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481-485

人心房肌细胞瞬间外向钾电流的特性<sup>1</sup>徐文洪, 李婉, 岳红文<sup>2</sup>, 王晓良<sup>3</sup>(中国医学科学院和中国协和医科大学 药物研究所,  
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4-氨基吡啶

A 372.2

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: 采用膜片箱全细胞记录法观察人心房肌通道电流的变化, 在用  $CdCl_2$   $0.1 \text{ mmol} \cdot L^{-1}$  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)  $10 \text{ mmol} \cdot L^{-1}$  (选择性的  $I_{to}$  的阻断剂) 能完全阻断此电流。其  $IC_{50}$  值为  $0.67 \text{ mmol} \cdot L^{-1}$ 。4-AP  $1 \text{ mmol} \cdot L^{-1}$  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。

Inhibitory effects of S-nitrosocaptopril on vasomotor tone<sup>1</sup>LIN Mo-Jun<sup>2</sup>, YANG Xin-Ping, WANG Jing, JIA Bing-Jun

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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 ( $3 \text{ nmol} \cdot L^{-1}$  -  $10 \mu\text{mol} \cdot L^{-1}$ ) produced concentration-dependent vaso-

relaxation 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 ( $10 \text{ nmol} \cdot L^{-1}$ ) 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  $1000 \text{ nmol} \cdot L^{-1}$  ( $P < 0.05$ ). 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,  $0.03 \text{ nmol} \cdot L^{-1}$ ) or L-arginine ( $3 \text{ nmol} \cdot L^{-1}$ ) did not significantly blocked the relaxing effect induced by CapNO. **CONCLUSION:** CapNO had a direct vasodilatory effect.

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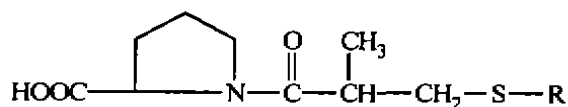
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Endothelium-derived relaxing factor is generally considered to be a labile NO-containing precursor, most likely *S*-nitrosothiols. Decomposition of the *S*-nitrosothiols by homolytic cleavage of the *S* - *N* bond leads to nitric oxide (NO) release and the corresponding biological effects<sup>[1-3]</sup>. 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<sup>[4-6]</sup>. Dr JIA Li recently developed synthetic techniques capable of producing crystals of an *S*-nitrosylated Cap, *S*-nitrosocaptopril (CapNO, 1-[(2*S*)-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**<sup>[7]</sup> New Zealand rabbit ( $\hat{\sigma}$ ,  $n = 5$ , 1.5 - 2.0 kg, Charles River Breeding Laboratories, Wilmington MA, USA) were exsanguinated 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 °C) 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  $\mu\text{mol} \cdot \text{L}^{-1}$ ) after submaximal 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  $\text{nmol} \cdot \text{L}^{-1}$ . Results were expressed in % of relaxation of Phe-induced tone.

**Perfusion pressure of the rat renal artery**<sup>[8]</sup> Sprague-Dawley rats ( $\hat{\sigma}$ ,  $n = 32$ , 210  $\pm$  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  $\text{g} \cdot \text{kg}^{-1}$ . Rectal temperature was maintained at 37 °C. 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 °C) by a peristaltic pump (DDB-300) at a rate of 0.8  $\text{mL} \cdot \text{min}^{-1}$ . 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 separately perfused into the renal artery at the same rate. The perfusion of one drug started 10 - 20 min after the effect of another drug disappeared 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 °C 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.  $^1\text{H}$  nuclear magnetic resonance (NMR) spectrum of CapNO showed a loss absorption band at 6.1 - 6.3  $\mu\text{mol} \cdot \text{L}^{-1}$  corresponding to the sulfur proton of Cap.

Phenylephrine, *L*-arginine, *N*<sup>G</sup>-methyl-*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, cumulative addition of CapNO from 3 nmol·L<sup>-1</sup> - 10 μmol·L<sup>-1</sup> produced concentration-dependent relaxation. The threshold concentration for CapNO in reducing tension was about 30 nmol·L<sup>-1</sup> (*n* = 5, *P* < 0.05), ED<sub>50</sub> value was (200 ± 27) nmol·L<sup>-1</sup>. In contrast, Cap at the same concentration had no effects (*n* = 5, Fig 1).

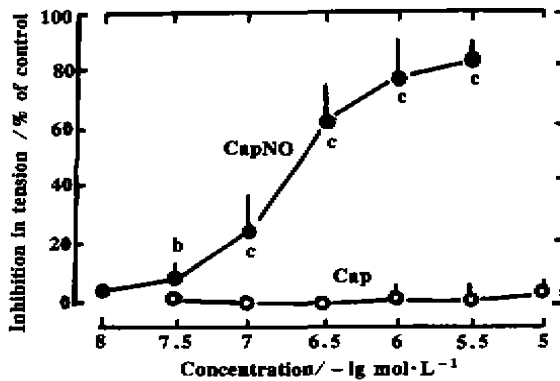


Fig 1. Inhibition of CapNO and Cap on tension of rabbit aortic rings precontracted with phenylephrine 100 - 200 nmol·L<sup>-1</sup>. *n* = 5 rabbits.  $\bar{x} \pm s$ . <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs control value (2.4 ± 0.1) g before drugs.

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<sub>r</sub>** When PP<sub>r</sub> 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<sup>-1</sup>) produced concentration-dependent decrease in PP<sub>r</sub> (*n* = 6, *P* < 0.05, Tab 1). However, Cap perfusion showed a reduction in PP<sub>r</sub> only at concentration of 1000 nmol·L<sup>-1</sup> (*n* = 6, *P* < 0.05, Tab 1), Cap at concentration of 10 and 100 nmol·L<sup>-1</sup> had no effects (*n* = 6, *P* > 0.05, Tab 1). The magnitude and duration of relaxation in PP<sub>r</sub> correlated well with perfusion

concentration. The duration of reduction in PP<sub>r</sub> induced by CapNO 10 - 1000 nmol·L<sup>-1</sup> 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,  $\bar{x} \pm s$ . <sup>a</sup>*P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs control. <sup>d</sup>*P* < 0.01 vs Cap.

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

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

Tab 2. Effects of L-NMMA and L-arginine on PP<sub>r</sub> evoked by CapNO. *n* = 7 rats,  $\bar{x} \pm s$ . <sup>c</sup>*P* < 0.01 vs before PP<sub>r</sub>. <sup>d</sup>*P* > 0.05 vs before change of PP<sub>r</sub> by CapNO.

|   | L-NMMA 0.03 nmol·L <sup>-1</sup> | L-arginine 3 nmol·L <sup>-1</sup> |
|---|----------------------------------|-----------------------------------|
| PP <sub>r</sub> (kPa)                                 |                                  |                                   |
| Before  | 11.3 ± 1.5                       | 12.0 ± 1.6                        |
| After   | 14.0 ± 2.0 <sup>c</sup>          | 11.2 ± 2.0 <sup>c</sup>           |
| Change %  | 17 ± 6                           | 6 ± 2                             |
| Change of PP <sub>r</sub> evoked by CapNO (% control) |                                  |                                   |
| Before  | 19 ± 5                           | 20 ± 6                            |
| After   | 20 ± 5 <sup>d</sup>              | 20 ± 9 <sup>d</sup>               |

**DISCUSSION**

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

dependent ( $30 \text{ nmol} \cdot \text{L}^{-1} - 10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ ) vasorelaxation in isolated rabbit aortic rings with intact endothelium. Denudation of endothelium did not alter the vasorelaxation to CapNO. This suggested 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<sub>r</sub> 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, an endogenous precursor for NO synthesis<sup>(9,10)</sup> did not significantly block the relaxing effect on PP<sub>r</sub> evoked by CapNO  $100 \text{ nmol} \cdot \text{L}^{-1}$ . 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<sup>(10,11)</sup>.

The relaxing potency of CapNO on both PP<sub>r</sub> 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<sup>(6,7)</sup>. 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 unfavourable factors for blood pressure and can contribute the development of hypertension<sup>(6,12)</sup>. CapNO corrected these two unfavourable factors and may have clinical implication for antihypertension.

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485-488

巯亚硝酰卡托普利的降低血管紧张性作用<sup>1</sup>

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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注压 (PP<sub>r</sub>)。 **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 ( $30 \text{ nmol} \cdot \text{L}^{-1} - 10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ ,  $P < 0.01$ ) 的舒张作用, 而相同浓度的 Cap 作用不显著; CapNO 降低 PP<sub>r</sub> 的作用亦呈浓度依赖性 ( $10 \text{ nmol} \cdot \text{L}^{-1} - 1000 \text{ nmol} \cdot \text{L}^{-1}$ ,  $P < 0.01$ ), 而 Cap 只在  $1000 \text{ nmol} \cdot \text{L}^{-1}$  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<sub>r</sub> 作用。 **结论:** CapNO 具有直接降低血管紧张性作用。