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Effects of captopril and enalaprilat on intracellular Ca^{2+} content in isolated cardiomyocytes from rats[1]

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KEY WORDS Fura-2; inbred SHR rats; inbred WKY rats; myocardium; verapamil; captopril; enalaprilat

AIM: To study the effects of ACEI captopril (Cap) and enalaprilat (Ena) on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cardiac myocytes isolated from SHR and WKY rats. **METHODS**: Using fluorescent probe Fura 2-AM combined with computer image processing technique to measure $[Ca^{2+}]_i$. **RESULTS**: Resting $[Ca^{2+}]_i$ was higher in SHR cardiac myocytes (174 ± 5 nmol·L^{-1}) than that in WKY rat myocytes (148 ± 15 nmol·L^{-1}, $P < 0.01$). Cap and Ena decreased the resting $[Ca^{2+}]_i$ in SHR myocytes (161 ± 11 and 166 ± 7 nmol·L^{-1}, respectively, $P < 0.05$) but not in WKY rat myocytes ($P > 0.05$). Both drugs inhibited $[Ca^{2+}]_i$ increment induced by KCl, NE, or Ang II in SHR and WKY rat myocytes except on KCl-induced $[Ca^{2+}]_i$ increment in WKY rat myocytes ($P > 0.05$). **CONCLUSION**: Cap and Ena had direct effects on pathological voltage-operated calcium channel in cardiac myocytes.

Angiotensin-converting enzyme inhibitors (ACEI) are widely used to treat hypertension and heart failure. Captopril (Cap) enhanced intracellular calcium ($[Ca^{2+}]_i$) handling of postinfarction failure myocardium[1] and had protective effect on ischemic and reperfused heart[2], reduced developed force in isolated heart muscle of guinea pig in a dose-dependent fashion[3] and exerted some effects on membrane current and contraction in single myocytes[4]. In the present study, we investigate the direct effects of captopril and enalaprilat on $[Ca^{2+}]_i$ and agonist-induced $[Ca^{2+}]_i$ increase in isolated cardiac myocytes from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats and the additive effect of Cap combined with verapamil (Ver) on $[Ca^{2+}]_i$.

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MATERIALS AND METHODS

SHR rats (12-wk-old) and normotensive WKY rats (BP: 24.8 ± 1.4 and 12.2 ± 1.6 kPa, respectively, Shanghai Institute of Hypertension) Noradrenaline (Shanghai Tian Feng Pharmaceutical Factory); angiotensin II, collengenase I type (Sigma); captopril (Sino-America Shanghai Squibb Pharmaceuticals Ltd); enalaprilat (Changzhou Pharmaceutical Factory); verapamil (Shanghai Tian Ping Pharmaceutical Factory); Fura 2-AM (Shanghai Institute of Physiology, Chinese Academy of Sciences), dissolved in Me_2SO. Isolated myocytes were incubated with Cap (1 μmol·L^{-1}, Ena (1 μmol·L^{-1}) or Cap + Ver (1 μmol·L^{-1}) for 30 min, before medication. The Ca^{2+} was assessed 2 min after exposure to the agonists, because the maximal influxes induced by agonists develop within 1 - 3 min.

Ca^{2+}-tolerant cardiac myocytes were isolated from SHR and WKY rat ventricles[5]. $[Ca^{2+}]_i$ was measured according to the method described in detail prediously[6].

Differences were analysed with t test.

RESULTS

Resting $[Ca^{2+}]_i$ was higher in SHR myocytes (174 ± 5 nmol·L^{-1}) than in WKY rat (148 ± 15 nmol·L^{-1}, $P < 0.01$). Cap, Ena, and Cap + Ver all decreased resting $[Ca^{2+}]_i$ in SHR myocytes (161 ± 11, 166 ± 7, and 155 ± 11 nmol·L^{-1}, respectively, $P < 0.05$). Cap alone or + Ver had no significant difference in lowering the $[Ca^{2+}]_i$ ($P > 0.05$). All these drugs had no significant effect on resting $[Ca^{2+}]_i$ in WKY rat myocytes ($P > 0.05$). KCl 40 mmol·L^{-1}, Ang II 1 μmol·L^{-1}, and NE 1 μmol·L^{-1} induced $[Ca^{2+}]_i$ elevations in SHR myocytes (202 ± 10, 187 ± 7, and 188 ± 13 nmol·L^{-1},

respectively, $P < 0.05$ *vs* SHR control). Cap, Ena, and Cap + Ver inhibited KCl (40 mmol·L^{-1}) NE (1 μmol·L^{-1}), and Ang II (1 μmol·L^{-1})-induced Ca^{2+} elevations in SHR myocytes ($P > 0.05$, *vs* Cap control, Ena control, Cap + Ver control, respectively), but the effect of Cap + Ver was not different from that of Cap alone ($P > 0.05$). Cap, Ena, and Cap + Ver inhibited NE- and Ang II-induced $[Ca^{2+}]_i$ elevations in WKY rat myocytes ($P < 0.05$). Cap + Ver also inhibited KCl-induced $[Ca^{2+}]_i$ increment (155 ± 11 nmol·L^{-1}, $P < 0.05$) but Cap or Ena had no such effect in WKY rat myocytes (Tab 1).

Tab 1. Effects of Cap 1 μmol·L^{-1}, Ena 1 μmol·L^{-1}, and Ver 1 μmol·L^{-1} on resting $[Ca^{2+}]_i$ and on KCl (40 mmol·L^{-1}), NE (1 μmol·L^{-1}), and Ang II (1 μmol·L^{-1})-induced $[Ca^{2+}]_i$ elevation (nmol·L^{-1}) in 6 rats. $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control (SHR), $^dP>0.05$ *vs* Cap (SHR), $^eP>0.05$ *vs* Ena (SHR), $^jP>0.05$ *vs* Cap + Ver (SHR), $^mP>0.05$ *vs* control (WKY), $^pP>0.05$, $^qP<0.05$ *vs* KCl (WKY), $^tP<0.05$ *vs* NE (WKY), $^wP<0.05$ *vs* Ang II (WKY).

Group	$[Ca^{2+}]_i$/ nmol·L^{-1}	Group	$[Ca^{2+}]_i$/ nmol·L^{-1}
Inbred SHR rat cardiomyocytes		Inbred WKY rat cardiomyocytes	
Control	174 ± 5	Control	148 ± 15
KCl	202 ± 10^c	Cap	146 ± 16^m
NE	188 ± 13^b	Ena	148 ± 17^m
Ang II	187 ± 7^c	Cap + Ver	147 ± 14^m
Cap	161 ± 11^b	KCl	173 ± 14
Cap + KCl	175 ± 18^d	Cap + KCl	172 ± 19^p
Cap + Ena	164 ± 11^d	Ena + KCl	167 ± 12^p
Cap + Ang II	166 ± 12^d	Cap + Ver + KCl	155 ± 11^q
Ena	166 ± 7^b	NE	180 ± 17
Ena + KCl	173 ± 24^e	Cap + NE	158 ± 14^t
Ena + NE	173 ± 8^e	Ena + NE	155 ± 19^t
Ena + Ang II	172 ± 12^e	Cap + Ver + NE	158 ± 15^t
Cap + Ver	155 ± 11^b	Ang II	176 ± 12
Cap + Ver + KCl	165 ± 10^j	Cap + Ang II	151 ± 18^w
Cap + Ver + NE	161 ± 7^j	Ena + Ang II	156 ± 14^w
Cap + Ver + Ang II	160 ± 11^j	Cap + Ver + Ang II	154 ± 17^w

DISCUSSION

In the present study, we found that resting free Ca^{2+} concentration was higher in SHR myocytes than in WKY rat myocytes, which indicated that voltage-operated calcium channel and Ca^{2+} influxes were altered in pathological condition such as hypertension. That Cap and Ena decreased the $[Ca^{2+}]_i$ in SHR myocytes but not in WKY rat myocytes indicated that Cap and Ena inhibited Ca^{2+} influxes in pathological state but had no effect on physiological calcium current. That ACEI decreased free Ca^{2+} concentration in ischemic and reperfused myocytes was partly due to its direct inhibitory effect on voltage-operated calcium channel. Cap and Ena also inhibited KCl-induced Ca^{2+} elevation in SHR myocytes but did not in WKY rat myocytes. However, Cap + Ver decreased Ca^{2+} concentration elevation induced by KCl in WKY rat myocytes. This was due to Ver effect but not to Cap. Cap (1 μmol·L^{-1}) and Ver (1 μmol·L^{-1}) have no additive effect with each other, but further study will be needed to show whether there is additive action in the lower doses. Cap + Ena inhibited NE-induced or Ang II-induced free Ca^{2+} elevation, but it is still needed to further study that this observed effect is the result of their direct blocking receptor itself, blocking receptor-operated calcium channel or the inhibition of calcium release from sacoplasmic reticulum.

In conclusion, this study demonstrated that calcium channel was altered in pathological condition such as hypertension and ACEI (Cap and Ena) had direct inhibitory effects on pathological voltage-operated calcium channel.

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卡托普利和依那普利拉对分离的大鼠心肌细胞内游离 Ca^{2+} 浓度的影响[1]

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关键词 Fura-2；近交 SHR 大鼠；近交 WKY 大鼠；心肌；维拉帕米；卡托普利；依那普利拉

目的：研究转换酶抑制剂卡托普利（Cap）和依那普利拉（Ena）对 SHR 和 WKY 大鼠心肌细胞内游离 Ca^{2+} 浓度的影响 **方法**：用荧光探针 Fura 2-AM 结合计算机图象处理技术测定分离心肌细胞内游离 Ca^{2+} 浓度 **结果**：SHR 心肌细胞内游离 Ca^{2+} 浓度（174 ± 5 nmol·L^{-1}）较 WKY 大鼠（148 ± 15 nmol·L^{-1}）高（$P < 0.01$） Cap 和 Ena 能明显降低 SHR 心肌细胞内游离 Ca^{2+} 浓度（分别为 161 ± 11 和 166 ± 7 nmol·L^{-1}，P 分别 < 0.05），但对 WKY 的无影响（$P > 0.05$）。两药均能明显降低 NE 和 Ang II 引起的 SHR 和 WKY 大鼠心肌细胞内 Ca^{2+} 升高，同时也能明显降低 KCl 引起的 SHR 细胞内 Ca^{2+} 升高（$P < 0.05$），但对 WKY 大鼠的无明显影响（$P > 0.05$） **结论**：Cap 和 Ena 对病理性电压依赖性 Ca^{2+} 通道有直接抑制作用。

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Effects of menidipine on dihydropyridines binding sites in cardiac and cerebral membranes from old rats with left ventricular hypertrophy[1]

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KEY WORDS menidipine; left ventricular hypertrophy; renovascular hypertension; heart; radioligand assay; brain; cell membrane

AIM: To study the effects of menidipine (Men) on the affinity and density of dihydropyridines (DHP) binding sites in the cell membranes of left ventricle (LV) and brain in elderly renovascular hypertensive rats (RVHR) with LV hypertrophy. **METHODS**: Renovascular hypertension was produced by clipping the left renal artery in 20-month-old rats. The affinity and density of DHP binding sites in the cell membranes of LV and brain were assessed by radioligand assay. **RESULTS**: Men (20 mg·kg^{-1}·d^{-1} ig for 9 wk) decreased markedly the systolic blood pressure and the LV weight ($P < 0.01$). Though not affecting the density of DHP receptor (B_{max}), Men markedly decreased the total number of DHP binding sites in hypertrophied LV (from 5.95 ± 0.62 to 4.0 ± 1.1 pmol·LV). Men also reduced B_{max} of DHP binding sites in the thalamus (from 522 ± 27 to 371 ± 24 pmol/g protein) and hippocampus (from 498 ± 26 to 332 ± 32 pmol/g protein). **CONCLUSION**: Men reversed the LV hypertrophy from renovascular hypertension accompanied with reduced total number of DHP binding sites in the cell membranes of LV and B_{max} of the cell membranes of thalamus and hippocampus from elderly LV hypertrophied rats.

Menidipine (Men) is a new calcium antagonist of dihydropyridines (DHP) exploited by our Laboratory. The effects of Men in improving the left ventricular pump function, MVO_2, CO_2 production, in reducing the peripheral vascular resistance and in increasing the coronary flow were stronger and lasting longer than those of nifedipine

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