BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Nov; 16 (6): 493—496

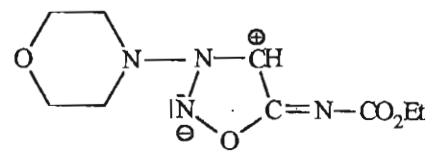
Effects of 3-morpholinosydnonimine-*N*-ethylcarbamide on hypoxia-induced mechanical and electric responses of isolated pig coronary artery[1]

YANG Xiao-Ping, WANG Fu-Xu, REN De-Cheng, LU Shi-Chen, FU Shao-Xuan, LI Yun-Shan (*Department of Pharmacology, Hebei Medical College, Shijiazhuang 050017, China*)

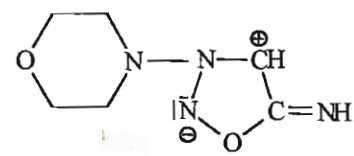
AIM: To study effects of 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1) on hypoxia-induced mechanical and electric activities of the isolated pig coronary artery. **METHODS**: Mechanical tension and membrane potential were measured simultaneously. **RESULTS**: Hypoxia initially caused a transient vascular smooth muscle cell membrane hyperpolarization followed by a membrane depolarization in isolated pig coronary artery. Subsequent addition of SIN-1 100 $\mu mol \cdot L^{-1}$ or verapamil (Ver) 10 $\mu mol \cdot L^{-1}$ led to membrane repolarization and relaxation of the vascular smooth muscle. Nitro-*L*-arginine (NLA) 0.2 $mmol \cdot L^{-1}$ and KCl 40 $mmol \cdot L^{-1}$ also induced membrane depolarization and vasoconstriction, which were similarly suppressed by SIN-1 or Ver. **CONCLUSION**: Hypoxic contractile response in isolated pig coronary artery is mediated by an increased Ca^{2+} influx via suppression of nitric oxide release.

KEY WORDS nitric oxide; verapamil; membrane potentials; coronary vessels; vascular smooth muscle; anoxia

Hypoxic contractile response of the isolated pig coronary artery might be related to the suppression of basal release of nitric oxide (NO) by endothelium[1]. NO released by endothelial cells can permanently hyperpolarizes the membrane of rat aorta smooth muscle cells and thereby may decrease the opening of voltage-dependent Ca^{2+} channels[2]. Severe hypoxia induced a depolarization and contraction of the human coronary artery[3]. It remains unclear whether hypoxia via inhibition of NO release can influence the membrane potential of the isolated pig coronary artery. Therefore, we observed effect of hypoxia on the electrical and mechanical activities of the isolated pig coronary artery, and roles of 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1, an active metabolite of molsidomine)[4,5], NO donor in these processes.



Molsidomine



SIN-1

MATERIALS AND METHODS

Coronary artery strip The right coronary arteries were taken from freshly slaughtered pig hearts. The helically cut strips of vessel (0.3—0.4 mm wide, 12—15 mm long) were mounted in a 2-mL bath that was continuously perfused with Krebs-Henseleit (K-H) solution 5 $ml \cdot L^{-1}$ (36.0 ± 0.5 ℃, pH 7.3—7.4) gassed with 95 % O_2 + 5 % CO_2. The functional endothelium was tested[1].

Mechanical and electric recording One end of

[1] Project supported by Natural Science Foundation of Hebei Province, № 393126.
Received 1994-08-01 Accepted 1995-04-04

the strip was firmly pinned on the silicon rubber in the bottom of the chamber with its intimal side upward, and another end was connected to a force transducer for 2 h at a resting tension of 1.5—2.0 g. A glass microelectrode (tip resistance 40—80 MΩ), filled with KCl 3 mol·L^{-1} was inserted into a smooth muscle cell from the intimal side. Successful impalements characterized by a sudden negative shift in voltage followed by a stable negative voltage, were maintained for 2 h of continuous recording. The electric signal was amplified by an amplifier (MEZ-8201). The tension and membrane potential were recorded simultaneously on a paper recorder.

Protocol Hypoxia was induced by 95 % N_2 + 5 % CO_2 for 40 min. Oxygenation was restored by K-H solution aerated with 95 % O_2+5 % CO_2. The hypoxic challenge was repeated twice at a 40-min interval of controlled oxygenation. In some experiments, after pretreatment with NLA for 30 min hypoxic challenge was performed. To study the effects of SIN-1 and Ver, after maximal response induced by hypoxia or NLA or KCl had reached, SIN-1 or Ver was injected into bath. Statistical significance between responses was evaluated by t test.

Drugs SIN-1 was from Casella AG (Frankfurt, Germany), NLA from Sigma and Ver from Tianjin He Ping Pharmaceutical Factory. All the drugs were prepared with freshly distilled water.

RESULTS

Effect of hypoxia on membrane potential and tension The resting membrane potential was 53 ± 4 mV (n=36). Hypoxia initially caused a transient membrane hyperpolarization (4.1 ± 2.0 mV, lasting 3.2 ± 0.6 min), but tension had no change. As hypoxia remained vascular smooth muscle cell developed sustained membrane depolarization and contraction. After hypoxia was remained for 7—8 min, it showed a maximal depolarization (−40 ± 3 mV, n=21) and a maximal contraction (502 ± 91 mg, n=21) (Fig 1).

Effects of SIN-1 and Ver on hypoxia As membrane depolarization and contraction induced by hypoxia had reached stable value,

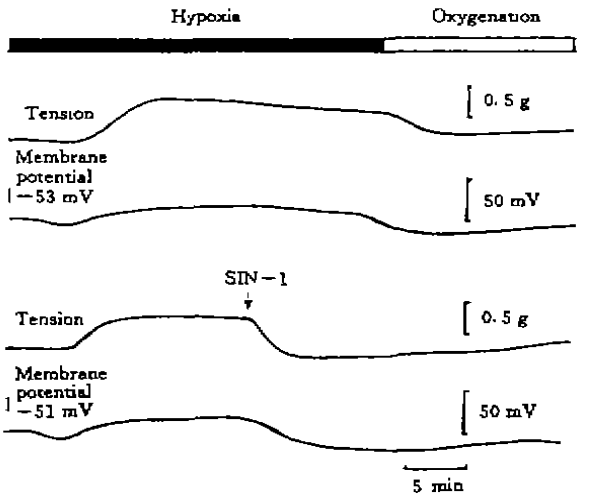


Fig 1. Effects of 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1, 100 μmol·L^{-1}) on hypoxia-induced depolarization and vasoconstriction of isolated pig coronary artery.

subsequent addition of SIN-1 100 μmol·L^{-1} led to a repolarization of the cell membrane to its resting level and vasorelaxation below basal tension. Addition of Ver 10 μmol·L^{-1} to the bath, the contractile response evoked by hypoxia was remarkably inhibited concomitant with membrane repolarization (Fig 1, Tab 1).

Effects of NLA and KCl on membrane potential and tension NLA 0.2 mmol·L^{-1} induced a sustained membrane depolarization and contraction of the isolated pig coronary artery, and steady values were obtained about 15 min after the onset of response. Subsequent addition of SIN-1 100 μmol·L^{-1} or Ver 10 μmol·L^{-1} developed a membrane repolarizing and relaxing response (Fig 2, Tab 1). KCl 40 mmol·L^{-1} evoked an obvious depolarization and contraction of the pig coronary artery. The depolarization induced by KCl was almost entirely repolarized by SIN-1 100 μmol·L^{-1} or Ver 10 μmol·L^{-1} accompaning vasorelaxation (Fig 2, Tab 1).

Effect of NLA on hypoxia Pretreatment

Tab 1. Effects of 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1) and verapamil on membrane depolarization and vasoconstriction induced by hypoxia, nitro-*L*-arginine (0.2 mmol·L^{-1}) and KCl (40 mmol·L^{-1}) in isolated pig coronary artery. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* resting membrane potetial; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* before.

Drug/ μmol·L^{-1}	Resting membrane potential/mV	Membrane potential/mV Before drugs	Membrane potential/mV After drugs	Contraction/mg Before drugs	Contraction/mg After drugs
Hypoxia					
Control	−55.0±1.8	−42.8±3.6^c	−42.0±3.2^d	550±114	548±89^d
SIN-1 (100)	−52.8±2.7	−41.7±2.7^c	−54.1±3.6^f	510±90	112±30$^{f@}$
Verapamil (10)	−53.5±3.8	−41.9±3.5^c	−51.6±4.0^f	526±78	52±21^f
Nitro-*L*-arginine					
Control	−54.8±2.2	−45.5±3.2^c	−44.0±2.5^d	448±93	450±60^d
SIN-1 (100)	−54.5±3.1	−46.1±1.7^c	−52.8±3.0^f	465±68	55±25^f
Verapamil (10)	−53.8±2.9	−44.8±3.4^c	−51.0±3.6^e	487±89	240±54^f
KCl					
Control	−54.0±2.0	−22.5±3.3^c	−23.5±2.6^d	1 117±193	1 100±217^d
SIN-1 (100)	−54.1±2.2	−21.8±1.7^c	−48.5±3.5^f	1 052±178	67±30^f
Verapamil (10)	−52.3±3.2	−24.0±2.6^c	−53.0±3.0^f	1 210±210	0

@: indicates relaxation below baseline.

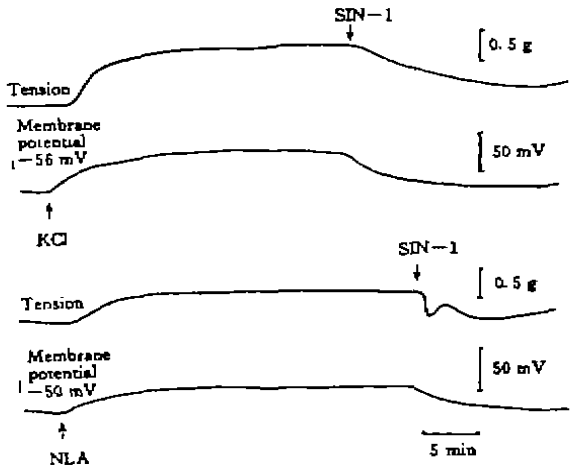


Fig 2. Effects of 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1, 100 μmol·L^{-1}) on KCl (40 mmol·L^{-1}) and nitro-*L*-arginine (0.2 mmol·L^{-1})-induced depolarization and vasoconstriction of isolated pig coronary artery.

with NLA 0.2 mmol·L^{-1} eliminated the mechanical and electric responses induced by hypoxia (Tab 2).

DISCUSSION

Results indicated that hypoxic vasoconstriction in pig coronary artery was mediated by membrane depolarization at the smooth muscle cell. This is consistant with the action of severe hypoxia on the human coronary artery[3]. It is generally accepted that higher K^+ open voltage-dependent Ca^{2+} channel and increase Ca^{2+} influx, and NLA (NO synthase inhibitor) reduce production of NO. The results that NLA induced depolarization and contraction, and can also abolish depolarization and vasoconstriction induced by hypoxia

Tab 2. Effect of nitro-*L*-arginine on hypoxia-induced depolarization and vasoconstriction of isolated pig coronary artery. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* resting membrane potetial; $^dP>0.05$, $^fP<0.01$ *vs* before hypoxia.

Drug/ μmol·L^{-1}	Resting membrane potential/mV	Membrane potential/mV Before drugs	Membrane potential/mV After drugs	Contraction/mg Before drugs	Contraction/mg After drugs
Control	−52.6±2.9	−53.6±2.4	−41.9±3.1^f	0	590±116
Nitro-*L*-arginine (0.2)	−54.0±2.7	−45.2±3.0^c	−42.7±2.6^{cd}	510±101	46±21^c

indicated that responses evoked by hypoxia would be relative to suppression of NO release. It had been reported that the smooth muscle cell membrane depolarization and basal $^{45}Ca^{2+}$ influx induced by NLA in rat aorta were consistently inhibited by SIN-1, NO donor or nisoldipine[2]. Our observations that SIN-1 and Ver induced membrane repolarizations and smooth muscle relaxations in hypoxia, NLA and high K^+-stimulated pig coronary artery strongly suggested that the membrane depolarization and vasoconstriction induced by hypoxia might be due to Ca^{2+} influx increased, whereas increased Ca^{2+} influx would be results of inhibition of NO release by endothelium.

ACKNOWLEDGMENT To Prof HE Rui-Rong for his invaluable suggestions and supplying SIN-1 and NLA.

REFERENCES

1 Yang XP, Wu ZJ, Xu YF, Dong W, Yang W, Fu SX, *et al*. Effects of inhibitor of endothelium-derived relaxing factor on hypoxic contraction of isolated pig coronary artery. Acta Pharmacol Sin 1994; **15**: 323—6.

2 Krippeit-Drews P, Morel N, Godfraind T. *Effects of nitric oxide on membrane potential and contraction of rat aorta.* J Cardiovasc Pharmacol 1992; **20** Suppl 12: S72—5.

3 Siegel G, Ruckborn K, Schnalke F, Grote J. Membrane physiological reactions of human arteriosclerotic coronary arteries to hypoxia. J Cardiovasc Pharmacol 1992; **20** Suppl 12: S217—20.

4 Noack E, Feelisch M. Molecular aspects underlying the vasodilator action of molsidomine. J Cardiovasc Pharmacol 1989; **14** Suppl 11: S1—5.

5 Schror K, Forster S, Wodtsch I, Schroder H. Generation of NO from molsidomine (SIN-1) *in vitro* and its relationship to changes in coronary vessel tone. J Cardiovasc Pharmacol 1989; 14 Suppl 11: S29—34.

484-486 4

3-Morpholinosydnonimine-*N*-ethylcarbamide 对缺氧诱发离体猪冠脉机械和电反应的影响

杨小平，王福旭，任德成，
吕士臣，傅绍萱，李蕴山
（河北医学院药理教研室，石家庄 050017，中国）

R965.2

目的：研究 3-Morpholinosydnonimine-*N*-ethylcarbamide (SIN-1)对缺氧诱发离体猪冠脉机械和电反应的影响. **方法**：同步记录机械张力和膜电位. **结果**：缺氧可诱发离体猪冠脉平滑肌细胞膜去极化和收缩；SIN-1 (100 μmol·L^{-1})和维拉帕米(Ver, 10 μmol·L^{-1})可使其复极化和松弛. SIN-1和 Ver 还可抑制左旋硝基精氨酸(NLA, 0.2 mmol·L^{-1})和 KCl (40 mmol·L^{-1})诱发的离体猪冠脉去极化和收缩反应. **结论**：缺氧收缩离体猪冠脉是其抑制一氧化氮释放、增加 Ca^{2+} 内流的结果.

关键词 一氧化氮；维拉帕米；膜电位；冠状血管；血管平滑肌；缺氧症

Information for authors

Acta Pharmacologica Sinica 1995 Jan; **16** (1): 3—16
Br Med J 1991 Feb 9; **301** (6772): 338—41
N Engl J Med 1991 Feb 7; **324** (6): 424—8

Reprints of any article published in Acta Pharmacologica Sinica may be purchased from the Institue for Scientific Information, 3501 Market Street, Philadelphia PA 19104, USA.

Fax: 1-215-386-6362. Phone: 1-215-386-0100.