CHANGES OF CONTRACTION RHYTHM DURING ISCHEMIA/REPERFUSION IN CULTURED CARDIAC MYOCYTES

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Abstract- **We analyzed the changes in fluctuation of contraction rhythm of cultured cardiac myocytes caused by simulated ischemia/reperfusion. Under the ischemia-like condition, the contraction intervals and the fluctuation of them increased as the ischemic time increased, and then myocytes stopped contraction by 3 hrs of the ischemia in many cases. The fluctuation temporarily decreased after reoxygenation compared with that before simulated ischemia, and then returned to the pre-ischemic level. The intracellular pH ($[pH]_i$) of myocytes gradually decreased through the ischemic period. However, the myocytes in an aggregate exhibited synchronized contraction with each other throughout the ischemic period. These results suggested that the increase in the fluctuation of contraction intervals during simulated ischemia was not caused by the decrease in the electrical interaction among myocytes and the extent of $[pH]_i$ decrease during ischemia was not so great that resulted in the electrical uncoupling of myocytes.**

I. INTRODUCTION

In the heart, a large amount of energy is consumed for the contraction activity of the cardiac myocytes. Therefore, deprivation of oxygen and metabolic substrates caused by cardiac ischemia directly affects the contraction activity at first, and finally results in the cardiac cell death if such an ischemic condition lasts for a long time. Cardiac arrhythmia caused by ischemia/reperfusion is an index of the pathological changes of the heart. Cultured cardiac myocytes of neonatal rat ventricle exhibit spontaneous contraction. Many studies concerning biochemical reactions of cultured cardiac myocytes during ischemia/reperfusion have been done so far. However, few studies have focused on the fluctuations of contraction rhythm during ischemia/reperfusion. This study aims at elucidating the mechanisms responsible for sinus arrhythmias caused by ischemia/reperfusion. For this, we used spontaneously contracting cultured cardiac myocytes from neonatal rats as an experimental model of sinuatrial node cells. We investigated how and why fluctuations in the contraction rhythm of cultured cardiac myocytes vary during simulated ischemia/reperfusion.

II. METHODOLOGY

A. Cell Culture

By the method proposed by Orita et al. [1], cardiac myocytes were prepared from 1-3 day old neonatal rat ventricles removed after decapitation. The ventricles were minced with scissors into fragments to be digested with 0.1 % collagenase (Wako Pure Chemical, Japan) at 37 °C for 15 min. The cells were resuspended in MCDB107 (Research Institute for the Functional Peptides, Japan). Finally, isolated myocytes were cultured at a density of about 3.0×10^5 cells/ml in a culture flask (3018, FALCON) at 37 °C, in 5 % CO_2 and 95 % air.

B. Ischemia/Reperfusion Protocol

The ischemiac condition was simulated by changing medium from normal to glucose-free MCDB107 and filling with N_2 gas in the culture flask. Reperfusion was also simulated by reoxygenation and changing the glucose-free medium to normal one. Recordings of contraction activity of myocytes were performed before (control), 1 hr and 3 hr after ischemia. Recordings were also done just and 1 hr after reperfusion.

C. Image Analysis

Images of beating myocytes were recorded with a CCD camera (C5985, Hamamatsu Photonics, Japan) through a phase-contrast microscope (IX-70, OLYMPUS, Japan). A small area (a square of about 20 pixels) of the myocyte was selected from the recorded images, and the video signals were digitized to an 8-bit number every video frame (30 frames/s) by a video capture board in a personal computer (Power Macintosh 7500/100, Apple, USA) for 5 min. The temporal variation of brightness in the selected area, reflecting the contraction rhythm of the cardiac myocyte, was calculated. Beat-to-beat intervals and the coefficient of variation were also calculated from the intervals between peaks of the data.

D. Measurement of Intracellular pH

Intracellular pH was measured using fluorescent dye, BCECF-AM (Dojin-do, Japan) [2]. Myocytes were loaded with 5 μM BCECF-AM in MCDB107 for 30 min at 37 °C and then washed out. The BCECF fluorescence was alternately excited with 490 and 450 nm wavelength lights. Emissions at 515 nm were measured for each excitation wavelength every 1 min through inverted microscope with CCD camera (C4880-80, Hamamatsu Photonics, Japan). The ratio of emission intensities at 490 and 450 nm excitation wavelengths was calculated and converted to a linear pH calibration scale. The pH calibration was done at the end of each experiment by the nigericin technique [3].

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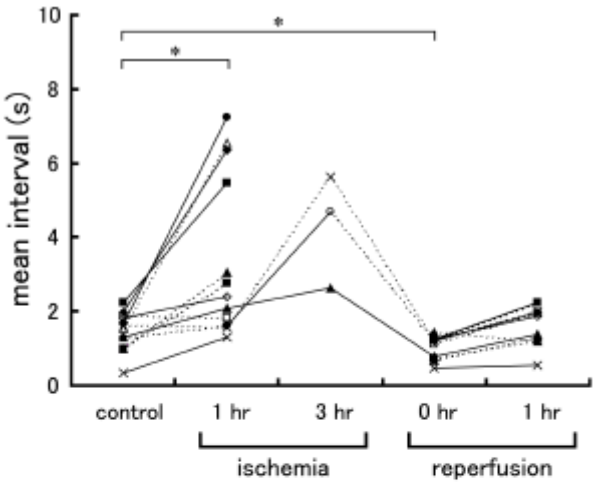

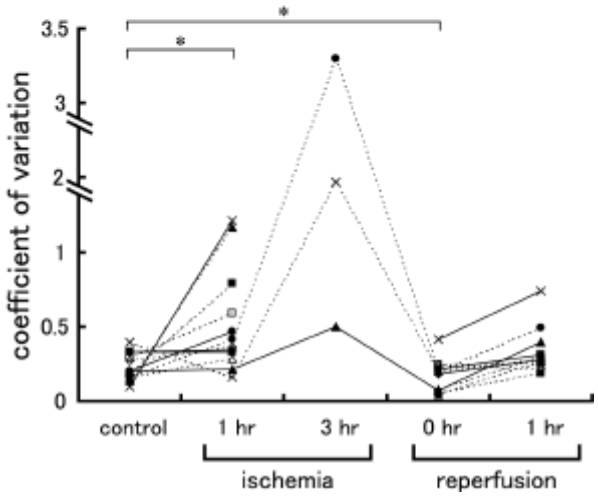

Fig. 1. Changes in mean contraction interval and coefficient of variation of them during and after simulated ischemia. Astarisks (*) indicate statistical significance ($p<0.01$) compared to the control.

III. RESULTS AND DISCUSSION

Figure 1 shows changes in the mean contraction interval and the coefficient of variation during ischemia and reperfusion. Under the ischemia-like condition, the contraction intervals and the fluctuation of them increased as the ischemic time increased. In many cases, myocytes stopped contraction by 3 hrs of the ischemia-like condition. When the contraction of myocytes persisted at that time, the mean contraction interval increased further. After reoxygenation, the fluctuation temporarily decreased compared with that before simulated ischemia, and then returned to the pre-ischemic level.

It is well known that myocardial ischemia diminishes intracellular pH ($[pH]_i$) of myocytes [4]. The decline of $[pH]_i$ leads to a decrease in gap junctional conductance [5-7]. In our previous study using cultured cardiac myocytes, the fluctuation of contraction intervals decreased as the strength of electrical coupling among cardiac myocytes increased [8]. Therefore, there is a possibility that the increase in the fluctuation during simulated ischemia observed in this study was caused by the decrease of the strength of coupling among myocytes. We then testified this possibility.

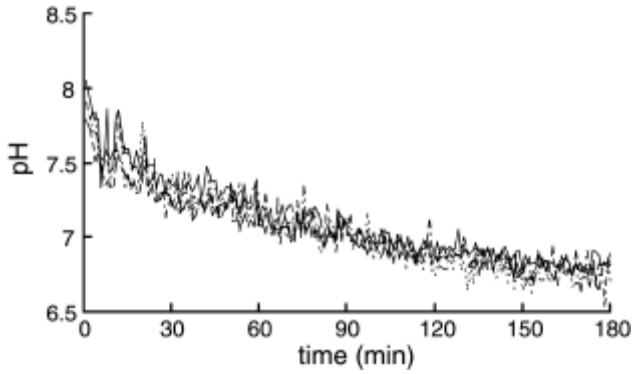

Fig. 2. Temporal change of intracellular pH of cardiac myocytes during simulated ischemia.

Figure 2 shows temporal changes in $[pH]_i$ of 4 myocytes. The $[pH]_i$ of all the myocytes gradually decreased through the ischemic period. The fluctuation of contraction intervals significantly increased at 1 hr of ischemia (Fig. 1). The $[pH]_i$ was about 7.0 at that time. Gap junctions among cultured cardiac myocytes possibly remains open at this value of $[pH]_i$ [6, 7].

To confirm whether gap junctions remained open during simulated ischemia, we analyzed the synchronization of contraction rhythms between aggregating myocytes. Figure 3 shows the cross correlograms between contraction activities of the myocytes in an aggregate. The peak value of the correlograms before and after 1 hr of ischemia was about 1.0, indicating that the myocytes in the aggregate exhibited synchronized contraction throughout the ischemic period. The present findings suggested that the increase in the fluctuation of contraction intervals during simulated ischemia was not caused by the decrease in the electrical interaction among myocytes and the extent of $[pH]_i$ decrease during ischemia was not so great that resulted in the electrical uncoupling of myocytes.

IV. CONCLUSION

The fluctuation of contraction intervals of cultured cardiac myocytes increased under the simulated ischemia. The extent of $[pH]_i$ decrease during ischemia was not so great that resulted in the electrical uncoupling of myocytes. Therefore the increase in the fluctuation during ischemia was not caused by the decrease in the electrical interaction among myocytes.

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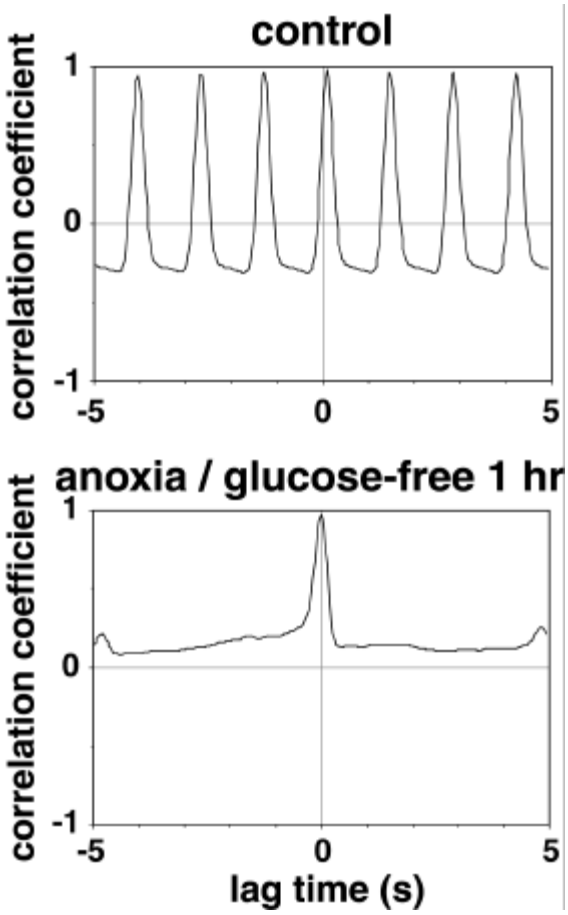

Fig. 3. Cross correlogram between contraction activities of two myocytes in an aggregate.

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