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Effects of captopril and enalapril on intracellular Ca^{2+} in vascular smooth muscle cell[1]

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KEY WORDS thoracic aorta; cultured cells; inbred WKY rats; inbred SHR rats; calcium; angiotensin II; captopril; enalapril; nifedipine; Fura-2

AIM: To determine whether angiotensin-converting enzyme inhibitors can affect Ca^{2+} handling in cultured aortic smooth muscle cells (ASMC) directly. **METHODS**: Cultured ASMC derived from rat aorta were loaded with the intracellular Ca^{2+} ($[Ca]_i^{2+}$) fluorescent indicator Fura 2-AM and digital image processing technique was used. **RESULTS**: Resting $[Ca^{2+}]_i$ was greater in ASMC from SHR *vs* WKY ($P<0.01$). KCl-, norepinephrine (NE)-, and angiotensin II (Ang)-induced $[Ca^{2+}]_i$ increases were enhanced in ASMC of SHR *vs* WKY (220 ± 6, 212 ± 8, and 215 ± 14 *vs* 199 ± 6, 202 ± 7, and 195 ± 7 $nmol \cdot L^{-1}$, respectively). Captopril (Cap) and enalapril (Ena) had no inhibitory effect on KCl-, NE-, and Ang-induced $[Ca^{2+}]_i$ increases in ASMC of WKY. Cap and Ena inhibited KCl-, NE-, and Ang-increased $[Ca^{2+}]_i$ in ASMC of SHR (210 ± 7, 194 ± 6, and 201 ± 6 $nmol \cdot L^{-1}$, respectively). Ena and nifedipine similarly decreased KCl-, NE-, and Ang-increased $[Ca^{2+}]_i$. **CONCLUSION**: Cap blocked KCl-, NE-, and Ang-increased ($[Ca^{2+}]_i$) via a voltage-dependent Ca^{2+} channel of which function and specificity was altered in ASMC of SHR.

Angiotensin-converting enzyme (ACE) inhibitors were widely used for treatment of hypertension. But the vasodilating action of ACE inhibitors cannot be fully explained in terms of a reduction of circulating angiotensin II (Ang)[1]. Though the inhibition of tissue ACE and accumulation of bradykinin which was known to induce endothelial synthesis and release of endothelium-dependent relaxing factor also involved in the hypotensive action[2,3], other non-ACE-dependent mechanisms cannot be excluded, such as a postjunctional blockade of α-adrenergic vasoconstriction[4], an inhibition of norepinephrine (NE) release from sympathetic neurons[5], and an inhibition of Na^+, K^+-ATPase[6]. But the resting $[Ca^{2+}]_i$ in aortic smooth muscle cells was lowered by long-term treatment with an ACE inhibitor[7]. This was observed after intact animals had been treated with an ACE inhibitor, which might also be an indirect consequence of ACE inhibition. So, we investigated the effects of captopril (Cap) and enalapril (Ena) on agonist-induced changes in $[Ca^{2+}]_i$ in ASMC of WKY and SHR rats to determine whether ACE inhibitors can affect $[Ca^{2+}]_i$ in ASMC directly.

MATERIALS AND METHODS

NE was from Shanghai Tian-feng Pharmaceutical Factory. Ang was from Shanghai Biochemical Pharmaceutical Factory. Cap and Ena were obtained from Chang-zhou Pharmaceutical Factory. Nifedipine (Nif) was obtained from Shanghai Tian-ping Pharmaceutical Factory. All drugs were freshly prepared in deionized water except of Nif which was dissolved in propyleneglycol. Fura 2-AM was from Shanghai Institute of Physiology, Chinese Academy of Sciences and dissolved in Me_2SO containing 0.05 % Triton X-100 (surfactant, Sigma). ASMC were incubated with Cap, Ena, and Nif for 10 min before application of the agonists. The increase in $[Ca^{2+}]_i$ was assessed 2 min after the agonists, because the maximal Ca^{2+} influxes induced by agonists developed within 1-3 min[8].

Cell culture Experiments were performed using passages 1-3 ASMC derived from thoracic aortae of 24 wk-old ♀ SHR and ♀ WKY ($n = 12$; weight, 298 ± 15 and 351 ± 7 g; systolic blood pressure, 24.7 ± 0.9, 14.1 ± 0.7 kPa, repectively.) supplied by Shanghai Institute of Hyperten-

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sion, Shanghai Second Medical University. ASMC were cultured[9]. The subculture was treated by 0.125 % pancreatin (Sigma). Cells grown on thin glass coverslips (9 mm × 10 mm) at a density of 4000-8000 cells (cm^2) were assayed 2 d later.

Measurement of $[Ca^{2+}]_i$ The medium was aspirated and cells were washed twice at 37 ℃ with 2 mL of HEPES-buffered solution (HBS) composed of (in $nmol \cdot L^{-1}$): NaCl 1, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, HEPES 10, and glucose 5. The cells were incubated for 30 min at 37 ℃ in the same buffer containing Fura 2-AM 10 $\mu mol \cdot L^{-1}$. At the end of loading, coverslips were washed twice and then put in a small glass cuvette on the stage of a fluorescence microscope. The fluorescence of Fura 2-loaded cells was measured at λ_{ex} 350 nm and λ_{em} 500 nm and images were recorded by photograph (exposure 10 s). By means of digital image processing of Fura-2 fluorescence[10], the grey values of images of Fura 2-loaded cells were obtained and then converted to $[Ca^{2+}]_i$ on calibration curve.

All data were analyzed with unpaired t test.

RESULTS

Resting $[Ca^{2+}]_i$ (198 ± 4 $nmol \cdot L^{-1}$, $n = 20$ cells of 5 rats, 4 cells/rat) was greater in ASMC of SHR than those of WKY (184 ± 8 $nmol \cdot L^{-1}$, $n = 20$ cells of 5 rats, 4 cells/rat, $P < 0.01$). KCl 20 or 80 $mmol \cdot L^{-1}$ resulted in no increase in $[Ca^{2+}]_i$ in ASMC of WKY (186 ± 7 or 184 ± 10 *vs* 183 ± 8 $nmol \cdot L^{-1}$, $n = 20$ cells of 5 rats, 4 cells/rats, $P > 0.05$) but induced increase in $[Ca^{2+}]_i$ in ASMC of SHR (216 ± 5 or 214 ± 11 *vs* 198 ± 4 $nmol \cdot L^{-1}$, $n = 20$ cells of 5 rats, 4 cells/rat, $P < 0.01$) *vs* control. Maximal response to KCl 40 $mmol \cdot L^{-1}$ was found in 2 groups of cells and greater in SHR than in WKY ASMC (220 ± 6 *vs* 199 ± 6 $nmol \cdot L^{-1}$, $n = 20$ cells of 5 rats, 4 cells/rat, $P < 0.01$).

Both NE and Ang induced a concentration-related increase in $[Ca^{2+}]_i$ which was greater in SHR than in WKY ASMC (Tab 1).

Both Cap and Ena diminished the KCl-induced $[Ca^{2+}]_i$ increase in ASMC of SHR, but had no effect on KCl response in ASMC of WKY. Nif markedly inhibited KCl-increased $[Ca^{2+}]_i$ in ASMC of WKY as well as in those of SHR (Tab 2).

A reduction of NE-increased $[Ca^{2+}]_i$ by Cap and Ena was seen in SHR and WKY ASMC (Tab 2). Nif had inhibitory effect on NE-increased $[Ca^{2+}]_i$ in ASMC of SHR, but not on response in ASMC of WKY (Tab 2). Both Cap and Ena significantly decreased Ang-increased $[Ca^{2+}]_i$ in SHR ASMC, but their inhibitory effects on Ang response in ASMC of WKY were less pronounced. The effect of Nif on Ang response was similar to that of Cap or Ena in 2 groups of cells.

Tab 1. Difference in norepinephrine and angiotensin II-produced Ca^{2+} influxes between vascular smooth muscle cells from normotensive WKY and SHR rats. $n = 20$ cells of 5 rats (4 cells/rat). $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* WKY.

Drugs/ $\mu mol \cdot L^{-1}$	WKY	SHR
	Ca^{2+}/ $nmol \cdot L^{-1}$	
Norepinephrine		
0.1	186 ± 5	211 ± 5^c
1	202 ± 7	212 ± 8^c
10	210 ± 7	229 ± 7^c
Angiotensin II		
0.1	187 ± 6	208 ± 12^b
1	195 ± 6	215 ± 14^b
10	209 ± 13	225 ± 7^b

Tab 2. Interaction between KCl (40 $mmol \cdot L^{-1}$) norepinephrine (NE, 1 $\mu mol \cdot L^{-1}$), and angiotensin II (Ang, 1 $\mu mol \cdot L^{-1}$)-produced Ca^{2+} influxes and captopril (Cap, 10 $\mu mol \cdot L^{-1}$), enalapril (Ena, 10 $\mu mol \cdot L^{-1}$), and nifedipine (Nif, 3 $\mu mol \cdot L^{-1}$) in vascular smooth muscle cells (ASMC) from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). $n = 10$ cells of 5 rats (2 cells/rats). $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Drugs	WKY	SHR
	Ca^{2+}/ $nmol \cdot L^{-1}$	
Control	199 ± 6	220 ± 6
Cap + KCl	197 ± 5^a	210 ± 7^c
Ena + KCl	199 ± 4^a	210 ± 3^c
Nif + KCl	191 ± 4^c	194 ± 4^c
Control	202 ± 7	212 ± 8
Cap + NE	190 ± 4^c	194 ± 6^c
Ena + NE	190 ± 5^c	199 ± 4^c
Nif + NE	200 ± 8^a	204 ± 3^c
Control	195 ± 7	215 ± 14
Cap + Ang	199 ± 5^a	201 ± 6^b
Ena + Ang	195 ± 5^a	192 ± 5^c
Nif + Ang	190 ± 8^a	205 ± 5^b

DISCUSSION

At single cell level, we demonstrated that besides the resting $[Ca^{2+}]_i$, KCl-, NE-, and Ang-induced increases in $[Ca^{2+}]_i$ were enhanced in ASMC of SHR rats *vs* those of WKY rats.

In ASMC of WKY rats, Cap and Ena did not alter KCl response, whereas Nif decreased KCl one, suggesting that voltage-dependent Ca^{2+} channel (VDC) is resistant to ACE inhibitors. Cap and Ena also had no effect on Ang response although they significantly reduced NE response, which may be due to a blockade of α-adrenoceptor[4]. The effects of Ang and NE were not blocked by Nif. These results reflect that receptor-operated calcium channel involved in mediating the Ca^{2+} entry by Ang[8] is also resistant to ACE inhibitors.

In ASMC of SHR rats, Cap inhibited not only NE-induced but also KCl- and Ang-induced increases in $[Ca^{2+}]_i$. Ena and Nif similarly decreased KCl-, NE-, and Ang-increased $[Ca^{2+}]_i$. These results supported the findings of Sada *et al*[7] that ACE inhibitor Cs-622 therapy caused a decrease in cytosolic free calcium of aorta in SHR rats. These data therefore indicated that Cap and Ena acted by blocking 3 agonist-increased $[Ca^{2+}]_i$ via VDC[8] whose function was altered. Interestingly, Enous R *et al* found[11] that ACE inhibitor perindopril directly decrease the calcium-dependent transient inward current in guinea pig ventricular myocytes that is responsible in part for ischemic and reperfusion-induced arrhythmias, while having no effect on physiologic calcium current. Our observation that Cap and Ena inhibited Ang-, and KCl-increased $[Ca^{2+}]_i$ in ASMC of SHR rats are similar to these findings, but not to that in WKY rats. Thus, it may be assumed that there is a VDC of altered function and specificity that can contribute toward the increased sensitivity to ACE inhibitors in the pathological states such as hypertension and myocardial ischemia.

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卡托普利和依那普利对血管平滑肌细胞内钙的影响[1]

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关键词 胸主动脉；培养的细胞；近交 WKY 大鼠；近交 SHR 大鼠；钙；血管紧张素 II；卡托普利；依那普利；硝苯地平；Fura-2

目的：检测 ACE 抑制剂对主动脉平滑肌细胞内 Ca^{2+} 的影响. **方法**：用荧光标计和图象处理技术. **结果**：SHR 细胞内 Ca^{2+} 以及 KCl, NE 和 Ang 在 SHR 细胞引起的 Ca^{2+} 增加多于 WKY 细胞. Cap 和 Ena 不影响 KCl 和 Ang 在 WKY 细胞的作用，但 Cap, Ena 和 Nif 抑制 KCl, NE 和 Ang 在 SHR 细胞的作用. **结论**：Cap 和 Ena 阻断功能和特异性已改变的电压依赖性钙通道.

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Orthogonal analysis of aggravating effects of α-, β-agonists and leukocytes on reperfusion-induced arrhythmias and ventricular fibrillation in Langendorff's rat heart

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KEY WORDS myocardial reperfusion injury; arrhythmia; ventricular fibrillation; phenylephrine; isoproterenol; cyclophosphamide; leukocyte

AIM: To study the effects of leukocyte (Leu), α-agonist (α-Ago), and β-agonist (β-Ago) on the arrhythmias induced by ischemia and reperfusion to determine which of the 3 factors was the most important one in exacerbating arrhythmias. **METHODS**: Arrhythmias were induced by the reduction and subsequent resumption of perfused flow in Langendorff's perfused rat hearts. Ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded on ECG, and the results were orthogonally analyzed. **RESULTS**: When Leu was present, the incidence of VF induced by ischemia-reperfusion was 80 %. The incidence in Leu-depleted hearts was 20 %, α-Ago and β-Ago elevated it to 60 % and 100 %, respectively. The results by orthogonal analysis demonstrated Leu or α-Ago + β-Ago increased VF incidence. With regard to arrhythmias, arrhythmia score was remarkedly increased by all of 3 factors and various combinations except β-Ago + Leu. **CONCLUSION**: Among these 3 factors, Leu was the most important one in facilitating reperfusion-induced arrhythmias.

Ischemia-reperfusion arrhythmias are related to the elevation of cAMP, the formation of lysophosphatides, the genesis of free oxygen radicals, disturbances of ionic homeostasis, leukocyte activation, and the stimulation of α- and β-adrenoreceptors[1-6]. Depletion of leukocyte in reflow blood produced a reduced incidence of arrhythmias induced by ischemia-reperfusion[5], which induced a significant release of endogenous catecholamines that intensify arrhythmias by the stimulation of α- and β-adrenoreceptors.

Isolated rat heart was used to study the effects of leukocyte, α-Ago and β-Ago on the arrhythmias induced by ischemia and reperfusion in the present experiment, and the results were orthogonally analyzed to determine which of the 3 factors was the most important in terms of exacerbating arrhythmias.

MATERIALS AND METHODS

Experimental protocol Sprague-Dawley rats (230 ± 20 g) were used to prepare the Langendorff's heart at a perfusion pressure of 9.3 kPa. The perfusate was composed (mmol · L^{-1}): NaCl 128, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 0.8, $NaCO_3$ 12.5, glucose 11.0, gassed with O_2 (pH 7.4 ± 0.5, 38 ℃). The heart was initially given 15-min aerobic perfusion, and global ischemia was produced by reducing the perfusion flow to one tenth for 10 min. Then the perfusion flow was resumed for 10 min (reperfusion).

Throughout the experimental period epicardial ECG was recorded to analyze the arrhythmia score[7] and the incidences

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