FASEB J 1991; 5: 331 – 7.
6 Liu QY, Wang XL. The blocking effects of six antiarrhythmic drugs on transient outward current in rat ventricular myocytes. Acta Pharm Sin 1997; 32: 183 – 7.
7 Mewes T, Ravens U.
L-type calcium currents of human myocytes from ventricle of non-failing and failing hearts and from atrium.
J Mol Cell Cardiol 1994; 26: 1307 – 20.
8 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ.
Improved patch-clamp technique for high-resolution current recording from cells and cell-free membrane patches.
Pflügers Arch 1981; 391: 85 – 100.
9 Liu QY, Wang XL. Imipramine blocks the transient outward current. Acta Pharmacol Sin 1997; 18: 351 – 5.
10 Wettwer E, Amos G, Gath J, Zerkowski H-R, Reidemeister J-C, Ravens U. Transient outward current in human and rat ventricular myocytes. Cardiovasc Res 1993; 27: 1662 – 9.
11 Castle NA, Slawsky MT.
Characterization of 4-aminopyridine block of the transient outward K^+ current in adult rat ventricular myocytes.
J Pharmacol Exp Ther 1993; 264: 1450 – 9.
12 Beuckelmann DJ, Nabauer M, Erdmann E.
Alterations of K^+ in isolated human ventricular myocytes from patients with terminal heart failure.
Circ Res 1993; 73: 379 – 85.

人心房肌细胞瞬间外向钾电流的特性[1]

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关键词 钾通道；膜片箝技术；心房；心肌；4-氨基吡啶

目的：研究人的心房肌细胞瞬间外向钾电流（I_{to}）的特性. **方法**：采用膜片箝全细胞记录法观察人心房肌通道电流的变化，在用 $CdCl_2$ 0.1 mmol·L^{-1} 阻断钙电流的情况下，细胞膜去极化引出瞬间外向钾电流（I_{to}）. **结果**：I_{to}为电压依赖的电流，迅速激活，迅速失活. 4-AP（4-氨基吡啶）10 mmol·L^{-1}（选择性的 I_{to}的阻断剂）能完全阻断此电流. 其 IC_{50}值为 0.67 mmol·L^{-1}. 4-AP 1 mmol·L^{-1}能使 I_{to}的激活曲线明显向正电位移动，使 I_{to}的激活电压提高，且较难激活 I_{to}. **结论**：在人心房肌细胞，I_{to}是一种主要的钾电流，它可被 4-AP 阻断.

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Inhibitory effects of *S*-nitrosocaptopril on vasomotor tone[1]

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KEY WORDS nitric oxide; *S*-nitrosocaptopril; captopril; renal circulation; thoracic aorta; angiotensin-converting enzyme inhibitor; vascular endothelium; phenylephrine; arginine; vasodilation

AIM: To study effects of captopril (Cap) and *S*-nitrosocaptopril (CapNO) on vascular tension. **METHODS**: Tension of rabbit aortic rings and perfusion pressure of rat renal artery (PP_r) were examined to evaluate the effects of CapNO. **RESULTS**: CapNO (3 nmol·L^{-1} – 10 μmol·L^{-1}) produced concentration-dependent vasorelaxation response in the rings of rabbit thoracic aortas. Endothelium denudation did not alter the relaxations to CapNO. In contrast, Cap had no vasorelaxing effect on the rings precontracted with phenylephrine. CapNO (10 nmol·L^{-1}) decreased rat PP_r *in vivo* ($P < 0.01$), and the effect was concentration-dependent and reversible. Cap showed a reduction in rat PP_r only at the concentration 1000 nmol·L^{-1} ($P < 0.05$). The relaxing potency of CapNO was 100 times higher than that of Cap in this respect. Pre-perfusion of rat renal arteries with N^G-monomethyl-*L*-arginine monoacetate (*L*-NMMA, 0.03 nmol·L^{-1}) or *L*-arginine (3 nmol·L^{-1}) did not significantly blocked the relaxing effect induced by CapNO. **CONCLUSION**: CapNO had a direct vasodilatory effect.

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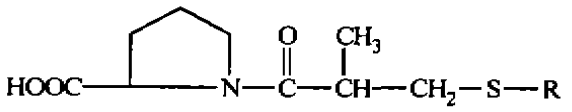
Endothelium-derived relaxing factor is generally considered to be a labile NO-containing precursor, most likely *S*-nitrosothiols. Decomposition of the *S*-nitrosothiols by homolytic cleavage of the *S* – *N* bond leads to nitric oxide (NO) release and the corresponding biological effects[1-3]. Due to a growing appreciation for NO-related responses that are not mediated directly by NO itself, there is increasing interest in compounds which generate NO in a controlled manner. Captopril (Cap) is an inhibitor of angiotensin converting enzyme (ACEI) containing a free sulfhydryl group that is not necessary for ACEI properties[4-6]. Dr JIA Li recently developed synthetic techniques capable of producing crystals of an *S*-nitrosylated Cap, *S*-nitrosocaptopril (CapNO, 1-[(2s)-3-nitrosomercapto-2-methyl-1-oxopropyl]-*L*-proline) that manifests both nitro-vasodilatory effect and ACEI activity. This study was to investigate effects of CapNO on vascular tension in comparison with that of Cap.



R = H Captopril
R = NO *S*-Nitrosocaptopril

MATERIALS AND METHODS

Rabbit aortic rings[7] New Zealand rabbit (♂, $n = 5$, 1.5 – 2.0 kg, Charles River Breeding Laboratories, Wilmington MA, USA) were exsanguinaled at common carotid arteries. The thoracic aortic rings (2.5 mm in length) were mounted in Krebs' solution (bubbled with 95 % O_2 + 5 % CO_2, 37 ℃) in 20 mL of organ chambers. Some experiments were performed using aortic rings in which the endothelium was removed by rubbing the lumen with a wooden applicator stick for 30 – 60 s. The absence of endothelium in these rings was confirmed by the lack of relaxation to acetylcholine (0.1 – 1.0 nmol · L^{-1}) after submaxmal contraction to an agonist.

Tension was measured isometrically, using Grass FTO3C transducers, and was displayed on model 7 Grass Polygraph. Rings were allowed to equilibrate for at least 1 h before experiments. Basal tension was maintained at approximately 2 g. Each ring was precontracted submaximally (30 % – 70 % of maximal tone) by phenylephrine (Phe) 100 – 200 nmol·L^{-1}. Results were expressed in % of relaxation of Phe-induced tone.

Perfusion pressure of the rat renal artery[8] Sprague-Dawley rats (♂, $n = 32$, 210 ± s 10 g, certificate of Grade Clean issued by Shanghai Medical Experimental Animal Management Committee) were purchased by Shanghai SPIPPR-PK Lab Animal Co and anesthetized by ip urethan 1 g · kg^{-1}. Rectal temperature was maintained at 37 ℃. The left renal artery was cannulated with PE 50 at its origin from aorta and perfused with Krebs' solution (bubbled with 95 % O_2 + 5 % CO_2, 37 ℃) by a peristaltic pump (DDB-300) at a rate of 0.8 mL · min^{-1}. Perfusion fluid was drained from a PE 50 tubing cannulated in the left renal vein.

The perfusing pressure of renal artery (PP_r) was measured using YM-3A pressure transducer and displayed on RM-6200 Polygraph (Nihon Kohden, Japan). Basal PP_r was maintained at approximately 80 % of carotid artery blood pressure. CapNO and Cap at the same concentration were seperately perfused into the renal artery at the same rate. The perfusion of one drug started 10 – 20 min after the effect of another drug dissapeared and PP_r recovered to the baseline.

Drugs CapNO synthesized by Dr JIA Li was in red crystalline powder that had no particular odor from sulfhydryl group. Its purity was > 97 % and mp was 49 – 52 ℃ when the red color of CapNO faded. The compound was soluble in water, acetone, ethanol, and when solubilized, produced a rose-colored solution. Infrared spectral (IR) analysis showed the presence of absorptions band corresponding to the *S*-NO band. ^1H nuclear magnetic resonance (NMR) spectrum of CapNO showed a loss absorption band at 6.1 – 6.3 μmol·L^{-1} corresponding to the sulfur proton of Cap.

Phenylephrine, *L*-arginine, N^G-monomethyl-*L*-arginine monoacetate (*L*-NMMA, Alexis, USA). Unless otherwise noted, all drugs were purchased from Sigma Chemical Co, USA and dissolved in Krebs' solution when using.

Analysis of data Data were expressed as $\bar{x} \pm s$, and compared using paired t test and ANOVA.

RESULTS

Effects of CapNO and Cap on endothelium-intact and endothelium-denuded rabbit aortic rings In Phe-precontracted rabbit aortic rings with intact endothelium, cummulative addition of CapNO from 3 nmol·L^{-1} – 10 μmol·L^{-1} produced concentration-dependent relaxation. The threshold concentration for CapNO in reducing tension was about 30 nmol·L^{-1} ($n = 5$, $P < 0.05$), ED_{50} value was (200 ± 27) nmol·L^{-1}. In contrast, Cap at the same concentration had no effects ($n = 5$, Fig 1).

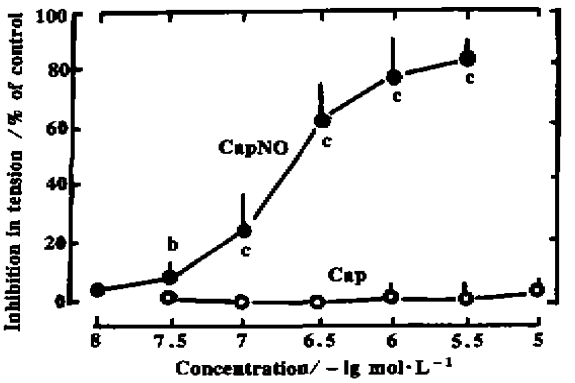


Fig 1. Inhibition of CapNO and Cap on tension of rabbit aortic rings precontracted with phenylephrine 100 – 200 nmol·L^{-1}. $n = 5$ rabbits. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs control value (2.4 ± 0.1) g before drugs.

The aortic rings denuded of endothelium still kept the same degree of relaxation induced by CapNO as endothelium-intact rings did (data not shown).

Effects of CapNO and Cap on the rat PP_r When PP_r induced by perfusion of the rat renal artery with Krebs' solution reached steady stable level, perfusion with progressive concentration of CapNO (10 – 1000 nmol·L^{-1}) produced concentration-dependent decrease in PP_r ($n = 6$, $P < 0.05$, Tab 1). However, Cap perfusion showed a reduction in PP_r only at concentration of 1000 nmol·L^{-1} ($n = 6$, $P < 0.05$, Tab 1), Cap at concentration of 10 and 100 nmol·L^{-1} had no effects ($n = 6$, $P > 0.05$, Tab 1). The magnitude and duration of relaxation in PP_r correlated well with perfusion concentration. The duration of reduction in PP_r induced by CapNO 10 – 1000 nmol·L^{-1} lasted for (24 ± 7), (33 ± 10), and (47 ± 20) min respectively. The duration of Cap at the same concentration was (16 ± 5), (19 ± 3), and (22 ± 4) min respectively.

Tab 1. Effects of CapNO and Cap on perfusion pressure in rat renal arteries. $n = 6$ rats, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control. $^fP < 0.01$ vs Cap.

Concentration (nmol·L^{-1})		Renal perfusion pressure (kPa) Before	After	Change	% Decrease of PPr
Cap	10	9.9 ± 1.1	9.6 ± 1.0	0.3 ± 0.3^a	2.7 ± 2.9
	100	10.7 ± 1.0	10.2 ± 1.5	0.5 ± 0.8^a	5 ± 7
	1 000	11.5 ± 2.4	10.5 ± 2.2	1.0 ± 0.7^b	9 ± 6
CapNO	10	10.9 ± 1.2	8.9 ± 0.6	1.9 ± 1.1^c	17 ± 8^f
	100	11.8 ± 1.9	8.8 ± 1.2	3.0 ± 1.1^c	25 ± 7^f
	1 000	11.3 ± 1.5	7.2 ± 1.2	3.7 ± 1.3^c	32 ± 10^f

Effects of *L*-NMMA and *L*-arginine on PP_r induced by CapNO The rate of increments in PP_r induced by preperfusion of renal artery with *L*-NMMA 0.03 nmol·L^{-1} for 5 min was (17 ± 6) % ($n = 7$, $P < 0.01$, Tab 2). The rate of reductions on PP_r induced by preperfusion with *L*-arginine 3 nmol·L^{-1} was (6 ± 2) % ($n = 7$, $P < 0.01$, Tab 2). There was no significant difference in reductions of PP_r evoked by CapNO 100 nmol·L^{-1}, between non-treatment and pretreatment with *L*-NMMA or *L*-arginine ($n = 7$, $P > 0.05$, Tab 2).

Tab 2. Effects of *L*-NMMA and *L*-arginine on PP_r evoked by CapNO. $n = 7$ rats, $\bar{x} \pm s$. $^cP < 0.01$ vs before PP_r. $^dP > 0.05$ vs before change of PP_r by CapNO.

	L-NMMA 0.03 nmol·L^{-1}	*L*-arginine 3 nmol·L^{-1}
PP_r(kPa)		
Before	11.3 ± 1.5	12.0 ± 1.6
After	14.0 ± 2.0^c	11.2 ± 2.0^c
Change %	17 ± 6	6 ± 2
Change of PP_r evoked by CapNO (% control)		
Before	19 ± 5	20 ± 6
After	20 ± 5^d	20 ± 9^d

DISCUSSION

The result of present study demonstrated that CapNO produced significantly concentration-

dependent (30 nmol · L^{-1} – 10 μmol · L^{-1}) vasorelaxation in isolated rabbit aortic rings with intact endotheium. Denudation of endothelium did not alter the vasorelaxation to CapNO. Thissuggested that vasorelaxation of CapNO was endothelium-independent. On the other hand, we also found that a regional infusion of CapNO into renal artery produced relaxations on PP_r in a concentration dependent manner. The magnitude and duration of relaxation correlated well with the graded increases in perfusion concentration. Pretreatment with *L*-NMMA, a competitive inhibitor of the NO synthase or *L*-arginine, a endogenous precursor for NO synthesis[9,10] did not significantly block the relaxing effect on PP_r evoked by CapNO 100 nmol· L^{-1}. This suggests CapNO is an exogenous NO donor. NO released by cleavage of *S* – *N* bond of CapNO induced relaxation of smooth muscle via activation of soluble guanylate cyclase and stimulation of cGMP production[10,11].

The relaxing potency of CapNO on both PP_r and tension of aortic rings was 100 times higher than that of Cap. Inhibitory rate of 98 % – 100 % induced by CapNO for ACE of rat lung tissue was similar to that of Cap[6,7]. Thus, the results indicated CapNO is a unique compound that inhibits vascular reactivity through activation of soluble guanylate cyclase and inhibition of ACE.

Increments of angiotensin Ⅱ and reductions in NO production and release from impaired or repaired endothelial cells are two unfarvourable factors for blood pressure and can contribute the development of hypertension[6,12]. CapNO corrected these two unfavourable factors and may have clinical implication for antihypertension.

REFERENCES

1 Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Circ Res 1983; 53: 557 – 73.

2 Singh RT, Hogg N, Joseph J, Kalyanaraman B. Mechanism of nitric oxide release from *S*-nitrosothiols. J Biol Chem 1996; 271: 18596 – 603.

3 Stamler JS, Jaraki O, Osborne J, Simon DI, Keaney J, Vita J, *et al*. Nitric oxide circulates in mammalian plasma primarily as an *S*-nitroso adduct of serum albumin. Proc Natl Acad Sci USA 1992; 89: 7674 – 7.

4 Jia L. Some advance in angiotensin converting enzyme inhibitor. Prog Physiol Sci 1985; 16: 229 – 34.

5 Jia L, Liu XJ, Pei RJ. The clinical trial and medical application prospect of nitric oxide and its donor. Chin Pharmacol Bull 1997; 13: 204 – 6.

6 Loscalzo J, Smick D, Andon N, Cooke J. *S*-nitrosocaptopril. Ⅰ. Molecular characterization and effects on the vasculature and on platelets. J Pharmacol Exp Ther 1989; 249: 726 – 9.

7 Jia L, Furchgott RF. Inhibition by sulfhydryl compounds of vascular relaxation induced by nitric oxide and endothelium-derived relaxing factor. J Pharmacol Exp Ther 1993; 267: 371 – 8.

8 Wang J, Jia BJ. Effects of pressor area of ventral surface of medulla oblongata on the vasotonicity of renal vessels of rats. Acta Physiol Sin 1993; 45: 359 – 67.

9 Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from *L*-arginine. Nature 1988; 333: 664 – 6.

10 Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43: 109 – 42.

11 Fung H-L, Chong S, Kowaluk E, Hough K, Kakemi M. Mechanisms for the pharmacologic interaction of organic nitrates with thiols. Existence of an extracellular pathway for the reversal of nitrate vascular tolerance by *N*-acetylcysteine. J Pharmacol Exp Ther 1988; 245: 524 – 30.

12 Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A. Defective *L*-arginine-nitric oxide pathway in offspring of essential hypertensive patients. Circulation 1996; 94: 1298 – 1303.

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巯亚硝酰卡托普利的降低血管紧张性作用[1]

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关键词 一氧化氮; 巯亚硝酰卡托普利; 卡托普利; 肾循环; 胸主动脉; 血管紧张素转换酶抑制剂类; 血管内皮; 苯福林; 精氨酸; 血管舒张

目的: 观察巯亚硝酰卡托普利(CapNO)和卡托普利(Cap)对血管紧张性的影响. **方法**: 记录家兔胸主动脉环张力和大鼠肾动脉灌流压(PP_r). **结果**: CapNO 对苯福林预收缩的内皮完整与去内皮主动脉环, 均呈浓度依赖性(30 nmol · L^{-1} – 10 μmol · L^{-1}, $P<0.01$)的舒张作用, 而相同浓度的 Cap 作用不显著; CapNO 降低 PP_r 的作用亦呈浓度依赖性(10 nmol · L^{-1} – 1000 nmol · L^{-1}, $P<0.01$), 而 Cap 只在 1000 nmol · L^{-1} 呈显著性变化; *N*-单甲基左旋精氨酸和左旋精氨酸预处理均不影响 CapNO 诱发的降 PP_r 作用. **结论**: CapNO 具有直接降低血管紧张性作用.