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Effect of iron overload on electrophysiology of slow reaction autorhythmic cells of left ventricular outflow tract in guinea pigs

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ABSTRACT

Objective: To investigate the electrophysiology effects and mechanism of iron overload on the slow response autorhythmic cells in the left ventricular outflow tract of guinea pigs.

Methods: Standard microelectrode cell recording techniques were adopted to observe the electrophysiological effects of different concentrations of Fe²⁺ (100 μmol/L, 200 μmol/L) on the left ventricular outflow tract autorhythmic cells. Heart tissues were perfused with FeSO₄ (200 μmol/L) combining with CaCl₂ (4.2 mmol/L), Verapamil, (1 μmol/L), and nickel chloride (200 μmol/L) respectively to observe the influences of these contents on electrophysiology of FeSO₄ (200 μmol/L) on the left ventricular outflow tract autorhythmic cells. **Results:** Fe²⁺ at both 100 μmol/L and 200 μmol/L could change the electrophysiological parameters of the slow response autorhythmic cells of the left ventricular outflow tract in a concentration-dependent manner resulting into decrease in V_{max}, A_{PA} and MDP, slower RPF and VDD, and prolonged APD₅₀ and APD₉₀ (*P* all <0.05). Besides, perfusion of increased Ca²⁺ concentration could partially offset the electrophysiological effects of Fe²⁺ (200 μmol/L). The L-type calcium channel (LTCC) blocker Verapamil (1 μmol/L) could block the electrophysiological effects of Fe²⁺ (200 μmol/L). But the T-type calcium channel (TTCC) blocker nickel chloride (NiCl₂, 200 μmol/L) could not block the electrophysiological effects of Fe²⁺ (200 μmol/L). **Conclusions:** Fe²⁺ can directly change the electrophysiological characteristics of the slow response autorhythmic cells of the left ventricular outflow tract probably through the L-type calcium channel.

1. Introduction

In clinic, there are some diseases such as thalassemia, aplastic anemia and myelodysplastic syndrome, which require long-term blood transfusion. Iron overload caused by blood transfusion can lead to myocardial injury and cause arrhythmia[1-4]. Our previous study found that the slow response autorhythmic cells in the ventricular outflow tract were closely related to the ventricular

arrhythmia[5,6]. There was no report of the electrophysiological effects and its mechanisms of iron overload on the autorhythmic cells in the ventricular outflow tract. In this experiment, exogenous iron was applied to the left ventricular outflow tract tissue to simulate the electrophysiological effects of iron poisoning on the slow response autorhythmic cells in order to explore the mechanism of ventricular arrhythmia induced by iron overload.

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2. Materials and methods

2.1. Experimental animals and reagents

A total of 64 guinea pigs comprising both males and females, weighing 250-350 g, were purchased from the Beijing Jinmuyang Experimental Animal Breeding Co. Ltd. [(animal license No.: SCXK (Beijing) 2010- 0001)]. The guinea pigs were randomly divided into eight groups as following: control group ($n = 8$), FeSO_4 (100 $\mu\text{mol/L}$) group ($n = 8$), FeSO_4 (200 $\mu\text{mol/L}$) group ($n = 8$), CaCl_2 (4.2 mmol/L)+ FeSO_4 (200 $\mu\text{mol/L}$) group ($n = 8$), Verapamil (1 $\mu\text{mol/L}$) group ($n = 8$), Verapamil (1 $\mu\text{mol/L}$)+ FeSO_4 (200 $\mu\text{mol/L}$) group ($n = 8$), nickel chloride (200 $\mu\text{mol/L}$) group ($n = 8$), and nickel chloride (200 $\mu\text{mol/L}$)+ FeSO_4 (200 $\mu\text{mol/L}$) group ($n = 8$). $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was produced by Tianjin Dingshengxin Chemical Co. Ltd. Verapamil by BioMol company. Nickel chloride ($\text{NiCl}_2 \cdot 7\text{H}_2\text{O}$) by Tianjin Kaixin Chemical Industry Co. Ltd.

2.2. Preparation of specimen

After the guinea pigs were anesthetized with ethyl carbamate, their chest were cut open and hearts removed. The hearts were quickly placed in O_2 saturated modified Locke solution. The tissue specimen of the left ventricular outflow tract was made^[7-9] and fixed with stainless steel needle on the silicone rubber in the perfusion chamber (1.5 cm \times 2 cm, the volume is about 4 mL). The O_2 saturated modified Locke solution (NaCl 157 mmol/L, KCl 5.6 mmol/L, CaCl_2 2.1 mmol/L, NaHCO_3 1.8 mmol/L, glucose 5.6 mmol/L, and PH7.3 to 7.4) were perfused at constant temperature of (35 \pm 1) $^\circ\text{C}$ and constant speed 10 mL/min. Animal breeding, care and all experiment procedures were performed in adherence to guidelines by Hebei North University animal experiment center and approved by Animal Ethics Committee.

2.3. Potential guidance

After the glass microelectrode was filled with the saturated KCl electrode solution, the DC resistance is 10-20 M Ω . The electrode was inserted into the lower part near the middle of the right and posterior flap. In most cases, the spontaneous slow action potential could be recorded directly by this way; otherwise the stimulation electrodes were placed at the heart tissue away from the valve in square wave stimulation of 2 ms, 1 Hz, and twice threshold intensity (YC-2 stimulator by Chengdu Instrument Factory); stimulation time ranged from a few seconds to a few minutes until a stable induced spontaneous rhythm appearing, stop stimulation began the experiment. The spontaneous slow reaction potential was amplified by SWF-1B type microelectrode amplifier (Chengdu instrument factory), and the results were input into a microcomputer by

RM6280C multi-channel physiological signal acquisition system (Chengdu Instrument Factory) to display electrical signals, and the parameters of spontaneous slow response potential to be analyzed.

2.4. Index observation

The following indexed were observed: Maximal diastolic potential (MDP), amplitude of action potential (APA), maximal rate of depolarization (V_{max}), velocity of diastolic depolarization (VDD), rate of pacemaker firing (RPF), 50% and 90% of duration of action potential (APD_{50} and APD_{90}).

2.5. Experimental process

Perfusion was conducted with the saturated O_2 modified Locke liquid, and when the spontaneous rhythm stabilized for 20 min, a set of normal slow spontaneous potential was collected as control group. Then, perfusion was conducted with drug of different concentrations and saturated O_2 modified Locke liquid. Potential changes were recorded at 0.5, 1, 2, 5 min, respectively after each perfusion. After each observation of the drug effect, the modified Locke solution saturated with O_2 was applied to washout for 20 min in order to observe the recovery of spontaneous slow reaction potential.

2.6. Experimental scheme

2.6.1. The electrophysiological effects of Fe^{2+} on the Slow reaction autorhythmic cells of the left ventricular outflow tract

Left ventricular outflow tract tissue was perfused with modified Locke solution containing FeSO_4 (100 $\mu\text{mol/L}$) and FeSO_4 (200 $\mu\text{mol/L}$), respectively to observe different concentrations of FeSO_4 on the electrophysiological changes of the slow reaction autorhythmic cells of the left ventricular outflow tract.

2.6.2. Effects of improved Ca^{2+} concentration in perfusion fluid on the electrophysiological effect of FeSO_4 (200 $\mu\text{mol/L}$)

The left ventricular outflow tract tissue was perfused with Locke solution, in which doubled concentration of Ca^{2+} (CaCl_2 , 4.2 mmol/L) and FeSO_4 (200 $\mu\text{mol/L}$) were added to observe the influences of high concentration of Ca^{2+} on electrophysiology of FeSO_4 (200 $\mu\text{mol/L}$).

2.6.3. Verapamil's effects on electrophysiological effect of FeSO_4 (200 $\mu\text{mol/L}$)

The left ventricular outflow tract tissue was perfused with Locke solution containing Verapamil (1 $\mu\text{mol/L}$) and FeSO_4 (200 $\mu\text{mol/L}$) in order to observe the effects of Verapamil on the electrophysiological effects of FeSO_4 (200 $\mu\text{mol/L}$).

2.6.4. Effects of nickel chloride on electrophysiological effects of FeSO₄ (200 μmol/L)

The left ventricular outflow tract tissue was perfused with Locke solution containing nickel chloride (200 μmol/L) and FeSO₄ (200 μmol/L) to observe the influences of nickel chloride on the electrophysiological effects of Fe²⁺ (200 μmol/L).

2.7. Statistical analysis

Software SPSS version 16.0 was used for statistical analysis. Measurement data was expressed as Mean ± SD. Self paired *t* test was used for comparison of different indexes before and after treatment. *P*<0.05 was considered as significant difference.

3. Results

3.1 The electrophysiological effects of Fe²⁺ on the Slow reaction autorhythmic cells of the left ventricular outflow tract

FeSO₄ (100, 200 μmol/L) can change the electrophysiological indexes of the slow response autorhythmic cells of the left ventricular

outflow tract in a concentration-dependent manner, make V_{max}, APA and MDP decreased, RPF and VDD slowed, APD₅₀ and APD₉₀ prolonged (*P*<0.05) (Table 1).

3.2. Influences of high concentration of Ca²⁺ on the electrophysiological effects of FeSO₄ (200 μmol/L)

Increased concentration of Ca²⁺ (CaCl₂, 4.2 mmol/L) could partially inhibit the electrophysiological effects of FeSO₄ (200 μmol/L) (Table 2).

3.3. Verapamil's effects on electrophysiological effect of FeSO₄ (200 μmol/L)

The LTCC channel blocker Verapamil (1 μmol/L) could block the electrophysiological effects of FeSO₄ (200 μmol/L) (Table 3).

3.4. Effects of nickel chloride on electrophysiological effect of FeSO₄ (200 μmol/L)

The TTCC channel blocker nickel chloride (NiCl₂, 200 μmol/L) could not block the electrophysiological effects of FeSO₄ (200 μmol/L) (Table 4).

Table 1

The electrophysiological effects of Fe²⁺ on the Slow reaction autorhythmic cells of the left ventricular outflow tract (Mean ± SD, n=8).

Group	VDD (mV/s)	RPF (bpm)	MDP (mV)	V _{max} (V/s)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)
Control	35.46±4.13	128.21±11.37	-57.62±2.63	10.53±1.25	58.69±4.67	142.12±10.81	178.38±11.35
FeSO ₄ (100 μmol/L) (5 min)	30.61±4.02 [*]	105.26±7.29 [*]	-54.28±2.35	8.56±1.58 [*]	52.31±3.25 [*]	159.85±11.32 [*]	192.78±12.86 [*]
FeSO ₄ (200 μmol/L) (5 min)	24.82±2.79 [#]	86.68±6.81 [#]	-50.98±3.62 [#]	6.89±1.16 [#]	45.72±3.38 [#]	191.39±12.14 [#]	229.94±13.13 [#]

^{*}*P*<0.05 vs. control; [#]*P*<0.05 vs. FeSO₄ (100 μmol/L).

Table 2

Effects of high concentration of Ca²⁺ on the electrophysiological effect of FeSO₄ (200 μmol/L) (Mean ± SD, n=8).

Group	VDD (mV/s)	RPF (bpm)	MDP (mV)	V _{max} (V/s)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)
Control	34.91±4.23	127.87±10.34	-56.76±2.77	10.21±1.09	57.88±4.51	141.56±12.29	177.29±12.12
FeSO ₄ (200 μmol/L) (5 min)	24.06±2.68 [*]	85.31±7.61 [*]	-49.81±3.73 [*]	7.01±1.03 [*]	46.83±3.23 [*]	191.41±11.08 [*]	229.81±13.43 [*]
FeSO ₄ (200 μmol/L)+Ca ²⁺ (4.2 mmol/L) (5 min)	31.85±3.26 [#]	110.73±5.25 [#]	53.35±3.22 [#]	8.62±1.17 [#]	53.46±3.06 [#]	163.32±11.33 [#]	195.78±10.68 [#]

^{*}*P*<0.05 vs. control; [#]*P*<0.05 vs. Fe²⁺ (200 μmol/L).

Table 3

Verapamil's effects on electrophysiological effect of FeSO₄ (200 μmol/L) (Mean ± SD, n=8).

Group	VDD (mV/s)	RPF (bpm)	MDP (mV)	V _{max} (V/s)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)
Control	35.67±3.38	129.07±9.56	-58.03±3.89	10.73±1.28	59.03±5.02	140.87±11.23	176.66±13.39
Verapamil (1 μmol/L) (5 min)	15.82±2.79 [*]	66.68±6.81 [*]	-40.98±3.62 [*]	5.89±1.16 [*]	38.72±3.38 [*]	206.39±15.14 [*]	239.94±17.13 [*]
Verapamil(1 μmol/L) + FeSO ₄ (200 μmol/L) (5 min)	15.09±2.93 [*]	65.93±7.08 [*]	-39.13±4.55 [*]	5.01±1.72 [*]	37.96±4.11 [*]	207.62±16.68 [*]	240.39±19.06 [*]

^{*}*P*<0.05 vs. control.

Table 4

Effects of nickel chloride on electrophysiological effects of FeSO₄ (200 μmol/L) (Mean ± SD, n=8).

Group	VDD (mV/s)	RPF (bpm)	MDP (mV)	V _{max} (V/s)	APA (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)
Control	33.83±3.96	125.58±12.39	-55.62±3.28	10.27±1.31	56.71±5.03	139.52±11.66	173.88±12.25
NiCl ₂ (200 μmol/L) (5 min)	14.12±2.64 [*]	64.51±6.63 [*]	54.89±4.76	7.34±1.22 [*]	45.87±4.12 [*]	158.21±13.84 [*]	194.27±14.32 [*]
NiCl ₂ (200 μmol/L)+FeSO ₄ (200 μmol/L) (5 min)	11.25±1.82 [#]	55.36±4.91 [#]	43.2±5.71 [#]	5.21±1.08 [#]	40.07±1.83 [#]	178.28±11.89 [#]	222.38±16.07 [#]

^{*}*P*<0.05 vs. control; [#]*P*<0.05 vs. NiCl₂ (200 μmol/L).

4. Discussion

Repeated blood transfusion, excessive consumption of high iron food and drugs can lead to the occurrence of iron overload, which can cause myocardial damage, arrhythmia and heart failure. Our previous studies showed that the electrophysiological characteristics of ventricular outflow tract resulting into slow response autorhythmic cells were one of the mechanisms of ventricular arrhythmias. In order to detect whether iron overload cause abnormal electrophysiological characteristics of slow response autorhythmic cells in ventricular outflow tract or not, we perfused the left ventricular outflow tract with different concentrations of FeSO_4 and detected changes in slow response action potential parameters of autorhythmic cells. Results showed decreased V_{max} , RPF, VDD and APA, and increased APD_{50} and APD_{90} . MDP decreased too Indicating that iron overload could change the electrophysiological characteristics of slow reaction autorhythmic cells in left ventricular outflow tract. Our previous studies showed that phase 0 depolarization of ion current in slow response autorhythmic cell is mainly calcium influx together with a small amount of sodium ions influx. Repolarization process was the efflux of potassium ions. Phase 4 automatic depolarization is mainly L-type calcium current ($I_{\text{Ca-L}}$) participation and progressive attenuation of potassium efflux. And hyperpolarization-activated inward ion current (I_p) participates in pacemaker current [10,11]. The comparison and analysis of the above-mentioned results concluded that Fe^{2+} may change the electrophysiological characteristics of the slow response cells of the left ventricular outflow tract by reducing the I_{Ca} . It was observed that electrophysiological effects of Fe^{2+} could be partially inhibited by increasing the Ca^{2+} concentration in the perfusion fluid, which further proved that Fe^{2+} plays a role through the calcium channel. Perfusion of LTCC blockers, Verapamil, and Fe^{2+} caused decrease of the electrophysiological effects of Fe^{2+} indicating that Verapamil could inhibit the electrophysiological effects of Fe^{2+} too. However, perfusion of TTCC blockers, nickel chloride could not decrease the electrophysiological characteristics of autorhythmic cells in the left ventricular outflow produced by Fe^{2+} , indicating that nickel chloride could not inhibit the electrophysiological effects of Fe^{2+} . The above-mentioned results showed that Fe^{2+} may change the electrophysiological characteristics of the left ventricular outflow tract through LTCC.

Results of this study showed that when iron overload occurs Fe^{2+} inflow, which goes directly through LTCC into autorhythmic cells in ventricular outflow tract, were in competition with the inflow of calcium ions and then lead to shrank L-type calcium current ($I_{\text{Ca-L}}$). This is probably the mechanism of electrophysiological characteristics of autorhythmic cells of left ventricular outflow tract produced by Fe^{2+} . Under the above-mentioned condition, the left ventricle of the heart are prone to conduction abnormalities, which in turn causes tachycardia arrhythmia. This might be one of the

mechanisms when ventricular arrhythmia occurs at iron overload.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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