Original Article
Changes of stretch-activated channel-transient receptor potential channels in atrial myocardium of rabbits with heart failure

Feng Cao[1,2], Teng Wang[1,2], Wenmao Ding[1,2], Zhe Li[1,2], Shaobo Shi[1,2], Xiaozhan Wang[1,2]

[1]Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, PR China; [2]Cardiovascular Research Institute, Wuhan University, Wuhan, PR China

Abstract: Transient receptor potential channels (TRPC) are shown recently to play an important role in the development of heart failure and arrhythmia. The purpose of the present study was to observe the changes of gene expression and protein level of transient receptor potential channel in isolated atrial myocardium after heart failure in rabbits. Twenty rabbits were randomly divided into two groups: control group (n=10, sham-operation rabbits: 5) and heart failure (n=10) group. Chronic heart failure model was produced by combined aortic regurgitation and coarctation of the abdominal aorta. Cardiac structure and function were detected via echocardiography 10 weeks later. The mRNA expression and protein level of TRPC1 of left atrial appendage were detected by RT-PCR and Western blotting, respectively. The left atria diameter, inter-ventricular septum, left ventricular post-wall dimension, left ventricular end diastolic and systolic diameter were also increased markedly in heart failure rabbit s, but their ejection fraction and left ventricular shorten fraction were decreased (P<0.05). Compared with the control group, the expression of TRPC1 mRNA and protein in heart failure group were markedly increased (P<0.05). The mRNA and protein expression of TRPC1 in atrial myocardia of rabbits with heart failure was significantly increased. TRPC1 may play a role in the vulnerability to atrial arrhythmias in dilated atria with heart failure.

Keywords: Heart failure, transient receptor potential channel, atrial arrhythmia

Introduction

Transient receptor potential (TRP) channels are a category of non-selective cationic channels, which has recently been studied extensively. TRP canonical (TRPC) channels are a group of principal members of TRP channels [1]. Studies have shown that TRPC channels are involved in the development of cardiomyotrophy, heart failure and cardiac arrhythmia [2, 3]. Furthermore, the channels also affect the cellular influx and efflux of several positively charged ions, including Ca^{2+}, K^+, Mg^{2+} and Na^+. However, so far it remains unclear whether the atrium level of type 1 TRPC (TRPC1) changes after heart failure. This study aimed to observe the atrium level of TRPC1 during heart failure and to probe the potential effects of TRPC1 in the development of atrial arrhythmia associated with heart failure.

Materials and methods

Experimental animals

Japanese white rabbits were procured from Center of Experimental Animals, Wuhan Institute of Biological Products. Twenty adult Japanese white rabbits, either male or female, with body weight from 2.0 to 2.2 kg, were divided into two groups at random, 10 rabbits in the heart failure group and 10 in the control group (among them, 5 rabbits received a mock operation), respectively.

Animal model

The animal model of heart failure was established by artificial aortic valve regurgitation in combination with abdominal aortic stenosis using the methods described in the literature [4]. After weighing the rabbits and administra-

tion of 3% pentobarbital anesthesia (30 mg/kg), the rabbits were stabilized in a supine position on the operating table. After sterilization procedures, a middle neck incision was made, followed by blunt separation of the right carotid artery. The distal end of the carotid artery was tied with a ligature and the proximal part was temporarily clamped. After cutting open the anterior wall, a 4F arterial catheter was inserted into the carotid artery and then sent to the aortic root to puncture through the aortic valve, thus resulting in traumatic aortic regurgitation. The successful operation was indicated by a 50% increase in pulse pressure. Two weeks after the operation, the abdominal aorta was isolated and then catheterized. The abdominal aorta, along with the inside catheter, was then ligated before the catheter was withdrawn. The ligation resulted in 50% reduction in the diameter of the abdominal aorta. In the mock operation received by rabbits in the control group, all the procedures were the same, except that no artificial aortic regurgitation and abdominal aortic stenosis were made. 800,000 units of Penicillin G were administered intramuscularly per day for three days postoperative to prevent infection. The animals were then further observed for 10 weeks.

Echocardiography study for cardiac structure and function

Ten weeks after the operation, echocardiography study was performed using GE Vivid7 Ultrasound diagnostic system, by measuring left atrial diameter (LAD), left ventricular end diastolic diameter (LVEDd), left ventricular inner diameter in systole (LVIDs), left ventricular posterior wall (LVPW) and inter-ventricular septum (IVS), ejection fraction (EF) and left ventricular short axis fractional shortening (FS).

RT-PCR examination of TRPC1 mRNA expression

Total RNA from heart tissue was extracted using TRIzol® Reagent (*Invitrogen*, Carlsbad, CA, USA). 50 mg of heart tissue was homogenized before 1.0 ml of TRIizol Reagent was added. The sample was incubated at room temperature for 15 minutes and then a 0.2 fold in volume of chloroform was added. The sample was centrifuged for 15 minutes at 12,000×g at 4°C after vortexing. 0.5 fold in volume of isopropyl alcohol was added and then the sample

was mixed and incubated at -20°C for 15 minutes. The sample tube was centrifuged at 4°C at 12,000×g for 10 minutes. The supernatant was taken and the RNA was precipitated with 1.0 ml of 75% ethanol by vortexing and subsequent centrifugation at 4°C at 7,500×g for 5 minutes. After discarding the supernatant, the RNA pellet was air dried. RNase-free sterile H_2O was used to completely dissolve the RNA. RT-PCR was employed to examine the expression of mRNA. TURBO DNA-*free*™ DNase (*Ambion,* Foster City, CA, USA) was utilized to eliminate genomic DNA contamination in RNA sample. An oligo (dT)$_{20}$ and SuperScript III Reverse Transcriptase (*Invitrogen*, Carlsbad, CA, USA) were used for cDNA synthesis. Subsequently, a 225 bp segment of TRPC1 cDNA was PCR amplified with a sense primer 5'-AGAAT-CCCGTAATCAACTC-3 and an anti-sense primer 5'-GTAATGGACCCAAGATAGAG-3'. The PCR was run for 35 cycles after an initial denaturing for 5 minutes at 95°C. Each PCR cycle was composed of 30 seconds of denaturing at 94°C, 30 seconds of annealing at 52°C and 30 seconds of primer extension at 72°C. The PCR products were further extended at 72°C for 5 minutes in the final stage and then stored at 4°C. The DNA samples were examined through 1.5% agarose gel electrophoresis. An automatic gel imaging and analysis system was employed to scan the gel after ethidium bromide staining; the intensities of DNA bands were then scored before performing semi-quantitative analysis.

Western blot detection of TRPC1 protein expression

Ten weeks after the operation, the left auricle of the heart was excised and flushed with saline before mincing into minute pieces. 1 ml of cold tissue lysis buffer was added before the tissue was homogenized. After centrifugation at 14,000 rpm at 4°C, the supernatant was mixed with 200 μl of 0.01 M Tris-Cl buffer (pH 7.6). The sample was centrifuged at 14,000 rpm at 4°C for 10 minutes. Again, the supernatant was taken. Bradford method was employed to quantify protein content before the sample was stored in a freezer at -70°C. 20 μg of total protein samples were mixed with 5x SDS loading buffer, boiled for 3 minutes and then loaded to a 10% SDS-polyacrylamide gel for electrophoresis. The samples were then transferred to a nitrocellulose membrane under constant elec-

Table 1. Results of echocardiography of rabbits in heart failure and control groups ($\bar{x} \pm s$)

	Control group	Heart failure
LAD (mm)	8.5±1.0	12.2±2.7*
LVPW (mm)	2.1±0.2	2.7±0.3*
IVS (mm)	2.2±0.2	2.9±0.3*
LVIDs (mm)	8.6±2.1	15.1±1.9*
LVIDd (mm)	12.9±3.2	18.2±2.1*
EF (%)	71.1±7.2	39.8±6.9*
FS (%)	38.9±5.8	21.3±6.7*

*Compared with control group, $P<0.05$.

trical current of 350 mA for one hour. The membrane was blocked with 5% non-fat milk in TBS buffer with 0.1% Tween-20 (TBST) for one hour and then incubated overnight with TPRC1 rabbit polyclonal antibody diluted at 1:500 in 5% non-fat milk in TBST at 4°C. After washing 3 times with TBST, the membrane was then incubated with a goat anti-rabbit IgG antibody conjugated with HRP diluted at 1:2500 in 5% non-fat milk in TBST for one hour. After washing with TBST, the membrane was finally incubated with enhanced chemiluminescent (ECL) reagent for one minute and exposed to Kodak film. Subsequently, the film was developed and the protein band intensity was quantified after scanning for protein expression levels in both the experimental and control groups.

Data analysis

SPSS17.0 statistical software was used for data analysis. Data were expressed by mean ± standard deviation (X ± S). Student t test was used to examine the difference in mean ± standard deviation between the two groups. Statistical significance of difference is indicated by $P<0.05$.

Results

Structural and functional changes in the hearts

The results of the echocardiography study were shown in **Table 1**. The heart failure group had a statistically significant increase in LAD, LVPW, IVS, LVIDs and LVIDd ($P<0.05$, for all indices), compared to the control group. However, the experimental group had a statistically significant decrease in both EF and FS ($P<0.05$, respectively), indicating the success in set-up of the heart failure models in this group.

Changes in TRPC1 mRNA expression after heart failure

The left auricular tissue mRNA expression level was 0.0357±0.0013 and 0.0983±0.0036 in the control group and in the heart failure group, respectively, as shown in **Figure 1**. The difference was of statistical significance ($P<0.05$).

Changes in TRPC1 protein content after heart failure

As shown in **Figure 2**, the TRPC1 protein content in left auricular tissue in the experimental group (0.4602±0.091) was markedly higher than that of the control group (0.1243±0.0082), and the difference was of statistical significance ($P<0.05$).

Discussion

In this study, we aimed to observe the changes in TRPC1 gene expression in atrial myocardial tissue in rabbits after heart failure using RT-PCR and Western blotting. As a result, we found enhanced expression of the TRPC1 gene at both mRNA and protein levels during heart failure.

TRP channels are a group of cationic ion channels located on the plasma membrane. TRPC1 was the first TRP channel identified in mammalian tissue, which is now known to be widely distributed among various tissues including brain, heart, taste buds, testes, ovary, and aortic endothelial cells [5]. While salivary glands were found to have high levels of expression, there is almost no expression in the liver and kidneys. The most recent literature has demonstrated that TRPC1 involves the activation of stretch-activated ion channels, which possess permeability to Na$^+$, K$^+$, and Ca^{2+} [6]. Those channels are particularly permeable to double-charged cationic ions, which can cause Ca^{2+} influx. When activated, they may enhance an early phase of repolarization during action potential or delay a late phase of repolarization. Therefore, TRPC1 is likely involved in the development of arrhythmia [7].

Studies have demonstrated that TRPC is expressed in human atrial and ventricular tissues [8, 9]. GsMTx-4 is a polypeptide isolated from spider venom. GsMTx-4 has been shown to block cationic ion channels and related currents. However, it did not affect potential-
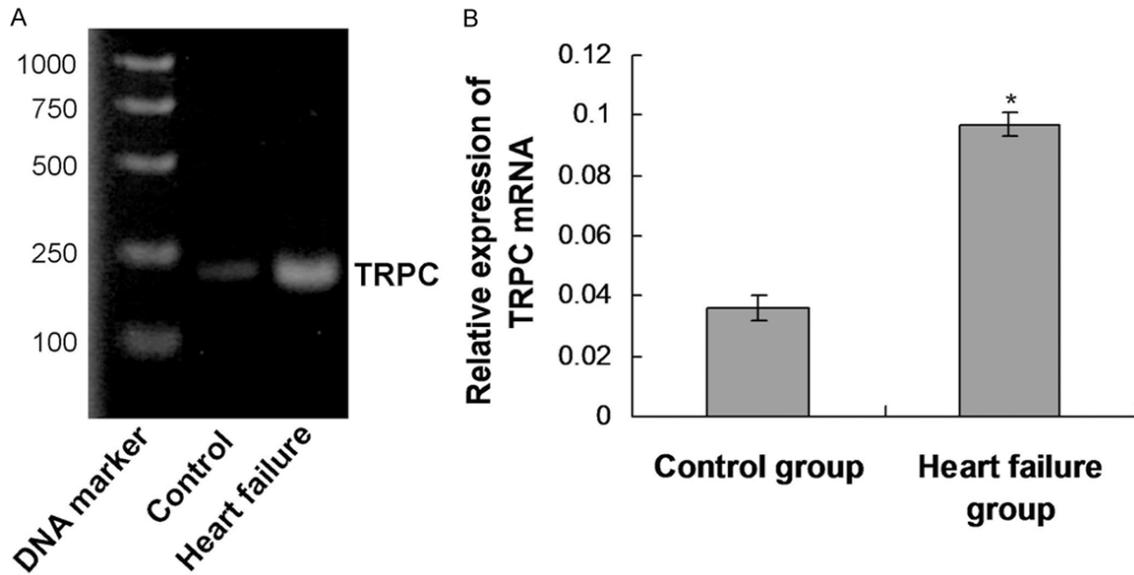
Figure 1. Expression of TRPC1 mRNA in atrial myocardia of rabbits obtained by RT-PCR. A. RT-PCR analysis for TRPC1 mRNA expression. B. Statistical analysis for the TRPC1 mRNA expression. $^*P<0.05$ represents the TRPC1 mRNA expression in heart failure group compared to control group.
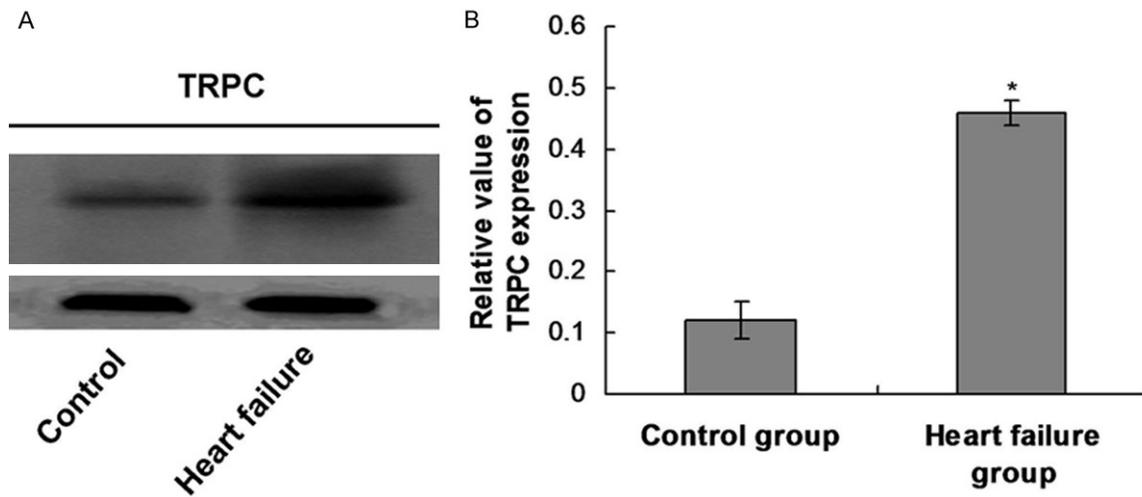
Figure 2. Expression of TRPC1 protein in atrial myocardia of rabbits obtained by western blot assay. A. Western blot assay for TRPC protein expression. B. Statistical analysis for the TRPC protein expression. $^*P<0.05$ represents the TRPC protein expression in heart failure group compared to control group.

dependent Ca^{2+} and K^+ channels and was not cross-reactive to potential-dependent channels. Thus, GsMTx-4 is a specific blocker for stretch-activated ion channels. Bode described that GsMTx-4 significantly reduced the occurrence of atrial fibrillation induced by stretching of the atrium [10]. Suchyna, et al, discovered that GsMTx-4 could decrease the depolarization potential after rapid heart pacing [11].

GsMTx-4 was also proven to reduce Ca^{2+} influx by blocking non-selective cationic ion channels. These studies indicated the important roles of stretch-activated ion channels in the development of atrial fibrillation in a dilated atrium.

It is widely accepted that mechanical burden has an important effect on the induction of arrhythmia. The electrical feedback to mechan-

ical force is mainly controlled by stretch-activated ion channels, but their activation may cause the development of stretch-induced arrhythmia. Franz, et al, discovered that TRPC1 has the characteristic functions of stretch-activated ion channels [12]. In their study in isolated rabbit hearts, rapid increase in intra-atrium pressure induced atrial fibrillation [13]. Furthermore, stretch-activated ion channel blocker Gd^{3+} and GsMTx-4 could inhibit the induction of atrial fibrillation. Research has found that stretching could modulate several ionic currents. In fact, prolongation of both plasma membrane depolarization and action potential is controlled by the currents of stretch-activated ion channels. Moreover, these currents have the tendency to cause arrhythmia [14]. In hypertrophic ventricular myocardial cells, stretch-activated ion channels are more reactive than normal ventricular myocardial cells [15]. It is postulated that TRPC1 may be involved in stretch-induced arrhythmia and that heart failure is likely to enhance the development of stretch-induced cardiac rhythmic disorders [16, 17].

Heart failure is an essential risk factor for the development of atrial fibrillation [18, 19]. During the disease progression of heart failure, the incidence rate of atrial fibrillation is as high as 10% to 35%. Ionic redistribution is one of the important pathophysiologic characteristics of heart failure, although the role TRPC and its clinical significance remain unclear [20]. In this study, we found that TRPC1 expression in atrial myocardial tissue at both mRNA and protein levels was markedly enhanced after heart failure. It is likely that TRPC1 participates in ion channel reconstruction and thus plays a role in the development of atrial arrhythmia. More detailed mechanisms of action remain to be further investigated. Finally, the relationship between atrial arrhythmia post heart failure and TRPC1 is still unclear and should be studied further.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Feng Cao, Department of Cardiology, Renmin Hospital of Wuhan University, Hubei Key Laboratory of Cardiology, 238 Jie Fang Road, Wuhan 430060, PR China. E-mail: cfengwh@sina.com

References

[1] dos Santos M, Bringhenti RN, Rodrigues PG, do Nascimento JF, Pereira SV, Zancan R, Monticielo OA, Gasparin AA, de Castro WP, Veronese FV. Podocyte-associated mRNA profiles in kidney tissue and in urine of patients with active lupus nephritis. Int J Clin Exp Pathol 2015; 8: 4600-4613.

[2] Venneekens R. Emerging concepts for the role of TRP channels in the cardiovascular system. J Physiol 2011; 589: 1527-1534.

[3] Inoue R, Jensen LJ, Shi J, Morita H, Nishida M, Honda A, Ito Y. Transient receptor potential channels in cardiovascular function and disease. Circ Res 2006; 99: 119-131.

[4] Ai X, Pogwizd SM. Connexin 43 downregulation and dephosphorylation in non-ischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A. Heart Circ Res 2005; 96: 54-63.

[5] Vassort G, Alvarez J. Transient receptor potential: a large family of new channels of which several are involved in cardiac arrhythmia. Can J Physiol Pharmacol 2009; 87: 100-107.

[6] Watanabe H, Murakami M, Ohba T, Ono K, Ito H. The pathological role of transient receptor potential channels in heart disease. Circ J 2009; 73: 419-427.

[7] Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. FASEB J 2009; 23: 297-328.

[8] 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.

[9] Kamkin A, Kiseleva I, Wagner KD, Bohm J, Theres H, Gunther J, Scholz H. Characterization of stretch-activated ion currents in isolated atrial myocytes from human hearts. Pflugers Arch 2003; 446: 339-346.

[10] Seol CA, Kim WT, Ha JM, Choe H, Jang YJ, Youm JB, Earm YE, Leem CH. Stretch-activated currents in cardiomyocytes isolated from rabbit pulmonary veins. Prog Biophys Mol Biol 2008; 97: 217-231.

[11] Bode F, Sachs F, Franz MR. Tarantula peptide inhibits atrial fibrillation during stretch. Nature 2001; 409: 35-36.

[12] 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 cat-

ion-selective stretch-activated channels. J Gen Physiol 2000; 115: 583-598.

[13] Franz MR, Bode F. Mechano-electrical feedback underlying arrhythmias: the atrial fibrillation case. Prog Biophys Mol Biol 2005; 82: 163-174.

[14] Ward ML, Williams IA, Chu Y, Copper 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.

[15] Kamkin A, Kiseleva I, Isenberg G. Stretch-activated currents in ventricular myocytes: amplitude and arrhythmogenic effects increase with hypertroph. Cardiovasc Res 2000; 48: 409-420.

[16] Sachs F. Stretch-Activated Ion Channels: What Are They? Physiology 2010; 25: 50-56.

[17] Watanabe H, Murakami M, Ohba T, Takahashi Y, Ito H. TRP channel and cardiovascular disease. Pharmacol Ther 2008; 118: 337-351.

[18] Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM. Heart disease and stroke statistics-2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008; 117: e25-e146.

[19] Li D, Melnyk P, Feng J, Wang Z, Petrecca K, Shrier A, Nattel S. Effects of experimental heart failure on atrial cellular and ionic electrophysiology. Circulation 2000; 101: 2631-2638.

[20] Sridhar A, Nishijima Y, Terentyev D, KHAN M, Terentyeva R, Hamlin RL, Nakayama T, Gyorke S, Cardounel AJ, Cames CA. Chronic heart failure and the substrate for atrial fibrillation. Cardiovasc Res 2009; 84: 227-236.