Clinical Research

Heterogeneous of potassium currents in free wall myocytes from the infarcted rabbit ventricle and regression effects of imidapril

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Abstract

Aim To define the heterogeneous changes of ion channels in the noninfarcted myocardium after myocardial infarction in rabbit and effects of imidapril.

Methods Rabbits with left coronary artery ligation were prepared and allowed to recover for 8 wk. Myocytes were isolated from subendocardial, midmyocardial and subepicardial regions of the noninfarcted left ventricular free wall. Ion currents were recorded with whole-cell patch clamp way.

Results The densities of the transient outward K+ currents (Ito) and the inward rectifier K+ currents (IK1) were greatly reduced in midmyocardium and subepicardium while two currents reduced gently in subendocardium. The densities of the delayed rectifier K+ currents (IK) were reduced in noninfarcted three layers similarly. Imidapril could reverse the changes of membrane currents in healed myocardial infarction cells and depress the dispersion of repolarization.

Conclusions The heterogeneities of K currents were enhanced in noninfarcted area. Normalization of heterogeneous changes of repolarizing after treatment with imidapril was observed. (J Geriatr Cardiol 2008; 5:)

Key Words myocardial infarction; heterogeneity; potassium channels; imidapril

Materials and methods

Agents

Collagenase type I, protease E, bovine serum albumin, egtazic acid, K2ATP, CdCl2, MgCl2, N-[2-hydroxyethyl]-piperazine-N’-[2-ethane-sulfonic acid] (HEPES), 4-aminopyridine(4-AP), Na-pyruvate and K-aspartate were purchased from Sigma Co., dofetilide from Pfizer Co., tetrodotxin (TTX) from Hebei Aquatic Product Research Institute, imidapril was provided as a gift from Tanabe Seiyaku Co. Inc., other reagents are of analytical grade.

Model of myocardial infarction

A rabbit model of myocardial infarction used in this study was previously described. Rabbits (2.0 to 2.5 kg) were anesthetized with pentobarbital (30 mg/kg, iv) and the left anterior descending coronary artery was occluded. The rabbits were then allowed to recover for 8 weeks, as the healed myocardial infarction group (HMI group). Another group tree days after surgery, received oral administration of imidapril (1.5 mg/kg/d) for 8 weeks, as the imidapril-treated group (IMI group). Animals undergoing an identical surgical procedure without coronary ligation served as the sham-operated group (sham group).
Isolation of Myocytes

Rabbit ventricular myocytes were isolated as described previously. The heart was suspended from a Langendorff column, and perfused with Tyrode’s solution containing 0.33 mg/mL collagenase, 0.025 mg/mL protease E for 25 min. The slices of tissue of Endo, M and Epi of the noninfarcted section in the left ventricular free wall were dissected with a surgical blade. The three layer tissue samples were minced and sequentially digested for 25 min in fresh enzymes solution (36 °C). Cells in each region were stored.

Solutions

The extracellular solution for \(I_{\text{to}}\) and \(I_{\text{K1}}\) currents measurements was Ca\(^{2+}\)-free Tyrode’s solution, which was omitted Ca\(^{2+}\) from Tyrode's. The pipette solution contained (in mmol/L) K-aspartate 85, KCl 45, Na-pyruvate 5, K\(_2\)ATP 3, MgCl\(_2\) 4, egtazic acid 10, HEPES 10 and glucose 11(pH 7.4). The extracellular solution for \(I_{\text{K}}\) (\(I_{\text{Ks}}\) and \(I_{\text{Kr}}\)) currents measurement was NMG solution (in mmol/L: N-me-thyl-D-glucamine 149, MgCl\(_2\) 5, HEPES 5). In this Na\(^+\)-free, K\(^+\)-free, CdCl\(_2\) 100 mmol/L-containing and 4-AP 5 mmol/L-containing external solution, pH adjusted to 7.4 with HCl. The \(I_{\text{Ca,L}}, I_{\text{K1}}, I_{\text{to}}, \) the Na\(^+\)-Ca\(^{2+}\) exchange current, and the Na\(^+\)-K\(^+\) pump current are negligible in this solution. The pipette solution for measuring \(I_{\text{K}}\) was similar to that of \(I_{\text{to}}\) and \(I_{\text{K1}}\) currents.

Electrophysiological Techniques

Currents were recorded using EPC-9 (HEKA, German). Stimuli output or data acquisition and processing were performed by Pulse-pulsefit software. Micropipettes had resistances from 2 to 4 M\(^\Omega\) when filled with normal Tyrode’s solution. Series resistance and capacitance were compensated and leak currents were subtracted.

Statistics

Data were mean ± S.D. A Student’s t-test was applied to compare the results of two different groups and ANOVA was used for multiple comparisons. P value < 0.05 was considered statistically significant. The steady-state activation and inactivation of \(I_{\text{Ca,L}}\) were fitted by Bolzmann equation \((I/I_{\text{max}} = 1/(1+\exp ((V_{\text{m}}-V_{\text{0.5}})/k))))\). \(V_{\text{0.5}}\) is instead of Half-maximal and k is instead of voltage and slope factor.

Results

Histological changes of hearts

Tab 1 summarized the characteristics of the rabbit healed infarction model used in the present study. Heart weight and heart weight to body weight ratios were significantly greater in HMI group than those in the sham group. The mean cell membrane capacitance in the noninfarction myocytes from infarcted hearts was greater than those from sham hearts. With imidapril treatment, the structural changes of infarcted hearts were significantly alleviated.

Transient outward potassium current (\(I_{\text{to}}\))

\(I_{\text{to}}\) was recorded by applying various pulses ranging from –40 mV to +50 mV for 300 ms from holding potential of –80 mV after depolarized to –40 mV for 25 ms to inactivate sodium current. The amplitudes of \(I_{\text{to}}\) Epi and M in HMI cells were small versus those of sham cells. At test potential of +50 mV, the densities of \(I_{\text{to}}\) in sham cells were 25.5 ± 2.1 pA/pF, Epi, n=20; 22.5 ± 1.8 pA/pF, M, n= 19 and12.7 ± 1.6 pA/pF, Endo, n=21 respectively. Compared with sham group, the magnitude of \(I_{\text{to}}\) was reduced by 54.4 %, Epi, n=21; 50.3 %, M, n=19 and 20.1 %, Endo, n=18 in HMI group. \(I_{\text{to}}\) densities after IMI treated increased to 18.4 ± 1.8 pA/pF, Epi, n=18; 17.7 ± 3.1 pA/pF, M, n=19; 9.7 ± 1.7 pA/pF, End, n=20. \(I_{\text{to}}\) relationships demonstrated that \(I_{\text{to}}\) density was markedly decreased in myocytes from infarcted hearts than those from sham hearts. Moreover, \(I_{\text{to}}\) densities were gradually reduced with potential shifting to the positive direction (Fig 1).

| Tab 1 Characteristics of hearts and cell membrane capacitance of isolated myocytes in Sham, HMI and IMI groups. means ±SEM. |
|---|---|---|---|
| n | Sham | HMI | IMI |
| Heart weight(g) | 15 | 9.4 ± 1.2 | 13.6 ± 2.3<sup>a</sup> | 10.3 ± 1.1<sup>b</sup> |
| Heart weight/body weight (g/kg) | 15 | 2.9 ± 0.3 | 3.8 ± 0.3<sup>a</sup> | 3.3 ± 0.2<sup>b</sup> |
| Cell membrane capacitance (pF) | 45 | 154 ± 12 | 223 ± 13<sup>a</sup> | 168 ± 10<sup>b</sup> |

<sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01 vs sham group. <sup>P</sup> < 0.05, <sup>a</sup>P < 0.01 vs HMI group.
The voltage dependence of $I_{\text{to}}$ activation was not markedly different in three group cells. $V_{0.5}$ of inactivation of HMI myocytes shifted to hyperpolarizing direction, but not $k_r$ of $I_{\text{to}}$ of Epi and M in HMI cells was delayed, while $k_r$ of Endo cells was slightly changed.

Rapidly component of delayed rectifier potassium current ($I_{Kr}$)

$I_{Kr}$ and $I_{Kr-tail}$ were recorded during 225 ms depolarizing pulses to 0 mV from a holding potential of -80 mV, and the tail current ($I_{Kr-tail}$) was determined upon repolarization to -40 mV. The densities of $I_{Kr-tail}$ in the noninfarction cells were similar to those of sham-operated cells. The densities of $I_{Kr-tail}$ were not striking difference in Epi, M and Endo cells. $I-V$ relationships demonstrated that $I_{Kr-tail}$ densities were in myocytes from infarcted hearts than those from sham hearts (data not show).

Slowly component of delayed rectifier potassium current ($I_{Ks}$)

$I_{Ks}$ and $I_{Ks-tail}$ were recorded by applying various pulses ranging from -20 mV to +50 mV for 7 s from holding potential of -80 mV, following by repolarizing to -30 mV. At +50 mV, the densities of $I_{Ks-tail}$ in sham cells were 0.9±0.1 pA/pF (Epi, n=17); 0.4±0.1 pA/pF (M, n=19) and 0.8±0.1 pA/pF (Endo, n=16). Compared with sham group, the densities of $I_{Ks-tail}$ in HMI cells were reduced by 25.3 %; 32.9 % (Epi), 24.0 %; 37.7 % (M) and 27.3 %; 34.7 % (Endo). But after IMI treated, the densities of $I_{Ks-tail}$ in HMI cells were enhanced to 0.7±0.1 pA/pF (Epi, n=19); 0.3±0.1 pA/pF (M, n=17) and 0.6±0.1 pA/pF (Endo, n=18) respectively. $I-V$ relationships demonstrated that $I_{Ks-tail}$ densities were less in myocytes from infarcted hearts than those from sham hearts (Fig 2).

Inward rectifier potassium current ($I_{ki}$)

To record $I_{ki}$, the cells were depolarized every 10 s from a holding potential of -80 mV, subsequently, from -100 mV at 10 mV increments up to +40 mV with the 225 ms pulses. When the current was normalized to membrane capacitance, the average current densities of Epi, 10.4±0.3 pA/pF, n=17; M, 11.9±0.4 pA/pF, n=20 and Endo, 8.7±0.1 pA/pF, n=17 in sham cells. $I_{ki}$ were reduced in Epi and M but not Endo of remote from the infarcted region of rabbit heart. The densities of the $I_{Ks-tail}$ were 6.2±0.6 pA/pF (Epi, n=17), 8.9±0.3 pA/pF(M, n=19), and 7.8±0.2 pA/pF(Endo, n=17) myocytes respectively. After treatment of IMI, compared with HMI group, the $I_{ki}$ of three layer myocytes remote from infarct were notably increased to Epi, 10.4±0.6 pA/pF, n=17; M, 11.2±1.5 pA/pF, n=20; Endo, 8.7±0.6 pA/pF, n=17 (Fig 3). Fig 3 also illustrated the $I-V$ curves of $I_{ki}$ densities in sham, HMI and IMI cells.

The acute effect of IMI on ion currents

Imi did not acutely affect either the shape of action potential or $I_{Ca-L}$ in all ventricular myocytes tests (data not show).

Discussion

Hypertrophy is a fundamental compensatory mecha-
nism sustaining cardiac output after infarction. Structural remodeling of the left ventricle after myocardial infarction involves the regions of infarct scar, border zone areas, as well as the noninfarcted remote myocardium. Regional remodeling is a strong risk factor for ventricular arrhythmias. In our study, the increment of cell size in the noninfarcted areas was found, implying that the myocardium developed remodeling.

A finding in our data was that a reduction of $I_{K}$ in M and Epi, with $I_{K}$ remaining unchanged in Endo. A possible consequence of these changes would be an alteration of the normal pattern of ventricular repolarization and increased vulnerability to ventricular arrhythmia. The observed regional changes in $I_{K}$ might cause, via an altered gradient in APD within the left ventricular wall, the dissociation of QRS complex and T-wave direction inversion in ECG recordings. The mechanisms underlying the decrease of $I_{K}$ in myocytes from failing hearts were not known. It should be recognized as a substantial change of electrical remodeling. The decrease in $I_{K}$ amplitudes and changes in their kinetics in infarcted tissue might be due to a decrease in functional channels or changes in their subunit composition.

In humans, $I_{K}$ play an important role in determining the cardiac action potential configuration. The importance of $I_{K}$ in the genesis of cardiac arrhythmias was suggested by its reduction in arrhythmogenic substrates such as heart failure, myocardial infarction and hypertrophy. Our data indicated that densities of $I_{K}$ were significantly decreased in noninfarcted three layer myocytes compared to normal. Heterogeneous changes in $I_{K}$ in infarcted hearts may impact on the effects of varying heart rate or neurohumoral modulation on repolarization. The homogeneous prolongation of QT interval in ECG recording in HMI cells was found because of reducing of $I_{K}$, which increased transmural dispersion of repolarization, or induce torsade de pointes in infarction heart. In this study, the densities of $I_{K}$ in the noninfarction cells were similar to those of sham-operated cells. The densities of $I_{K}$, however, were not strikingly different in Epi, M and Endo cells in each group. We guessed that $I_{K}$ contributed seldom to the abnormal changes in HMI hearts. $I_{K}$ was decreased or unchanged in different studies.

We observed a reduction of $I_{K}$ in three layer myocytes from infarction rabbit cells compared with sham cells. $I_{K}$ were reduced in Epi and M but not Endo of remote from the infarcted region of rabbit heart. The change in $I_{K}$ may be one of increase factors of transmural dispersion of repolarization.

Long-term treatment with imidapril after MI could decrease experimental animals’ mortality, the mechanism was not only restraining the constitution remodeling and preserving the heart function, but also relating with regressing the electric remodeling. Among factors, the renin-angiotensin system plays an important role in the regulation of cardiac myocyte growth. This study demonstrated normalization of cell membrane capacitance of ventricular myocytes after regression of infarction heart with chronic imidapril treatment. Furthermore, normalization of potassium current abnormality was associated with normalization of APD, which probably of the drug explains the reducing the transmural heterogeneity of repolarization. This improvement would seem to be mediated by a reduction in LV myocardial infarction remodeling rather than a direct antiarrhythmic effect, since transmural dispersion of APD did not change after infarction and sham cells treated with drug directly. Cells isolated from infarction rabbits treated with imidapril had adjacent to normal cell membrane capacitance, action potential duration, and membrane current densities. It probably suggested that the lower incidence of ventricular arrhythmia and sudden death by imidapril treated might be due in part to prevention of transmural dispersion.

References

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