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Apamin-Sensitive K^+ Current Upregulation in Volume-Overload Heart Failure is Associated with the Decreased Interaction of CK2 with SK2

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Abstract Recent studies have shown that the sensitivity of apamin-sensitive K^+ current (I_{KAS}, mediated by apamin-sensitive small conductance calcium-activated potassium channels subunits) to intracellular Ca^{2+} is increased in heart failure (HF), leading to I_{KAS} upregulation, action potential duration shortening, early after depolarization, and recurrent spontaneous ventricular fibrillation. We hypothesized that casein kinase 2 (CK2) interacted with small conductance calcium-activated potassium channels (SK) is decreased in HF, and protein phosphatase 2A (PP2A) is increased on the opposite, upregulating the sensitivity of I_{KAS} to intracellular Ca^{2+} in HF. Rat model of volume-overload HF was established by an abdominal arteriovenous fistula procedure. The expression of SK channels, PP2A and CK2 was detected by Western blot analysis. Interaction and colocalization of CK2 with SK channel were detected by co-immunoprecipitation analysis and double immunofluorescence staining. In HF rat left ventricle, SK3 was increased by 100 % ($P < 0.05$), and SK2 was not significantly changed. PP2A protein was increased by 94.7 % in HF rats ($P < 0.05$), whereas the level of CK2 was almost unchanged. We found that CK2 colocalized with SK2 and SK3 in rat left ventricle. With anti-CK2α antibody, SK2 and SK3 were immunoprecipitated, the level of precipitated SK2 decreased by half, whereas precipitated SK3 was almost unchanged. In conclusion, the increased expression of total PP2A and decreased interaction of CK2 with SK2 may underlie enhanced sensitivity of I_{KAS} to intracellular Ca^{2+} in volume-overload HF rat.

Keywords Heart failure · SK channels · Protein kinase CK2 · Protein phosphatase 2A · Apamin-sensitive K^+ current

Introduction

Heart failure is associated with a high prevalence rate and mortality rate (Hunt et al. 2009; Hjalmarson 1999). Malignant arrhythmia, such as ventricular tachycardia (VT) and ventricular fibrillation (VF), occurs frequently. A molecular mechanism of arrhythmia genesis is electric remodeling, resulting from the abnormality of the ion channels expression and function, which are significantly changed under HF condition.

The existence of SK channels has been documented within the last few years, and their functional roles are being elucidated gradually (Xu et al. 2003; Tuteja et al. 2005; Lu et al. 2007; Ozgen et al. 2007; Li et al. 2009; Diness et al. 2010, 2011; Zhang et al. 2008; Lu et al. 2009; Marionneau et al. 2005). Three subtypes of SK channels (SK1-3) are expressed in both atria and ventricle of human and rat hearts (Xu et al. 2003; Tuteja et al. 2005; Lu et al. 2007; Ozgen et al. 2007; Li et al. 2009; Diness et al. 2010, 2011; Zhang et al. 2008; Lu et al.

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2009; Marionneau et al. 2005; Ni et al. 2013; Chua et al. 2011). SK2 and SK3 that are more sensitive to apamin (a neurotoxin which selectively blocks SK channels) (Zhang et al. 2008; Castle et al. 1989) mediates I_{KAS}. Activation of SK channels in ventricular myocytes requires Ca^{2+} to be released from sarcoplasmic reticulum (SR) (Terentyev et al. 2014).

In congestive heart failure, cellular Ca^{2+} handling is altered. SR Ca^{2+} stores are reduced, SR Ca^{2+}-ATPase (SERCA) is downregulated, relaxation of the systolic Ca^{2+} transient is slowed and diastolic Ca^{2+} is increased (Bers 2008). Previous studies have shown that the sensitivity of I_{KAS} to intracellular Ca^{2+} is increased in HF, leading to I_{KAS} upregulation, action potential duration (APD) shortening, early after-depolarization, and recurrent spontaneous ventricular fibrillation (SVF) (Ni et al. 2013; Chua et al. 2011; Gui et al. 2013).

Recent studies have documented that the catalytic and regulatory subunits of CK2 and PP2A interact with cytoplasmic N and C terminals of SK channel to form a multiprotein complex in the plasma membrane form rat brains. Within the complex, CK2 phosphorylates calmodulin (CaM, β-subunit of SK channel) reduces the affinity of CaM to intracellular Ca^{2+}, and accelerates channel deactivation. On the opposite, PP2A dephosphorylates CaM and recovers the channel's activity (Allen et al. 2007; Bildl et al. 2004). But not all SK channel may always be associated with CK2 and PP2A. Therefore, we study the normal and heart failure rats to test the hypotheses that CK2 interacted with SK channel is decreased in HF, and PP2A is increased on the opposite, which raises the sensitivity of I_{KAS} to intracellular Ca^{2+} in HF.

Materials and Methods

Establishment of Rat Volume-Overload HF Model

Male Sprague–Dawley rats (140–160 g) were purchased from the Laboratorial Animal Center of Xi'an Jiaotong University (Shaanxi, China). The investigation conformed with the Guide for the Care and Use of laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1985). Rats were divided randomly into two groups, sham-operation group (SO group, $n = 11$) and heart failure group (HF group, $n = 12$). Rat model with HF was established as we had reported before (Ni et al. 2013; Du et al. 2007). Briefly, rats of HF group were subjected to abdominal arterio-venous fistula (volume overload) to induce HF. And SO group rats were operated in an identical way, but a fistula was not established. Eight weeks after operation, echocardiography was performed to confirm HF formation (Grimm et al. 1999; Mulder et al. 2004). Hearts were rapidly excised and left ventricles' free walls were flash frozen in liquid nitrogen.

Isolation of Single Cardiomyocytes

Cardiomyocytes were isolated as described previously (Ni et al. 2013), with some modification. Rats were injected with heparin sodium (400 IU/kg, i.p.) to anti-coagulate, and 20 min later, were anesthetized with sodium pentobarbital (40–50 mg/kg, i.p.). The hearts were excised quickly and mounted on a Langendorff perfusion apparatus within 60 s and perfused with Ca^{2+}-free Tyrode's solution containing (in mM): NaCl, 140; KCl, 5.4; $MgCl_2$, 1.0; $CaCl_2$, 1.8; HEPES, 10.0, Glucose, 10.0 (pH 7.2–7.4 with NaOH). Then, a 15 min perfusion with the same buffer containing 179 IU/mL collagenase type I (Sigma), 0.01 % protease (Sigma) and 0.06 % bovine serum albumin with 0.06 mmol/L Ca^{2+} was performed. The digestion was terminated with the followed Ca^{2+}-free Tyrode's solution. The perfusion was conducted at a constant temperature of 37 °C. The heart was removed from the perfusion apparatus and left ventricle (LV) was then dissected mechanically to obtain cardiomycytes. Isolated cardiocytes were stored in Kraftbrühe (KB) solution at room temperature of 22–24 °C. At least 80 % of the isolated cardiomyocytes were rod shaped with clear striation.

Immunofluorescence Confocal Microscopy

The isolated rat left ventricular myocytes were incubated in ice cold 4 % paraformaldehyde for 30 min, permeabilized with 0.1 % Triton-X 100 at room temperature for 5 min, and blocked with 5 % albumin from bovine serum (BSA) for at 1 h at room temperature. Then, they were incubated with rabbit anti-CK2, PP2A antibody and goat anti-SK3 (1:50 dilution; Santa Cruz Biotechnology, USA), SK2 antibody (1:50 dilution; Abcam, UK) overnight at 4 °C. This was followed by 2 h incubation with donkey anti-rabbit Alexa Fluor 594-labeled (1:100 dilution; invitrogen, USA) and donkey anti-goat Alexa Fluor 488-labeled fluorescent secondary antibody (1:100 dilution; invitrogen, USA). The fluorescence images were captured by a confocal laser scanning microscope (Model TCS Laser Scanning Confocal Microscope System, Leica, Zeiss, Germany). Negative control cells were incubated with secondary antibodies without primary antibodies.

Western Blot Analysis

Protein samples were extracted from the LV homogenates of HF and SO rats. Protein was quantified

using a Protein Assay Kit (Bio-Rad). Homogenates (30 μg) were separated by 10 % SDS-PAGE gel and transferred to PVDF membranes under 100 V at 4 °C for 2 h. The transferred membrane was blocked by 5 % skim milk and 0.1 % Tween-20 in Tris-buffered saline for 1 h, and then incubated with rabbit polyclonal anti-SK2 (1:200 dilution, Alomone Labs, Jerusalem, Israel), rabbit polyclonal anti-CK2α (1:500 dilution, Merck Millipore, Darmstadt, Germany) or rabbit polyclonal anti-PP2Ac antibodies (1:500 dilution, Merck Millipore, Darmstadt, Germany) overnight at 4 °C. And then the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody (1:20,000 dilution, Santa Cruz Biotechnology, USA). The relative level of the protein was normalized by reprobing the membrane with β-actin antibody (1:500 dilution, Santa Cruz Biotechnology, USA). All protein bands were detected by a chemiluminescence (ChemiDoc XRS, BioRad), and the intensities of the bands were quantified by densitometry.

Co-Immunoprecipitation (Co-IP)

For co-IP detection, homogenates of LV tissues were extracted in 0.5 mL RIPA buffer (20 mM Tris/HCl, PH7.6; 150 mM NaCl; 20 mM KCl; 1.5 mM $MgCl_2$; 1 mM PMSF) with protease inhibitor cocktail (0.1–0.3 μM Aptotinin, 1 μM Pepstatin, 100 μM Leupeptine). Samples of 500 μg of protein were incubated with anti-CK2α (2 μg, Santa Cruz Biotechnology, USA) and anti-PP2Ac antibodies (2 μg, Santa Cruz Biotechnology, USA) on the rotator for 4 h at 4 °C and then precipitated with 20 μg protein A/G Plus-agarose beads (Santa Cruz Biotechnology, USA) on the rotator overnight at 4 °C. The immunoprecipitates were collected by centrifuging the samples at 15,000 rpm for 5 min at 4 °C. The beads were then washed five times with 1 mL lysis buffer, repeating centrifugation step above each time. The supernatant was discarded. The beads were resuspended in 2× loading buffer, and then the samples were boiled for 5 min and centrifuged at 15,000 rpm for 10 min. The supernatant was analyzed by Western blot analysis. Anti-SK2, anti-SK3, anti-CK2α, or anti-PP2Ac antibodies were used for Western blot analysis.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical significance between the SO and HF group was observed with unpaired Student's t test. A result with P value of 0.05 or less was considered statistically significant.

Results

Volume Overload Induced HF in Rats

Eight weeks after the operation, most HF group rats exhibited obvious clinical signs of HF, such as lethargy, appetite loss, and tachypnea. Echocardiographic data showed a significant left ventricular dilation and systolic dysfunction in HF group compared with SO group. As showed in Table 1, with HF, LV end-diastolic diameter (LVEDD) increased by 37.9 % ($P < 0.01$), end-diastolic volume (LVEDV) increased by 145.8 % ($P < 0.01$), LV ejection fraction (EF) decreased by 11.3 % ($P < 0.01$), mean fractional shortening (FS) decreased by 13.1 % ($P < 0.05$), indicating the volume overloaded heart failure rat model was established successfully.

SK2, SK3 Protein Expression in Control and HF Rat LV

In order to detect the expression of apamin-sensitive subtypes SK2 and SK3, Western blot analysis was performed. Whole cell protein was extracted from LV of HF ($n = 6$) and SO ($n = 6$) rats. SK2 channel bands displayed a band with molecular mass of 60 kDa and SK3 channel bands were detected at 80 kDa. SK2 channel protein in HF was not significantly changed compared with that in SO group (SK2: HF 0.064 ± 0.012 vs. SO 0.049 ± 0.014, $P > 0.05$; Fig. 1). SK3 was doubled in HF group (SK3: HF

Table 1 Echocardiographic data of sham-operation (SO) and volume-overload heart failure (HF) rats

Group	SO ($n = 11$)	HF ($n = 12$)
LVEDD (mm)	7.47 ± 0.97	10.30 ± 1.26**
LVESD (mm)	4.23 ± 0.84	6.42 ± 1.41**
PWTd (mm)	1.42 ± 0.22	1.53 ± 0.27
PWTs (mm)	2.30 ± 0.16	2.77 ± 0.45**
IVST (mm)	1.71 ± 0.21	1.72 ± 0.39
LVEDV (mL)	0.96 ± 0.32	2.30 ± 0.75**
LVESV (mL)	0.44 ± 0.82	0.68 ± 0.49
EF (%)	80.81 ± 4.27	71.64 ± 8.80**
FS (%)	43.90 ± 5.07	38.13 ± 6.95*
HR (bpm)	358.73 ± 49.38	355.67 ± 38.47
LVM (mg)	649.59 ± 147.79	1181.06 ± 324.58**

Values are mean ± SD

No. of rats (n), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), diastolic posterior wall thickness (PWTd), systolic posterior wall thickness (PWTs), interventricular septal thickness (IVST), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), ejection fraction (EF), fractional shortening (FS), heart rate (HR), left ventricular mass (LVM)

* $P < 0.05$ vs. SO; ** $P < 0.01$ vs. SO

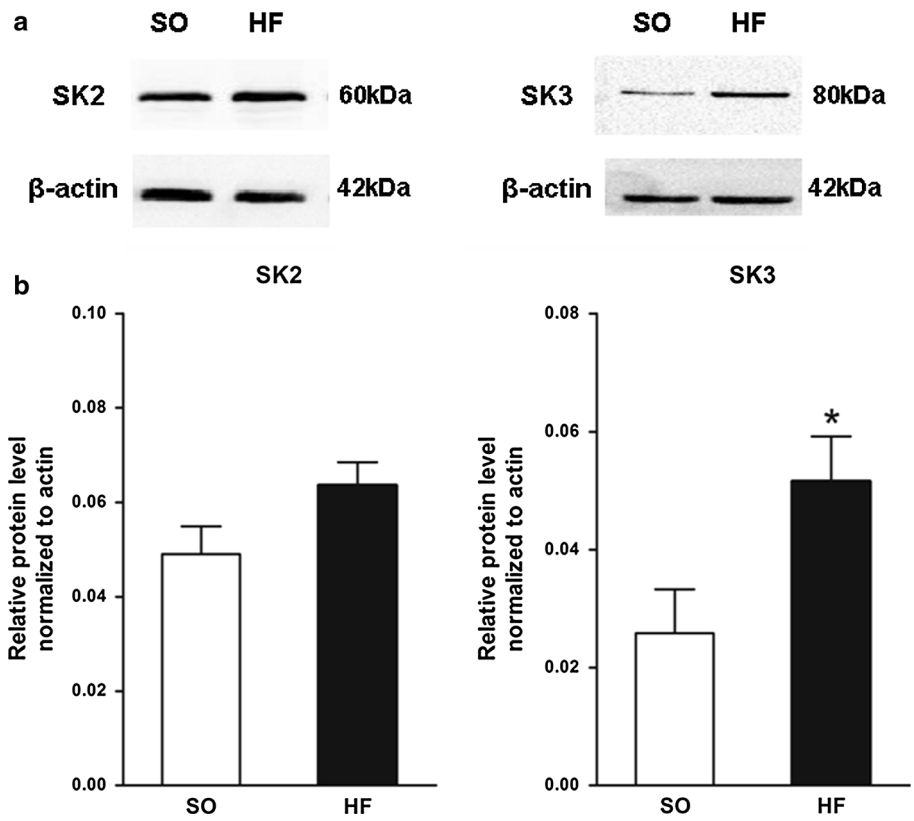

Fig. 1 Expression of SK2 and SK3 in sham-operated (SO) and heart failure (HF) rat left ventricle tissue. **a** Representative immunoblot of SK2 and SK3 obtained in SO and HF rat LV tissue. **b** Relative protein level of SK2 and SK3 in SO and HF rat LV tissue, normalized to β-actin. SK3 was increased twofold in HF versus SO rats. Protein was extracted from 6 SO and 6 HF rat LV, $^*P < 0.05$ vs. SO group

0.052 ± 0.019 vs. SO 0.026 ± 0.018, $P < 0.05$; Fig. 1). The results were normalized to β-actin.

CK2, PP2A Protein Expression in Control and HF Rat LV

CK2 is a protein kinase, which phosphorylates CaM, reduces the affinity of CaM to intracellular Ca^{2+}, and accelerates channel deactivation. PP2A is a phosphatase, which has the inverse effect. According to Allen's and Bildl's studies, CK2 and PP2A were coassembled with SK channels and regulate channel gating (Allen et al. 2007; Bildl et al. 2004). In order to detect the expression of CK2 and PP2A, Western blot analysis was performed in LV homogenates from HF and SO rat hearts. With heart failure, PP2A protein was increased by 94.7 % (PP2A: HF 1.48 ± 0.14 vs. SO 0.76 ± 0.13, $P < 0.01$; Fig. 2), whereas the level of CK2 was almost unchanged (CK2: HF 1.06 ± 0.37 vs. SO 0.94 ± 0.48, $P > 0.05$; Fig. 2). The results indicated that the increased PP2A and unchanged CK2 in HF might play a role in the dephosphorylation and phosphorylation of SK channel, and regulate the activity of SK channel.

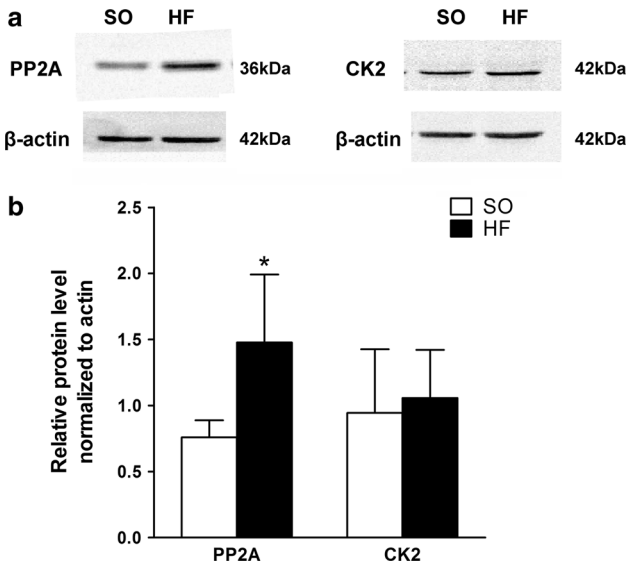

Fig. 2 Expression of PP2A and CK2 in sham-operated (SO) and heart failure (HF) rat left ventricle tissue. **a** Representative immunoblot of PP2A and CK2 obtained in SO and HF rat LV. **b** Relative protein level of PP2A and CK2 in SO and HF rat LV tissue, normalized to β-actin. PP2A was increased by 94.7 % in HF versus SO rats. Protein was extracted from 6 SO and 6 HF rat hearts, $^*P < 0.05$ vs. SO group

Interaction of SK Channels with CK2, PP2A

In order to further determine whether enhanced sensitivity of SK channel to intracellular Ca^{2+} in HF is related to the interaction of PP2A and CK2 with SK2, co-immunoprecipitation analysis was performed in HF and SO rat LV tissue.

CK2 antibodies were used to isolate CK2 from homogenates by binding to protein A/G Plus-agarose beads, and then followed by immunoblotting analysis of SK channel protein. Immunoblotting images revealed precipitated SK2 and SK3 reactive bands (Fig. 3a). Quantitation revealed that the level of precipitated SK2 was decreased by 52 % in normalized HF rat LV tissue versus SO (SK2: HF 0.48 vs. SO 1.00; Fig. 3b), whereas the level of precipitated SK3 decreased slightly (SK3: HF 0.72 vs. SO 1.00; Fig. 3b), normalized to precipitated CK2. However, the SK2 or SK3 was not detected in co-immunoprecipitation by using PP2A antibodies.

The colocalization of CK2, PP2A with SK channels was further confirmed by immunofluorescence staining in control and HF rat LV myocytes. Figure 4a shows representative confocal images where SK2 (green) was present throughout the cell membrane, and it colocalized with CK2 (red, arrow, the part distributed in membrane) that was decreased by half in HF myocytes (consistent with the immunoprecipitation data). PP2A (red) was distributed both throughout the membrane and in cytosol as showed in Fig. 4b. The part distributed in the membrane (arrow) was also colocalized with SK2, and was almost increased twofold in HF. The colocalization of SK3 with CK2 and PP2A is shown in Fig. 5. SK3 (green) was also present throughout the myocyte membrane, and the colocalization with CK2 had no obvious change, whereas the colocalization with PP2A was increased in HF myocytes. No labeling was detected when the primary antibodies were omitted in negative control samples (data not shown).

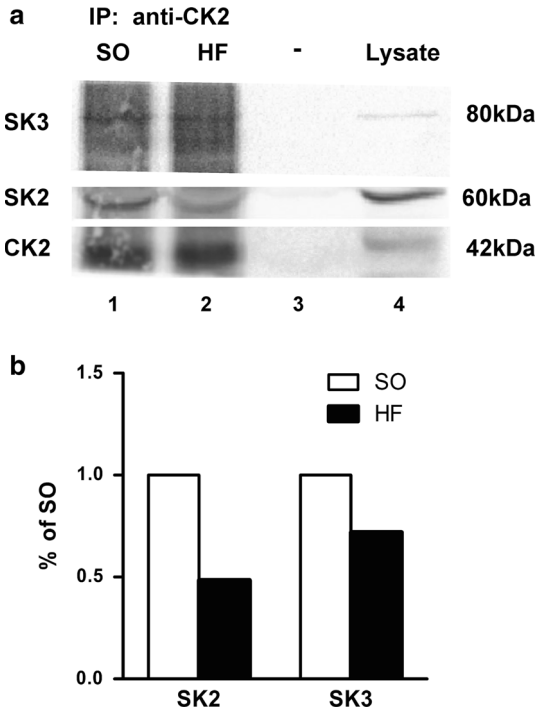

Fig. 3 Coimmunoprecipitation of SK2, SK3, and CK2 from sham-operated (SO) and heart failure (HF) rat left ventricle tissue. **a** Protein precipitated (IP) with anti-CK2α antibody (anti-CK2) from preparations of HF and SO rat heart was separated by SDS-PAGE, blotted to PVDF membrane, and probed with anti-SK2, anti-SK3, or anti-CK2α antibodies as shown in lanes 1–2. Negative control in lanes 3 excluded nonspecific SK2 binding to CK2. Lane 4 was a result obtained using rat LV tissue lysate confirming the presence of SK2, SK3 and CK2 proteins. **b** Relative protein level of SK2 and SK3 (normalized to immunoprecipitated CK2)

Discussion

In this study, the expression and association of SK channels, PP2A and CK2 are evaluated. The results indicate that decreased interaction of CK2 with SK2 may enhance sensitivity of I_{KAS} to intracellular Ca^{2+} in volume-overload HF rat LV. It is consisted with the conclusion that expression of total PP2A is increased relative to CK2. It seems that the affection of the abnormal balance of protein kinase and phosphatase in the increasing sensitivity of I_{KAS} to intracellular Ca^{2+} and the density of I_{KAS}, lead to APD shortening, early after-depolarization and recurrent SVF.

Repolarization Reserve in Heart Failure

Cardiac action potentials (APs) are driven by ionic currents flowing through specific channels and exchangers across cardiomyocyte membranes. Once initiated by rapid Na^+ entry during phase 0, the APD is determined by the balance between inward depolarizing currents, carried mainly by Na^+ and Ca^{2+}, and outward repolarizing currents carried mainly by K^+. K^+ currents play a major role in repolarization. The loss of a K^+ current can impair repolarization, but there is a redundancy of K^+ currents so that when one K^+ current is dysfunctional; other K^+ currents increase to compensate, a phenomenon called "repolarization reserve" (Michael et al. 2009). Repolarization reserve protects repolarization under conditions that increase inward current or reduce outward current, threatening the balance that governs APD. This protection comes at the expense of reduced repolarization reserve, potentially resulting in unexpectedly large APD prolongation and arrhythmogenesis, when an additional repolarization-suppressing intervention is superimposed. In HF, reduction in I_{to}, I_{Ks}, and I_{K1} leads to APD prolongation; I_{Kr} remains largely

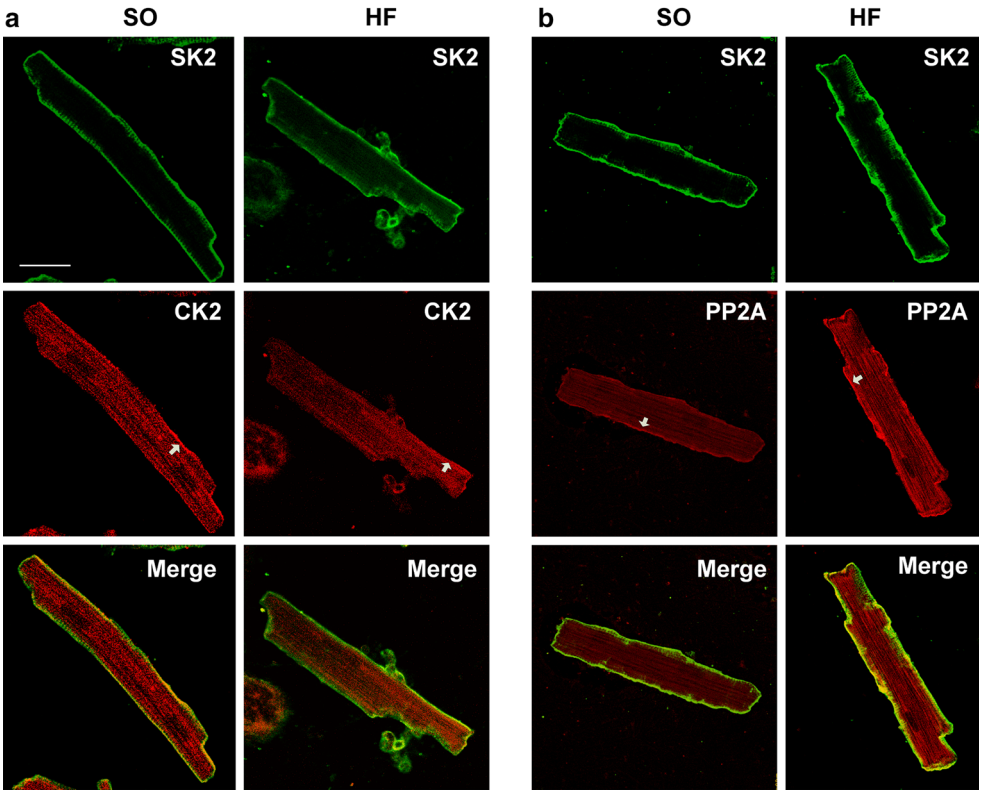

Fig. 4 Colocalization of SK2 and CK2 or PP2A in sham-operated (SO) and heart failure (HF) rat cardiomyocytes. Rat isolated cardiomyocytes double labeled with anti-SK2 (*green channel*) and anti-CK2 (*red channel*) antibodies (**a**) and with anti-SK2 (*green channel*) and anti-PP2A (*red channel*) antibodies (**b**). In each case, the *green and red channels* are shown separately, as well as merged. *Arrow* points to the part distributed in the membrane. *Scale bar* is 25 μm (Color figure online)

unchanged during HF. Changes in ion channel properties appear as part of an adaptive response to maintain function in the face of disease-related stress. However, if the stress is maintained the adaptive ion channel changes may themselves lead to dysfunction, in particular cardiac arrhythmias.

Expression of SK3 is Increased in HF LV and SK2 is not Significantly Changed

SK channel was first to be found in neuron, and could produce an after hyperpolarization following a neural action potential, which protects the cell from the deleterious effects of continuous tetanic activity. Recently, several studies showed that SK channels are also expressed in cardiomyocytes. In our study, SK2 channel protein in HF rats is not significantly changed and SK3 is increased in HF rats versus SO. It supports the results of previous studies (Ni et al. 2013). SK channel plays an important role in repolarization of ventricular myocytes (Xu et al. 2003; Tuteja et al. 2005). According to the Su-Kiat's study, the density of I_{KAS} [SK2 and SK3 channels mediate I_{KAS} (Zhang et al. 2008; Castle et al. 1989)] is significantly increased in tachycardia-induced HF rabbit myocytes, leading to APD shortening, and increasing the risk of recurrent SVF (Chua et al. 2011). The increasing density of I_{KAS} may act as a repolarization reverse. It protects the heart by keeping the balance that governs APD; however, it is at the expense of reduced repolarization reserve, and increased risk of arrhythmias. Therefore, it is important to find the mechanism that regulates SK channels, and it may be a novel therapeutic target.

As the activation of SK channel depends on intracellular Ca^{2+}, it is considered as an important link between Ca^{2+} and electrical events of cardiomyocyte. Recently, many studies have confirmed that SK channels play an important role in atrial fibrillation (AF), and that NS8593 (a non-selective inhibitor of SK channels, primarily inhibiting SK2 and SK3, but inhibits all SK channels when intracellular Ca^{2+} is 500 nM) and UCL1684 (inhibiting SK2 and SK3 channels) could terminate or prevent AF occurrence in various cardiac preparations. These effects are considered to be associated with the apparent increasing of intracellular Ca^{2+} (Ozgen et al. 2007; Li et al. 2009; Diness et al. 2010, 2011; Skibsbye et al. 2011; Nattel 2009; Ellinor et al. 2010; Yu et al. 2012). Similarly, disfunction of Ca^{2+} channel, Ca^{2+} handling and Na^+/Ca^{2+} exchanger (Bers 2008; Arai et al. 1994; Baartscheer et al. 2003), leading to calcium overload in HF. However, our previous study finds that a highly elevated level of intracellular Ca^{2+} in SO rat cardiomyocytes is unable to increase I_{KAS} as large as in HF. It demonstrates that upregulation of I_{KAS} in HF not only relies on the Ca^{2+} elevation, but also on the sensitivity of I_{KAS} to intracellular Ca^{2+} (Ni et al. 2013).

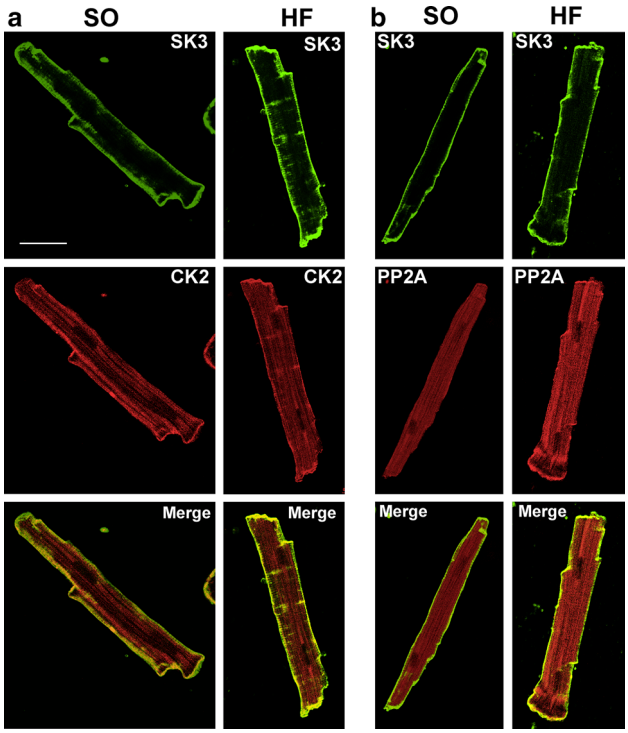

Fig. 5 Colocalization of SK3 and CK2 or PP2A in sham-operated (SO) and heart failure (HF) rat cardiomyocytes. Rat isolated LV myocytes double labeled with anti-SK3 (*green channel*) and anti-CK2 (*red channel*) antibodies (**a**) and with anti-SK3 (*green channel*) and anti-PP2A (*red channel*) antibodies (**b**). In each case, the *green and red channels* are shown separately, as well as merged. *Scale bar* is 25 μm (Color figure online)

Colocalization of CK2 and SK2 Channel is Decreased in HF

In health and disease, kinase/phosphatase balance governs cardiac excitability. The balance of protein phosphorylation is tightly related to the competing activities of protein kinases and phosphatases. Abnormal regulation of protein phosphorylation is highly associated with mechanical dysfunction and arrhythmias in cardiovascular diseases, including sinus node disease, atrial fibrillation, myocardial infarction, and heart failure (Reiken et al. 2001; He et al. 2011; Chelu et al. 2009; Swaminathan et al. 2011; Bers 2011; Shan et al. 2010; DeGrande et al. 2013). As a phosphatase, the activation of PP2A could be enhanced by sympathetic nervous system, which is an important mechanism in developing HF. Furthermore, pharmacological inhibitors of kinase activity have significantly enhanced our ability to treat cardiovascular disease phenotypes (Reiken et al. 2003; Shelton et al. 2009).

Recent studies have shown that CaM is constitutively bound to the intracellular C terminus of SK channel and serves as the Ca^{2+} sensor. The catalytic and regulatory subunits of CK2 and PP2A interact with the cytoplasmic N and C termini of the channel protein form a polyprotein complex. Within the complex, CK2 phosphorylates CaM at threonine 80, reduces the Ca^{2+} sensitivity, and accelerates channel deactivation (Bildl et al. 2004), PP2A counterbalances the effects of CK2, dephosphorylating CaM and recovering the channel's activity (Allen et al. 2007).

The expression of PP2A and CK2 is studied in the LV of HF rat, and the level of PP2A is increased twofold in HF, but no significant change is found in the level of CK2. It indicates that increased PP2A and unchanged CK2 in HF may play a role in the phosphorylation of CaM and activity of SK channels. To further determine it, co-immunoprecipitation and immunofluorescence staining are performed. Interaction and colocalization between SK channels and CK2 are first to be reported in rat cardiac tissue. Interaction of CK2 and SK2 in HF is found to be significantly decreased, and the interaction of CK2 and SK3 is unchanged. However, SK channel protein is not detected by immunoprecipitation using PP2A antibodies. Maybe the interaction between SK channels and PP2A is relatively weak. But the immunofluorescence staining could show that PP2A is distributed both throughout the membrane and in cytosol, and the part distributed in the membrane was also colocalized with SK2, and was increased in HF. Our observations suggest that although total CK2 is unchanged in HF LV, the decreased interaction of CK2 with SK2 contributes to enhance the sensitivity of I_{KAS} to intracellular Ca^{2+}. On the contrary, SK3 may not play a major role in enhancing the sensitivity, or SK3 may be regulated by other ways which remain unknown. It may provide theoretical basis for search novel the therapeutic target.

Limitations

The observations strongly suggest that decreased levels of colocalized CK2 (with SK2) may contribute to decreased SK2 phosphorylation and increased sensitivity of I_{KAS} to intracellular Ca^{2+} in HF LV; however, the electrophysiological effect needs further verification. Furthermore, because SK2 and SK3 are both sensitive to apamin, and there is no specific inhibitor to distinguish them, it is difficult to know which subtype contributes more to I_{KAS} in HF myocytes.

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Complicance with Ethical Standards

Ehical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in

accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent Informed consent was obtained from all individual participants included in the study.

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