Environmental Medicine, Vol. 46 No.1, 2, 2002 (page 89 ~ 91)

Altered I_f Channel Gene Expression in Mouse Hearts after Myocardial Infarction

Haruki TAKEMURA,[1,2] Noriko NIWA,[2] Mayumi HOJO,[2] Jong-Kook LEE[2]
Kenji YASUI,[2] Yuichi UEDA[1] and Itsuo KODAMA[2]
[1] Department of Thoracic Cardiovascular Surgery
Graduate School of Medicine, Nagoya University, Nagoya 466-8550, Japan
[2] Department of Circulation
Division of Regulation of Organ Function
Reserch Institute of Enviromental Medicine, Nagoya 464-8601, Japan

Abstract: I_f channels play a key role in the pacemaker activity of sinus node in adult heart. Four subtypes of (HCN1-4), which encode I_f channel, have been identified. In the present study, we quantitatively estimated the expression of HCN1-4 mRNA in mouse ventricular myocardium 4 weeks after myocardial infarction (MI) by a real time PCR. In sham-operated control mice, HCN2 mRNA was expressed most abundantly (915.2±225.0molecules / 10^5 GAPDH molecules) in the ventricle . The expressions of mRNAs for HCN1, HCN3 and HCN4 were less abundant (15.2±5.0, 9.7±1.1, 48.2±25.3 molecules / 10^5 GAPDH molecules respectively). In mice after MI, HCN2 mRNA increased significantly by 2.7 fold compared to controls. There was no significant difference of HCN1, HCN3 and HCN4 mRNA between two groups. There is an upregulation of HCN2 mRNA in compensated hypertrophied muscle in MI. This might be responsible for an enhancement of abnormal automaticity in the hearts after MI.

Key words: I_f, HCN gene, mouse, myocardial infarction, hypertrophy

In the pathologic conditions such as MI, hypertrophy and heart failure, various remodelings of cardiac ion channels have been reported.[1] This remodeling might contribute to an arrhythmogenesis.[2]

I_f channel exists in the sinus node and His-purkinje system in adult heart, which play a role in the formation of pacemaker potential.[3] Four subtypes of genes (HCN1-4) coding I_f channels have been recognized.[4] Major genes expressed in the normal human heart are HCN2 and 4 genes.[5] An enhancement of I_f currents has been demonstrated in ventricular hypertrophy[4,6] and heart failure.[4,7] However, underlying molecular mechanisms for the I_f current upregulation remains unclear.

In present study, we analyzed mRNA expression of HCN1-4 in compensated hypertrophied interventricular septum (IVS) after MI.

Materials and Methods

1) Animals

Female ICR mice, weighting 28–33g (9–11 weeks old) were used for the surgery.

2) Procedure for construction of MI

Mice were randomly divided to two groups and underwent either left anterior descending coronary artery ligation or sham operation. Mice were anesthetized with pentobarbital (50mg/Kg,i.p.). The thoracotomy was performed under artificial respiration. After the pericardial sac was opened and the heart was exteriorized through the intercostal space, the left anterior descending artery was ligated using 8–0 prolene with an atraumatic needle (Ethicon). Infarction was identified from discoloration of the ventricle. Then, the thorax was closed and the skin sutured with 5–0 prolene. Sham operated animals were subject to similar surgery, except that no ligation was placed on coronary arteries.

3) Total RNA extraction and reverse transcription

At 4 weeks after the operation, the IVS in MI mice showed hypertrophy of surviving ventricular myocardium, compared with sham-operated mice as control. Total RNA was extracted from the IVS using the AGPC method. After treatment with DNase I, single-stranded cDNA was synthesized by an oligo d(T) primer and SuperScript II RNase H-reverse transcriptase (Gibco BRL).

4) Quantification of mRNA expression for I_f channel

The real-time PCR assay was performed to estimate the expression of HCN1-4 genes (Perkin Elmer, ABI Prism 7700). The primers and probes were designed as shown in Table 1. PCR product was subcloned using TA cloning and sequenced. cDNA molecules of PCR product (10^3–10^7 molecules) were amplified for the determination of standard curve. GAPDH gene was used as an endogenous control.

5) Data analysis

Data are presented as mean ± SEM. Statical analysis of data were performed using nonpaired t test. Differences were considered significant at $p<0.05$.

Reprint requests to: Dr. Itsuo KODAMA at the above address.

Table 1 Sequence of PCR primers and sequence-specific probes for HCN1-4 and GAPDH

Target Sequence	Accession No.	Primer	Sequence (5′-3′)	Position	Amprico Length
HCN1	AJ225123	HCN1 sense	CAAGACAGCAGAGCACTTCGT	935-956	106
		HCN1 probe	TCTCAGTCTCTTGCGGTTATTACGCCTTTC	977-1066	
		HCN1 antisense	AGATACATACACCAGTGGGAAGAG	1017-1040	
HCN2	AJ225122	HCN2 sense	TCGTCTTCAACGTGGTCTCG	688-907	101
		HCN2 probe	TGAACTTCCGCACCGGCATTGTTATTGAG	736-764	
		HCN2 antisense	ACTTCACGGAGATCATCCTGGAC	766-788	
HCN3	AJ225124	HCN3 sense	TGATCTGATCTCCTCCATCCCT	620-641	88
		HCN3 probe	TGGAGCTGGAGCCACGACTAGATG	664-687	
		HCN3 antisense	TGAGGTCTACAAAACGGCA	689-707	
HCN4	AF064874	HCN4 sense	TGACTTGGTCCTCAACTTCCGC	391-412	84
		HCN4 probe	TCGTGGTGGAGGACAACACAGAAATCAT	420-447	
		HCN4 antisense	GCAGAGGATCAAGATGAAGT	457-476	
GAPDH	M32599	GAPDH sense	CTTCACCACCATGGAGAAGGC	343-363	238
		GAPDH probe	CCTGGCCAAGGTCATCCATGACAACTTT	517-344	
		GAPDH antisense	CTCATGACCACAGTCCATGCC	560-580	

Results and Discussion

Each mRNA expression of HCN subtype genes (HCN1-4) in IVS in MI mice and sham mice was quantified by real-time PCR at 4 weeks after operation.

Figure 1 illustrates the quantitative data of HCN1-4. In sham-operated mice, HCN2 mRNA was dominantly expressed in the ventricle (338.8±71.1 molecules / 10^5 GAPDH molecules, n=3). mRNA expressions of HCN1, HCN3 and HCN4 were relatively small (HCN1, 20.5±6.1; HCN3, 5.8 ±1.7 and HCN4, 12.3±1.1 molecules / 10^5 GAPDH molecules). In MI mice, HCN2 mRNA significantly increased by 2.7 fold (915.2±225.0 molecules / 10^5 GAPDH molecules, p=0.03, n=4), compared to sham MI. HCN1, HCN3 and HCN4 genes were not changed (15.2±5.0; 9.7±1.1 and 48.2±25.3 molecules / 10^5 GAPDH molecules, n=4, respectively).

In this study, we have found that HCN2 mRNA expression was upregulated in the compensated hypertrophied IVS of MI mice. In rat cardiac hypertrophy and human failing heart with ischemic or dilated cardiac cardiomyopathy, the I_f current was shown to be increased in the ventricle. HCN2 gene upregulation might contribute to the enhancement of I_f current in the diseased heart. However, the remodeling of ion channel in the pathological heart is generally due to the re-capitalization of the fetal gene.[9-11] Yasui et al.[12] have reported that the dominant subtype gene for I_f channel in the ventricle is switched from HCN4 to HCN2 with embryonic development. The increase of HCN2 mRNA expression in compensated hypertrophied ventricle after MI in this experiment was not compatible with the fetal gene expression theory in remodeling. Further investigation is required for I_f channel remodeling in diseased heart.

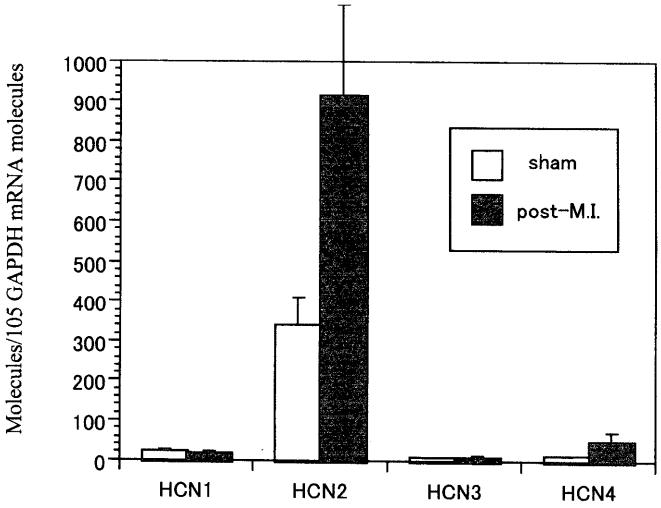

Fig. 1 Quantification of expression of HCN1-HCN4 mRNA assessed by real-time PCR in the interventricular septum of mice at 4 weeks after coronary artery ligation (post-MI) and sham operation (sham). Each bars represents mean (±SEM, n=3–4). GAPDH gene was used as an endogenous control.

References

1) Cerbai E, Barbieri M, Mugelli A. Occurrence and properties of the hyperpolarization-activated current, I_f, in ventricular myocytes from normotensive and hypertensive rats during aging. Circulation. 1996; 94: 1674–1681.
2) Cerbai E, Barbieri M, Porciatti F, et al. Ionic channels in hypertrophy and heart failure: relevance for arrhythmogenesis. New Trends Arrhyth. 1995; 9: 135–139.
3) Hoppe UC, Beuckelmann DJ. Characterization of hyperpolarization-activated inward current in isolated human atrial myocytes. Cardiovasc Res. 1998; 38: 788–801.
4) Shi W, Wymore R, Yu H,et al. Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues. Circ Res. 1999; 85: 1–6.
5) Ludwing A, Zong X, Hofmann F, et al. Structure and function of cardiac pacemaker channels. Cell Phisiol Biochem. 1999: 179–186.
6) Cerbai E, Barbieri M, Mugelli A. Characterization of the hyperpolarization-activated current, I_f in ventricular myocytes isolated from hypertensive rats. J Physiol (Lond) 1994; 481: 585–591.
7) Hoppe UC, Jansen E, Sdkamp M, et al. Hyperpolarization-activated inward current in ventricular myocytes from normal and failing human hearts. Circulation. 1998; 97: 55–65.
8) Hoppe UC, Beuckelmann DJ. Characterization of hyperpolarization-activated inward current in isolated human atrial myocytes. Cardiovasc Res. 1998; 38: 788–801.
9) van Bilsen M, Chien KR. Growth and hypertrophy of the heart: towards an understanding of cardiac specific and inducible gene expression. Cardiovasc Res 1993; 27: 1140–1149.
10) Robinson RB, Yu H, Chang F, et al. Developmental change in the voltage-dependence of the pacemaker current, I_f, in rat ventricle cells. Pflugers Arch 1997; 433: 533–535.
11) Brochu RM, Clay JR, Shrier A. Pacemaker current in single cells and in aggregates of cells dissociated from the embryonic chick heart. J Physiol (Lond) 1992; 454: 503–515.
12) Yasui K, Liu W, Opthof T, et al. I_f current and spontaneous activity in mouse embrionic ventricular myocytes. Circ Res. 2001; 88: 536–542.

Received June 3, 2002; accepted June 10