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

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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 (APD_{90}) 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 ($P<0.01$). APD_{90} of three myocardial layers in the hypokalemic group were significantly prolonged than those in the control group ($P<0.01$). $\triangle APD_{90}$ 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. $\triangle APD_{90}$ and TDR in the hypokalemic group were significantly higher than those in the control group ($P<0.05$), and the increase in APD_{90} 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 $\triangle Cx43$ 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 myocardial 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.

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2. Materials and methods

2.1. Experimental animals

A total of 30 health rabbits were provided by Experimental Center of Hainan Medical Uinversity, 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, $MgCl_2$ 1, $CaCl_2$ 1.8, NaH_2PO_4 1, HEPES 5, Glucose 10, pH 7.4 set with NaOH. Hypokalemic tyrode's solution (mmol/L): KCl 1.5, NaCl 115, $MgCl_2$ 1, $CaCl_2$ 1.8, NaH_2PO_4 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 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) Electrod making and location for monophasic action potential of the three layers of myocardium. The reference electrode was fixed in the aortic root, simple electrod 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 10 mm from apex, 5 mm from ventricular septal part. Homemade disk adjusted the needle insertion depth. The simple electrode of subendocardial myocardium was inserted in the left ventricular chamber, precisedly adjusted the electrode location, electrode under epicardial myocardium was fixed in the left ventricular of 0.5 mm under the epicardium, the electrode of midcardial was fix 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 monoaction 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[1], from the epicardial surface to the endocardial direction, left ventricular epicardial myocardium tissue was sliced serially 10 times with 20 μ m thick of each section (a total of 200 μ m), 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 concentratio, protein extract was transfered 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 incubationa 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 determinating 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; $\triangle APD_{90}$: APD_{90} difference between left ventricular midcardial and subendocardial myocardium; Transmural dispersion of repolarization (TDR): difference between the longest APD_{90} and shortest APD_{90} among three layers myocardium; Cx43 protein expression in three layers of myocardium; $\triangle$Cx43: 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 hypokalemic 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 ($P<0.01$). $\triangle APD_{90}$ 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.

Observasion indexes	Hypokalemic group	Control group
VFT (V)	7.00 ± 1.49 *	13.40 ± 2.95
Endo–APD_{90} (ms)	246.90 ± 15.04 *	203.10 ± 16.07
Mid–APD_{90} (ms)	285.00 ± 13.77 *	226.80 ± 20.31
Epi–APD_{90} (ms)	232.10 ± 16.59 *	187.70 ± 17.27
$\triangle APD_{90}$ (ms)	38.10 ± 10.29 *	23.70 ± 5.68
TDR (ms)	52.90 ± 14.55 #	36.10 ± 12.44

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.

Observasion indexes	Hypokalemic group	Control group
Endo–Cx43	0.40 ± 0.06 *	0.57 ± 0.07
Mid– Cx43	0.22 ± 0.44 *	0.45 ± 0.05
Epi– Cx43	0.36 ± 0.06 *	0.52 ± 0.08
$\triangle$Cx43	0.18 ± 0.05 #	0.11 ± 0.07

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 autorhythmicity and excitability ofmyocardial 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 torepolarization heterogeneity of 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 ($P < 0.01$). $\triangle APD_{90}$ in hypokalemi group was increased to 38.10 +/− 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 (Cx) is the main protein molecules involved in MVA. Studies have shown that Cx is widely involved in various works of the heart cells and the surface membrane of electrical conduction system. Multiple Cx 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 Cx is GJ protein molecular basis. On the myocardial cell membrane surface, there are many known Cx phenotypes, including Cx40, Cx43 and Cx45, Cx43 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 Cx43 can promote electric coupling through regulation of ion exchange between ventricular muscle cells. Some scholars mutated normal mice gene encoding Cx43 to form an invalid heterozygote[4], detected conduction velocity of left ventricular myocardial cells in mice in vitro. The results show that, after nonsense mutations, left ventricular myocardial Cx43 expression was decreased by 50%, and the left ventricular myocardial cell conduction velocity was decreased by 38%, further confirmed that the Cx43 was current main medium between left ventricular myocardial cells.

Further researches also found that under some pathophysiological conditions, the occurrence of ventricular arrhythmia is also associated with GJ, especially the Cx43 refactoring. Previous studies have shown that after myocardial infarction, left ventricular myocardium Cx43 will arranged abnormally, which can lead to electrical conduction between myocardial cell heterogeneity. This may be an important anatomical basis of ventricular arrhythmias[5]. Reduction of Cx43 content in left ventricular myocardial cells decreases electrical coupling between cells, then make electrical conductivity change in the heart, transmission delay, increase in the discrete degree of monophase action potential duration, and the conduction of left ventricular myocardial tissue become more uneven. Left ventricular myocardial biopsy of patients with severe coronary artery disease in left heart function showed that the occurrence of ventricular arrhythmia is associated with the

Cx43 reduce[6]. For heart failure patients, it also has been confirmed that ventricular arrhythmia is associated with the expression of Cx43, Cx43 cut, which will make coupling loss between myocardial cells, and lead the potential duration more uneven[7].

Our experiment results further show that compared with normal control group, relative grey value of Cx43 in hypokalemic group (0.22 ± 0.04, 0.36 ± 0.06, 0.40 ± 0.06 in epicardial, midcardial, and endocardial tissues respectively) were significantly reduced ($P < 0.001$), and the decrease in midmyocardial tissue, Cx43 protein was more significant. Cx43 relative gray−scale in the epicardial and endocardial tissues were increased compared to normal control group, indicating that hypokalemic perfusion can weaken Cx43 protein expression in the left ventricular myocardial especially in midmyocardial tissues, make the heterogeneity of left ventricular myocardial Cx43 expression more outstanding. This may be an important mechanism for discrete deploration in the left ventricular myocardial cells.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Yamada KA, Kanter EM, Green KG, Saffitz JE. Transmural distribution of connexins in rodent hearts. *J Cardiovascu Electrophysiol* 2004; **15**: 710–714.

[2] Wang SQ. The relationship of potassium content in early acute myocardial infarction with ventricular arrhythmia and infarct location. *Crit Relat Med J* 1998; **4**(4): 155.

[3] Antzelevitch C, Sicouri S, Litovsky SH, Lukas A, Krishnan SC, Di Diego JM, et al. Heterogeneity within the ventricular wall: electophysiology and pharmacology of epicardial, endocardial and M cell. *Circ Res* 1991; **69**(6): 1427–1449.

[4] Thomas SA, Schuessler RB, Berul Cl, Beardslee MA, Beyer EC, Mendelsohn ME, et al. Disparate effects of deficient expression of comiexin43 on atrial and ventricular conduction: evidence for chamber−specific molecular determinants of conduction. *Circulation* 1998; **97**: 686–691.

[5] Wen H, Jiang H, Lu Z, He B, Hu X, Chen J, et al. Carvedilol ameliorates the decreases in connexin 43 and ventricular fibrillation threshold in rats with myocardial infarction. *Tohoku J Exp Med* 2009; **218**: 121–127.

[6] Kaprielian RR, Gunning M, Dupont E, Sheppard MN, Rothery SM, Underwood R, et al. Downregulation of immunodetectable connexin43 and decreased gap junction size in the pathogenesis of chronic hibernation in the human left ventricle. *Circulation* 1998; **97**: 651–660.

[7] Ai X, Zhao W, Pogwizd SM. Connexin43 knockdown or overexpression modulates cell coupling in control and failing rabbit left ventricular myocytes. *Cardiovasc Res* 2010; **85**: 751–762.