Slowing of atrioventricular conduction in mice lacking SK2 channel

Qian Zhang[1], Ziming Dong[2,*], Valeriy Timofeyev[3]

[1]*Department of Physiology, Basic Medical College, Zhengzhou University, Zhengzhou, Henan* 450052, *China*; [2]*Department of Pathophysiology, Basic Medical College, Zhengzhou University, Zhengzhou, Henan* 450052, *China*; [3]*Division of Cardiovascular Medicine, University of California, Davis, CA*95616, *USA*

Received December 13, 2006

Abstract

SK2 channel in the atrioventricular node (AVN) was examined by using SK2 channel-deficient (SK2 + /Δ) mice. In the present study, results showed that the PR and RR intervals were prolonged in SK2 + /Δ mice. The spontaneous action potentials(APs) recorded from the AVN exhibited a significant decrease in the beating frequency and a prolongation of AP duration in the SK2 + /Δ mice compared with the WT littermates, suggesting that the deficit causes sinus bradycardia and slower atrioventricular conduction without affecting the excitability of the sinoatrial node. Immunofluorescence confocal investigation showed further that the Ca^{2+}-activated K^+ channel is not only expressed in the working myocytes, and also in the AV conduction system. The findings demonstrate that the SK2 channel expressed in the heart contributes to AVN autorhythmicity and atrioventricular conduction. [Life Science Journal. 2007;4(1):37-39] (ISSN: 1097-8135).

Keywords: automatic activity; Ca^{2+}-activated K^+ channel; atrioventricular node; conduction; gene knockout

1 Introduction

The atrioventricular node (AVN) plays an critical role in generating the correct timing between atrial and ventricular contraction. Abnormality in atrioventricular (AV) conduction can give rise to arrhythmias[1]. Small conductance Ca^{2+}-activated K^+ (SK) are present in most neurons and mediate the afterhyperpolarizations following action potential (AP)[2,3]. Evidence indicated that the presence of SK2 channel, a subtype of SK channel, in cardiac myocytes plays a crucial role in cardiac AP profile and is involved in many physiological processes[4,5]. The functional significance of different ionic channels in the generation and regulation of cardiac automaticity is currently subject of an extensive research effort.

2 Materials and Methods

2.1 Animals

All animal care and procedures were approved by the University of California, Davis Institutional Animal Care and Use Committee. Creation of SK2 knockout mice have been recently described[6]. The transgenic line was backcrossed onto C57Bl/6J mice for greater than 7 generations before they were used. SK2-deficient mice(SK2 + /Δ) and corresponding wild type(WT) animals from either sex were used. The age of two groups ranged from 12 - 18 weeks. Transgene presence was confirmed using PCR of tail tip and expression was assessed by immunoblotting of cardiac homogenates as described previously[6]. All chemicals were purchased from Sigma Chemical Co.

2.2 Electrocardiographic (ECG) recordings

ECG recordings were obtained using Bioamplifier (BMA831, CWE, Incorporated, Ardmore, PA). The animals were placed on a temperature-controlled warming blanket at 37 ℃. Four consecutive 2-minute epochs of ECG data were obtained from each animal. The rate-corrected QT interval (QTc) was calculated using modified Bazett's formula as previously described[7].

2.3 AV node tissue preparation and recording

Preparations were performed according to Hancox[8]. In Brief, the different transgenic mice were anesthetized with pentobarbital(60 mg/kg). The heart was excised rapidly and the right atrium was opened under a dissecting microscope to expose the coronary sinus, the triangle of Koch. The final preparation included the entire AV node region and surrounding atrial. The strip of the tissue was continuously superfused with Tyrode's solution contained (mmol/L) NaCl 138, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2, NaH_2PO_4 0.33, glucose 10, and HEPES 10, pH 7.4.

For electrophysiological recording, spontaneous APs were recorded from isolated AVN preparations by

*Corresponding author. Email: ziming-dong@163.com

using microelectrode techniques with 3 mol/L KCl microelectrodes at 33 ℃.

2.4 Immunohistochemistry

Immunofluorescence labeling was performed on isolated single AVN cells from WT and SK2 + /Δ mice as described previously[9]. Cells fixed with 4% formaldehyde and then permeabilized using 0.2% Triton X-100. After they were blocked with 1% BSA in PBS, the cells were incubated overnight at 4 ℃ with the primary antibody. The following primary antibodies were used: (1) anti-SK2 (Sigma, 1:100), a polyclonal antibody raised in rabbit; (2) anti-neurofilament 160 kD (NF 160, Chemicon, 1 : 100), a monclonal antibody raised in mouse; immunofluorescence labeling for confocal microscopy was performed by treatment with FITC-conjugated goat anti-rabbit antibody (Sigma, 1 : 250) and Texas red-conjugated donkey anti-mouse antibody (Calbiochem, 1:250). Control experiments performed by incubation with secondary antibody only under the same experimental condition. Immunofluorescence-labeled samples were examined with a Pascal Zeiss confocal laser scanning microscope.

2.5 Statistics

Data are expressed as means ± SE. Statistical comparison was performed by Student t test, with a value of $P<0.05$ considered significant.

3 Results

3.1 SK2 + / mice prolonged AV conduction

To estimate the influence of the SK2 channel on electrophysiological characteristics of the heart action, we performed surface ECG recordings from anesthetized WT and SK2 + /Δ mice. The RR interval was significantly increased from 134.97 ± 7.8 ms in the WT mice to 153.8 ± 6.1 ms in SK2 + /Δ ($P<0.05$). The PR interval was prolonged from 35.1 ± 3.42 ms in WT mice to 42.50 ± 2.30 ms in SK2 + /Δ mice ($P<0.05$), pointing to a sinus bradycardia (R-R interval) with a significant prolongation of AV conduction (PR interval). The QTc interval (54.1 ± 5.95 and 58.4 ± 3.52 for WT and SK2 + /Δ mice) was not significantly modified. The other ECG parameters were not significantly modified (data not shown).

3.2 AVN preparation from SK2 + /Δ mice showed slower automaticticity

The spontaneous activity of the AVN preparation was studied to test whether the slowing P-R interval observed in the ECG from the SK2 + /Δ mice was associated with dysfunction of AVN. Figure 1 showed the characteristic spontaneous APs recorded from the regions within and around the AVN at 33℃. APs recorded from the regions within the AV node can be identified by the presence of slow diastolic depolarization and a very slow upstroke of phase 0. The SK2 + /Δ mice showed a significant slow spontaneous activities of the AVN compared with WT mice (Figure 1). Analysis of the AP (Table 1) displayed a significant prolongation of the cycle length in SK2 + /Δ mice compared with WT mice ($P<0.05$), and significant wider APD_{50} and APD_{80} in SK2 + /Δ mice compared with WT mice ($P<0.05$, respectively). The DDR decreased in the AVN cells, but no significant difference between WT and SK2 + /Δ groups. The findings indicated that the present of the AV node was dysfunction and the automaticticity of AVN cells decreased.

Figure 1. AVN spontaneous APs recording

Table 1. Action potential parameters measured in AVN cells from WT and SK2 + /Δ mice

	n	MDP (mV)	CL (ms)	APD_{50} (ms)	APD_{80} (ms)	DDR (mV/s)	V_{max} (V/s)
WT	9	49.18 ± 2.09	365.64 ± 27.62	33.82 ± 1.40	71.72 ± 2.56	26.98 ± 2.98	4.17 ± 0.53
SK2 + /Δ	9	48.11 ± 1.84	441.50 ± 25.94*	40.00 ± 2.14 *	85.80 ± 4.17 *	19.74 ± 3.41	3.72 ± 0.75

*$P<0.05$, compared with WT. Cycle length, CL; the maximum diastolic potential, MDP; the maximum upstroke velocity, V_{max}; the rate of diastolic depolarization, DDR; AP duration at 50% and 80% repolarization, APD_{50} and APD_{80}, respectively.

3.3 Expression of SK2 channel protein in AV node

At the mouse AV node, immunohistochemistry was used to investigate the expression of SK2 channel in the WT and SK2 + /Δ mice. To help distinguish different myocytes, NF160 was immunolabeled as marker which is expressed in the AV conduction system[10]. Figure 2 showed SK2 (green) protein was strongly expressed in the WT AVN cell(a). A reduced level of the SK2 labeling was detected in the SK2 + /Δ mouse(b). The control experiment eliminated the positive labeling further confirming that the labeling seen in AV nodal cell (c) was epitope-specific. In addition, the mouse atrial myocytes were more intensely labeled compared with ventricular myocytes.

4 Discussion

The present study shows for the first time the role and distribution of the SK2 channel at the AV node. The major phenotypic effects of the lack of SK2 in AVN were prolonged the PR and RR intervals. Electrophysiological study indicated a slow autorhythmicity through a prolongation of the APD and dysfunction of the AVN.

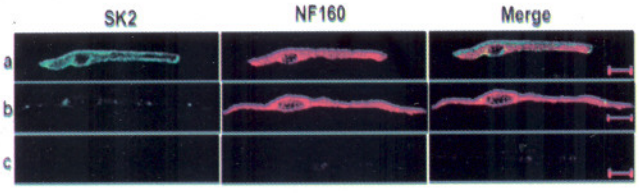

Figure 2. Confocal photomicrographs of double staining with anti-SK2 (green) and anti-NF 160 (red) antibodies in single AVN cell. The scale bar is 20 μm.

The small-conductance K_{Ca} channels are encoded by at least three genes, *SK1*, *SK2*, *SK3*[11,12]. Electrophysiological studies and molecular cloning techniques have documented the expression of multiple types of voltage-gated K^+ channels in cardiac myocytes isolated from different species and from different regions of the heart[13]. The importance of the channel is proved by the fact that the late phase of the cardiac AP is susceptible to abnormal excitation, e.g. early after depolarization and arrhythmias[5]. In the previous, we documented that lack of SK2 channel prolonged the membrane repolarization, particularly in atria. *In vivo* electrophysiological studies showed evidence of AVN dysfunction and atrial arrhythmias (data didn't show).

In the present study, ECGs and electrophysiological recordings document both bradycardia and slowing of the atrioventricular conduction in SK2 + /Δ mice, whereas the lack of SK2 channels did not significantly modify the function of the SAN cells. The electrophysiological results indicated further that the loss of the SK2 channel slowed down the beating rate of AVN cell because of the prolonged APD through a delayed AP repolarization. The slowing of the AV conduction in the SK2 + / Δ mice is also caused by dysfunction in the excitability of the AVN. We could not completely reject the damage of AVN cell during the surgery. The exact reasons for this finding have to be further accessed.

In this study, the expression of SK2 channel is not only detected in the mouse working myocytes, but also in the conduction system. Application of immunohistochemical imaging has shown that the expression of ion channels can provided new insights into how the AV node works at the celluar level.

References

1. De Carvalho AP, De Almeida DF. Spread of activity through the atrioventricular node. Cir Res 1960; 8:801 – 9.
2. Vergara C, Latorre R, Marrion NV, *et al*. Calcium-activated potassium channels. Curr Opin Neurobiol 1998; 8: 321 – 9.
3. Kohler M, Hirschberg B, Bond CT, *et al*. Small-conductance, calcium-activated potassium channels from mammalian brain. Science 1996; 273:1709 – 14.
4. Xu YF, Tuteja D, Zhang Z, *et al*. Molecular identification and functional roles of a Ca^{2+}-activated K^+ channel in human and mouse hearts. J Bio Chemistry 2003; 278:49085 – 94.
5. Lu L, Zhang Q, Timofeyev V, *et al*. Function coupling of SK2 to L-type Ca^{2+} channel via α-actinin2 is critical for normal cardiac functions. Cir Res 2007; 100: 112 – 20.
6. Chris TB, Herson PS, Strassmaier T, *et al*. Small conductance Ca^{2+}-activated K^+ channel knock-out mice reveal the identity of calcium-dependent afterhyperpolarization currents. J Neuroscience 2004; 24:5301 – 6.
7. Mitchell GF, Jeron A, Koren G. Measurement of heart rate and Q-T interval in the conscious mouse. Am J Physiol 1998; 274:H747 – 51.
8. Hancox JC, Levi AJ, Lee CO, *et al*. A method for isolating rabbit atrioventricular node myocytes which retain normal morphology and function. Am J Phsiol 1993; 265:H755 – 66.
9. Spitzer KW, Sato N, Tanaka H, *et al*. Electrotonic modulation of electrical activity in rabbit atrioventricular node myocytes. Am J Physiol 1997; 273:H767 – 76.
10. Vitadello M, Vettore S, Lamar E, *et al*. Neurofilament M mRNA is expressed in conduction system myocytes of the developing and adult rabbit heart. J Mol Cell Cardiol 1996; 28: 1833 – 44.
11. Stocker M. Ca^{2+}-activated K^+ channels: molecular determinants and function of the SK family. Nat Rev Neurosci 2004; 5:758 – 70.
12. Tuteja D, Danyan XU, Timofeyev V, *et al*. Differential expression of small-conductance Ca^{2+}-activated K^+ channels SK1, SK2, and SK3 in mouse atrial and ventricular myocytes. Am J Physiol Heart Circ Physiol 2005; 289(6):H2714 – 23.
13. Pennefather P, Lancaster B, Adams PR, *et al*. Two distinct Ca-dependent K currents in bullfrog sympathetic ganglion cells. Proc Natl Acad Sci USA 1985; 2: 3040 – 4.