nitric oxide on electrical responses of circular smooth muscle isolated from the guinea-pig stomach antrum

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Abstract

The effects of endogenous and exogenous nitric oxide (NO) on electrical activity were investigated in circular smooth muscle preparations isolated from the guinea-pig stomach antrum. The actions of endogenous NO were evaluated from the effects of inhibition of NO synthesis by Nω-nitro-L-arginine (nitroarginine), while those of exogenous NO were assessed from the effects of SIN-1, an NO donor. Antral circular smooth muscle generated slow potentials periodically at a frequency of about 1 cycle per min (cpm), and unitary potentials were also generated in a random fashion in the interval between slow potentials. Application of nitroarginine (10^-5 M) increased the frequency of slow potentials, with no significant alteration of the resting membrane potential and amplitude of slow potentials. Frequency analysis of unitary potentials revealed that nitroarginine also increased the spectral density at 0.01–1 Hz frequency. The refractory period for the generation of slow potentials evoked by depolarizing pulses was about 10 s, but was decreased to 6 s by nitroarginine. In the presence of nitroarginine, SIN-1 (10^-9–10^-7 M) reduced the amplitude and frequency of slow potentials: low concentrations (<10^-8 M) reduced only the frequency of slow potentials, while higher concentrations (10^-8–10^-7 M) reduced both the amplitude and frequency of slow potentials, in a concentration-dependent manner, before abolishing the slow potentials. The power spectrum of the unitary potentials indicated that SIN-1 (>10^-8 M) reduced the spectral density at 0.01–1 Hz frequency. The refractory period for the generation of slow potentials was increased again to about 10 s by SIN-1. Thus, the excitatory effects of nitroarginine could be antagonized by SIN-1, suggesting that the inhibitory effects of endogenous NO are comparable to those of exogenous NO produced by SIN-1. The results also suggested that the effects of NO on smooth muscle are insignificant and NO selectively inhibits the activity of intramuscular interstitial cells of Cajal (ICC-IM).

Key words: gastric muscle, nitric oxide, slow potential, unitary potential, SIN-1
Introduction

Gastric smooth muscle is spontaneously active with rhythmic generation of slow waves or action potentials (Tomita, 1981). Spike potentials may be generated by activation of voltage-gated L-type Ca-channels, since these potentials are blocked by Ca-antagonists. However, the cellular mechanisms involved in the generation of slow waves remain unclear, and the contribution of voltage-gated L-type Ca-channels in the generation of these potentials may be negligibly small, since several types of Ca-antagonists do not prevent generation of slow waves. Slow waves may originate in interstitial cells distributed in the myenteric region (ICC-MY) (Sanders, 1996; Huizinga et al., 1997; Sanders et al., 1999; Suzuki, 2000; Hirst and Ward, 2003; Takaki, 2003). ICC-MY form gap junctions with both surrounding ICC-MY and smooth muscle cells (Komuro et al., 1996). Pacemaker potentials produced in ICC-MY are propagated to smooth muscle cells in an electrotonic manner (Dickens et al., 1999).

In isolated smooth muscle preparations of the guinea-pig gastric antrum, three types of electrical responses are recorded; slow waves recorded from circular smooth muscle, driving potentials (or pacemaker potentials) recorded from ICC-MY and square-shaped potentials (follower potentials) recorded from longitudinal smooth muscle cells (Dickens et al., 1999). Each of these potentials is synchronized, and the pacemaker potentials appear prior to either slow waves or follower potentials, indicating that ICC-MY are indeed pacing the rhythmic activities of circular and longitudinal smooth muscle cells (Dickens et al., 1999). However, isolated circular muscle bundles, in the absence of ICC-MY, also produce regenerative potentials (slow potentials) rhythmically (Suzuki and Hirst, 1999), possibly due to summation of unitary potentials produced in interstitial cells of Cajal distributed within muscle bundles (ICC-IM, Edwards et al., 1999). These two types of potentials, slow potentials and slow waves, are generated in circular smooth muscle under different conditions. Although both potentials are voltage-sensitive, with depolarization accelerating and hyperpolarization inhibiting their generation (Nose et al., 2000; Fukuta et al., 2002), the frequency is much higher for slow waves (4–6 cycle per min, cpm) than for slow potentials (0.1–2 cpm) (Suzuki and Hirst, 1999; Nose et al., 2000). Significant difference between these two types of potentials also appears in their sensitivity to caffeine, as low concentrations of caffeine (1 mM) abolish slow potentials but only reduces the amplitude of slow waves by about half (Dickens et al., 1999; Suzuki and Hirst, 1999; Nose et al., 2000).

Gastric smooth muscle is innervated by cholinergic excitatory nerves, nitrergic inhibitory nerves and non-adrenergic non-cholinergic (NANC) inhibitory nerves, which when electrically stimulated, respectively elicit atropine-sensitive excitatory junction potentials (EJP), nitroarginine-sensitive inhibitory junction potentials (IJP) with slow time course and apamin-sensitive IJP with fast time course (Ohno et al., 1996; Suzuki et al., 2003; Teramoto and Hirst, 2003). The EJP accelerates the generation of slow potentials, possibly by reducing the refractory period for the generation of slow potentials due to activation of protein kinase C (PKC), while slow IJP, possibly produced by neurogenic nitric oxide (NO) reduces the frequency of spontaneous activity due to inhibition of PKC (Lee et al., 2004). The inhibitory effects of neurogenic NO appear mainly on ICC-IM, as the inhibition of generation of unitary potentials (Suzuki et al., 2003; Teramoto and Hirst, 2003).
Experiments were carried out to compare the effects of endogenous and exogenous NO on electrical responses recorded from circular muscle bundles of the guinea-pig stomach antrum. All electrical responses examined were abolished by 1 mM caffeine, thereby confirming that these activities all originated from ICC-IM (Suzuki and Hirst, 1999; Edwards et al., 1999; Kito and Suzuki, 2003b). The effects of endogenous NO were assessed from the effects of nitroarginine. Exogenous NO was supplied by adding SIN-1, an NO-donor (Kukovetz and Holzmann, 1985), to the superfusate, in the presence of nitroarginine. The results indicated that both endogenous and exogenous NO inhibits the activities of circular muscle cells, by inhibiting the activity of intramuscular interstitial cells.

Methods

Male albino guinea-pigs (200–300 g in weight) were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane, Maruishi Pharm., Osaka, Japan), and exsanguinated by decapitation. All animals were treated ethically according to the Guidelines for the Care and Use of Laboratory Animals of Nagoya City University Medical School, as accredited by the Physiological Society of Japan. The stomach was excised, and opened by cutting along the small curvature in Krebs solution. The mucosal layers were removed by cutting with fine scissors, and smooth muscle tissue was excised from the antrum region.

A segment of circular muscle (about 1.5 mm width and 3 mm long) was isolated and a single circular muscle bundle preparation (250–300 µm long) made by removing the longitudinal muscle layer with the attached myenteric plexus. This was pinned out with the serosal side uppermost on the silicone rubber plate fixed at the bottom of the recording chamber (10 mm wide, 20 mm long, 2 mm deep, the volume about 1 ml). The preparations were superfused with warmed (35°C) and oxygenated Krebs solution, at a constant flow rate of about 2 ml/min.

Experiments were carried out in the presence of 1 µM nifedipine throughout, so as to minimize muscle movements. Conventional microelectrode techniques were used to record electrical activities of smooth muscle cells, and as reported previously (Suzuki and Hirst, 1999; Suzuki et al., 2002a; Kito et al., 2002b), they were usually recorded from two cells simultaneously using two glass capillary microelectrodes (outer diameter, 1.2 mm, inner diameter 0.6 mm; Hilgenberg, Germany) filled with 0.5 M KCl (the tip resistances ranging between 150 and 300 MΩ). In some experiments, responses were recorded using single electrodes. Electrical responses recorded with a high input impedance amplifier (Axoclamp-2B, Axon Instruments, Inc., Foster City, California, U.S.A.) were displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Osaka, Japan) and also stored on a personal computer for later analysis. Frequency analysis of unitary potentials was carried out along the methods reported previously (Edwards et al., 1999).

The ionic composition of the Krebs solution was as follows (mM): Na+ 137.4, K+ 5.9, Ca2+ 2.5, Mg2+ 1.2, HCO3– 15.5, H2PO4– 1.2, Cl– 134, and glucose 11.5. The solutions were aerated with O2 containing 5% CO2, and the pH of the solutions was maintained at 7.2–7.3.

Drugs used were caffeine, Nω-nitro-L-arginine (nitroarginine) and nifedipine (all from Sigma, St. Louis, MO, USA) and SIN-1 hydrochloride (3-morpholinosydnonimine HCl, Calbiochem, San
Diego, California, U.S.A.). Nifedipine and SIN-1 were dissolved in dimethyl sulphoxide (DMSO) to make stock solutions, and added to Krebs solution to make the desired concentrations just prior to use. Other drugs tested were dissolved in distilled water. The final concentration of the solvent in Krebs solution did not exceed 1/1000. Addition of these chemicals to Krebs solution did not alter the pH of the solution.

Experimental values were expressed as the mean value ± standard deviation (SD). Statistical significance was tested using the Student’s t-test, and probabilities of less than 5% (P<0.05) were considered to be significant.

Results

Effects of Nω-nitro-L-arginine on electrical activities of circular smooth muscle

In the presence of 10⁻⁶ M nifedipine, isolated circular smooth muscle tissue of the stomach antrum had resting membrane potentials ranging between –58 and –72 mV (mean, –65.1 ± 3.3 mV, n=20), and all preparations examined showed rhythmic generation of slow potentials, with associated random generation of unitary potentials between them. The frequency of slow potentials ranged from 0.1 to 2.3 cycle per min (cpm) (mean, 0.97 ± 0.59 cpm, n=20), while their amplitude ranged from 21.5 mV to 38.0 mV (mean, 30.8 ± 4.8 mV, n=20). These electrical activities were abolished reversibly by 1 mM caffeine (data not shown). These properties were similar to those reported previously (Suzuki and Hirst, 1999).

Experiments were carried out to test the effects of inhibiting the biosynthesis of nitric oxide (NO) with Nω-nitro-L-arginine (nitroarginine), a known inhibitor of NO synthase (Moncada et al., 1991), on electrical activities of antral circular smooth muscle bundle preparations. The abolition of slow potentials by 1 mM caffeine was confirmed in all preparations examined (data not shown). Figure 1 shows one of the typical effects of nitroarginine on spontaneous electrical activity of circular smooth muscle. Application of nitroarginine (10⁻⁵ M) increased the frequency of slow potentials, with no significant change in the resting membrane potential (Fig. 1, A & B). In this preparation, only the frequency, but not the amplitude and duration of slow potentials, was significantly increased by nitroarginine (Fig. 1, C, D & E). The data obtained from 20 preparations isolated from different animals are summarized in Fig. 2, and shows the effect of nitroarginine on the resting membrane potential (Fig. 2A), and on both the amplitude (Fig. 2B) and frequency (Fig. 2C) of the slow potentials. These results indicated that, although each parameter varied between preparations, nitroarginine increased the frequency of slow potentials significantly, but did not change either the resting membrane potential or the amplitude of the slow potentials (frequency, 2.1 ± 1.0 cpm, n=20, P<0.05; resting membrane potential, –64.8 ± 3.1 mV, n=20, P>0.05; amplitude of slow potentials, 31.9 ± 4.7 mV, n=20, P>0.05).

The effects of nitroarginine on unitary potentials were evaluated by frequency analysis. The spectral density of unitary potentials plotted as a function of the frequency showed that nitroarginine increased the density at 0.1–1 Hz frequency (Fig. 3). Thus, the results indicate that nitroarginine increases the generation of unitary potentials.

Slow potentials have a refractory period for their generation, and this may be one of the important factors which determine the frequency of spontaneous activity (Nose et al., 2000).
Attempts were made to observe the effects of nitroarginine on the refractory period for the generation of slow potentials by penetrating two microelectrodes into different cells in isolated small segments of circular muscle. Responses of the two cells were synchronized, indicating that both cells were electrically coupled. In the preparation shown in Fig. 4, application of a depolarizing current pulse (intensity, 3 nA; duration, 2 s) to one electrode at about 4.5 s after...
Fig. 3. Frequency analysis of the effects of nitroarginine on unitary potentials recorded from antral circular muscle bundles, in the absence (Control, filled circles) and presence of $10^{-5}$ M nitroarginine (open circles). Nifedipine $10^{-6}$ M was present throughout.

Fig. 4. Effects of nitroarginine on the refractory period of slow potentials. In a segment of antral circular muscle, electrical responses were recorded from two cells simultaneously. A muscle cell was stimulated by a depolarizing current pulse (2 s duration, 3 nA) applied to one electrode, and electrical responses evoked were recorded from another cell via the second electrode. Electrical stimulus was applied at about 5 s (Aa), 8 s (Ab) and 4 s (B) after cessation of a slow potential, in the absence (A) and presence of $10^{-5}$ M nitroarginine (B). The relationship between the time for stimulating the muscle cell after cessation of slow potentials and the amplitude of the evoked responses recorded in the absence (filled circles) and presence of $10^{-5}$ M nitroarginine (open circles) is summarized in D. SP, amplitude of spontaneously generated slow potentials in the absence (filled circle) and presence of $10^{-5}$ M nitroarginine (open circle) (mean ± S.D., $P>0.05$). Nifedipine $10^{-6}$ M was present throughout.
cessation of a slow potential produced an electrotonic potential followed by a group of unitary potentials to the second cell (Fig. 4Aa). Stimulation of the muscle cell at 8 s after a slow potential evoked an electrotonic potential with a following slow potential (Fig. 4Ab). Repeated stimulation of muscle with depolarizing pulses at different times following cessation of slow potentials indicated that a minimum latency (equivalent to the refractory period) of about 5 s was required for the generation of a response which would result in a following slow potential. In the presence of nitroargnine, however, stimulation of a muscle cell muscle about 4 s after a cessation of a slow potential resulted in the generation of another slow potential during electrotonic depolarization (Fig. 4B). The relationship between the time for pulse stimulation after cessation of slow potentials and the amplitude of the responses evoked by the pulse showed that the refractory period was about 5 s in the absence of nitroargnine, and it was reduced to about 3.5 s in the presence of nitroargnine, with no significant increase in amplitude of the evoked slow potentials (Fig. 4D). In 7 experiments, the refractory period was reduced from 9.5 ± 3.2 s to 5.5 ± 2.4 s by nitroargnine (P<0.05). The amplitude of the spontaneously generated slow potentials was not significantly changed by nitroargnine (Fig. 4D). These results suggest that NO is involved as one of the endogenous factors that increases the refractory period for the generation of slow potentials.

**Effects of SIN-1 on electrical responses of circular muscle tissue**

Experiments were carried out to test the effects of SIN-1, a known NO donor (Kukovetz and Holzmann, 1985), on circular smooth muscle bundle preparations, all in the presence of 10^{-5} M nitroargnine. One of the typical effects of SIN-1 on electrical responses is shown in Fig. 5, where the exposure of tissue to solution containing 10^{-8} M SIN-1 reduced the amplitude and frequency of slow potentials (Fig. 5, A and B). Increasing the concentration of SIN-1 to 10^{-7} M abolished the slow potentials and reduced unitary potential generation (Fig. 5C). In this particular preparation, the effects of different concentrations of SIN-1 (10^{-9} to 10^{-7} M) on the resting membrane potential (Fig. 6A), and on the amplitude (Fig. 6B), duration (Fig. 6C) and frequency of slow potentials (Fig. 6D) are summarized. The results indicated that the threshold concentration of SIN-1 required for inhibiting the frequency of slow potentials (equal to 3 × 10^{-9} M) was about 10 times lower than that required for the inhibition of the amplitude of slow potentials (equal to 3 × 10^{-8} M), i.e., SIN-1 inhibited the frequency of slow potentials at concentrations lower than that required to inhibit the amplitude of slow potentials. Slow potentials were nearly abolished in the presence of 3 × 10^{-8} M SIN-1, while the resting membrane potential was not altered by SIN-1 (up to 10^{-7} M). The duration of slow potentials was not reduced significantly in solutions containing SIN-1 concentrations up to 10^{-8} M (Fig. 6C).

The effects of SIN-1 on slow potentials were examined in 6 different preparations, and the variation of the effects is summarized in Fig. 7. The inhibition of slow potentials appeared between 3 × 10^{-8} and 10^{-6} M in 4 preparations, and only partial inhibition was observed in one preparation. In SIN-1 sensitive preparations, the inhibitory effects of SIN-1 on the frequency of slow potentials appeared at concentrations much lower than that for the amplitude. The inhibition of slow potentials by SIN-1 was reversible, and recovered after 10–15 min wash-out
**Fig. 5.** Electrical responses recorded from a segment of antral circular smooth muscle, in the absence (Control, A) and presence of SIN-1 (B, $10^{-8}$ M; C, $10^{-7}$ M). All responses were obtained from the same cell. Nitroarginine $10^{-5}$ M and nifedipine $10^{-6}$ M were present throughout.

**Fig. 6.** Summary of the effects of SIN-1 on the resting membrane potential (A) and slow potentials (B, amplitude; C, duration; D, frequency) recorded from a segment of antral circular smooth muscle. Mean ± S.D. (n=10–12). cpm, cycle per min. *: significantly different from 0 M SIN-1 ($P<0.05$). Nitroarginine $10^{-5}$ M and nifedipine $10^{-6}$ M were present throughout.
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Frequency analysis of unitary potentials indicated that SIN-1 (10^{-8} M) reduced the power spectrum of unitary potentials at 0.01–1 Hz frequency (Fig. 8). The inhibitory actions of SIN-1 on unitary potentials were reversible, and recovered after 10–15 min wash-out (data not shown).

By applying the two-electrode recording method to a small segment of circular smooth muscle preparations, electrotonic potentials produced by rectangular inward current pulses (1–2 s duration, 0.5–3 nA intensity) applied via one electrode were recorded in the other. The amplitude of electrotonic potentials produced by different intensities of current pulse was linear, either in the absence or presence of 10^{-8}–10^{-7} M SIN-1, and the current-voltage relationship was not significantly altered by SIN-1 (data not shown). Both the amplitude and time course of the electrotonic potentials were similar in the absence and presence of SIN-1 (Fig. 9, A & B). The input resistance calculated from these electrotonic potentials was 5.2 ± 0.3 MΩ (n=8) in control.
Fig. 8. Frequency analysis of unitary potentials recorded in the absence (filled circles) and presence of $10^{-8}$ M SIN-1 (open circles). Nitroarginine $10^{-5}$ M and nifedipine $10^{-6}$ M were present throughout.

Fig. 9. Effects of SIN-1 on electrotonic potentials produced by rectangular current pulses. In a segment of antral circular smooth muscle, membrane potentials were recorded from two cells simultaneously. A current pulse (0.6 nA intensity, 1 s duration) was applied to one electrode, and the electrotonic potential produced was recorded from the second electrode, in the absence (A) and presence of $3 \times 10^{-7}$ M SIN-1 (B). Upper and lower traces in A and B are change in membrane potential and stimulating current monitor, respectively. Mean amplitude and time constant of electrotonic potentials, measured in the absence and presence of SIN-1 (n=10–15 for each) are shown in graphs C and D, respectively. Nitroarginine $10^{-5}$ M and nifedipine $10^{-6}$ M were present throughout.
and the value was not significantly changed in the presence of $3 \times 10^{-7}$ M SIN-1 ($5.1 \pm 0.2$ MΩ, n=8, $P>0.05$). These results indicate that SIN-1 does not alter ionic conductance of the membrane in smooth muscle.

The effects of SIN-1 on the refractory period for slow potentials were examined. As shown in Fig. 10A, in the presence of $10^{-5}$ M nitroarginine, stimulation of the muscle with a depolarizing pulse (2 s duration, 0.8 nA intensity) at about 10 s after cessation of a spontaneously generated slow potential produced an electrotonic potential with a following slow potential. However, in the co-application of $3 \times 10^{-8}$ M SIN-1 with nitroarginine, stimulation of muscle with similar latency produced only an electrotonic potential, with no following slow potential generation (Fig. 10B). It required a delay of over 14 s for the depolarizing current pulse to elicit the regenerative amplitude of slow potentials (Fig. 10C). The relationship between the time for stimulating the muscle cell with a depolarizing current pulse after cessation of slow potentials and the amplitude of the evoked responses, obtained in the absence (filled circles) and presence of $3 \times 10^{-4}$ M SIN-1 (open circles), were plotted on the same scale (D). Mean values (± S.D.) of the amplitude of spontaneously generated slow potentials, in the absence (filled circle) and presence of SIN-1 (open circle) are shown at the left-hand side of the graph (both were significantly different). Nitroarginine $10^{-5}$ M and nifedipine $10^{-6}$ M were present throughout.

Fig. 10. Effects of SIN-1 on the refractory period of slow potential generation. In a segment of antral circular smooth muscle, a rectangular current pulse (2 s duration, 0.8 nA intensity) was applied at about 10 s (A and B) or at about 14 s (C) after cessation of a spontaneously slow potential. Responses were recorded in the absence (A) and presence of $3 \times 10^{-7}$ M SIN-1 (B and C). Traces A–C were recorded from the same cell. The relationship between the time for stimulating the muscle cell with a depolarizing current pulse after cessation of slow potentials and the amplitude of the evoked responses, obtained in the absence (filled circles) and presence of $3 \times 10^{-4}$ M SIN-1 (open circles), were plotted on the same scale (D). Mean values (± S.D.) of the amplitude of spontaneously generated slow potentials, in the absence (filled circle) and presence of SIN-1 (open circle) are shown at the left-hand side of the graph (both were significantly different). Nitroarginine $10^{-5}$ M and nifedipine $10^{-6}$ M were present throughout.
Discussion

The present experiments were designed to investigate the effects of endogenous and exogenous NO on electrical responses (slow potentials and unitary potentials) in circular smooth muscle isolated from the guinea-pig stomach antrum. The actions of endogenous NO were evaluated from the effects of inhibiting the biosynthesis of NO using nitroarginine, a known inhibitor of NO synthase. The effects of exogenous NO were examined by applying SIN-1, a known NO donor (Kukovetz and Holzmann, 1985). Antral circular smooth muscle preparations showed periodic generation of slow potentials and random generation of unitary potentials in the interval between slow potentials. These electrical activities were abolished by 1 mM caffeine, indicating that they are initiated by ICC-IM (Suzuki and Hirst, 1999; Edwards et al., 1999; Kito and Suzuki, 2003b). That is, the present experiments were carried out to observe the effects of NO on electrical activities of ICC-IM indirectly, by recording responses from smooth muscle cells. The results indicated that nitroarginine increased and SIN-1 decreased these spontaneous activities, suggesting that NO has inhibitory actions on the activity of ICC-IM.

Caffeine has inhibitory actions on many types of smooth muscle, but the cellular mechanism of the inhibition is controversial. In general, the inhibitory actions of caffeine are considered to be produced by the elevation of the tissue concentration of cyclic AMP (cAMP), as a result of the inhibition of phosphodiesterase activity (Arnoud, 1987). However in isolated stomach of the guinea-pig, caffeine inhibits spontaneous activities with no associated elevation of cAMP contents (Nakamura et al., 2004), as is also the case in the guinea-pig ileum (Prestwich and Bolton, 1995). Possible involvement of the inhibitory actions of caffeine on inositol 1,4,5-trisphosphate (IP3) receptors is also reported in a variety of tissues (Parker and Ivorra, 1991; Toescu et al., 1992; Berridge, 1993; Somlyo and Somlyo, 1994; Maes et al., 1999). Comparing the effects of caffeine on slow waves and slow potentials indicates that slow potentials are abolished by 1 mM caffeine, while only a partial inhibition is elicited for slow waves (Dickens et al., 1999; Suzuki and Hirst, 1999; Suzuki, 2000). Increasing the concentration of caffeine to 3 mM inhibits the plateau component of pacemaker potentials, and this allows visualization of the first component of pacemaker potentials (Hirst and Edwards, 2001). As the plateau component of pacemaker potentials may be formed by Ca2+-activated Cl– conductance (Kito et al., 2002a; Kito and Suzuki, 2003), it is reasonable to consider that the inhibition by caffeine may be due to the reduced release of Ca2+ from internal stores in ICC-MY.

The present experiments indicated that the excitation by nitroarginine and inhibition by SIN-1 of spontaneous activity of gastric muscle appeared with no significant change in the resting membrane potential. NO has inhibitory actions on some classes of K-channels (Kuriyama et al., 1998). Therefore, the results suggest that the inhibition of K-channels by NO does not occur in gastric smooth muscle. Alternatively, the concentration of NO required to modulate spontaneous electrical activities of gastric muscle was much lower than that for modulating K-channels. An absence of any change in the resting membrane potential during excitation of nitrergic nerves has been reported (Suzuki et al., 2003; Teramoto and Hirst, 2003), and these results suggest that endogenous NO may have no significant effect on the K-channels.
distributed in gastric smooth muscle. The observations made in the present experiments agree with the concept that NO selectively inhibits the activity of ICC-IM in gastric muscle (Suzuki et al., 2003; Teramoto and Hirts, 2003; Lee et al., 2004). The absence of any change in membrane potential, input resistance and time constant of the membrane during inhibition of slow potentials by SIN-1 also supports this concept. In addition, the absence of any change in electrotonic potentials during the inhibition of slow potentials by SIN-1 indicates that NO makes no significant alteration to the intercellular couplings through gap junctions.

In many tissues, NO produces inhibitory actions through elevated production of cyclic GMP (cGMP) due to activation of guanylate cyclase (Ignarro, 1991). Possible involvement of cGMP in the nitrergic inhibitory responses is suggested from the antagonism by oxadiazolo quinoxalin-1-one (ODQ), an inhibitor of guanylate cyclase, of nerve-stimulation induced responses in gastrointestinal muscle (Ward et al., 1992; Jun et al., 2003; Teramoto and Hirst, 2003). NO supplied from SIN-1 also activates guanylate cyclase and increases production of cGMP in many types of smooth muscle tissues (Rinaldi and Cingolani, 1983; Ignarro, 1991; Ward et al., 1995; Kito and Suzuki, 2003a; Jun et al., 2003). Although the present experiments did not test the effects of ODQ on gastric activity produced by endogenous and exogenous NO, the observed results suggest that the inhibition of antral activity by NO may be causally related to the elevated production of cGMP.

Spectral density of unitary potentials was increased by nitroarginine and decreased by SIN-1, confirming that NO inhibits the activity of ICC-IM (Suzuki et al., 2003; Teramoto and Hirst, 2003). Unitary potentials with properties similar to those of ICC-IM are also generated in ICC-MY in the stomach of guinea-pig (Kito et al., 2002c). However, the sensitivity of electrical activity to SIN-1 is very low in ICC-MY (Kito and Suzuki, 2003b). Thus, although the general properties of unitary potentials in both ICC-IM and ICC-MY are similar, their responsiveness to NO is different. Slow potentials generated in circular muscle are formed by the summation of unitary potentials (Edwards et al., 1999). Therefore, the modulation of slow potentials by nitroarginine and SIN-1 may be primarily related to the frequency of unitary potentials. The generation of slow potentials has a refractory period (Nose et al., 2000), and the activity of protein kinase C (PKC) is causally related to the refractory period (Kito et al., 2002b). PKC is also taking an important role for determining the frequency of spontaneously generated slow potentials (Suzuki et al., 2002c; Nakamura and Suzuki, 2003) and also the excitation of gastric muscles evoked by intramuscular nerve stimulation (Lee et al., 2004). Thus, the present results could be extrapolated to indicate that NO modulates the activity of ICC-IM by acting on PKC. If this is the case, the concentration of NO produced endogenously in the gastric muscle tissue may be of the order of $10^{-9}$–$10^{-8}$ M.

Ca-activated Cl-conductances may have a central role in the formation of pacemaker potentials (Tokutomi et al., 1995; Kito et al., 2002a) or slow potentials (Hirst et al., 2002). Significant inhibition by SIN-1 of the frequency of slow potentials before reducing their amplitude and duration suggests that this chemical has the ability to inhibit pacemaking mechanisms first in ICC-IM, and that its inhibitory actions on Ca-activated Cl-channels may be weak. Although ICC-IM take the place of pacemaker cells in isolated circular muscles, their physiological importance appears to be as the mediator of neuronal signals from enteric nerves...
to smooth muscle (Ward and Sanders, 2001). Therefore, the inhibition by NO of the activity of ICC-IM could effectively modulate nerve-mediated regulation of gastric smooth muscle.

In summary, both endogenous and exogenous NO reduces spontaneous electrical activities in antrum muscle of the stomach, by inhibiting the generation of unitary potentials and increasing the refractory period for slow potential generation, with no alteration of the resting membrane potential. As these potentials have been considered to be generated in ICC-IM, the site of actions of NO may be mainly on ICC-IM.

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