Original Paper

Upregulation of TRPC1 Contributes to Contractile Function in Isoproterenol-induced Hypertrophic Myocardium of Rat

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Key Words

Transient receptor potential canonical channel 1 • Stretch-activated ion channels • Hypertrophic myocardium • Contractile function

Abstract

Aims: The transient receptor potential canonical channel 1 (TRPC1) is a crucial component of the stretch-activated ion channels (SACs). The objective of this research was to demonstrate the contribution of TRPC1 in maintaining cardiac contractile function in the hypertrophic myocardium. **Methods:** Hypertrophic rat hearts were induced by injecting isoproterenol intraperitoneally, and the expressions of TRPC1/3/6 and Na⁺/Ca²⁺ exchanger 1 (NCX1) proteins were analyzed by Western blot. The intracellular calcium images, the action potential of myocardium, the length-dependent contractile force of ventricle muscle and the cardiac output of isolated heart were investigated. **Results:** The expression of TRPC1 was increased in the hypertrophic myocardium. After being stretched, the ascendant amplitude of the increase in the intracellular calcium ion concentration ([Ca²⁺]_i) in the hypertrophic myocardium was higher than that in the normal myocardium. The increase of the APD₅₀ and the amplitude of the membrane potential depolarization were more significant in the hypertrophic myocardium after the activation of SACs. When the heart preparations were perfused with Tyrode's solution, there was no difference in the cardiac systolic function between the cardiac hypertrophy group and the control group. Gadolinium, a SACs blocker, reduced the length-dependent contractile force and suppressed the ascending limb of the Frank-Starling curves in the hypertrophic heart. **Conclusions:** The upregulation of TRPC1 contributes to the contractile function in the hypertrophic myocardium by increasing [Ca²⁺]_i through the SACs.

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Introduction

The transient receptor potential canonical channels (TRPCs) are a class of cation channels that regulate Ca^{2+} influx in the myocardium [1]. TRPC1 and TRPC6 are considered to be participants of the stretch-activated ion channels (SACs), which are permeable to Na^+ , K^+ and Ca^{2+} . The stretch-activated current (I_{SAC}) increases with the upregulation of TRPC1 [2, 3]. The TRPC3 channels colocalize with the $\text{Na}^+/\text{Ca}^{2+}$ exchanger 1 (NCX1), and the TRPC3-NCX1 signaling complex is a pivotal mechanism for the control of cardiac Ca^{2+} homeostasis during cardiomotility [4, 5]. When the heart muscle is stretched, the contractile force increases. The main explanation for this mechanism is that stretching augments the effect of changes in the overlap between the thin and thick filaments [6]. Gadolinium chloride (Gd^{3+}), a SACs blocker, reduced the ventricle contractile force more obviously in the longer muscle lengths than in the shorter muscle lengths [7]. The data indicated that the cations entering from the SACs contribute to the length-dependent contractile force, but the role of the SACs on cardiac systolic function remains controversial [8].

The expression of TRPC1 was increased in the hypertrophied myocardium [9, 10], and the amplitude of I_{SAC} in the ventricular myocytes was increased with hypertrophy [11], but the significance of TRPC1 for cardiac contractile function remains unclear. In this study, we investigated the role of TRPC1 on length-dependent contractile function in the isoproterenol-induced hypertrophic myocardium of rat, and our results demonstrated that upregulation of TRPC1 contributes to contractile function in the hypertrophic myocardium by increasing intracellular calcium ion concentration ($[\text{Ca}^{2+}]_i$) through the SACs.

Materials and Methods

Rat model of cardiac hypertrophy

The Animal Ethics Committee of Huazhong University of Science and Technology approved the study protocol. The investigation conforms to 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).

Male Sprague-Dawley rats (250 – 280 g) were randomly divided into the cardiac hypertrophy (CH) group and the control group. The CH group rats were administered isoproterenol 5 mg/kg for 5 days, once daily by intraperitoneal injection [12]. The control group rats received saline solution (0.9% NaCl). All the rats were kept under standard conditions with free access to food and water for 5 weeks since the first injection [13].

Morphological examination

The rats were weighed and anesthetized. The hearts were excised and weighed in an analytical balance. All the samples from identical part of every rat left ventricles were prepared for the paraffin sections, and then stained with hematoxylin and eosin for the light microscopic evaluation. The mean myocyte diameter was calculated from each section with software Image-Pro Plus 6.0 (Media Cybernetics, USA).

Western blot analysis

The expression of TRPC1/3/6 and NCX1 proteins were determined by Western blot. The proteins were extracted from the identical part of the rat left ventricles in the CH and control groups. The protein samples (70 μg) were separated by 8% SDS-PAGE. Polyclonal rabbit anti-TRPC1, anti-TRPC3 and anti-TRPC6 antibodies (Alomone Labs, Israel), anti-NCX1 and anti-GAPDH antibodies (Santa Cruz Biotechnology, USA) were used.

Calcium imaging

The ventricular myocytes were freshly isolated, using a collagenase–protease dispersion method [9]. The myocytes were used within 6 h of harvesting, and only typical Ca^{2+} -tolerant, rod-shaped cells were selected for analysis. The cells were incubated with Tyrode's solution containing 5 $\mu\text{mol/L}$ Fura-2/AM (Invitrogen, USA) for 30 min at room temperature. After being washed three times and incubated in

isotonic (1.0 T) extracellular solution for 30 min, the cells were illuminated using a calcium imaging system (TILL Photonics, Germany). The fluorescence signal was recorded as previously described [14].

After the 1.0 T extracellular solution was replaced by the hypotonic (0.6 T) extracellular solution, the cells swelled because of the hyposmotic solution and the cytomembrane was stretched [15, 16]. The changes in $[Ca^{2+}]_i$ were displayed as a ratio of the fluorescence relative to the intensity before the application of the hypotonic extracellular solution (F/F_0). The protocol was repeated three times with the new myocyte chambers as follows: (i). in the extracellular solution containing the L-type Ca^{2+} channel inhibitor, verapamil (Ver 0.1 $\mu\text{mol/L}$); (ii). in the extracellular solution containing the SACs blocker, gadolinium chloride (Gd^{3+} 10 $\mu\text{mol/L}$) [17]; (iii). in the extracellular solution containing the NCX inhibitor, nickel chloride (Ni^{2+} 5.0 mmol/L) [18]. The extracellular solutions contained (mmol/L): 65 NaCl, 5 KCl, 2.5 $CaCl_2$, 0.5 $MgSO_4$, 10 glucose, 10 HEPES, 130 (1.0T) or 17 (0.6T) mannitol (pH 7.4). An osmolarity of 296 mOsm/liter was taken as isotonicity (1.0 T), and 178 mOsm/liter was taken as hypotonicity (0.6 T) [15].

Action potential recording

The papillary muscles were excised from the right ventricles, both ends of the muscle were tied a knot, and the working muscle between two knots was 3.0 mm. One knot was fixed in the center of a perspex chamber, and the other knot was attached to a micrometer for length adjustments. The preparations were perfused with Tyrode's solution (8 ml/min, 37 °C) containing (mmol/L): 137 NaCl, 2.7 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 12 $NaHCO_3$, 0.4 NaH_2PO_4 , 5.6 glucose (pH 7.4), saturated with 95% O_2 and 5% CO_2 . The action potential (AP) was recorded through a glass microelectrode inserted into the myocyte, using a standard microelectrode recording technique [19]. The glass microelectrode contained a 3 mol/L KCl solution and had a tip resistance of 10-15 M Ω . The muscles were stimulated with a pair of platinum electrodes at the pacing rate of 0.2 Hz. The AP signals were placed into a microelectrode amplifier and recorded by a computer system (Chengyi, China). The muscles were first set to the original length without stretch (L_0). Then, the papillary muscles were stretched to an increase of 10% of the length [11]. The length of 110% L_0 was defined as 1.1 L_0 . The experiments were repeated to observe the effects of 10 $\mu\text{mol/L}$ Gd^{3+} and 0.1 $\mu\text{mol/L}$ Ver on the AP.

Contractile force of myocardium

The preparations of left ventricle papillary muscle were mounted in a muscle chamber and perfused in Tyrode's solution. The muscles were stimulated with a pair of platinum electrodes at the pacing rate of 0.2 Hz to obtain isometric force. The signals were recorded by a force transducer (Chengyi, China). The contractile force of myocardium was observed at the lengths of L_0 and 1.1 L_0 . The experiment was repeated to observe the effects of 10 $\mu\text{mol/L}$ Gd^{3+} and 0.1 $\mu\text{mol/L}$ Ver on the cardiac contractility of the preparations before and after being stretched.

Isolated working heart preparation

The Langendorff models of rat hearts were prepared as previously described [20] and perfused with Tyrode's solution. The perfusion fluid was guided through the left atrial cannula to the left ventricle (the basic ventricular preload was 10 cmH₂O filling pressure), which ejected the fluid out against an afterload of 80 cmH₂O. The cardiac output was recorded at different ventricular preloads. Frank-Starling curves of the relationship between the ventricular preload and the stroke volume were drawn. The changes of curves were observed when the SACs were blocked by 10 $\mu\text{mol/L}$ Gd^{3+} .

Statistical analysis

The experimental results were analyzed with analysis of variance. The results are expressed as the means $\pm$ standard error of the mean (SE). The values of $P < 0.05$ were considered to be statistically significant.

Results

Expression of TRPC1, TRPC3, TRPC6 and NCX1 in hypertrophic myocardium

The overall mortality after 5 weeks of isoproterenol administration was 30%. Cardiac hypertrophy was observed in the ventricles. The mean myocyte diameter of the CH group

Table 1. Diameter of cardiomyocyte, body and heart weights after 5 weeks of isoproterenol administration. Values are the means $\pm$ SE. Cardiac index = heart weight / body weight, LVW = left ventricle weight, BW = body weight, RVW = right ventricle weight, LAW = left atrium weight, RAW = right atrium weight; n = 10 per group, * $P < 0.05$ vs. control

	Control	Cardiac hypertrophy
Diameter of cardiomyocyte (μm)	13.06 $\pm$ 1.08	16.51 $\pm$ 1.28 *
Body weight (g)	406.50 $\pm$ 11.79	365.10 $\pm$ 13.75 *
Cardiac index (mg/g)	2.39 $\pm$ 0.14	3.08 $\pm$ 0.18 *
LVW/BW (mg/g)	1.76 $\pm$ 0.08	2.25 $\pm$ 0.15 *
RVW/BW (mg/g)	0.45 $\pm$ 0.07	0.59 $\pm$ 0.09 *
LAW/BW (mg/g)	0.08 $\pm$ 0.02	0.11 $\pm$ 0.03 *
RAW/BW (mg/g)	0.09 $\pm$ 0.02	0.12 $\pm$ 0.03 *

Fig. 1. Western blot analyses of TRPC1/3/6 and NCX1 protein expression in hearts. The statistical data are shown by the relative density ratio of TRPC1/3/6 and NCX1 to the GAPDH band. The data presented are representative of ten separate hearts from the control (Con) and cardiac hypertrophy (CH) groups. * $P < 0.05$ vs. Con.

was more than that of the control group. The biometric parameters in the surviving rats were presented in Table 1. The atria and ventricles were significantly hypertrophied in the CH group of rats. The Western blot analysis revealed that the expression of TRPC1 was upregulated in the CH group, while the levels of TRPC3, TRPC6 and NCX1 remained stationary. The data were consistent with previous reports [10, 21], and were shown in Fig. 1.

Increase of intracellular calcium induced by stretching cytomembrane

After the 1.0 T extracellular solution was replaced by the 0.6T extracellular solution, the cytomembrane was stretched by hyposmotic cell swelling, the SACs were activated and $[\text{Ca}^{2+}]_i$ was increased. The ascendant amplitude of $[\text{Ca}^{2+}]_i$ increase in the hypertrophic myocardium was obviously higher (CH 30.75 $\pm$ 4.16% vs. control 11.65 $\pm$ 1.43%, $P < 0.05$). The data of typical cells were shown in Fig. 2. The administration of Gd^{3+} and Ni^{2+} suppressed the increase of $[\text{Ca}^{2+}]_i$ in both groups, and Ver had no effect.

Fig. 2. Hyposmotic cell swelling induced Ca^{2+} influx. The ascendant amplitude of $[\text{Ca}^{2+}]_i$ in the cardiac hypertrophic (CH) myocardium was obviously higher than control (Con) group in the empty bathing solution (EBS). Gd^{3+} (10 $\mu\text{mol/L}$) and Ni^{2+} (5.0 mmol/L) suppressed the increase of $[\text{Ca}^{2+}]_i$ in both Con and CH groups separately, and verapamil (Ver 0.1 $\mu\text{mol/L}$) had no effect. The numbers above the bar graphs denote the number of cells analyzed. An osmolarity of 296 mOsm/liter was taken as isotonicity (1.0 T), and 178 mOsm/liter was taken as hypotonicity (0.6 T). * $P < 0.05$ vs. Con; # $P < 0.05$ vs. EBS.

Fig. 3. Action potential of myocardium. (A) The cardiac hypertrophy (CH) cells had longer action potential duration (APD) than control (Con) group in the empty bathing solution (EBS). The APD was prolonged, and the resting membrane potential (RMP) was depolarized when the preparation was stretched to 110% of the original length ($1.1L_0$). The administration of Gd^{3+} (10 $\mu\text{mol/L}$) decreased the APD and hyperpolarized the RMP. (B) The increases of the APD_{50} induced by stretching (ΔAPD_{50}). (C) The changes in RMP. n=10 per group, * $P < 0.05$ vs. Con.

Although Gd^{3+} has an inhibitory effect on the $\text{Na}^+-\text{Ca}^{2+}$ exchanger current, most of the NCX1 are not blocked by Gd^{3+} at the concentration of 10 $\mu\text{mol/L}$ [22]. The results confirmed that Na^+ was the main carrier of the stretch-activated currents [11, 23], the intracellular Ca^{2+} increased predominantly through the NCX1, and the activity of the SACs increased in the hypertrophic myocardium.

Action potential of myocardium

After the action potential duration was to 50% repolarization (APD_{50}), the membrane potential was under -30 mV, and the main Ca^{2+} entrance — L-type Ca^{2+} channels were

Fig. 4. The contribution of the SACs to the length-dependent contractile force of myocardium. (A) After the muscle length increased 10% from the original length (L_0), the developed contractile force (F_{dev}) between the control (Con) and the cardiac hypertrophy (CH) group was undifferentiated in the empty bathing solution (EBS), but the difference of F_{dev} between the Con and CH groups became obvious in the presence of Gd^{3+} (10 μ mol/L). (B) The summary of data showing the changes in F_{dev} . Gd^{3+} suppressed F_{dev} in the CH group, and its efficacy was further enhanced by 0.1 μ mol/L verapamil (Ver). $n = 10$, $*P < 0.05$ vs. Con; $\# P < 0.05$ vs. Ver absence.

inactivated [24]. Thus the APD_{50} of ventricular myocytes, which best reflects the contribution of the L-type Ca^{2+} current [19], were measured and reported in Fig. 3. The results revealed that the hypertrophic myocardium had longer action potential duration (APD) than normal (before stretched, APD_{50} CH 37.22 ± 1.23 ms vs. control 18.14 ± 0.78 ms; $n=10$, $P < 0.05$), mainly because of the significantly decreased transient outward K^+ current density in the hypertrophied cells [25]. The APD_{50} were prolonged by stretching in both groups because of the stretch-activated inward currents [23], and shortened by Gd^{3+} . The increases of APD_{50} induced by stretching (ΔAPD_{50}) in the CH group were more significant than those in the control group (Fig. 3B). The administration of Gd^{3+} decreased the APD and restrained the prolongation induced by stretching.

The resting membrane potential (RMP) was depolarized during stretching. The amplitude of depolarization in the CH group was more significant than that in the control group (Fig. 3C). Significant Na^+ entry through the TRPCs may cause membrane depolarization and activation of the L-type Ca^{2+} channels, contributing to the Ca^{2+} influx [26]. Thus the increased activity of the SACs contributed to the Ca^{2+} influx for the contractile force in the hypertrophic myocardium. The administration of Gd^{3+} hyperpolarized the RMP and restrained the depolarization induced by stretching in both groups. In our observation, neither the AP configuration nor the APD was obviously affected by 0.1 μ mol/L Ver.

Role of SACs in length-dependent contractile force

When the muscles were stretched from L_0 to $1.1L_0$, the developed contractile force (F_{dev}) of the control and the CH groups was undifferentiated in the Tyrode's solution. After the SACs were blocked by Gd^{3+} , the increases of F_{dev} were different (Fig. 4), and obviously decreased in the hypertrophic myocardium. The administration of 0.1 μ mol/L Ver decreased the basal contraction force but did not affect the increase of F_{dev} in both groups. But Ver enhanced the effect of Gd^{3+} in suppressing the increase of F_{dev} , especially in the hypertrophic myocardium.

Role of SACs in the Frank-Starling effect

The Frank-Starling curves of control and CH hearts were similar when perfused with Tyrode's solution, but the ascending limb of curves in the CH group was decreased in the presence of 10 μ mol/L Gd^{3+} . Compared with the control group, a more serious decline

Fig. 5. The Frank-Starling curves for the control (Con) and the cardiac hypertrophy (CH) group hearts. The ascending limb of curves in the CH heart was significantly suppressed by Gd^{3+} ($10 \mu\text{mol/L}$). $n = 10$, * $P < 0.05$ vs. Con; # $P < 0.05$ vs. Con Gd^{3+} .

induced by Gd^{3+} was observed in the CH group (Fig. 5). The results demonstrated that the SACs enhanced the Frank-Starling effect in the hypertrophic heart, and that the contribution of the SACs to the systolic function was more significant in the hypertrophic myocardium.

Discussion

Normal cardiac systolic function obeys the Frank-Starling mechanism: when the heart muscle is stretched, it increases the force of contraction so that cardiac output matches the venous return on a nearly beat-to-beat basis [8]. The ascending limb of the Frank-Starling curves declines with the occurrence of systolic dysfunction. Many studies indicated that cations entering from the SACs contributed to the length-dependent contractile force [7-9]. The experiments confirmed that the main carrier of I_{SAC} in rat cardiac myocyte was Na^+ , and in the range of strain available, the change in I_{SAC} was approximately linear with both sarcomere length and strain [11, 23]. The amplitude of I_{SAC} in the ventricular myocyte increased with hypertrophy [11], and the increased $[Na^+]$ caused Ca^{2+} influx through NCX1, especially in the myocardium with prolonged APD in heart failure [27, 28].

The intracellular Ca^{2+} increased when the myocardium was stretched, which indicated that the SACs, types of mechanosensitive, non-selective cation channels contributed to the cardiac systolic function in addition to the effect of changes in overlap between the thin and thick filaments [6, 7, 9]. The TRPCs, NCX1 and L-type Ca^{2+} channel are involved in the intracellular Ca^{2+} transient as follows: the TRPCs are functionally coupled to NCX1, and the substantial Na^+ entry through the TRPC-encoded non-selective cation channels, which causes Ca^{2+} influx to extrude Na^+ from the cell. The activation of the TRPC channels may induce membrane depolarization and cause the opening of the L-type Ca^{2+} channel [26, 29]. TRPC1 is hypothesized to be the primary component of the SACs by the following mechanism: I_{SAC} was increased when TRPC1 was upregulated [2], reducing the expression of TRPC1 by the antisense morpholinos inhibited Ca^{2+} influx [30]; the homozygous TRPC1 knockout mice cardiomyocytes failed to respond to the different forms of stretching (swelling and positive pressure), as indicated by the lack of SACs [31]. Isoproterenol was used to induce the necrosis of rat myocardium and cardiac dysfunction, and the surviving cell hypertrophied because of the increased workload [32]. The expression of TRPC1 increased in the hypertrophic myocardium, and we considered that the activity of SACs increased due to TRPC1 upregulation, because TRPC3/6 and NCX1 were unchanged.

The APD prolongation and membrane depolarization caused by the inward currents from the SACs promoted substantial Ca^{2+} entry into the cell through the NCX1 and L-type Ca^{2+} channel [26, 28]. The amplitude of the APD prolongation and membrane depolarization were more significant in the hypertrophic myocardium, which facilitated more Ca^{2+} influx compared with the normal myocardium. The contractile functions of the CH and control groups were similar before the SACs were blocked. In the presence of Gd^{3+} , the length-dependent contractile force was significantly depressed in the CH group, and the ascending limb of Frank-Starling curves in the CH group declined dramatically. The above results

demonstrated that the increased $[Ca^{2+}]_i$ through the SACs had a significant contribution to maintaining the cardiac contractile function in the hypertrophic myocardium, and decompensation occurred when the SACs were blocked.

Although isoproterenol-induced cardiac hypertrophy is similar to hypertrophic cardiomyopathy, the findings of our study are limited to the model we used because the changes of the TRPCs vary in cardiovascular diseases. Different membrane channel protein families (including the TRPCs, TRPMs and Piezos, etc) have been proposed as participants in forming the mechanosensitive Ca^{2+} permeant cation channel [33-35]. Multiple independent studies have shown that TRPC1 is the primary component of the SACs, but the accurate structure of the SACs is still unclear; the role of TRPC1 in forming the SACs may not be exactly identical in different cells [33]. Changes in a specific protein expression (TRPC1) and a channel activity (SACs) correlating with a specific pathological process does not mean that the two are the same.

We conclude that the hypertrophic myocardium expresses more TRPC1 to enhance the capacity of the SACs and facilitate Ca^{2+} influx to maintain the contractile force when the preload increases, which is a type of compensatory mechanism to avoid heart failure occurring. These findings may provide a new perspective on the physiological function of the TRPCs in the myocardial cell. It suggests that promoting the activation of the TRPCs could be a potential therapeutic target for cardiac systolic dysfunction.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

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References

- 1 Minke B: The history of the drosophila TRP channel: the birth of a new channel superfamily. *J Neurogenet* 2010;24:216-233.
- 2 Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP: TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol* 2005;7:179-185.
- 3 Spassova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL: A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A* 2006;103:16586-16591.
- 4 Goel M, Zuo CD, Sinkins WG, Schilling WP: TRPC3 channels colocalize with Na^+/Ca^{2+} exchanger and Na^+ pump in axial component of transverse-axial tubular system of rat ventricle. *Am J Physiol Heart Circ Physiol* 2007;292:H874-H883.
- 5 Eder P, Probst D, Rosker C, Poteser M, Wolinski H, Kohlwein SD, Romanin C, Groschner K: Phospholipase C-dependent control of cardiac calcium homeostasis involves a TRPC3-NCX1 signaling complex. *Cardiovasc Res* 2007;73:111-119.
- 6 Allen DG, Jewell BR, Murray JW: The contribution of activation processes to the length-tension relation of cardiac muscle. *Nature* 1974;248:606-607.
- 7 Lab MJ, Zhou BY, Spencer CI, Horner SM, Seed WA: Effects of gadolinium on length-dependent force in guinea-pig papillary muscle. *Exp Physiol* 1994;79:249-255.
- 8 Cannell MB: Pulling on the heart strings: a new mechanism within Starling's law of the heart? *Circ Res* 2009;104:715-716.
- 9 Ward ML, Williams IA, Chu Y, Cooper PJ, Ju YK, Allen DG: Stretch-activated channels in the heart: contributions to length-dependence and to cardiomyopathy. *Prog Biophys Mol Biol* 2008;97:232-249.
- 10 Ohba T, Watanabe H, Murakami M, Takahashi Y, Iino K, Kuromitsu S, Mori Y, Ono K, Iijima T, Ito H: Upregulation of TRPC1 in the development of cardiac hypertrophy. *J Mol Cell Cardiol* 2007;42:498-507.
- 11 Kamkin A, Kiseleva I, Isenberg G: Stretch-activated currents in ventricular myocytes: amplitude and arrhythmogenic effects increase with hypertrophy. *Cardiovasc Res* 2000;48:409-420.

- 12 Krenek P, Kmecova J, Kucerovala D, Bajuszova Z, Musil P, Gazova A, Ochodnický P, Klimas J, Kyselovic J: Isoproterenol-induced heart failure in the rat is associated with nitric oxide-dependent functional alterations of cardiac function. *Eur J Heart Fail* 2009;11:140-146.
- 13 Takeshita D, Shimizu J, Kitagawa Y, Yamashita D, Tohne K, Nakajima-Takenaka C, Ito H, Takaki M: Isoproterenol-induced hypertrophied rat hearts: does short-term treatment correspond to long-term treatment? *J Physiol Sci* 2008;58:179-188.
- 14 Hu ZL, Huang C, Fu H, Jin Y, Wu WN, Xiong QJ, Xie N, Long LH, Chen JG, Wang F: Disruption of PICK1 attenuates the function of ASICs and PKC regulation of ASICs. *Am J Physiol Cell Physiol* 2010;299:C1355-C1362.
- 15 Clemo HF, Baumgarten CM: Swelling-activated Gd^{3+} -sensitive cation current and cell volume regulation in rabbit ventricular myocytes. *J Gen Physiol* 1997;110:297-312.
- 16 Suchyna TM, Johnson JH, Hamer K, Leykam JF, Gage DA, Clemo HF, Baumgarten CM, Sachs F: Identification of a peptide toxin from *Grammostola spatulata* spider venom that blocks cation-selective stretch-activated channels. *J Gen Physiol* 2000;115:583-598.
- 17 Lamberts RR, Van Rijen MH, Sipkema P, Fransen P, Sys SU, Westerhof N: Coronary perfusion and muscle lengthening increase cardiac contraction: different stretch-triggered mechanisms. *Am J Physiol Heart Circ Physiol* 2002;283:H1515-H1522.
- 18 Wasserstrom JA, Vites AM: The role of Na^+ - Ca^{2+} exchange in activation of excitation-contraction coupling in rat ventricular myocytes. *J Physiol* 1996;493:529-542.
- 19 Fauconnier J, Bedut S, Le Guennec JY, Babuty D, Richard S: Ca^{2+} current-mediated regulation of action potential by pacing rate in rat ventricular myocytes. *Cardiovasc Res* 2003;57:670-680.
- 20 Muller-Strahl G, Hemker J, Zimmer HG: Comparison between left and right heart function in the isolated biventricular working rat heart. *Exp Clin Cardiol* 2002;7:7-19.
- 21 Nakajima-Takenaka C, Zhang GX, Obata K, Tohne K, Matsuyoshi H, Nagai Y, Nishiyama A, Takaki M: Left ventricular function of isoproterenol-induced hypertrophied rat hearts perfused with blood: mechanical work and energetics. *Am J Physiol Heart Circ Physiol* 2009;297:H1736-H1743.
- 22 Zhang YH, Hancox JC: Gadolinium inhibits Na^+ - Ca^{2+} exchanger current in guinea-pig isolated ventricular myocytes. *Br J Pharmacol* 2000;130:485-488.
- 23 Zeng T, Bett GC, Sachs F: Stretch-activated whole cell currents in adult rat cardiac myocytes. *Am J Physiol Heart Circ Physiol* 2000;278:H548-H557.
- 24 Yuan W, Ginsburg KS, Bers DM: Comparison of sarcolemmal calcium channel current in rabbit and rat ventricular myocytes. *J Physiol* 1996;493:733-746.
- 25 Yang X, Salas PJ, Pham TV, Wasserlauf BJ, Smets MJ, Myerburg RJ, Gelband H, Hoffman BF, Bassett AL: Cytoskeletal actin microfilaments and the transient outward potassium current in hypertrophied rat ventricular myocytes. *J Physiol* 2002;541:411-421.
- 26 Wang YX, Zheng YM: Molecular expression and functional role of canonical transient receptor potential channels in airway smooth muscle cells. *Adv Exp Med Biol* 2011;704:731-747.
- 27 Kang TM, Hilgemann DW: Multiple transport modes of the cardiac Na^+ / Ca^{2+} exchanger. *Nature* 2004;427:544-548.
- 28 Weber CR, Piacentino VR, Houser SR, Bers DM: Dynamic regulation of sodium/calcium exchange function in human heart failure. *Circulation* 2003;108:2224-2229.
- 29 Chen JB, Tao R, Sun HY, Tse HF, Lau CP, Li GR: Multiple Ca^{2+} signaling pathways regulate intracellular Ca^{2+} activity in human cardiac fibroblasts. *J Cell Physiol* 2010;223:68-75.
- 30 Kerstein PC, Jacques-Fricke BT, Rengifo J, Mogen BJ, Williams JC, Gottlieb PA, Sachs F, Gomez TM: Mechanosensitive TRPC1 channels promote calpain proteolysis of talin to regulate spinal axon outgrowth. *J Neurosci* 2013;33:273-285.
- 31 Seth M, Zhang ZS, Mao L, Graham V, Burch J, Stiber J, Tsiokas L, Winn M, Abramowitz J, Rockman HA, Birnbaumer L, Rosenberg P: TRPC1 channels are critical for hypertrophic signaling in the heart. *Circ Res* 2009;105:1023-1030.
- 32 Marques FD, Ferreira AJ, Sinisterra RD, Jacoby BA, Sousa FB, Calviari MV, Silva GA, Melo MB, Nadu AP, Souza LE, Irigoyen MC, Almeida AP, Santos RA: An oral formulation of angiotensin-(1-7) produces cardioprotective effects in infarcted and isoproterenol-treated rats. *Hypertension* 2011;57:477-483.
- 33 Maroto R, Kurosky A, Hamill OP: Mechanosensitive Ca^{2+} permeant cation channels in human prostate tumor cells. *Channels (Austin)* 2012;6:290-307.
- 34 Wei C, Wang X, Chen M, Ouyang K, Song LS, Cheng H: Calcium flickers steer cell migration. *Nature* 2009;457:901-905.
- 35 Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A: Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* 2010;330:55-60.