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Effects of praeruptorine C on the intracellular free calcium in normal and hypertrophied rat ventricular myocytes

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KEY WORDS praeruptorine C; calcium; left ventricular hypertrophy; renovascular hypertension; Fura-2; cell size

AIM: To study the intracellular free calcium ($[Ca^{2+}]_i$) in normal and hypertrophic left ventricular myocytes isolated from adult rat hearts and the effects of praeruptorine C (Pra-C) on them. **METHODS**: $[Ca^{2+}]_i$ of single myocyte was measured with Fura 2-AM. **RESULTS**: The resting $[Ca^{2+}]_i$ was 87 ± 4 nmol·L^{-1} in normal left ventricular myocytes, 123 ± 7 nmol·L^{-1} in hypertrophied myocytes. After exposure to KCl (20, 40, and 60 mmol·L^{-1}), the $[Ca^{2+}]_i$ were increased by 66 %, 141 %, and 268 % in normal myocytes, and 77 %, 185 %, and 243 % in hypertrophic myocytes, respectively. Pra-C (1, 10, and 100 μmol·L^{-1}) concentration-dependently inhibited the $[Ca^{2+}]_i$ elevation caused by KCl (35 mmol·L^{-1}) or norepinephrine (20 μmol·L^{-1}) in both normal and hypertrophied myocytes. All of the effects of Pra-C were similar to that of nifedipine. **CONCLUSION**: $[Ca^{2+}]_i$ of hypertrophied myocytes was higher than that of normal ones and Pra-C decrease the $[Ca^{2+}]_i$ elevation in left ventricular myocytes resulted from its calcium channel blockade.

Ca^{2+} overload in left ventricular hypertrophied (LVH) myocardium was recognized to contribute to the myocardial structural and functional abnormalities[1]. The ventricular thickness and left ventricular wet weight (LVWW)/body weight (BW) were regarded as the determination standard for the LVH[2]. Yet, no investigations were found about the cell dimensions and the level of $[Ca^{2+}]_i$ in single isolated hypertrophied ventricular myocytes caused by renovascular hypertension. Praeruptorine C {Pra-C, 2-methyl-10-(acetyloxy)-9, 10-dihydro-8,8-dimethyl-2-oxo-2*H*,8*H*-benzo[1,2-*b*: 3,4-*b*]dipyran-9-ol ester of 2-butenolc acid} isolated from Qian-Hu — the roots of *Peucedanum praeruptorium* Dunn, was found to increase coronary flow[3], inhibit the contraction of aorta induced by $CaCl_2$ or KCl, and protect the myocardial reperfused injury[4]. In the present study, we tried to set a new concept to describe the myocardial hypertrophy and evaluate any difference in $[Ca^{2+}]_i$ between normal and hypertrophic left ventricular myocytes at rest and under stimulation. The effects of Pra-C were investigated.

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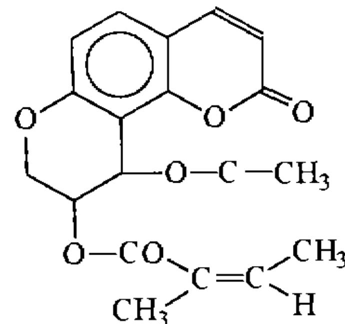

Praeruptorine C

MATERIALS AND METHODS

Adult Sprague-Dawley rats (♂, $n = 20$) weighting 209 ± 23 g were used. Systolic blood pressure (SBP) was measured in conscious rat with tail-cuff method using a BP recorder. Renovascular hypertension (two kidney-one clip) was made[5]. Hypertension was defined as SBP≥20 kPa.

Isolation of ventricular myocytes The sham-operated and hypertensive rats at 8 wk after SBP elevation[6] were decapitated. The heart was put into ice-cold Joklik modified Eagle's medium (MEM, Sigma Chemistry Co) for suspension culture[6,7]. The Langendorff heart was perfused[7]. The softened and paled ventricle was minced in Ca^{2+}-free MEM and dissociated into a single cell suspension, which was filtered through nylon gauze. Myocytes in the filtrates were

collected by sedimentation under gravity, and resuspended in fresh warm MEM (containing Ca^{2+} 30 μmol·L^{-1} and 2 % BSA). Ca^{2+} concentration of the MEM was increased gradually to 1 mmol·L^{-1} in 30 min.

Measurement of cell size The cell preparation was put into a small cell-pool with a coverslips in the bottom. Under inverted microscopy, the cell length and width were measured by using computer software MICC (Dongnan University). The cell size = length × width.

Fura 2-AM loading and measurement of $[Ca^{2+}]_i$ Myocytes were loaded with Fura 2-AM 5 μmol·L^{-1} (Sigma) in MEM at 37 ℃ for 25 min, followed by 3 times washes with Krebs' solution[7].

The equipment was AR-CM-MIC cations measurement system (SPEX Corporation, USA), with computer software DM3000. Only one cell was measured each time. The $[Ca^{2+}]_i$ was calculated according to the equation[8]: $[Ca^{2+}]_i$ (nmol·L^{-1}) = $K_d \times Sb_1/Sb_2 \times (R - R_{min})/(R_{max} - R)$.

Pra-C, supplied by the Department of Phytochemistry, Jiangsu Institute of Botany, was white-colored and dissolved in polyethylenglycol 400.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Unpaired t test was used to compare between groups.

RESULTS

SBP and cell size The SBP, whole ventricular wet weight (WVWW), and LVWW of hypertensive rats were increased compared with control rats, especially the ratio of LVWW/BW (Tab 1). These results indicated that the LVH had established 8 wk after the elevation of SBP in our model.

Tab 1. Systolic blood presure, body weight, and whole ventricular wet weight in normal and hypertensive adult rats. $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ vs control.

		Control	Hypertensive
SBP, kPa	Before	14.1±1.1	13.4±1.5^a
	After	13.8±2.2	27.0±1.4^c
WVWW, mg		1.31±0.13	1.73±0.12^c
LVWW, mg		0.6±0.05	1.11±0.27^c
LVWW/BW, mg/g		1.16±0.15	2.95±0.5^c

Of the left ventricular hypertrophied myocytes, the cell length was increased by 18 %, and the size was increased by 41 % ($P < 0.01$). In the length of the right ventricular cells, there were slight changes between normal and LVH rats (Tab 2).

Tab 2. Size of left ventricular myocyte (LVM) and right ventricular myocyte (RVM). LVM size = length × width. $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control.

		Control	Hypertensive left ventricular hypertrophy
LVM	Length/μm	108±11	128±11^b
	Width/μm	21±3	30±4^c
RVM	Length/μm	101±7	108±14^a
	Width/μm	21±7	22±1^a
LVM	Size/μm^2	2 320±394	3 724±598^c

Pra-C on resting $[Ca^{2+}]_i$ The resting $[Ca^{2+}]_i$ of normal myocytes was 87±4 nmol·L^{-1} in Krebs' solution containing $CaCl_2$ 1.0 mmol·L^{-1}. In the hypertrophic cells, it was 123±7 nmol·L^{-1}, which was higher than that of normal ones ($P < 0.01$). Preincubated with Pra-C 100 μmol·L^{-1}, there was no change in $[Ca^{2+}]_i$ in both normal and hypertrophic myocytes, just similar as that of nifedipine.

Pra-C on KCl-induced $[Ca^{2+}]_i$ elevation In the presence of Ca^{2+} 1.0 mmol·L^{-1}, KCl 20, 40, and 60 mmol·L^{-1} increased the $[Ca^{2+}]_i$ by 66 %, 141 %, and 268 % in the normal myocytes, while 77 %, 185 %, and 243 % in hypertrophic myocytes, respectively (Fig 1).

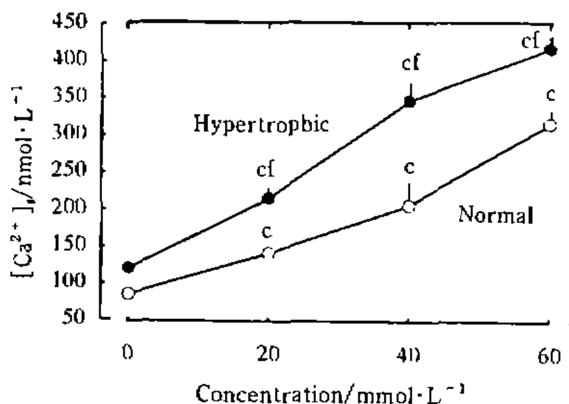

Fig 1. KCl-induced $[Ca^{2+}]_i$ elevations in normal and hypertrophied ventricular isolated from adult rat hearts. $n = 6$, $\bar{x} \pm s$. $^cP < 0.01$ vs before KCl; $^fP < 0.01$ vs normal myocytes.

In the normal and hypertrophic myocytes, the Pra-C depressed the KCl effects in a concentration-dependent manner as the nifedipine did (Fig 2).

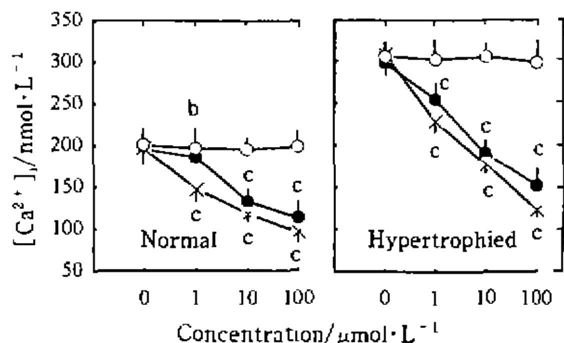

Fig 2. Effects of Pra-C (●) and Nif (×) on KCl (35 mmol·L^{-1})-induced elevations of $[Ca^{2+}]_i$ in ventricular myocytes isolated from adult rat hearts. $n = 6$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* solvent control (○).

Pra-C on norepinephrine-induced $[Ca^{2+}]_i$ elevation Norepinephrine 10 and 20 μmol·L^{-1} increased the $[Ca^{2+}]_i$ by 80 % and 119 % in the presence of extracellular Ca^{2+} 1.0 mmol·L^{-1} in hypertrophic myocytes, but 90 % and 139 % in normal ones, respectively. Pra-C concentration-dependently inhibited the effects of norepinephrine on $[Ca^{2+}]_i$ both in normal and hypertrophied myocytes (Fig 3).

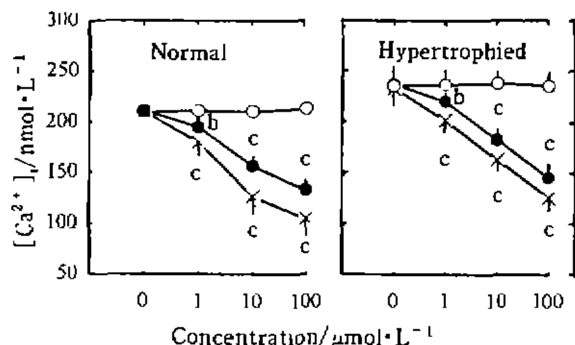

Fig 3. Effects of Pra-C (●) and Nif (×) on norepinephrine (20 μmol·L^{-1})-induced elevations of $[Ca^{2+}]_i$ in ventricular myocytes isolated from adult rat hearts. $n = 6$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* solvent control (○).

DISCUSSION

In these experiments, the $[Ca^{2+}]_i$ of hypertrophied myocytes was higher than that of normal ones at rest and under stimulated condition, indicating that there were calcium over load and abnormal in calcium transfer in the adult rat myocytes with LVH. The resting $[Ca^{2+}]_i$ determined in these experiments for isolated normal rat ventricular myocytes (87 ± 4 nmol·L^{-1}) was lower than that (137 nmol·L^{-1}) measured with Quin-2 as Ca^{2+} indicator[9]. But our estimate was remarkably close to the value (84 ± 3 nmol·L^{-1}) obtained using Fura-2 in single cultured neonatal heart cell[10]. Pra-C had no effects on the resting $[Ca^{2+}]_i$ in normal and hypertrophied myocytes, which was similar to that of nifedipine. It suggested that Pra-C did not alter the plasma membrane permeability to Ca^{2+}.

In this investigation, KCl and norepinephrine induced signifcantly $[Ca^{2+}]_i$ elevation in both normal and hypertrophic left ventricular myocytes. Pra-C also depressed the effect of KCl in cultured neonatal heart cells[11]. Pra-C inhibited KCl-induced $[Ca^{2+}]_i$ elevation in a concentration-dependent manner accounting for its Ca^{2+} channel blockade[12]. Pra-C did not inhibited $[Ca^{2+}]_i$ elevation induced by ouabain, which suggested that it did not affect the Na^+/Ca^{2+} exchange[11]. Pra-C depressed the effects of norepinephrine on $[Ca^{2+}]_i$ in adult ventricular myocytes, just as that of nifedipine, indicating that Pra-C act on the receptor-operated channel[13].

In this study the cell dimensions of normal left ventricular myocytes were similar to those reported by Powell *et al*[14], but cell length and size was increased in cells isolated from renovascular hypertensive rat, suggesting that there was hypertrophic in ventricular myocyte itself.

There were not only increase in the cell dimensions but also the elevation in $[Ca^{2+}]_i$ at rest and under stimulated condition in hypertrophied ventricular myocytes isolated from renovascular hypertension adult rat hearts. Pra-C decreased the $[Ca^{2+}]_i$ elevation induced by KCl and norepinephrine referring to its effect of calcium blockade.

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前胡丙素对分离的成年鼠正常及肥厚心室肌细胞内游离钙的影响

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关键词 前胡丙素；钙；左室肥厚；肾血管性高血压；Fura-2；细胞大小

目的：研究分离的成年大鼠正常及肥厚左室肌细胞$[Ca^{2+}]_i$及前胡丙素的作用. **方法**：用 Fura 2-AM 测定单细胞$[Ca^{2+}]_i$. **结果**：外钙为 1.0 mmol·L^{-1}时，正常左室肌细胞静息钙 87 ± 4 nmol·L^{-1}，肥厚细胞 123 ± 7 nmol·L^{-1}. 肥厚心肌细胞中，加入 KCl 20，40，60 mmol·L^{-1}，$[Ca^{2+}]_i$增加 29 %，78 %和 185 %，幅度高于正常细胞. 前胡丙素 1，10，100 μmol·L^{-1}浓度依赖地抑制 KCl 及去甲肾上腺素诱导$[Ca^{2+}]_i$增加. 作用与硝苯啶相似. **结论**：肥厚心肌细胞静息钙高于正常细胞；前胡丙素抑制激动剂引起的$[Ca^{2+}]_i$升高源于其钙通道阻断作用.

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