Korean J Physiol Pharmacol
Vol 4: 33−40, February, 2000

The Effect of NO Donor on Contraction, Cytosolic Ca^{2+} Level and Ionic Currents in Guinea-pig Ileal Smooth Muscle

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This study was designed to clarify the mechanism of the inhibitory action of a nitric oxide (NO) donor, 3-morpholino-sydnonimine (SIN-1), on contraction, cytosolic Ca^{2+} level ($[Ca^{2+}]_i$), and ionic currents in guinea-pig ileum. SIN-1 (0.01 ~ 100 μM) inhibited 25 mM KCl- or histamine (10 μM)-induced contraction in a concentration-dependent manner. SIN-1 reduced both the 25 mM KCl- and the histamine-stimulated increases in muscle tension in parallel with decreased $[Ca^{2+}]_i$. Using the patch clamp technique with a holding potential of −60 mV, SIN-1 (10 μM) decreased peak Ba currents (I_{Ba}) by 30.9±5.4% (n=6) when voltage was stepped from −60 mV to +10 mV and this effect was blocked by ODQ (1 μM), a soluble guanylyl cyclase inhibitor. Cu/Zn SOD (100 U/ml), the free radical scavenger, had little effect on basal I_{Ba}, and SIN-1 (10 μM) inhibited peak I_{Ba} by 32.4±5.8% (n=5) in the presence of Cu/Zn SOD. In a cell clamped at a holding-potential of −40 mV, application of 10 μM histamine induced an inward current. The histamine-induced inward current was markedly and reversibly inhibited by 10 μM SIN-1, and this effect was abolished by ODQ (1 μM). In addition, SIN-1 markedly increased the depolarization-activated outward K^+ currents in the all potential ranges. We concluded that SIN-1 inhibits smooth muscle contraction mainly by decreasing $[Ca^{2+}]_i$ resulted from the inhibition of L-type Ca^{2+} channels and the inhibition of nonselective cation currents and/or by the activation of K^+ currents via a cGMP-dependent pathway.

Key Words: Intestinal smooth muscle relaxation, SIN-1, $[Ca^{2+}]_i$, Ionic currents, Guinea-pig ileum

INTRODUCTION

Evidences from functional and morphological studies support the involvement of nitric oxide (NO) in nonadrenergic noncholinergic inhibitory neurotransmission in a number of intestinal smooth muscles (Furness et al, 1992; Rand & Li, 1995). Recent evidence also supports the thought that NO may be the primary inhibitory neurotransmitter in many gastrointestinal smooth muscles including guinea-pig ileum (Shuttleworth et al, 1991; Osthaus & Galligan, 1992).

NO is generally thought to cause smooth muscle relaxation by stimulation of soluble guanylate cyclase leading to an increase in guanosine 3′ : 5′ -cyclic monophosphate (cyclic GMP), with subsequent activation of cyclic GMP-dependent kinases leading to relaxation (Ignarro, 1990). Generally, NO induces smooth muscle relaxation through cyclic GMP-dependent mechanisms by (1) causing activation of K^+-channels and the resulting inhibition of voltage-operated Ca^{2+}-channels (Archer et al, 1994), (2) directly inhibiting voltage-operated Ca^2 channels (Lorenz et al, 1994), (3) activating the Ca^{2+} pump on the plasma membrane (Rashatwar et al, 1987; Yoshida et al, 1991), and (4) decreasing the sensitivity of contractile elements to Ca^{2+} (Karaki et al, 1997).

NO may have a role in regulating the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in smooth muscle cells. In this context, voltage-dependent (L-type) Ca^{2+}

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channels could be possible targets for the action of NO. It appears that L-type Ca^{2+} channels are known to be modulated by several intracellular second-messenger systems, including cGMP-dependent protein kinase pathway. 8-Br-cGMP or the NO-releasing agents sodium nitroprusside (SNP) and SNAP have been reported to lead to a decrease of Ca^{2+} channel activity (Clapp & Gurney, 1991; Ishikawa et al, 1993; Tewari & Simard, 1997). Furthermore, SNP has been shown to inhibit Ca^{2+} entry through endothelin-1-activated nonselective cation channels in rat aortic smooth muscle cells (Minowa et al, 1997).

Based on these findings, we now assume that NO induces relaxation mainly by suppressing cytosolic Ca^{2+} concentration in smooth muscle cells. In order to elucidate the precise mechanisms of the relaxant effect of NO donor, 3-morpholino-sydnonimine (SIN-1) on intestinal smooth muscle, we examined its effects on $[Ca^{2+}]_i$ and muscle tension in intact guinea-pig ileum. We also used whole-cell voltage clamp technique in freshly dispersed ileal smooth muscle cells and determined the effects of SIN-1 on the inward and outward currents.

METHODS

Tissue preparations

Male guinea-pigs (300~400 g) were killed by a sharp blow to the neck and exsanguination. The abdomen was opened and a section of ileum was isolated promptly and placed in physiological salt solution (PSS). Lumen of ileum was cleaned gently with PSS. A 7 mm diameter glass pipette was inserted in the lumen of the ileum and longitudinal muscle layer was separated from the underlying circular muscle layer. Segments of longitudinal muscle layer, about 10 mm long, was used for experiments.

Measurements of muscle tension

Each strip was attached to a holder under a resting tension of 0.5 g. After equilibration for 1 h in a PSS, each strip was repeatedly exposed to 40 mM KCl solution until the responses became stable. The high K^+ solution was prepared by replacing NaCl with equimolar KCl. These solutions were saturated with 100% O_2 at 37°C to maintain pH at 7.2. Muscle contraction was recorded isometrically with a force-displacement transducer and recorded on a pen-recorder.

Fura-2 loading and simultaneous measurements of tension and $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured according to the method described by Kwon et al (1993) using the fluorescent Ca^{2+} indicator, fura-2. Muscle strips were exposed to the acethoxymethyl ester of fura-2 (fura-2/AM, 5 μM) in the presence of 0.02% cremophor EL for 5~6 hr at room temperature. After loading, the muscle strips were washed with PSS at 37°C for 20 min to remove uncleaved fura-2/AM and was held horizontally in a temperature-controlled, 7 ml organ bath. One end of the muscle strip was connected to a force-displacement transducer to monitor the muscle contraction. The muscle strips were illuminated alternately (48 Hz) at two excitation wavelengths (340 and 380 nm). The intensity of 500 nm fluorescence (F340 and F380) was measured by using a fluorimeter (CAF-100; Jasco, Tokyo). The ratio of F340 to F380 (F340/F380) was calculated as an indicator of $[Ca^{2+}]_i$. The absolute Ca^{2+} concentration was not calculated in this experiment because the dissociation constant of the fluorescence indicator for Ca^{2+} in cytosol may be different from that obtained in vitro (Karaki, 1989). Therefore, the increase in ratio induced by 40 mM K^+ was considered as a reference response (100%).

Preparation of cells for patch clamp analysis

The muscle layers of ileum were cut into small pieces, placed in Ca^{2+}-free PSS. The Ca^{2+}-free PSS was replaced PSS containing 30 μM Ca^{2+} (low Ca^{2+} PSS). 30 min incubation at 37°C was carried out in fresh low-Ca^{2+} PSS containing collagenase (0.3 mg/ml), papain (0.3 mg/ml) and bovine serum albumin (1 mg/ml). After this enzyme digestion, tissue fragments were suspended in a fresh 120 μM Ca^{2+}-containing PSS and gently agitated. The resulting suspension was centrifuged at 600×g for 2 min and the cells were resuspended in a 0.5 mM Ca^{2+}-containing PSS. Cell suspension was placed on glass cover-slips and stored in a moist atmosphere at 4°C. Experiments were carried out at room temperature (22~24°C).

Whole-cell voltage clamp

Whole-cell membrane current and potential were recorded at room temperature using standard patch-clamp techniques. Patch pipette had a resistance of 3~6 MΩ when filled with a pipette solution. Membrane currents were measured with an Axopatch 1 C voltage-clamp amplifier (Axon Instrument). Command pulses were applied using an IBM-compatible computer and pCLAMP (version 5.5) software. The data were filtered at 2 kHz and displayed on a oscilloscope (Tektronix), a computer monitor and a pen recorder (Universal Oscillograph, Harvard).

Solutions

PSS used for the bath solution had following composition (mM): NaCl 126; KCl 6; $CaCl_2$ 2; $MgCl_2$ 1.2; glucose 14; HEPES 10.5 (pH 7.2 with NaOH). The patch pipette solution for outward K^+ currents had the following composition (mM): KCl 134, $MgCl_2$ 1.2; ATP 1; GTP 0.1; EGTA 0.05, glucose 14; HEPES 10.5 (pH 7.2 with KOH). Inward currents were isolated by the suppression of K^+ currents using pipette solution contained (mM): filled with CsCl 134, glucose 14, EGTA 0.05, HEPES 10.5 and Na_2 ATP 4 (pH 7.2 with CsOH). In experiments dealing with Ca^{2+} currents, $CaCl_2$ in the bath solution was replaced by $BaCl_2$.

Statistics

The results of the experiments are expressed as mean±S.E. mean; n: represents the number of strips of ileum. Student's t test was used for statistical analysis of the results and $p<0.05$ was considered to be significantly different.

RESULTS

The effect of SIN-1 on muscle-contraction

Fig. 1 shows the inhibitory effect of cumulative application of SIN-1 on the sustained contractions induced by high K^+ (25 mM) and histamine (10 μM) in guinea-pig ileum. Contractions induced by 10 μM histamine and 25 mM KCl showed similar sensitivity to SIN-1.

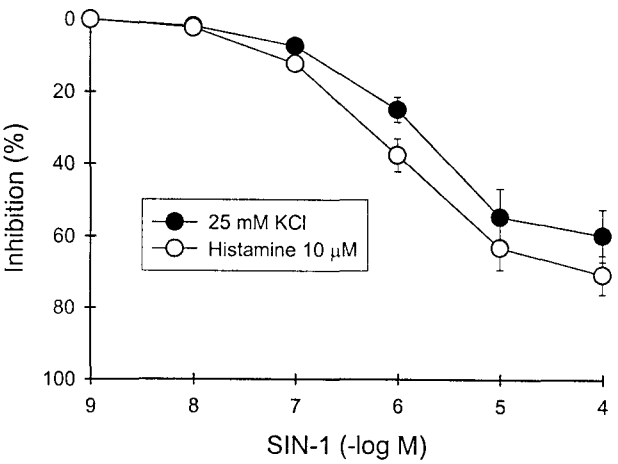

Fig. 1. Concentration-response curves of guinea-pig ileal smooth muscle strips precontracted with 25 mM KCl (●) and 10 μM histamine (○) to SIN-1. 100% represents the contractile tension before addition of SIN-1. Each points represents mean of 6 experiments and SE mean is shown by vertical bar when it is greater than symbol.

The effect of SNP on muscle-contraction and $[Ca^{2+}]_i$

As shown in Fig. 2, histamine (10 μM) and high K^+ (25 mM) induced sustained increases in the muscle tension and $[Ca^{2+}]_i$ in the muscle loaded with fura-2. Approximately 5 min after the application of a stimulant, the levels of muscle tension and $[Ca^{2+}]_i$ reached a plateau. In the presence of histamine, addition of 10 μM SIN-1 inhibited the plateau $[Ca^{2+}]_i$ to 68.2±5.7% (n=7) and muscle tension to 87.4± 6.3% (n=7). Addition of 10 μM SIN-1 also inhibited high K^+-induced increase in $[Ca^{2+}]_i$ to 23.4±5.4% (n=6) and muscle tension to 72.1±6.6% (n=6). The effects of SIN-1 were removed by washout (not shown).

Effects of SIN-1 on the inward Ca^{2+} and nonselective cation currents

The effects of 10 μM SIN-1 on Ca^{2+} currents through voltage-dependent Ca^{2+} channels that were examined in single ileal smoth muscle cells in a solution containing 2 mM Ba^{2+} (Fig. 3A). When Ba^{2+} was used instead of Ca^{2+} as the charge carrier, Ba^{2+} currents (I_{Ba}) were elicited by depolarization from a holding potential of −60 mV to a test potential of +10 mV for 80 ms at 0.1 Hz. Application of 10 μM SIN-1 reduced the peak I_{Ba} amplitude (30.9±5.3%, n=6). After washout of SIN-1, I_{Ba} recovered almost

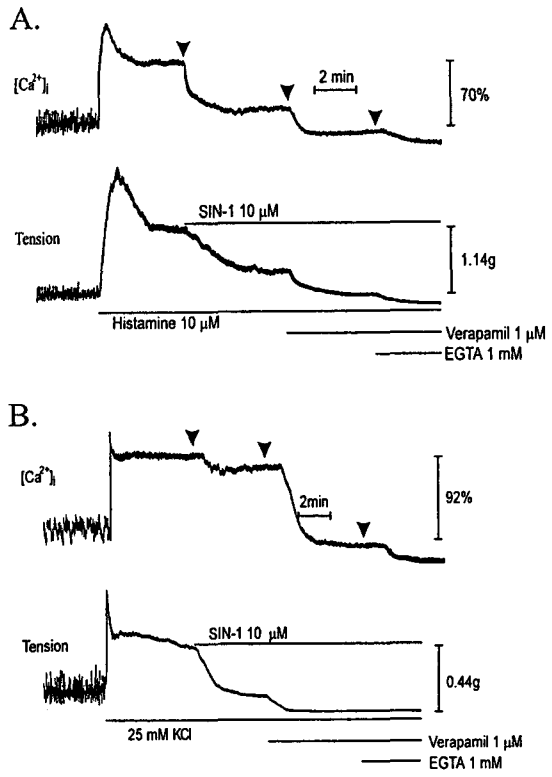

Fig. 2. The effect of SIN-1 on $[Ca^{2+}]_i$ (upper trace) and muscle tension (lower trace) in ileum in the presence of histamine (10 μM)(A) or 25 mM KCl (B). 100% represents the 40 mM KCl-induced increases in $[Ca^{2+}]_i$ measured at 5 min after application of high K^+. When $[Ca^{2+}]_i$ and muscle tension induced by histamine or 25 mM KCl reached a steady state level, 10 μM SIN-1 and 1 μM verapamil were cumulatively added. Then 1 mM EGTA was added.

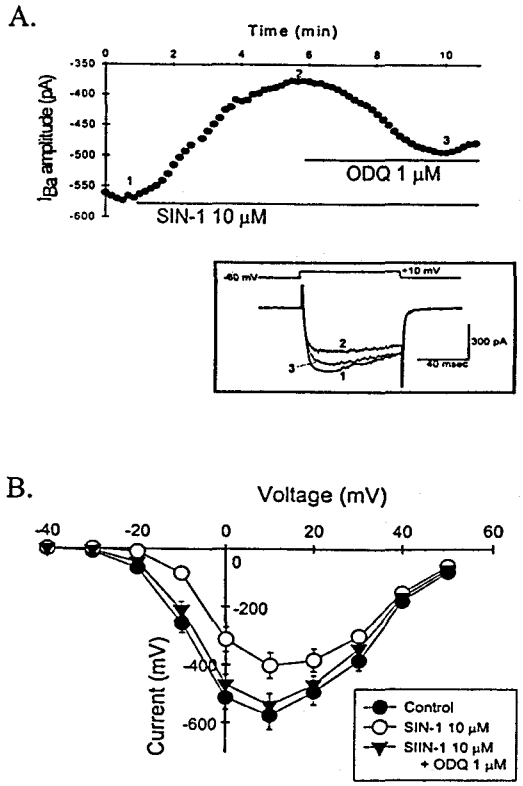

Fig. 3. Effect of SIN-1 on Ba currents (I_{Ba}) in the presence of ODQ in ileal smooth muscle cells. I_{Ba} was elicited by 80 ms depolarization from -60 mV to $+10$ mV at 0.1 Hz. (A) time course of change in peak amplitude of I_{Ba}. SIN-1 (10 μM) was applied during the period indicated by a horizontal bar. I_{Ba} was significantly inhibited, and then recovered by 1 μM ODQ. Inset: current traces which were recorded at the times corresponding indicated by 1, 2 and 3 in (A). B: Current-voltage relationships for the peak I_{Ba}. Control (●), in the presence of 10 μM SIN-1 (○) and in the presence of 10 μM SIN-1 and 1 μM ODQ (▼).

completely. ODQ (1 μM), a soluble guanylate cyclase inhibitor, showed no effect on basal I_{Ba} itself (data not shown), but it almost completely abolished the SIN-1-induced inhibition of I_{Ba} (Fig. 3A and 3B).

Fig. 4 shows the effects of 10 μM SIN-1 on I_{Ba} in ileal smooth muscle cells. Cu/Zn SOD (100 U/ml), the free radical scavenger, did not change either basal I_{Ba} or SIN-1-induced inhibition of I_{Ba} (Fig. 4A and 4B). Application of 10 μM SIN-1 reduced the peak I_{Ba} amplitude (32.4$\pm$5.8%, n=5).

In ileal smooth muscle cells clamped at a holding potential of -40 mV, application of 10 μM histamine induced a slow inward current with an increase in baseline fluctuation (Fig. 5A) Application of SIN-1 (10 μM) following onset of the sustained current produced a rapid reversal of the response to histamine, inhibiting the sustained current by 78.8$\pm$9.2% (n=6, Fig. 5A). The effects of SIN-1 was removed by washout (not shown). In cells treated with the selective inhibitor of soluble guanylyl cyclase, ODQ (1 μM), SIN-1 (10 μM) no longer inhibited the sustained current activated by histamine (Fig. 5B).

Effects of SIN-1 on the outward K^+ currents

Fig. 6A shows 10 superimposed current traces elicited by 900-ms test pulses that ranged from -40 to $+50$ mV in 10-mV increments from the holding

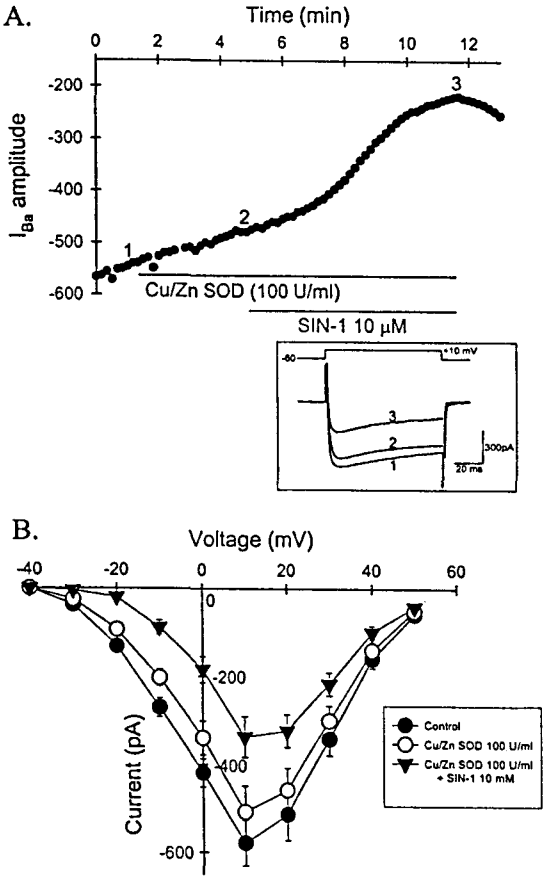

Fig. 4. Effect of SIN-1 on Ba currents (I_{Ba}) in ileal smooth muscle cells. I_{Ba} was elicited by 80 ms depolarization from −60 mV to +10mV at 0.1 Hz. (A) time course of change in peak amplitude of I_{Ba}. Cu/Zn SOD (100 U/ml) was applied during the period indicated by a horizontal bar. I_{Ba} was not affected, and 10 μM SIN-1 significantly inhibited I_{Ba} even in the presence of Cu/Zn SOD. Inset: current traces which were recorded at the times corresponding indicated by 1, 2 and 3 in (A). B: Current-voltage relationships for the peak I_{Ba}. Control (●), in the presence of 100 U/ml Cu/Zn SOD (○) and in the presence of 100 U/ml Cu/Zn SOD and 10 μM SIN-1 (▼).

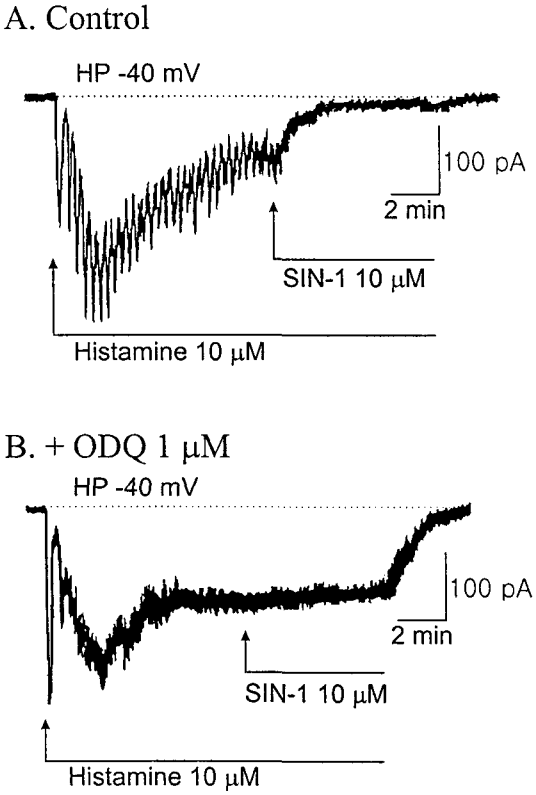

Fig. 5. Typical tracing of the effect of SIN-1 on histamine-induced inward currents in ileal smooth muscle cells. After whole cell was made, current was clamped at −40 mV under voltage clamp method. After histamine (10 μM)-induced inward current was reached to a steady-state, SIN-1 (10 μM) was applied to the bathing medium (A). In panel B, the same protocol was performed in the presence of 1 μM ODQ.

potential of −60 mV. Cells were dialyzed intracellularly with a KCl-rich solution containing 0.05 mM EGTA. The threshold for activation of the net outward currents was −30 mV. Larger depolarizing voltage steps elicited faster and larger outward currents that slowly decreased during maintained depolarization. SIN-1 (10 μM) increased the net outward currents (Fig. 6B). The current amplitude at the last step voltage (from −60 mV to +50 mV) increased from 1865±283 to 2837±293 pA (n=7) with the addition of SIN-1 to the bath. These effects were recovered after washout of SIN-1. Fig. 6C shows average I-V relationships for the net whole-cell current in control conditions or in the presence of 10 μM SIN-1.

DISCUSSION

In the guinea-pig ileal smoth muscle, SIN-1 inhibited the increase in $[Ca^{2+}]_i$ and muscle tension induced by stimulation with histamine or high K^+-deploarization. In smooth muscles, at least three types of channel regulation could produce the inhibitory responses of $[Ca^{2+}]_i$ caused by SIN-1: i) inhibition of voltage-dependent Ca^{2+} channel (L-type Ca^{2+} channel); ii) inhibition of non-selective cation channel

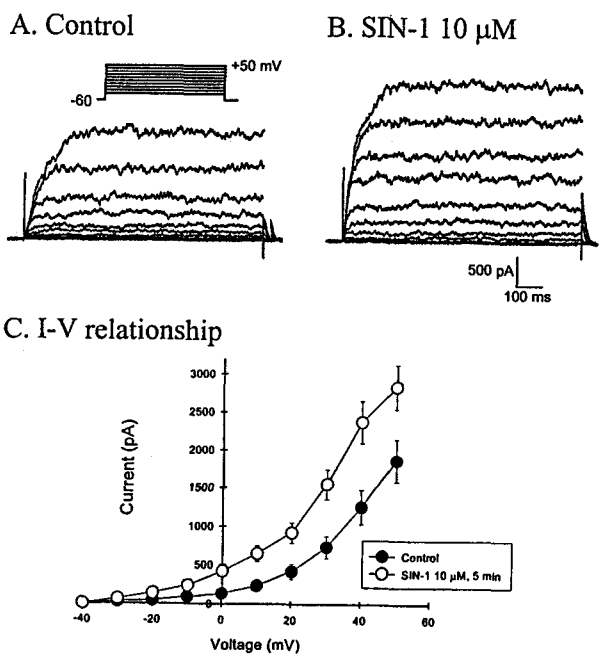

Fig. 6. The effect of SIN-1 on the outward K^+ currents recorded from ileal smooth muscle cells. In each panel the cell was held at −60 mV, and test depolarization with durations of 900 ms were applied from −40 to +50 mV in 10-mV increments. Currents were recorded using a pipette containing 0.05 mM EGTA. (A): Whole cell currents under control conditions. (B): Effect of 10 μM SIN-1 on the whole cell currents. (C): Current-voltage relationships recorded when maximum effects were seen at 5 min after addition to bath. ●; Control, ○, 10 μM SIN-1.

which mediates membrane depolarization or directly passes Ca^{2+} into cytoplasm, or iii) an increase in voltage- or Ca^{2+}-dependent K^+ channel.

Intestinal smooth muscle cells contain at least two types of voltage-dependent Ca^{2+}-channels (T- and L-types) (Yoshino et al, 1989). Since nifedifine inhibited the Ca^{2+} channel current in intestinal smooth muscles, it is suggested that the L-type Ca^{2+} current is the predominant current component (Nilius et al, 1985; Nowycky et al, 1985). SIN-1 partially but significantly decreased the inward current in the guinea-pig ileal smooth muscle was recovered after removal of SIN-1 (Fig. 3). Previous studies showed that NO decreases $[Ca^{2+}]_i$ primarily by inhibiting Ca^{2+} entry through voltage-gated Ca^{2+} channels in other smooth muscles (Clapp & Gurney, 1991; Blatter & Wier, 1994). These findings suggest that the inhibition of L-type Ca^{2+} currents may be at least partly responsible for the decrease in $[Ca^{2+}]_i$ in the guinea-pig ileal smooth muscle. Akbarali & Goyal (1994) reported that NO donor, SNP has a direct inhibitory effect on L-type Ca^{2+} currents in opossum esophageal circular muscle cells. However, this mechanism may not be responsible for the inhibition in ileum, since ODQ, a soluble guanylate cyclase inhibitor (Garthwaite et al, 1995), almost completely restored the SIN-1-induced inhibition of I_{Ba} current (Fig. 3)

In addition to releasing NO, SIN-1 also releases superoxide anions which could potentially react with NO to form peroxynitrite (Feelisch et al, 1989). To ensure that the cyclic GMP-dependent component of the relaxation was not due to peroxynitrite, experiments were also carried out in the presence of Cu/Zn SOD. The presence of SOD did not affect both the basal peak I_{Ba} amplitude or the SIN-1-evoked inhibition of peak I_{Ba} (Fig. 4). This indicates that superoxide anion was not involved in the SIN-1-evoked smooth muscle relaxation.

As has been reported by Inoue & Isenberg (1990), whole-cell recordings of patch clamp technique showed that acetylcholine activates nonselective cation channels in freshly dispersed guinea-pig ileal smooth muscle cells. The present results showed that SIN-1 reversibly inhibited this cation current activated by histamine (Fig. 5). Because the effect of SIN-1 can be antagonized by ODQ, it is likely that this mechanism is also mediated by guanylyl cyclase system coupled to NO. In vascular smooth muscles, it has also been reported that both NO donors and cGMP inhibit the nonselective cation currents (Minowa et al, 1997; Zhang et al, 1998). These results indicate that SIN-1 reduces the histamine-induced inward current by inhibiting the activity of a nonselective cation channels in the ileum.

An increase in the K^+ current that balances the inward current during the stimulation would tend to decrease the level of depolarization, which in turn inhibits L-type Ca^{2+} channel. In the present experiment, SIN-1 increased the outward K^+ current in all potential ranges (Fig. 6). Previously, it has been reported that both nitrovasodilator and cGMP induce outward currents by activating charybdotoxin-sensitive K^+ channels in vascular smooth muscle (Archer et al, 1994). He & Goyal (1993), using single cells of the guinea-pig ileum, showed an increase in outward current when SNP or 8-Br-cGMP was added to the bath solution. Our studies, however, do not preclude a direct action of SNP on KCa channels

(Kwon et al, 1998).

In summary, we demonstrated that SIN-1, through cyclic GMP-dependent signaling pathways, inhibits smooth muscle contration by decreasing $[Ca^{2+}]_i$ resulted from the inhibition of nonselective cation currents and the inhibition of Ca^{2+} currents in guinea-pig ileal smooth muscle.

ACKNOWLEDGEMENT

This work was supported by the Faculty Research fund of Yonsei University College of Medicine for 1996.

REFERENCES

Akbarali HI, Goyal RK. Effect of sodium nitroprusside on Ca^{2+} currents in opossum esophageal circular muscle cells. *Am J Physiol* 266: G1036−G1042, 1994

Archer SL, Huang JMC, Hampl V, Nelson DP, Shultz PJ, Weir EK. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc Natl Acad USA* 91: 7583−7587, 1994

Blatter LA, Weir WG. Nitric oxide decreases $[Ca^{2+}]_i$ in vascular smooth muscle by inhibition of the calcium current. *Cell Calcium* 15: 122−131, 1994

Clapp LH, Gurney AM. Modulation of calcium movements by nitroprusside in isolated vascular smooth muscle cells. *Pflugers Arch* 418: 462−470, 1991

Feelisch M, Ostrowski J, Noack E. On the mechanism of NO release from sydnonimines. *J Cardiovasc Pharmacol* 14: 513−522, 1989

Furness JB, Pompolo S, Shuttleworth CWR, Burleigh DE. Light- and electron-microscopic immunochemical analysis of nerve fibre types innervating the taenia of the guinea-pig caecum. *Cell Tiss Res* 270: 125−137, 1992

Garthwaite J, Southam E, Boulton CL, Nielsen EB, Schmidt K, Mayer B. Potent and selective inhibition of nitric oxide-sensitive gyanylyl cyclase by 1H-[1,2,4] oxadaizolo[4,3-a]quinoxalin-1-one. *Mol Pharmacol* 48: 184−188, 1995

He XD, Goyal PK. Nitric oxide involvement in the peptide VIP-associated inhibitory junctional potential in the guinea-pig ileum. *J Physiol* 461: 485−499, 1993

Ignarro LJ. Haem-dependent activation of guanylate cyclase and cyclic GMP formation by endogenous nitric oxide: A unique transduction mechanism for transcellular signalling. *Pharmacol Toxicol* 67: 1−7, 1990

Inoue R, Isenberg G. Effect of membrane potential on acetylcholine-induced inward current in guinea-pig ileum. *J Physiol* 424: 57−71, 1990

Ishikawa T, Hume JR, Keef KD. Regulation of Ca^{2+} channels by cAMP and cGMP in vascular smooth muscle cells. *Circ Res* 73: 1128−1137, 1993

Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K, Miyamoto S, Nakazawa H, Won KJ, Sato K. Calcium movement, distribution, and functions in smooth muscle. *Pharmacol Rev* 49: 157−230, 1997

Kwon S-C, Ozaki H, Hori M, Karaki H. Isoproterenol changes the relationship between cytosolic Ca^{2+} and contraction in guinea pig taenia caecum. *Jpn J Pharmacol* 61: 57−64, 1993

Kwon S-C, Rim SJ, Kang BS. Modulation of outward potassium currents by nitric oxide in longitudinal smooth muscle cells of guinea-pig ileum. *Korean J Physiol Pharmacol* 2: 225−232, 1998

Lorenz JN, Bielefeld DR, Sperelakis N. Regulation of calcium channel current in A7r5 vascular smooth muscle cells by cyclic nucleotides. *Am J Physiol* 266: C1656−C1663, 1994

Minowa T, Miwa S, Kobayashi S, Enoki T, Zhang XF, Komuro T, Masaki T. Inhibitory effect of nitrovasodilators and cyclic GMP on ET-1-activated Ca^{2+}-permeable nonselective cation channel in rat aortic smooth muscle cells. *Br J Pharmacol* 120: 1536−1544, 1997

Nilius B, Hess P, Lansman JB, Tsien RW. A novel type of cardiac calcium channel in ventricular cells. *Nature* 316: 443−436, 1985

Nowycky MC, Fox AP, Tsein RW. Three types of neuronal calcium channel with different calcium antagonist sensitivity. *Nature* 316: 440−443, 1985

Osthaus LE, Galligan JJ. Antagonists of nitric oxide synthesis inhibit nerve-mediated relaxations of longitudinal muscle in guinea pig ileum. *J Pharmacol Exp Ther* 260: 140−145, 1992

Rand MJ, Li CG. Nitric oxide as a neurotransmitter: Nature of transmitter and mechanism of transmission. *Annu Rev Physiol* 57: 659−682, 1995

Shuttleworth CWR, Murphy R, Furness JB. Evidence that nitric oxide participates in non-adrenergic inhibitory transmission to intestinal muscle in the guinea-pig. *Neurosci Lett* 130: 77−80, 1991

Tewari J, Simard JM. Sodium nitroprusside and cGMP decrease Ca^{2+} channel availability in basilar artery smooth muscle cells. *Pflugers Arch* 433: 304−311, 1997

Yoshida Y, Sun H-T, Cai J-Q, Imai S. Cyclic GMP-dependent protein kianse stimulate the plasma membrane Ca^{2+}-pump ATPase of vascular smooth muscle via phosphorylation of a 240 kDa protein. *J Biol Chem* 266: 19819−19820, 1991

Yoshino M, Someya T, Nishio A, Yazawa K, Usuki T, Yabu H. Multiple types of voltage-dependent Ca cha-

nnels in mammalian intestinal smooth muscle cells. *Pflugers Arch* 414: 401−409, 1989

Zhang X-F, Komuro T, Miwa S, Minowa T, Iwamuro' Y, Okamoto Y, Ninomiya H, Sawamura T, Masaki T. Role of nonselective cation channels as Ca^{2+} entry pathway in endothelin-1-induced contration and their suppression by nitric oxide. *Eur J Pharmacol* 352: 237−245, 1998