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Ca^{2+} sensitivity of contractile system and Ca^{2+} release from sarcoplasmic reticulum in skinned myocardium from rats with pressure overload LV hypertrophy and heart failure[1]

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KEY WORDS congestive heart failure; left ventricular hypertrophy; calcium; sarcoplasmic reticulum; contractile proteins; trabecular meshwork; caffeine

ABSTRACT

AIM: To explore the possible subcellular mechanisms underlying the decreased contractility of myocardium in left ventricle failured hearts caused by pressure overload. **METHODS**: Left ventricle pressure overload hypertrophy (LVH) and congestive heart failure (CHF) models were created in rats by ascending aortic banding. Left ventricle trabeculae skinned fibers were prepared by treatment with saponin 500 or 50 mg·L^{-1}. Relative Ca^{2+}-activated tensions ($T_{6.0}$% and $T_{5.6}$%) of saponin (500 mg·L^{-1})-skinned fibers were taken as the indices of Ca^{2+} sensitivity of contractile protein, caffeine-induced contracture of saponin 50 mg·L^{-1}-skinned fibers was an index of Ca^{2+} release from sarcoplasmic reticulum (SR). **RESULTS**: 1) There was no significant difference in relative Ca^{2+} activated tensions among CHF, LVH, and Sham-operated group in basic situation ($P > 0.05$). After treatment with caffeine 10 mmol·L^{-1}, the increase in value of δT % were significantly higher in LVH and CHF compared with that in Sham-operated control ($P < 0.01$). 2) The amplitudes of caffeine (5 and 10 mmol·L^{-1})-induced contracture were (0.66 ± 0.14) and (1.20 ± 0.27) g/mm^2 in control group, 19.8 % and 25.8 % lower in LVH ($P < 0.05$), 78.8 % and 80.9 % much more lower in CHF ($P < 0.01$). **CONCLUSION**: Decreased SR Ca^{2+} release was the main factor responsible for depressed contractility in failured myocardium while the Ca^{2+} sensitivity of contractile protein might not be involved.

INTRODUCTION

Contractility of myocardium is determined by 1) availability of intracellular calcium to myofilaments, mainly from calcium release of sarcoplasmic reticulum (SR) via ryanodine receptor (RyR); 2) responsiveness of contractile system to calcium. Cardiac hypertrophy induced by long term pressure overload is known as an early compensated stage with maintenance of myocardium contractile force, which is followed by later decompensated stage of heart failure characterized by depressed myocardial contractility. Abnormality of Ca^{2+} release from SR and Ca^{2+} sensitivity of contractile protein are supposed to be 2 subcellular mechanisms underlying the suppressed contractility of failured myocardium.

Recently, investigations on the SR Ca^{2+} release channel-RyR protein function and gene encoding RyR expression have been well documented[1,2,3,4]. It has been shown that the activity and density of Ca^{2+} release channel in SR are abnormal in ischemic failured human heart[1] and different animal pathological models[2]. But little is known about the abnormal change of SR Ca^{2+} release function and contractile protein Ca^{2+} sensitivity on the level of skinned myocardium.

In this paper we used saponin-skinned left ventricle trabeculae fibers from rats with left ventricular hypertrophy (LVH) and heart failure caused by

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experimental ascending aortic stenosis, to explore how the above functions change during cardiac hypertrophy and heart failure, which is in charge of the decreased contractility of failured heart.

MATERIALS AND METHODS

Preparation of pathological animal model Heart failure and hypertrophy rat models were made according to the method of Feldman with small modification[5]. Wistar ♂ rats of 4-wk-old (weighing 50 – 70 g) (Grade Ⅱ, Certificate № 022) were chosen for operation. Silver clip (0.9 mm internal diameter) was used to create ascending aorta stenosis, animals of age-matched controls underwent a same left thoracotomy without placement of the clip. All rats were fed normal rat chow and water *ad lib*. Eighteen weeks after operation, rats with aortic stenosis showing clinical signs associated with heart failure (eg, despnea, cyanopathy) were denoted as congestive failure group (CHF), while those without clinical changes were denoted as LVH group.

Preparation of skinned left ventricular trabeculae CHF, LVH, and Sham-operated rats were killed at the end of period and hearts were quickly removed to oxygenated Krebs-Henseleit buffer. Left ventricular trabeculae (< 1 mm in diameter) were isolated carefully and transferred to the perfusion chamber with one end of the fiber attached to isometric force transducer (Statham UC2, Gould, USA). The fibers were subjected to skinning procedure previously described in detail[6]. After skinning procedure, the permeability of sarcolemma and membrane of SR of the trabeculae were checked by MgATP removal and caffeine-induced contracture, respectively[7].

Solutions Three basic solutions were used as previously described[6]: 1) Ca^{2+} free relaxing solution, 2) skinning solution containing saponin 50 or 500 mg·L^{-1}, 3) activating solution containing different concentration of free Ca^{2+} was expressed as pCa (negative lg of the Ca^{2+} concentration). pCa 6.0, pCa 5.6, and pCa 4.5 were used in this experiment. All chemicals were purchased from Sigma Chemical Co. Solution pH was adjusted to 7.0 with KOH 1 mol·L^{-1} and checked by a digital pH meter (Model 5986-62, Cole Parmer, Germany).

Measurement of Ca^{2+} sensitivity of contractile protein After incubation in saponin 500 mg·L^{-1} skinning solution, both sarcolemma and the membrane of SR were perforated. Fibers were exposed to activating solutions of pCa 6.0, pCa 5.6, and pCa 4.5 sequentially and the Ca^{2+}-activated forces were recorded and revised by muscle cross-area (mm^2). The Ca^{2+}-activated tension ($T_{6.0}$ and $T_{5.6}$) expressed as the % of maximal tension (T_{max}) obtained at pCa 4.5 was taken as the index of Ca^{2+} sensitivity of contractile protein.

Measurement of Ca^{2+} release from SR After incubation in saponin (50 mg·L^{-1}) skinning solution, the sarcolemma of trabeculae became perforated but the membrane of SR remained intact. Fibers were exposed to caffeine 25 mmol·L^{-1} to empty the Ca^{2+} store in SR[8]. After reloading SR by pCa 7.0 solution for 3 min, fibers were exposed to caffeine 5 or 10 mmol·L^{-1} and contracture force was recorded and revised by CSA. The amplitude of caffeine-induced contracture tension was taken as the index of the function of Ca^{2+} release from SR.

Statistical analysis All values were presented as $\bar{x} \pm s$. Differences between groups were compared by ANOVA and Newman-Keuls *q* test.

RESULTS

Ca^{2+} sensitivity of contractile protein In preparations skinned by saponin 500 mg·L^{-1}, Ca^{2+}-activated forces induced by pCa 6.0, pCa 5.6, and pCa 4.5 were recorded in basic situation and under the treatment of caffeine 10 mmol·L^{-1}. The data of T_{max} were similar in three groups: (0.84 ± 0.23) g/mm^2 in CHF, (0.91 ± 0.28) g/mm^2 in LVH and (0.97 ± 0.31) g/mm^2 in Sham control group ($P > 0.05$) (Fig 1).

The relative Ca^{2+}-activated tension induced by pCa 6.0 ($T_{6.0}$%) and pCa 5.6 ($T_{5.6}$%) in CHF, LVH, and Sham groups were calculated. No significant difference was observed among these groups in basic situation ($P > 0.05$) (Tab 1).

Caffeine 10 mmol·L^{-1} increased Ca^{2+}-activated forces in three groups at different extents (Fig 1). Under the treatment with caffeine 10 mmol·L^{-1}, the increase in value of relative Ca^{2+}-activated tension ($\delta T_{6.0}$%) and ($\delta T_{5.6}$%) were significantly higher in CHF and LVH groups compared with that in Sham-

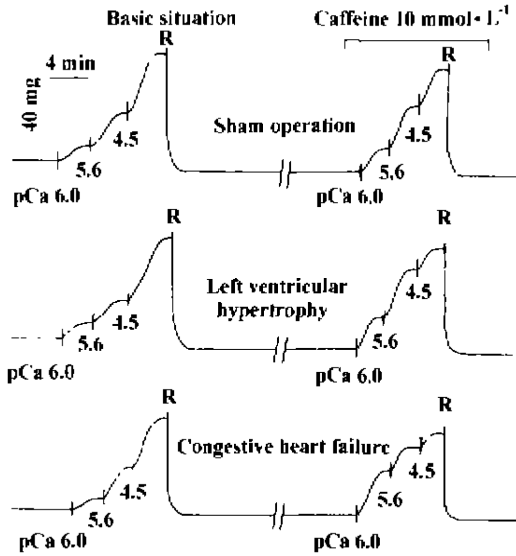

Fig 1. Ca^{2+} activated forces in saponin (500 mg·L^{-1})-skinned ventricular trabeculae fibers from age-matched rat hearts (R: relaxing solution, pCa: − lg [Ca^{2+}]). $n = 8$.

Tab 1. Relative tension activated by pCa 6.0 ($T_{6.0}$%) and pCa 5.6 ($T_{5.6}$%) in saponin (500 mg·L^{-1})-skinned trabeculae and increase in value (δT%) treated with caffeine 10 mmol·L^{-1}. $n = 8$. $^cP < 0.01$ *vs* Sham.

	$T_{6.0}$% −	$\delta T_{6.0}$% + Caffeine	$T_{5.6}$% −	$\delta T_{5.6}$% + Caffeine
Sham	21.2±5.3	11.2±3.3	45.6±9.9	21.0±2.9
LVH	19.3±3.8	21.8±2.6^c	43.8±6.2	31.6±3.6^c
CHF	18.1±4.6	21.5±3.9^c	39.2±7.1	35.5±3.3^c

Sham: sham-operated control. LVH: left ventricular hypertrophy. CHF: congestive heart failure.

operated control ($P < 0.01$) (Tab 1).

Ca^{2+} release function of SR After reloading SR with pCa 7.0 solution, typical caffeine-induced contractures were produced by the treatment of caffeine 5 and 10 mmol·L^{-1} in LVH and Sham control groups, but not in CHF group. The amplitudes of contracture induced by caffeine 5 and 10 mmol·L^{-1} in Sham group were (0.66±0.14) and (1.20±0.27) g/mm^2. The values in LVH group were (0.53±0.10) and (0.89±0.15) g/mm^2, which were lower than that in Sham control ($P < 0.05$). The values in CHF group were (0.14±0.07) and (0.23±0.08) g/mm^2, which were much more decreased compared with that in Sham control and in LVH ($P < 0.01$) (Fig 2).

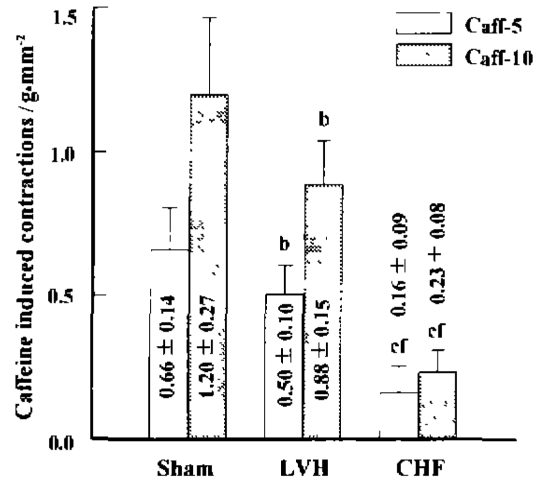

Fig 2. Comparison of the amplitude of caffeine-induced contractures in saponin 50 mg·L^{-1} skinned left ventricular trabeculae from sham operated control (Sham), hypertrophy (LVH) and heart failure (CHF) groups. $n = 8$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* Sham; $^fP < 0.01$ *vs* LVH. (Caff-5: caffeine 5 mmol·L^{-1}, Caff-10: caffeine 10 mmol·L^{-1})

DISCUSSION

The myocytes Ca^{2+} responsiveness was decreased apparently in the end stage of right ventricular hypertrophy in rat hearts[9]. In the present paper, we observed that in basic situation, there was no change on myocardial Ca^{2+} sensitivity in left ventricular hypertrophic and failured hearts compared with that in age-matched control, this result was similar to those of hypertrophy in guinea pig[10] and ferret hearts[11]. From the results, we can infer that abnormality of Ca^{2+} sensitivity of contractile protein was not involved in the mechanisms underlying the depressed contractility in left ventricular failured hearts. But interestingly, we found that the Ca^{2+}-activated tension in pathological myocardium was more sensitive to caffeine. Further study is needed to explain the difference of responsiveness to caffeine between pathological and normal preparations.

In the preparations with intact SR membrane, caffeine-induced contractures deeply decreased in failured hearts compared with those in hypertrophic or age-matched control group, indicating that the abnormality of SR Ca^{2+} release function existed in the end stage of failured hearts in rats. This abnormal

change of SR Ca^{2+} release might be the main factor responsible for the depressed contractility in heart failure. There are 2 possible explanations on the reduced SR Ca^{2+} release function: a down-regulated number and function of RyR protein, or reduced available stored Ca^{2+} in SR due to decreased SR Ca^{2+} uptake[12]. Plenty of evidences showed that the gene expression of SR Ca^{2+}-ATPase decreased in failured hearts[3,5,13,14] which supported the possibility of the reduced stored Ca^{2+} in SR due to decreased SR Ca^{2+} uptake. Whether the gene expression of SR Ca^{2+} release channel changed in this situation still remained controversy[3,4]. Further researches on the molecular mechanisms of decreased SR Ca^{2+} release are necessary.

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压力超负荷左心室肥大和心衰大鼠心肌收缩系统 Ca^{2+} 敏感性和肌浆网 Ca^{2+} 释放[1]

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关键词　充血性心力衰竭；左心室肥大；钙；肌浆网；收缩蛋白质类；小梁网；咖啡因

目的：探讨压力超负荷左心衰心肌收缩能力下降的亚细胞机制．**方法**：采用升主动脉缩窄法制备左心室压力超负荷心肌肥大(LVH)和充血性心衰(CHF)大鼠病理模型；采用皂苷 500 或 50 mg·L^{-1} 制备左室肌小梁蜕膜标本，皂苷 500 mg·L^{-1}蜕膜标本的相对 Ca^{2+} 激活张力($T_{6.0}$% 和 $T_{5.6}$%)和皂苷 50 mg·L^{-1}蜕膜标本的咖啡因挛缩幅值分别是收缩系统 Ca^{2+} 敏感性和肌浆网 Ca^{2+} 释放的指标．**结果**：1)基础状态下，LVH、CHF 和同龄假手术对照大鼠的相对 Ca^{2+}激活张力不存在显著差异($P>0.05$)；但变力因素咖啡因 10 mmol·L^{-1} 干预后 Ca^{2+}激活张力的增量 δT% 在 LVH 组和 CHF 组明显高于对照组($P<0.01$)；2)咖啡因 5 和 10 mmol·L^{-1}引发的挛缩幅值在对照组为(0.66 ± 0.14)和(1.20 ± 0.27) g/mm^2，LVH 组略低于对照组 19.8 %和 25.8 % ($P<0.05$)，CHF 组大幅低于对照组 78.8 %和 80.9 % ($P<0.01$)．**结论**：导致衰竭心肌收缩能力低下，可能主要源于肌浆网 Ca^{2+} 释放功能的显著下降而非收缩系统的 Ca^{2+} 敏感性改变．

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