降钙素基因相关肽介导的离体大鼠心脏缺血预处置[1]

肖洲生，李元建[2]，邓汉武
(湖南医科大学药理教研室，长沙 410078，中国)

关键词 降钙素基因相关肽；心肌再灌注损伤；心功能试验；肌酸激酶

目的：研究降钙素基因相关肽(CGRP)在离体大鼠心脏缺血预处置(PC)中的介导作用。方法：离体大鼠心脏缺血 5 min 后再灌 5 min，重复三次，然后缺血 30 min，再灌 30 min。结果：PC 显著改善再灌时心功能，降低室性心律失常发生率和减少心肌 CK 释放(对照、缺血再灌和预处置组 CK 活性分别为 0.30±0.07、2.03±0.49 和 0.92±0.4 $U \cdot min^{-1}/g$ wet wt)。CGRP 受体拮抗剂 $CGRP_{8-37}$ ($0.1\ \mu mol \cdot L^{-1}$)能取消 PC 所致的心功能改善，室性心律失常发生率降低和 CK 释放减少(预处置和 $CGRP_{8-37}$ 处理组 CK 活性分别为 0.92±0.4 和 2.55±0.32 $U \cdot min^{-1}/g$ wet wt^{-1}，$P<0.01$)。结论：CGRP 是一种内源性心肌保护物质，在大鼠心脏的 PC 中起重要作用

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 448-450

Effect of captopril on intracellular pH in vascular smooth muscle cells[1]

QI Jian-Hua, ZHANG Lu[2], WANG Jun, GU Pei-Kun, JIN Zheng-Jun, HUANG Ming-Zhi[3]
(*Department of Pharmacology, [2]Department of Computer Applications to Medicine, [3]Shanghai Institute of Hypertension, Shanghai Second Medical University, Shanghai 200025, China*)
WANG Xin-Ming[4]
(*Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS thoracic aorta; cultured cells; inbred SHR rats; inbred WKY rats; hydrogen; sodium; angiotensin Ⅱ; captopril

AIM: To observe the effect of captopril (Cap) on intracellular pH (pH_i) in aortic smooth muscle cells (ASMC). **METHODS:** Cultured ASMC derived from rat and rabbit aortae were loaded with the fluorescent dye BCECF and pH_i was determined using digital image processing method. **RESULTS:** The pH_i of untreated SHR and WKY rats were 7.37±0.29 and 7.19±0.31, respectively. Oral Cap decreased pH_i (7.11±0.26, $P<0.05$) and exaggerated pH_i response to angiotensin Ⅱ (Ang-Ⅱ, $0.1\ \mu mol \cdot L^{-1}$) in ASMC of SHR rats *vs* WKY rats (0.14±0.05 *vs* 0.21±0.05 pH units, $P<0.01$). Cap *in vitro* had no effect on Ang-Ⅱ induced intracellular alkalinization *in* ASMC of rabbits. **CONCLUSION:** Oral Cap inhibits $Na^+ - H^+$ exchange activity in ASMC of SHR rats.

The $Na^+ - H^+$ antiport is involved in the pathogenesis of hypertension[1]. An augmented activity of this ion transport system is demonstrated in human hypertension and in inbred SHR rats[2,3].

Antihypertensive treatment with angiotensin-converting enzyme (ACE) inhibitor captopril (Cap) reduces cellular Na^+ and Ca^{2+} which are linked to $Na^+ - H^+$ exchanger in patients with essential hypertension and SHR rats[3-5]. Little is known about the effect of Cap on intracellular pH (pH_i) and its modulator $Na^+ - H^+$ exchanger[1] although the effect of enalapril on this antiport has been examined[2]. The present study was undertaken to determine whether oral Cap affected $Na^+ - H^+$ antiport in aortic smooth muscle cells (ASMC) of SHR rats. We also examined the effect of Cap *in*

[1] Project in part supported by the National Natural Science Foundation of China, № 39270779, and the Science and Technology Foundation of Development of Shanghai Advanced School, № 93CB30.
[4] Correspondence to Prof WANG Xin-Ming
Received 1995-01-11 Accepted 1996-01-02

vitro on pH_i changes in ASMC of rabbits.

MATERIALS AND METHODS

Materials The molecular probe 2′,7′-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF) was from Sigma. Angiotensin Ⅱ (Ang-Ⅱ) was from Sigma. Cap was obtained from Changzhou Pharmaceutical Factory. Medium-199 was from Nissui.

Solutions The HEPES-buffered solution (HBS) contained: NaCl 1, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, HEPES 10, and glucose 5 mmol·L^{-1}. A 0.2 mmol·L^{-1} stock solution of BCECF was prepared in Me_2SO.

Preparation of rats[5] SHR (♂, 8 wk old, $n = 10$, weighing 150 ± 13 g) and WKY rats (♂, 8 wk old, $n = 5$ weighing 206 ± 15 g) were obtained from Shanghai Institue of Hypertension and divided into 3 groups: Cap-treated SHR group (SHR_{cap}, $n = 5$) received Cap at 100 mg·kg^{-1}·d^{-1} via the drinking water for 16 wk; untreated SHR ($n = 5$) and WKY group ($n = 5$) given tap water. Systolic blood pressure (SBP) was determined in conscious, restrained rats by the tail-cuff method. At the end of the dosing period, the aorta was excised.

Cell culture ASMC were cultured[6] for 7 − 8 d of rat aortae or at passage level 9 − 11 derived from rabbit aortae. Cells of rabbits grown on coverslips at a density of (1 − 5) × 10^5 cells·L^{-1} were made quiescent in 0.4 % Medium-199 supplemented with Cap or vehicle for 48 h prior to experiment[7]. The pH_i changes was assessed 2 min or 6 − 7 h after exposure to Ang-Ⅱ.

Measurement of pH_i Cells grown coverslips were rinsed twice with HBS and loaded with BCECF 2 μmol·L^{-1} for 15 min at 37 ℃. After loading, the cells on coverslips were washed twice with HBS and then placed in a small glass cuvette on the stage of fluorescence microscope. The fluorescence of BCECF loaded cells was monitored at λ_{em} 530 nm with λ_{ex} 450 nm and recorded by photograph. The grey values of images were measured using digital imaging method[6,8] and then converted to pH_i on calibration curve.

Group t-test was used for statistic comparison.

RESULTS

Blood pressure of rats The SBP values of WKY, untreated SHR and SHR_{cap} were 16.0 ±0.6, 26.8 ± 0.9, and 15.8 ± 0.4 kPa ($n = 5$ rats), respectively. SBP of untreated SHR was higher than that of the other two groups. There was no significant difference in SBP between WKY and SHR_{cap}.

Basal pH_i and pH_i response to Ang-Ⅱ in ASMC of rats There was no significant difference in basal pH_i between untreated SHR and WKY (7.37 ±0.29 *vs* 7.19 ± 0.31, $n = 20$ cells of 5 rats, 4 cells/rat). But basal pH_i was higher in ASMC of untreated SHR compared with SHR_{cap} (7.11 ±0.26, $n = 20$ cells of 5 rats, 4 cells/rat, $P<0.05$). Ang-Ⅱ 0.1 μmol·L^{-1} produced an intracellular alkalinization in ASMC of untreated-SHR, but had no apparent response in ASMC from both SHR_{cap} and WKY rats (Tab 1).

Tab 1. Angiotensin Ⅱ (0.1 μmol·L^{-1})-induced changes in pH_i in aortic smooth muscle cells of rats. $n = 20$ cells of 5 rats (4 cells/rat), $\bar{x} \pm s$. $^cP<0.01$ *vs* untreated-SHR rats.

	pH changes (pH units)
Untreated-SHR rats	0.21 ± 0.05
Cap treated-SHR rats	0.14 ± 0.05^c
WKY rats	0.11 ± 0.09^c

Effect of Cap on Ang-Ⅱ-induced pH_i changes in ASMC of rabbits Basal pH_i was 7.13 ± 0.29 ($n = 40$ cells of 4 rabbits, 10 cells/rabbit. Ang-Ⅱ 0.1 − 10 μmol·L^{-1} induce intracellular alkalinization in a concentration-dependent manner (7.34 ±0.3, 7.45 ± 0.31, and 7.59 ± 0.27, $n = 20$ cells of 4 rabbits, 5 cells/rabbit). Cap 0.1 − 10 μmol·L^{-1} had no effect on Ang-Ⅱ (1 μmol·L^{-1})-induced intracellular alkalinization (7.52 ± 0.21, 7.49 ±0.34, and 7.38 ± 0.28 *vs* 7.45 ± 0.31, $n = 20$ cells of 4 rabbits, 5 cells/rabbit, $P>0.05$).

DISCUSSION

Our results revealed that antihypertensive therapy with Cap for 16 wk decreased pH_i and exaggerated Ang-Ⅱ-induced intracellular alkalinization in ASMC of SHR rats. It was reported that pH_i and vasoconstrictor-induced pH_i changes were regulated by $Na^+ - H^+$ exchanger[1,3] of which activity was enhanced in SHR rats[2,3]. These results indicate that oral Cap decreases enhanced $Na^+ - H^+$ exchange activity in ASMC of SHR. These results did not agree with studies by Rossopf *et al*[2] who demonstrated that elevated platelet $Na^+ - H^+$ exchange activity was refractory to antihypertensive treatment with enalapril for 6 wk. Perhaps, the difference may be due to different duration of therapy or different type of ACE

inhibitors. Our results also showed that Ang-Ⅱ induced a concentration-related intracellular alkalinization in ASMC of rabbits, but pH_i response to Ang-Ⅱ remained unchanged when ASMC were incubated with Cap. This shows that Na^+-H^+ exchanger in ASMC of normotensive animals is resistant to Cap.

The results of this study demonstrated that oral cap caused a decrease in enhanced $Na^+ - H^+$ exchange activity in ASMC of SHR rats and Cap *in vitro* did not affect $Na^+ - H^+$ exchanger in ASMC of rabbits. We can not tell whether there is difference in $Na^+ - H^+$ exchanger between normotensive animals and hypertensive animals and whether Cap can directly interfere with the $Na^+ - H^+$ antiport in ASMC of SHR rats or not.

REFERENCES

1 Grinstein S, Rotin D, Mason MJ. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. Biochim Biophys Acta 1989; **988**: 73 - 97

2 Rosskopf D, Siffert G, Osswald U, Witte K, Dusing R, Akkerman JWN, *et al*. Platelet $Na^+ - H^+$ exchanger activity in normotensive and hypertensive subjects: effect of enalapril therapy upon antiport activity.
J Hypertens 1992; **10**: 839 - 47.

3 Okada K, Ishikawa S, Saito T. Enhancement of intracellular sodium by vasopressin in spontaneously hypertensive rats. Hypertension 1993; **22**: 300 - 5

4 Niutta E, Cusi D, Colombo R, Tripodi G, Pellizzoni M, Pati P, *et al*. Antihypertensive effect of Captopril, canrenoate potassium, and atenolol. Relations with red blood cell sodium transport and renin.
Am J Hypertens 1988; **1** (4 Pt 1): 364 - 71.

5 Sada T, Koike H, Ikeda M, Sato K, Ozaki H, Karaki H. Cytosolic free calcium of aorta in hypertensive rats: Chronic inhibition of angiotensin converting enzyme
Hypertension 1990; **16**: 245 - 51.

6 Qi JH, Zhang L, Wang J, Wei PJ, Gu PK, Jin ZJ. Effects of captopril and enalapril on intracellular Ca^{2+} in vascular smooth muscle cells.
Acta Pharmacol Sin 1996; **17**: 142 - 5

7 Berk BC, Rao GN. Angiotensin Ⅱ-induced vascular smooth muscle cell hypertrophy: PDGF A-chain mediates the increase in cell size. J Cell Physiol 1993; **154**: 368 - 80

8 Wei PJ, Zhang L, Li GZ, Gu PK, Jin ZJ
The effect of berbamine on intracellular calcium in isolated cardiomyocytes: a digital image processing study.
Acta Pharmacol Sin 1992; **13**: 84 - 5.

卡托普利对血管平滑肌细胞内 pH 影响[1]

齐建华，章 鲁[2]，王 军，顾培坤，金正均，黄明智[3]（上海第二医科大学药理教研室，[2]计算机医学应用教研室，[3]上海市高血压研究所，上海 200025，中国）
王新明（中国科学院上海细胞生物学研究所，上海 200031，中国）

关键词 胸主动脉；培养的细胞；近交 SHR 大鼠；近交 WKY 大鼠；氢；钠；血管紧张素Ⅱ；卡托普利

目的：观察 Cap 对主动脉平滑肌细胞（ASMC）pH 的影响。**方法**：荧光指示剂 BCECF 标记培养的大鼠或家兔 ASMC 用计算机图象处理技术确定 pH 值。**结果**：SHR 大鼠 ASMC pH 相似 WKY 大鼠 口服 Cap 降低 ASMC pH（$P < 0.05$）和对 Ang-Ⅱ的增强碱化反应（$P < 0.01$）。体外 Cap 处理不影响 Ang-Ⅱ引起家兔 ASMC 碱化 **结论**：口服 Cap 抑制 SHR 大鼠 ASMC $Na^+ - H^+$ 交换。