Effects of hypokalemia on transmural dispersion of ventricular repolarization in left ventricular myocardium

Jiang-Hua Zhong¹², Shi-Juan Lu², Mo-Shui Chen², Zi-Bin Chen², Liu Wang², Ping-Sheng Wu¹

¹Department of Cardiology, Affiliated South Hospital of South Medical University, Guangzhou 440100, China
²Department of Cardiology, Haikou People’s Hospital, Haikou 570102, China

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ABSTRACT

Objective: To observe effects of hypokalemia on transmural heterogeneity of ventricular repolarization in left ventricular myocardium of rabbit, and explore the role of hypokalemia in malignant ventricular arrhythmia (MVA). Methods: A total of 20 rabbits were randomly divided into control group and hypokalemic group. Isolated hearts in the control group were simply perfused with modified Tyrode’s solution, and were perfused with hypokalemic Tyrode’s solution in hypokalemic group. Ventricular fibrillation threshold (VFT), 90% monophasic action potential repolarization duration (APD90) of subepicardial, midmyocardial and subendocardial myocardium, transmural dispersion of repolarization (TDR) and Cx43 protein expression in three layers of myocardium were measured in both groups. Results: VFT in the control group and the hypokalemic group were (13.40±2.95) V, and (7.00±1.49) V, respectively. There was a significant difference between two groups (P<0.01). APD90 of three myocardial layers in the hypokalemic group were significantly prolonged than those in the control group (P<0.01). ΔAPD90 in the hypokalemic group and the control group were (38.10±10.29) ms and (23.70±5.68) ms, and TDR were (52.90±14.55) ms and (36.10±12.44) ms, respectively. ΔAPD90 and TDR in the hypokalemic group were significantly higher than those in the control group (P<0.05), and the increase in APD90 of midmyocardium was more significant in the hypokalemic group. Cx43 protein expression of all three myocardial layers were decreased significantly in the hypokalemic group (P<0.01), and ΔCx43 was significantly increased (P<0.05). Reduction of Cx43 protein expression was more significant in the midmyocardium. Conclusions: Hypokalemic can increase transmural heterogeneity of Cx43 expression and repolarization in left ventricular myocardium of rabbit, and decrease VFT and can induce MVA more easily.

1. Introduction

Severe hypokalemia can induce or increase the occurrence of ventricular arrhythmia, which is more significant in pathological conditions. Many organic heart diseases such as hypertension, left ventricular hypertrophy and heart failure are likely to trigger fatal ventricular arrhythmia. Because of intensive control of blood pressure and heart function improvement and constantly use of diuretic, hypokalemia is the most common side effects of the use of diuretics clinically.

The present study aims to research the effects of hypokalemia on transmural heterogeneity of ventricular repolarization in left ventricular’s three layers of myocardium and on Cx43 expression, to discuss electrophysiology and protein mechanism of its prone ventricular arrhythmia, for clinical prevention and treatment of the organic heart disease death.
2. Materials and methods

2.1. Experimental animals

A total of 30 health rabbits were provided by Experimental Center of Hainan Medical University, male and female unlimited, weighting 2.0 to 3.0 kg.

2.2. Reagent preparation

Standard Tyrode’s solution (mmol/L): NaCl 115, KCl 5.4, MgCl₂ 1, CaCl₂ 1.8, NaH₂PO₄ 1, Glucose 10, pH 7.4 set with NaOH. Hypokalemic tyrode’s solution (mmol/L): KCl 15, NaCl 115, MgCl₂ 1, CaCl₂ 1.8, NaH₂PO₄ 1, HEPES 5, Glucose 10, pH 7.4 set with NaOH. Tyrode’s solution were modulated by 95%O₂ and 5%CO₂ to pH 7.4.

2.3. Animal grouping

Experimental rabbits were randomly divided into control group and hypokalemia group by half, the isolated hearts of control group were perfused with simple improved Tyrode’s solution, hypokalemia group was given with hypokalemia solution, tyrode’s perfusion.

2.4. Animal preparation

One g/kg urethane via ear marginal vein anesthesia was performed on all the rabbits, the heart was quickly isolated into Tyrode’s perfusion at 4 ℃ for cardiac arrest, after aorta intubation, Langendorff perfusion was conducted (36.5–37.5 ℃) saturated with pure oxygen, constant temperature and constant pressure of 8.52 to 8.78 kpa.

2.5. Electrophysiological experiments

(1) Electrodi making and location for monophasic action potential of the three layers of myocardium. The reference electrode was fixed in the aortic root, simple electrode detecting monophasic action potentials were made from polytetrafluoroethylene wrapped by silver of 0.3 mm diameter. The simple electrodes of subepicardial, midmyocardial and subendocardial myocardium were fixed respectively in medical syringe needle, inserted at the location about 2.0 mm from epicardial surface. (2) Experimental parameters: Simple electrodes fixed in three layers of myocardial myocardium were respectively connected to the biological signal acquisition and processing system, the parameter was set as: filter wave 500–1 000 Hz, time 0.1. Along the boundary of right ventricular free wall and crest, the sinoatrial node were destroyed using scissors, until a slow sinoatrial rhythm was detected. About 20 min after perfusion in each group, the myocardium monophasic potentials of were synchronously recorded if the parameters were stable at that time.

2.6. Tissue protein extract of left ventricular three–layer myocardium

Left ventricular free wall was sheared after electrophysiology experiment and cryopreserved in liquid nitrogen. Through rapid frozen section method, from the epicardial surface to the endocardial direction, left ventricular epicardial myocardium tissue was sliced serially 10 times with 20 μm thick of each section (a total of 200 μm), by the same way, left ventricular epicardial myocardium tissue was sliced from the endocardial surface to the epicardial direction, and midcardial myocardium tissue was isolated about 3 mm from epicardial surface. The three layers of myocardial tissue were preserved in eppendorf tube. Homogenate were centrifuged with 12 000 rpm/min for 30 min at 4 ℃, supernatant liquid was taken for measuring the concentration of histones.

2.7. Western blot detection of Cx43 expression

According to the sample concentration, protein extract was transferred onto PVDF membrane after SDS PAGE, then blocked in 5% TBST skim milk for 2 h at room temperature, then added Cx43 monoclonal antibody at 4 ℃ overnight, followed by incubulation with horseradish peroxidase labeled secondary antibody for 2 h at room temperature, PVDF membrane was imaged on X–ray film, using enhanced chemiluminescence method. With gel image analysis system to get protein signal images for determining the optical density values of Cx43 and internal reference beta actin, their ratio is calculated as the expression level of Cx43 protein.

2.8. The observed indicators

MAP parameter: the horizontal distance from MAP phase 0 to 90% amplitude were repolarized; ΔAPD₉₀: APD₉₀ difference between left ventricular midcardial and subendocardial myocardium; Transmural dispersion of repolarization (TDR): difference between the longest APD₉₀ and shortest APD₉₀ among three layers myocardium; Cx43 protein expression in three layers of myocardium; ΔCx43: Cx43 difference between left ventricular midcardial and subendocardial myocardium.

2.9. Statistics analysis

In this completely random design tests, all the data are calculated and analyzed as mean±SD using SPSS 13.0 statistical software, for each experimental data lines, normality test was applied; for compare data between the two groups, independent sample t test was used, with α =0.05 (double side) as the inspection level, P < 0.05 for statistical significance.
3. Results

3.1. Monophasic action potential indexes

Ventricular fibrillation threshold (VFT) of control and hypokalemic group were 13.4 V and 7.0 V, respectively ($P<0.01$), indicating hypokalemia perfusion decreased the VFT in left ventricular myocardium as shown in Table 1. APD$_{90}$ in three layers of left ventricular myocardium was significantly increased in hypokalemic group ($P<0.01$). $\Delta$APD$_{90}$ of hypokalemic group was significantly increased to (38.10±10.29) ms ($P<0.01$), indicating hypokalemia induced a increase of transmural heterogeneity in ventricular repolarization of left ventricular myocardium. TDR was significantly increased ($P<0.05$) in hypokalemic group (52.90 ms), compared with that of control group (36.10 ms), indicating hypokalemia further increased dispersion of transmural repolarization in left ventricular myocardium (Table 1).

Table 1
Monophasic action potential indexes.

<table>
<thead>
<tr>
<th>Observation indexes</th>
<th>Hypokalemic group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFT (V)</td>
<td>7.00±1.49 *</td>
<td>13.40±2.95</td>
</tr>
<tr>
<td>Endo–APD$_{90}$ (ms)</td>
<td>246.90±15.04 *</td>
<td>203.10±16.07</td>
</tr>
<tr>
<td>Mid–APD$_{90}$ (ms)</td>
<td>285.00±13.77 *</td>
<td>226.80±20.31</td>
</tr>
<tr>
<td>Epi–APD$_{90}$ (ms)</td>
<td>323.10±16.59 *</td>
<td>237.40±17.27</td>
</tr>
<tr>
<td>$\Delta$APD$_{90}$ (ms)</td>
<td>38.10±10.29 *</td>
<td>23.70±5.68</td>
</tr>
<tr>
<td>TDR (ms)</td>
<td>52.90±14.55 #</td>
<td>36.10±12.44</td>
</tr>
</tbody>
</table>

Endo: subendocardial myocardium; Mid: midmyocardial myocardium; Epi: subepicardial myocardium; * $P<0.01$, compared with control group; # $P<0.05$, compared with control group.

3.2. Cx43 expression in left ventricular myocardium

Cx43 expression in control group, hypokalemic group showed relative grey values in subendocardial, midmyocardial and subepicardial myocardium with a significant decrease (0.22±0.04, 0.36±0.06, 0.40±0.06, respectively) ($P<0.01$), Cx43 protein expression of myocardial myocardium was decreased more obviously, relative grey values in subendocardial, and subepicardial myocardium increased with comparison to the control, indicating hypokalemia induced a increase of transmural heterogeneity in ventricular repolarization of left ventricular myocardium.

Table 2
Cx43 expression.

<table>
<thead>
<tr>
<th>Observation indexes</th>
<th>Hypokalemic group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endo–Cx43</td>
<td>0.40±0.06 *</td>
<td>0.57±0.07</td>
</tr>
<tr>
<td>Mid–Cx43</td>
<td>0.22±0.44 *</td>
<td>0.45±0.05</td>
</tr>
<tr>
<td>Epi–Cx43</td>
<td>0.36±0.06 *</td>
<td>0.52±0.08</td>
</tr>
<tr>
<td>$\Delta$Cx43</td>
<td>0.18±0.05 #</td>
<td>0.11±0.07</td>
</tr>
</tbody>
</table>

Endo: subendocardial myocardium; Mid: midmyocardial myocardium; Epi: subepicardial myocardium; * $P<0.01$, compared with control group; # $P<0.05$, compared with control group.

4. Discussion

In clinical patients, hypokalemia is a common electrolyte disorder, but due to its prone to trigger severe ventricular arrhythmias draw more attention by the clinicians. The reasons for hypokalemia mainly include insufficient potassium intake, too much potassium lost, and a large number of potassium transfers from extracellular into the cells.

Hypokalemia has multifaceted impact on ventricular muscle electrophysiology. First of all, it can increase the excitability of ectopic pacemakers’ potential within ventricular muscle, then trigger ectopic rhythm activity easily. In addition, hypokalemia can lead to a slow pace of electrical conduction, which tends to turn back. Hypokalemia therefore, can increase auto- rhythmicity and excitability of myocardial muscle, at the same time, can reduce the myocardial conductivity, thus trigger ventricular arrhythmia easily. Previous experiments showed that hypokalemia can reduce VFR; on the contrary, the rise of potassium concentration could improve VFR. Large size of the clinical sample showed, the occurrence of malignant ventricular arrhythmias in patients with acute myocardial infarction is closely related to the potassium concentration[2]. Severe hypokalemia can cause fatal arrhythmia, myocardial infarction, heart failure and other pathological conditions, but the electrophysiological mechanism is not entirely clear.

In recent years, with the understanding of left ventricular midmyocardial cells, people put forward the concept of electrophysiology transmural heterogeneity of ventricle muscle cells[3]. The left ventricular myocardium, from the anatomical point of view, in addition to the subepicardial and endoepicardial myocardium cells on the cross sectional; there is a layer of midmyocardial cells with unique electrophysiological properties. Compared to the subepicardial and endoepicardial myocardium cells, stimulated by stable frequency of electronic triggers, monophasic action potential, specially the monophasic action potential repolarization duration in midmyocardial cells are significantly extended. This difference can lead to the difference in the electrophysiological properties of the left ventricular myocardial cells on cross section, and the extension of monophasic action potential in midmyocardial cells can induce early afterdepolarization, delay after depolarization and electrophysiological activity basis of the left ventricular myocardial cells. This extremely uneven depolarization of three layers can induce turn–back formation easily in the left ventricular myocardial cross section to form malignant ventricular arrhythmia (MVA), such as cutting–edge reverse type of ventricular tachycardia, ventricular flutter and ventricular fibrillation.

Our results also showed that VFT in hypokalemic group is 7.0 V, decreased more significantly than the normal control group ($P<0.01$). It suggested that the hypokalemia perfusion reduced left ventricular myocardial VFT, making it prone to ventricular fibrillation. Compared with normal control group, left ventricular myocardial APD$_{90}$ in hypokalemic group were...
significantly longer ($P < 0.01$). $\Delta A P D_{90}$ in hypokalemic group was increased to $38.10 \pm 10.29$ ms ($P < 0.01$), TDR ($52.90$ ms) was also increased significantly ($P < 0.05$) compared with normal control group ($36.10$ ms), suggesting that hypokalemia perfusion can extend APD of the three myocardial layers in normal rabbits, especially the prolonged midmyocardial APD has expanded across deploration dispersion, and uniformity between the three–layer myocardium was increased after expanded dispersion.

It has been confirmed that gap junction protein (C43) is the main protein molecules involved in MVA. Studies have shown that C43 is widely involved in various works of the heart cells and the surface membrane of electrical conduction system. Multiple C43 in myocardial cell surface composition has special biological characteristics of gap junction channels (GJ), and adjacent cardiac muscle cells are closely connected to each other by GJ, electrical and chemical signals pathway between each other cell, to maintain normal electrical coupling and mechanical coupling between myocardial cells. Thus, GJ is normal anatomical basis of electrical activity of myocardial cells, the C43 is GJ protein molecular basis. On the myocardial cell membrane surface, there are many known C43 phenotypes, including C40, C43 and C45, C43 phenotype which are mainly expressed in left ventricular myocardial cell. They are important protein molecules between ventricular muscle cells, as a mediator of mainn electric current conduction between ventricular muscle cells. Myocardial gap junction channels composed by C43 can promote electric coupling through regulation of ion exchange between ventricular muscle cells. Some scholars mutated normal mice gene encoding C43 to form an invalid heterozygote[4], detected conduction velocity of left ventricular myocardial cells decrease by 50%, and the left ventricular myocardial conduction velocity of left ventricular myocardial cells become more uneven[7].

We declare that we have no conflict of interest.

References