Experimental study on the function of cardiomyocytes

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Abstract. This paper aimed at investigating the properties of cultured cardiomyocytes using microcirculatory and molecular technology to culture cardiomyocytes from different parts of the neonate Wistar rat’s heart and record their spontaneous pulsation under time-lapse video microscopy, then analyze their activity and inspect their survival rate and apoptotic rate under natural and nitric oxide conditions. The pulsation frequency in cardiomyocytes of different parts in heart are: 78.5 ± 11.0 beats/min in the atrium, 88.4 ± 6.3 beats/min in the left ventricle, 90.3 ± 7.9 beats/min in the right ventricle and 115.3 ± 11.4 beats/min in the cardiac apex, respectively, with an average frequency of 81.3 beats/min. Different concentrations of nitric oxide showed no effect on the frequency of cardiomyocyte pulsation. The survival rates of the above cardiomyocytes are 96.0%, 95.0%, 95.0%, and 95.3% respectively and 95.0% for the whole heart. The apoptotic rates are 1.3%, 1.1%, 4.8%, and 1.8% respectively and 5.1% for the whole heart. Different concentrations of nitric oxide had no effect on these results. Our study showed that cultured myocardial cells from different parts of the heart displayed various pulsation frequencies, and the frequency of the cardiac apex is the highest while the atrium is lowest. We also found that there is no statistically significant difference in the survival rates and apoptotic rates of different parts of the heart, and that nitric oxide has no effect on the beating frequency, survival rates or apoptotic rates of the cardiomyocytes in vitro.

Keywords: Cardiomyocytes, beating rate, nitric oxide

1. Introduction

Cardiovascular disease is one of the major diseases throughout the world, and myocardial ischemia is the early phenomena of heart disease in clinical trials [1]. Due to the complicated effects of hemodynamics, hormones and neural reactions in vivo, the myocardial ischemic model cannot reflect the effects of some factors or elements during this process accurately, which influences the reliability and accuracy of the research data. Recently, cell culture technology has been widely used in studies of the function of human organs. A large number of tests have shown that cardiomyocytes primarily cultured in vitro could reflect the function of the heart, while cells at neonatal stage are thought to be an effective model for the study of myocardial ischemia [2].

In this study, we used molecular techniques to study the relationship between cardiomyocytes with different beating frequencies, cell apoptosis and cell death under ischemic conditions and also to explore the function of nitric oxide.

The current tendency is to combine research techniques from different medical fields to study the function of cardiomyocytes from different angles [3–5], to expound on the function of cardiomyocytes from a molecular level, reveal the pathophysiological processes that take place in cells during ischemia, thus opening up new ways to prevent heart diseases.

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2. Materials and methods

2.1. Animal and apparatus

We used pure-bred Wistar rats aged 1–2 days (provided by the Institute of Medical Experimental Animals, Chinese Academy of Medical Sciences, Peking Union Medical College, China). We used DMEM/F12 medium (Gibco, USA), Fetal calf serum (Gibco, USA), 5′-BrdU (Sigma, USA), and L-AA (Beijing Chemical Reagents Co., China). As experimental apparatus, we used a Time Lapse Recorder-8050 (National, Japan), a 5000-series television camera (National, Japan), an inverted phase-contrast microscope (Nikon, Japan), a water-jacketed incubator (Forma Scientific Model, USA), monitor (JVC, Japan), and centrifuge (Heraeus, Germany).

2.2. Procedures

2.2.1. Cell cultivation [6,7]

We divided the cardiomyocytes into two groups: Group I consisted of cells from the whole heart, Group II of cells from the atrium, left ventricle, right ventricle and cardiac apex, respectively. After we had cut the whole heart into pieces, we rinsed the pieces with iced PBS several times to clean red blood cells, digested them with 0.08% trypsin for 6 minutes at 37°C and abandoned the supernatant. Then we added another 0.08% trypsin for 6 minutes and translocated the supernatant into a new tube with the same volume of iced DMEM/F12 medium containing 10% FCS to neutralize the trypsin, and digested the remained tissue with the trypsin 8–10 times to reach full digestion. We centrifuged the supernatants for 10 minutes at 1500 rpm and collected the digested cells, observed the living state, then seeded the cells into 25 cm² cultivation flasks, and incubated at 37°C 5% CO₂ air. We collected the medium after 90 minutes, centrifuged for another 10 minutes at 1500 rpm, and the cells left are cardiomyocytes. We added 0.1 mM 5-BrduR into the medium and cultured the cells at 37°C, with 5% CO₂ air. All the above steps were carried out under sterile conditions. Finally, we identified the cardiomyocytes with HE dye and α-actin dye.

2.2.2. Time-lapse video microscopy and image analysis

We added different concentrations of L-AA to the medium, and placed the flask on the stage of the inverted phase-contrast microscope housed inside a temperature-controlled chamber. We recorded the action state of the selected cells using a time-lapse recorder 8050 for a continuous 4 hours, then analyzed the data.

2.2.3. Cell necrosis rate and cell apoptotic rate [8,9]

We blended the 4% Trypan Blue and 1.8% sodium chloride equally to produce 2% Trypan Blue solution, then mixed together a drop of cell suspension and a drop of Trypan Blue, deposited this for 2 minutes and counted 150 cells with the cell arithmometer. For cell apoptotic rats, we referred to the manual from the Beijing Baosai Biological Reagent Co.

3. Results

3.1. The culture and identity of cardiomyocytes

The cardiomyocytes from the whole heart and each part of the neonatal Wistar rats began to grow along the flask after 6 hours’ culture, and their shape changed from circles to polygons. Observed at
normal display speed, we found that some cells exist individually, beat with their immobile frequency and protrude around, while some cells couple together and take on a rhythmic synchronous beating (Figs 1–3).

Figure 2 shows that the cardiomyocytes hold out pseudopod, have 1~2 nuclei agreeing with the hypothesis that cardiomyocytes are polynucleus cells. Figure 3 shows that the α-actin dye is positive, which proves that the cells cultured in this experiment are cardiomyocytes.

Fig. 1. Cardiomyocytes observed under an inverted phase-contrast microscope. (A: 10 × 10; B: 10 × 10.)

Fig. 2. Cardiomyocytes with HE dye. (A: 10 × 10; B: 10 × 10.)

Fig. 3. Cardiomyocytes with α-action dye. (A: 10 × 10; B: 10 × 10.)
3.2. The beating frequency of different cardiomyocytes and the effect of L-AA

Table 1 indicates that there are differences in the beating frequency of cardiomyocytes from different parts of the heart: there are significant statistical differences between cardiac apex and atrium, cardiac apex and left ventricle, cardiac apex and right ventricle \( (P < 0.01) \). There are also statistic differences between atrium and left ventricle and atrium and right ventricle \( (P < 0.05) \), while there is no statistical difference between left and right ventricles. Under normal conditions, different concentrations of L-AA have no effect on the beating frequency of each part of the heart (Table 1).

3.3. The necrosis rate of different cardiomyocytes and the effect of L-AA

Table 2 shows that there are no significant differences in necrosis rate among cardiomyocytes from different parts of the heart under normal conditions, while different concentrations of L-AA have no effect on the necrosis rate of each part of the heart \( (P > 0.05) \). Living cells could not be dyed, while dead cells could be dyed blue by Trypan Blue (Table 2).

<table>
<thead>
<tr>
<th>Normal condition</th>
<th>Arginine (1 mmol/l)</th>
<th>Arginine (5 mmol/l)</th>
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<tr>
<td>Atrium</td>
<td>78.5 ± 11.0</td>
<td>81.5 ± 9.2</td>
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<tr>
<td>Left ventricle</td>
<td>88.4 ± 6.3*</td>
<td>87.6 ± 10.1*</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>90.3 ± 10.9*</td>
<td>93.3 ± 9.2*</td>
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<tr>
<td>Cardiac apex</td>
<td>115.34 ± 1.36**</td>
<td>120.1 ± 9.3**</td>
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<tr>
<td>Whole heart</td>
<td>81.3 ± 7.3</td>
<td>85.0 ± 6.1</td>
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\*\( P < 0.05 \), \*\*\( P < 0.01 \).

Table 2

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<tbody>
<tr>
<td>Atrium</td>
<td>4.0%</td>
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</tr>
<tr>
<td>Left ventricle</td>
<td>5.0%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>5.0%</td>
<td>5.2%</td>
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<tr>
<td>Cardiac apex</td>
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<td>4.8%</td>
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Table 3

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<tr>
<td>Atrium</td>
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<td>Left ventricle</td>
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<tr>
<td>Cardiac apex</td>
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<tr>
<td>Whole heart</td>
<td>5.1%</td>
<td>6.3%</td>
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\( P > 0.05 \).
3.4. The apoptotic rate of different cardiomyocytes and the effect of L-AA

Table 3 shows that there are no significant differences in apoptotic rate among cardiomyocytes from different parts of the heart under normal conditions, while different concentrations of L-AA have no effect on the apoptotic rate of each part of the heart \( (P > 0.05) \) (Table 3).

4. Discussion

Cardiomyocytes consist of working cells and autorhythmic cells. Although working cells have excitability and contractility, they have no autonomy, and cannot beat spontaneously without extraneous stimulation. Autorhythmic cells have autonomy, thus could beat spontaneously without extraneous stimulation. Because we have not distinguished working cells from autorhythmic cells, the beating cells here are a mix of these two kinds of cells.

As we know, the autorhythmic signal starts from the sinoatrial node, and transfers to the Purkinje system via the atrioventricular node and atrioventricular bundle. We found in our experiment that the beating frequency of cardiomyocytes in the atrium is slowest in the original part of the signal and fastest in the final parts of signal transfer, so that there is a significant statistical difference between different parts of the heart. We proposed that the differences in beating frequency of cardiomyocytes in our experiment may be related to the metabolic level of cells per se, which might influence the physiological function of cardiomyocytes. The cardiac apex lies in the bottom of the ventricle which has a high metabolism, so the beating frequency of this area is highest in our experiment; the atrium lies at the top of the ventriculus with a low metabolism, so the beating frequency of this area is lowest.

Nitric oxide is a new kind of signal transfer gas which exerts a profound influence on the cardiovascular system, immunity system and nerve system [10]. In a normal heart, nitric oxide is synthesized by endothelial NO synthase through a series of reactions. NO can then activate Gc, increase the level of cGMP and relax the smooth muscle. Moreover, it has been found that nitric oxide participates in every phase of ischemic preconditioning as a signal transfer molecule under ischemic/reperfusion conditions, playing an important role in this process [11–13].

As we know, myocardial ischemic injury is the early presentation of heart disease in clinical trials. It can induce not only cell necrosis but also cell apoptosis. As one means of death, apoptosis occurs mainly in the left ventricle and may lead to ventricle dysfunction or heart failure [14], suggesting that the apoptosis of cardiomyocytes might be one means of the loss of cardiomyocytes in heart failure [14,15]. Although the active factor for apoptosis is not yet clear, we believe that this process must involve many factors that work in coordination [16]. We found in our experiment that, under normal conditions, there is no significant difference in necrosis rate and apoptotic rate among cardiomyocytes from different parts of the heart, while different concentrations of L-AA have no effect on those results; but under ischemic conditions, nitric oxide can enhance the tolerance of the myocardium to long-term ischemic stress, that is, ischemic preconditioning. It can reduce the severity of ischemia and lethal arrhythmia, recover heart function, delay myocardial infarction and reduce infarcted heart size [17–19]. We speculate that these results may help to clarify the effect of nitric oxide as an endogeneous signal molecule that can regulate the cardiovascular function of cardiomyocytes under ischemic conditions.

Acknowledgement

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References


