EFFECTS OF HYPOXIA AND REOXYGENATION ON TISSUE ATP LEVEL AND ELECTRICAL AND MECHANICAL FUNCTION OF ISOLATED GUINEA PIG VENTRICULAR MUSCLES.

TOSHIO ASANO,** KOKI SHIGENOBU AND YUTAKA KASUYA**

Department of Pharmacology, Pharmaceutical Research and Development Laboratory, Asahi Chemical Ind. Co., Ltd.,* 6-2700 Asahi-machi, Nobeoka, Miyazaki, 882, Japan and Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo,** Bunkyo-ku, Tokyo, 113, Japan

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The myocardial ATP level in guinea pig ventricular muscles was determined during hypoxia and reoxygenation and compared with the cardiac electrical and mechanical function. The action potential duration (APD), contractile force (CF) and tissue ATP level were depressed gradually depending on the N₂ concentration in the medium. Time course studies revealed that the membrane electrical activity and the ATP content did not show parallel evolution, although changes in CF coincided with changes in ATP level.

Keywords — cardiac action potential; heart muscle; action potential duration; right ventricular papillary muscle; hypoxia; ATP

INTRODUCTION

Hypoxia or ischemia reduces the action potential duration (APD), the contractile force (CF) and the tissue ATP level of guinea pig ventricular muscle. Changes in APD and CF have been correlated with the availability of ATP derived from glycolysis. On the other hand, it has been suggested that the effect of hypoxia on the inhibition of the electrical and mechanical function may be due not only to a decrease in the ATP level but also to other factors. The effects of changes in the tissue ATP level on cardiac electrical and mechanical function in isolated myocardium remains unclear. Therefore, we carried out the present in vitro study to determine the effects of hypoxia and reoxygenation on cardiac function and the tissue ATP level in guinea pigs.

MATERIALS AND METHODS

Male guinea pigs (250—350 g) were killed by a sharp blow and the heart was quickly removed. Papillary muscle preparations obtained from the right ventricle were mounted horizontally in an organ bath containing 30 ml of physiological salt solution (PSS) maintained at 37 ± 0.5°C and aerated by bubbling with an oxygen mixture (95% O₂; 5% CO₂). The PSS had the following composition (mM): NaCl 135, KCl 5, CaCl₂ 2, MgCl₂ 1, NaHCO₃ 15, glucose 5.5. The muscle was stimulated at 1 Hz through platinum electrodes. The electrical and mechanical activities were recorded simultaneously by the procedure described in an earlier report. ATP content was determined in right ventricular strips. A ventricular muscle was placed in the same organ bath. Electrical stimulation with 5 ms rectangular current pulses was applied via bipolar platinum electrodes at a frequency of 1 Hz. The stimulus intensity was 10 times greater than the threshold strength to produce the action potential. A small piece of ventricular muscle was dissected out during hypoxia and reoxygenation and rapidly frozen by immersing it in liquid
nitrogen. After 10-min centrifugation at 3000 rpm, the precipitate was dissolved with 1N NaOH to determine the protein content as described by Bradford.\(^7\) The potassium perchlorate precipitate was removed by further centrifugation and the ATP content of the neutralized supernatant was determined by the luciferin-luciferase method.\(^8\)

**RESULTS**

We subjected isolated guinea pig ventricular muscles to extensive, moderate or mild hypoxia and found that the changes in APD, CF and the tissue ATP level were dependent on the N\(_2\) concentration of the bathing solution (Fig. 1). Three different hypoxic conditions were induced as described in the figure legend. Hypoxia produced a marked shortening of APD without inducing significant effects in the resting and overshoot potential or + V max as described by us previously.\(^6\) These results are in good agreement with data of others.\(^2,9\) When the bath was bubbled with 95% N\(_2\):5% CO\(_2\) (extensive hypoxia), APD50, APD90, CF and the ATP level declined to 25, 35, 0 and 20% of the control in 60 min, respectively. These values decreased more slowly and to a lesser extent when the N\(_2\) concentration of the hypoxic buffer was further decreased. When time courses were compared during extensive hypoxia, the decrease in CF and the ATP level was found to precede the decrease in APD; CF declined along with the ATP level. We obtained similar results during extensive, moderate, mild hypoxia. Upon varying the N\(_2\) concentration in the gas mixture bubbled through the perfusion buffer, significant differences were observed in the rates of decline in APD, CF and ATP. The decline in CF and ATP was significantly faster than the decline in APD50 and APD90 during mild hypoxia; the decline in CF always coincided with the decline in the ATP level.

In an attempt to relate more clearly the tissue ATP level, APD and CF, we carried out experiments in which these parameters were measured during reoxygenation after extensive hypoxia.

**FIG. 1. Time Course of Hypoxia Inhibition of APD50, APD90, CF and the ATP Level in Isolated Guinea Pig Ventricular Muscles Bathed in the Presence of Three Different N\(_2\) Concentrations Obtained by Mixing an Oxygen-mixture (95% O\(_2\): 5% CO\(_2\)) and a Nitrogen-mixture (95% N\(_2\): 5% CO\(_2\)) in Different Ratios**

Extensive hypoxia = nitrogen mixture only, moderate hypoxia = 2:1 ratio of the oxygen and nitrogen mixtures; mild hypoxia = 1:1 ratio of the oxygen and nitrogen mixtures. APD50 and APD90 represent the time required for the membrane potential to repolarize to the level of 50 and 90%, respectively, of the total height of the action potential. In several experiments, the pH of the solution was continuously monitored and maintained at 7.4 throughout the experiment. Note that the decrease in APD50 and APD90 was always preceded by a decrease in CF and the ATP level. These values were not significantly altered during 3 h in the normal condition (oxygen mixture only). The number of experiments in shown in parentheses. Vertical bars indicate the S.E. of the mean.

- APD 90, ○ APD 50, ▲ ATP, □ CF.
As shown in Fig. 2, upon returning to the control condition after 60 min of extensive hypoxia, there was a marked and rapid increase in APD50 and APD90. After 15 min of reoxygenation, both APD50 and APD90 quickly returned to the control level; on the other hand, only small increases in CF and the ATP level were observed. The decline in APD was relatively slow, whereas the restoration of APD was rapid.

**DISCUSSION**

By utilizing three different degrees of hypoxic conditions, we were able to show that changes in APD, CF and tissue ATP levels are fairly dependent upon the N₂ concentration of the bathing solution.

In our study, APD and the ATP content do not show parallel evolution. This result is apparently not in agreement with the working hypothesis proposed by others¹,²) that the cardiac action potential duration is dependent on the slow inward current and that this current can be influenced by metabolism. Two possibilities may be considered for explaining the results. First, only a small amount of ATP may be enough to maintain electrical activity of the cell membrane, accounting for the slow decrease in APD during mild and moderate hypoxia. Second, certain factor(s) other than changes in the ATP level may influence the decline and recovery of APD. The latter explanation has been favoured in previous reports.³⁻⁵) However, we have no other data to prove or disprove this. Further study on this point is needed.

The relative decreases in ATP appeared to be less than the decrease in CF. However, the depression and recovery of the tissue ATP level coincided with the depression and recovery of CF under three hypoxic conditions and upon reoxygenation. These findings are similar to those of others¹,²,⁸) and suggest that hypoxia may induce a severe decline in the ATP level in some compartments, leading to severe CF depression. Although no complete explanation for the various effects of hypoxia on ventricular muscle is presently available, it seems likely that changes in CF are closely related to changes in the ATP level.

**FIG. 2. Restoration of APD50 and APD90 and Accompanying Partial Recovery of CF and the ATP Level in Guinea Pig Ventricular Muscles during Reoxygenation**

Reoxygenation was begun 60 min after extensive hypoxia. While APD50 and APD90 fully recovered to the control level within 15 min, CF and the ATP level recovered only partially. Vertical bars indicate the S.E. of the mean from 6 experiments.

- APD90, ○ APD50, □ CF.

**REFERENCES**


