BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Mar; 18 (2): 140 - 142

Effects of captopril and enalaprilat on intracellular Ca^{2+}, Na^+ contents and pH in hypoxic and reoxygenated cardiomyocytes

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KEY WORDS captopril; enalaprilat; calcium; sodium; cell hypoxia; Fura-2; BCECF; SBFI; verapamil

AIM: To study the mechanisms of captopril (Cap) and enalaprilat (Ena) protective effects on hypoxic and reoxygenated cardiac myocytes. **METHODS**: Using fluorescent probes Fura 2-AM, BCECF/AM, SBFI/AM combined with computer image processing techniques to measure intracellular ion concentrations. **RESULTS**: $[Ca^{2+}]_i$ (165 ± 8 nmol·L^{-1}) and $[Na^+]_i$ (9.2 ± 0.8 mmol·L^{-1}) were higher but $[pH]_i$ (6.7 ± 0.3) was lower in hypoxic and reoxygenated myocytes (196 ± 14 nmol·L^{-1}, 9.3 ± 1.3 mmol·L^{-1}, 6.61 ± 0.19, respectively) than in normal ones. Cap and Ena reduced $[Ca^{2+}]_i$ (149 ± 11 and 152 ± 10 nmol·L^{-1} respectively) and intracellular acidosis (7.11 ± 0.22 and 7.2 ± 0.4, respectively) during hypoxia. Cap also decreased $[Na^+]_i$ in hypoxic myocytes (8.1 ± 0.9 mmol·L^{-1}). During reoxygenation, Cap decreased $[Ca^{2+}]_i$ and $[Na^+]_i$ but Ena had no significant effect on them. Cap or Ena had no additive effect when combined with verapamil (Ver). **CONCLUSION**: Cap and Ena protected hypoxic and reoxygenated cardiomyocytes, but the mechanisms were not the same.

Angiotensin-converting enzyme inhibitors (ACEI) optimized reperfusion injury after acute myocardial infarction[1,2], which was not correlated with its inhibition of the Ang II production[3]. Different ACEI had different protective effects[4]. But the exact mechanisms of ACEI protective effects on cardiac myocytes are not clear yet.

Intracellular ionic imbalance played an important role on the cardiac myocyte injury[5]. Captopril had direct effect on intracellular Ca^{2+} concentration ($[Ca^{2-}]_i$) through blocking pathological calcium channel[6]. But there were few reports about ACEI effects on intracellular Na^+ ($[Na^+]_i$), $[Ca^{2+}]_i$ and pH ($[pH]_i$) alterations in hypoxic and reoxygenated cardiac myocytes. In the present study, we elucidated the mechanisms of captopril (Cap) and enalaprilat (Ena) protective effects on hypoxic and reoxygenated cardiac myocytes and investigated whether they had the additive effects with calcium channel blocker verapamil (Ver).

MATERIALS AND METHODS

Materials Wistar rats (24-week-old, Shanghai Medical University); collagenase I type (Sigma); captopril (Sino-America Shanghai Squibb Pharmaceuticals Ltd); enalaprilat (Chang Zhou Pharmaceutical Factory); verapamil (Shanghai Tian Ping Pharmaceutical Factory); BCECF/AM, SBFI/AM, pluronic F-127, sulfinpyrazone, BCECF, SBFI, Fura-2 (Sigma); Fura 2-AM (Shanghai Institute of Physiology, Chinese Academy of Sciences).

Methods and Design Ca^{2+}-tolerant cardiac myocytes isolated from heart ventricles were prepared[7]. Isolated single cardiac myocytes were divided into 5 groups:

1) Normal control group: gassed with humidified 95 % O_2 + 5 % CO_2 for 40 min;

2) Hypoxic group: gassed with humidified 95 % N_2 + 5 % CO_2 for 20 min;

3) Hypoxia + drugs: further divided into 4 subgroups: a) Cap 1 μmol·L^{-1}, b) Ena 1 μmol·L^{-1}, c) Cap + Ver 1 μmol·L^{-1}, d) Ena + Ver 1 μmol·L^{-1}. Then gassed with humidified 95 % N_2 + 5 % CO_2 for 20 min;

4) Reoxygenated group: 20 min-hypoxic cells were gassed with humidified 95 % O_2 + 5 % CO_2 for 20 min;

5) Reoxygenation + drugs: Further divided into 4 subgroups: a) Cap 1 μmol·L^{-1}, b) Ena 1 μmol·L^{-1}, c) Cap + Ver 1 μmol·L^{-1}, d) Ena + Ver 1 μmol·L^{-1}. Then gassed with humidified 95 % O_2 + 5 % CO_2 for 20 min.

After hypoxia and reoxygenation all cells were washed twice with Krebs' solution and measured for $[Ca^{2+}]_i$[7] and

Received 1995-11-08 Accepted 1996-08-27

$[pH]_i$[8]: The cells were incubated with BCECF/AM (final concentration 2 $\mu mol \cdot L^{-1}$) for 10 min at 22 ℃, and under a fluorescent microscope (λ_{ex} 500 nm and λ_{em} 530 nm for BCECF). $[Na^+]_i$ measurement[9]: the final concentration of SBFI/AM was 5 $\mu mol \cdot L^{-1}$. The cells were placed under the fluorescent microscope (λ_{ex} 340 nm and λ_{em} 500 nm) by digital image processing of SBFI fluorescence. The ion concentrations and pH were obtained on the calibation curves.

Statistics Data were presented as $\bar{x} \pm s$ and analysed with t test.

RESULTS

During hypoxia there were higher $[Ca^{2+}]_i$ and $[Na^+]_i$ in hypoxic myocytes (165 ± 8 nmol·L^{-1} and 9.2 ± 0.8 mmol·L^{-1}, respectively) than in normal myocytes (135 ± 13 nmol·L^{-1} and 8.1 ± 0.6 mmol·L^{-1}, respectively, $P < 0.01$ and $P < 0.05$). $[pH]_i$ was lower in hypoxic myocytes than in normal ones (6.7 ± 0.3 and 7.4 ± 0.2 respectively, $P < 0.01$). Both Cap 1 $\mu mol \cdot L^{-1}$ and Ena 1 $\mu mol \cdot L^{-1}$ inhibited $[pH]_i$ decrement (7.1 ± 0.2 and 7.2 ± 0.4, respectively) and decreased the $[Ca^{2+}]_i$ elevations (149 ± 11 and 152 ± 10 nmol·L^{-1}, respectively, $P < 0.05$) in hypoxic cardiac cells but did not restored them to the normal ($P < 0.05$). Cap also inhibited the $[Na^+]_i$ increment (8.1 ± 0.9 mmol·L^{-1}, $P < 0.05$) but Ena had no significant effect on it. Under the concentration of 1 $\mu mol \cdot L^{-1}$, there were no additive effects of Cap + Ver or Ena + Ver ($P > 0.05$). During reoxygenation $[pH]_i$ was still lower (6.61 ± 0.19, $P < 0.05$) and $[Ca^{2+}]_i$ and $[Na^+]_i$ still increased (196 ± 14 nmol·L^{-1} and 9.3 ± 1.3 mmol·L^{-1}, respectively). Cap 1 $\mu mol \cdot L^{-1}$ and Ena 1 $\mu mol \cdot L^{-1}$ had no significant effects on $[pH]_i$ (6.9 ± 0.3 and 6.8 ± 0.3 respectively, $P > 0.05$), but Cap decreased $[Ca^{2+}]_i$ and $[Na^+]_i$ (161 ± 14 nmol·L^{-1} and 7.9 ± 0.4 mmol·L^{-1}, respectively). Ena had no such effects (173 ± 24 nmol·L^{-1} and 8.4 ± 1.1 mmol·L^{-1} respectively, $P > 0.05$). Cap or Ena combined with Ver 1 $\mu mol \cdot L^{-1}$ had no additive effect ($P > 0.05$) (Tab 1).

Tab 1. Effects of captopril (Cap), enalapril (Ena), Cap + verapamil (Ver), Ena + Ver (1 $\mu mol \cdot L^{-1}$ respectively) on $[Ca^{2+}]_i$, $[Na^+]_i$, and $[pH]_i$ in cardiac myocytes during hypoxia and reoxygenation (n = the number of rats). H: hypoxia; R: reoxygenation. $^bP < 0.05$, $^cP < 0.01$ vs control; $^eP < 0.05$, $^fP < 0.01$ vs hypoxia; $^gP > 0.05$ vs H + Cap; $^jP > 0.05$ vs H + Ena; $^mP > 0.05$, $^nP < 0.05$, $^oP < 0.01$ vs R; $^pP > 0.05$ vs R + Cap.

	$[Ca^{2+}]_i$/ nmol·L^{-1}	$[Na^+]_i$/ mmol·L^{-1}	$[pH]_i$
Rats	6	5	7
Control	135 ± 13	8.1 ± 0.6	7.37 ± 0.19
Hypoxia	165 ± 8^c	9.2 ± 0.8^b	6.7 ± 0.3^c
H + Cap	148 ± 11^{eh}	8.1 ± 0.9^e	7.11 ± 0.22^f
H + Cap + Ver	143 ± 8^{fg}	7.9 ± 0.9^{eg}	7.1 ± 0.3^{fg}
H + Ena	152 ± 19^{eh}	8.5 ± 1.4^d	7.2 ± 0.4^f
H + Ena + Ver	144 ± 9^{fj}	8.3 ± 1.2^{dj}	7.3 ± 0.3^{fj}
Reoxygenation	196 ± 14^c	9.3 ± 1.3^b	6.61 ± 0.19^b
R + Cap	161 ± 14^c	7.9 ± 0.4^n	6.9 ± 0.3^m
R + Cap + Ver	151 ± 10^{co}	7.7 ± 1.2^{no}	6.8 ± 0.5^m
R + Ena	173 ± 24^m	8.4 ± 1.1^m	6.8 ± 0.3^m
R + Ena + Ver	176 ± 17^m	8.1 ± 1.5^m	6.8 ± 0.4^m

DISCUSSION

In the present study, we found that during hypoxia $[Ca^{2+}]_i$ and $[Na^+]_i$ elevated but $[pH]_i$ decreased, which were detrimental to the cardiac myocytes. Cap and Ena inhibited the $[Ca^{2+}]_i$ elevations by blocking the pathological calcium channels, through which some outer Ca^{2-} entered the cells[10]. In addition, Cap and Ena decreased the progression of intracellular acidosis, which was also one of the mechanisms of their protective effects on hypoxic cardiac myocytes. But further study will still be needed to investigate whether ACEI inhibited anaerobic glycolysis or decrease the myocytes' requirement for energy due to ACEI effects on $[Ca^{2+}]_i$.

During reoxygenation, $[Ca^{2+}]_i$ and $[Na^+]_i$ further increased and $[pH]_i$ was not restored to the normal. Cap and Ena had no significant effects on $[pH]_i$. Under the condition of 1 $\mu mol \cdot L^{-1}$, Cap decreased $[Ca^{2+}]_i$ and $[Na^+]_i$ significantly but Ena had no such effects. It was impossible for Cap to inhibit $[Ca^{2+}]_i$ elevation through its directly blocking calcium channels because in reoxygenated cardiac myocytes, $[Ca^{2+}]_i$ elevation was not by the way of calcium channels but by the way of Na^+-Ca^{2+} exchange[10] and nonspecific ion channels which was induced by free radicals[11]. Cap 1 $\mu mol \cdot L^{-1}$ had no effect on the Na^+-Ca^{2+} exchange

because $[Na^+]_i$ did not change significantly compared with reoxygenation group. So Cap reduced $[Ca^{2+}]_i$ and $[Na^+]_i$ through its ability to scavenge free radicals[11]. Either during hypoxia or during reoxygenation, both Cap and Ena (1 μmol $\cdot L^{-1}$) had no additive effects when combined with Ver (1 μmol $\cdot L^{-1}$), which suggested that it was not necessary for ACEI combined with Ver to treat ischemic heart injury with general doses, but it is still needed to investigate whether they had additive effects with the use of other concentrations.

In conclusion, both Cap and Ena protected hypoxic cardiac myocytes by the way of diminishing $[Ca^{2+}]_i$ and $[pH]_i$ alterations. Cap also protected reoxygenated cardiac myocytes through decreasing $[Ca^{2+}]_i$ and $[Na^+]_i$.

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卡托普利和依那普利拉对缺氧和复氧心肌细胞内 Ca^{2+}, Na^+ 和 pH 值的影响

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关键词 卡托普利; 依那普利拉; 钙; 钠; 细胞缺氧; Fura-2; BCECF; SBFI; 维拉帕米

目的: 探讨卡托普利(Cap)和依那普利拉(Ena)保护缺氧和复氧心肌细胞作用机制. **方法**: 采用荧光探针结合图象处理技术测定细胞内离子浓度. **结果**: 缺氧和复氧心肌细胞内 Ca^{2+} (分别为 165 ±8和 196 ± 14 nmol $\cdot L^{-1}$) 和 Na^+ (分别为 9.2 ±0.8和 9.3 ± 1.3 mmol $\cdot L^{-1}$) 高于正常而 pH 低于正常(分别为 6.7 ± 0.3 和 6.61 ± 0.19). Cap 和 Ena 减少缺氧细胞内 Ca^{2+} 浓度并减轻细胞内酸化. Cap 也减少缺氧细胞内 Na^+ 浓度(8.1 ± 0.9 mmol $\cdot L^{-1}$). Cap 减少复氧细胞内 Ca^{2+} 和 Na^+ 浓度, 但 Ena 无此作用. Cap 或 Ena 与 Ver 合用无协同作用. **结论**: Cap 和 Ena 对缺氧和复氧心肌细胞有保护作用, 但机制并不完全一致.