Experimental Studies

Effects of Hypoxia and Reoxygenation on Steady-state and Potentiated Contractions in Papillary Muscle of Guinea Pigs

Jun Asayama, M.D., Tetsuya Tatsumi, M.D., Hiroshi Miyazaki, M.D., Yasuhiko Yamahara, M.D., Takashi Matsumoto, M.D., Ryuta Sakai, M.D., Miho Inoue, M.D., Itsuki Omori, M.D., Daisuke Inoue, M.D., and Masao Nakagawa, M.D.

SUMMARY
We studied the effects of hypoxia and reoxygenation on steady-state contractions and potentiated contractions of papillary muscles of guinea pigs. Isometric tension was measured while 120 min periods of hypoxia and reoxygenation were repeated twice. Reoxygenation after the first period of hypoxia induced a gradual recovery in steady-state contractions and a rapid recovery in potentiated contractions from the first hypoxia-induced contractile depression. After the second period of hypoxia, steady-state and potentiated contractions decreased progressively. During the second period of reoxygenation, the recovery of steady-state and potentiated contractions was very poor and the marked elevation of diastolic tension did not decrease. There were no good correlations between hypoxic depression just before reoxygenation and the recovery of both potentiated contraction and steady-state contraction at 120 min of reoxygenation. The recovery from the hypoxia-induced depression was poor in the preparations with marked elevation in diastolic tension.

From these findings, we conclude that hypoxia-induced depression is progressively worsened by an additional episode of hypoxia and that diastolic tension is one of the determinants of the low contractile level achieved by steady-state and potentiated contractions in the severely hypoxic state. The degree of hypoxia-induced depression does not determine redevelopment of force with reoxygenation.

Key Words:
Hypoxia  Reoxygenation  Steady-state contraction  Potentiated contraction

From the Second Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto.
Address for correspondence: Jun Asayama, M.D., Second Department of Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan.
Received for publication May 24, 1991.
Accepted August 6, 1991.

73
Reperfusion after a brief period of coronary artery occlusion results in delayed recovery of regional function despite the absence of myocardial necrosis.\textsuperscript{1,2} The mechanism for this transitory stunned myocardium remains unclear. However, it is now well recognized that the positive inotropic effect of catecholamines and Ca\textsuperscript{2+},\textsuperscript{3,4} and postextrasystolic potentiation (PESP)\textsuperscript{5} is not abolished in postischemic stunned myocardium.

The purpose of this study was to investigate whether the degree of hypoxia-induced contractile depression determines redevelopment of force following reoxygenation, and whether an additional period of hypoxia in the incompletely recovered muscle deteriorates force in hypoxic muscle.

Previously, we reported that the maximum PESP ("potentiated contraction") of guinea pig papillary muscles is obtained at a postextrasystolic interval of 3 to 6 sec.\textsuperscript{6,7} Then, we used potentiated contraction and steady-state contraction as parameters of contractile function during hypoxia and reoxygenation.

\textbf{Materials and Methods}

\textbf{Animal preparation}

Thirteen guinea pigs of either sex, weighing between 300 and 350 g, were used. Isolated papillary muscles were prepared as previously described.\textsuperscript{6,7} Briefly, the heart was quickly removed and placed in normal physiological saline solution (see below) at room temperature.\textsuperscript{6-8} Papillary muscles (1-1.5 mm in diameter) were isolated from the right ventricle under a microscope, and a small loop of virgin silk suture was tied on each end. Muscles were mounted horizontally in a 2-ml experimental chamber, with one end fixed and the other fastened to an isometric force transducer (ME-4021, MEC Commercial). Muscles were stretched to about 120\% of slack length.

\textbf{Stimulation protocol}

Muscles were paced at a basic cycle length of 1.5 sec using field stimulation applied through a bipolar electrode positioned on the surface of the muscle. An extra-stimulus with a coupling interval of 0.6 sec was given after trains of 37 pulses, a postextrasystolic interval of 5.4 sec was set, and the programmed trains were repeated using a programmable pulse generator (Cardiac stimulator BC-02A, Fukuda Denshi). The duration of one cycle was just 1 min as shown in Fig. 1. (The postextrasystolic interval of 5.4 sec was chosen since the maximum potentiated contraction was obtained at a postextrasystolic interval of 3 to 6 sec in the condition of a basic cycle length
Solutions
Preparations were superfused at a constant flow rate (Micro tube pump MP-3, EYELA) with prewarmed oxygenated solutions. The control superfusate was a normal Tyrode’s solution containing (in mM) NaCl 137, KCl 4, CaCl$_2$ 1.8, MgCl$_2$ 1, NaH$_2$PO$_4$ 0.33, Na$_2$HPO$_4$ 2.44 and glucose 12, bubbled with 100% O$_2$ at pH=7.4 and 30°C.

Protocol
The influence of a combination of hypoxia and the absence of glucose on steady-state contractions and potentiated contractions was investigated after an equilibration period of at least 60 min in normal Tyrode’s solution. The hypoxic solution was prepared by gassing the Tyrode’s solution with 100% N$_2$. Muscles remained for 120 min in the hypoxic glucose-free solution before 120 min reoxygenation in the presence of glucose. The cycle of 120 min of hypoxia and reoxygenation was repeated in 13 rats.

Data analysis and measurements
Data were subjected to analysis of variance followed by Duncan’s multiple range test to determine statistical significance. Statistical analysis for paired data was performed with Student’s t-test. Values are presented as mean±SEM. To allow comparisons between papillary muscles of different sizes, the tension of either steady-state contraction or potentiated contraction at baseline was arbitrarily assigned a value of 100%. Before going into results, it is necessary to define % hypoxic depression of contraction, % recovery of contraction from hypoxia-induced depression, and % resting tension, terms which were used for convenience. As can be seen from Fig. 1,
% hypoxic depression of steady-state contraction and % hypoxic depression of potentiated contraction are calculated by \((a-c)/a \times 100\%\) or \((a-i)/a \times 100\%\), and \((b-d)/b \times 100\%\) or \((b-j)/b \times 100\%\), respectively. Percent recovery of steady-state contraction and % recovery of potentiated contraction are calculated by \((f-c)/(a-c) \times 100\%\) or \((l-i)/(a-i) \times 100\%\), and \((g-d)/(b-d) \times 100\%\) or \((m-j)/(b-j) \times 100\%\), respectively. Percent resting tension was calculated by \((e/a) \times 100\%\), \((h/a) \times 100\%\), \((k/a) \times 100\%\) or \((n/a) \times 100\%\).

**Results**

Figure 2 shows a representative time course of developed tension. Figure 3 shows the relative changes in steady-state contractions, potentiated contractions, and diastolic tension in 13 preparations. Hypoxia induced a gradual decrease in steady-state contractions and potentiated contractions and an increase in diastolic tension. Reoxygenation induced a rapid decrease in diastolic tension, a gradual recovery in steady-state contractions, and a rapid recovery in potentiated contractions. These time-dependent changes of steady-state contractions, potentiated contractions and diastolic tension were significant (F ratio; 114.8, 93.0, 13.2). The second period of hypoxia progressively worsened the recovery of steady-state and potentiated contractions at 120 min of reoxygenation. At 120 min of hypoxia, there were no statistical differences in decrease in tension between the two types of contractions. After reoxygenation the recovery of potentiated contractions was better than that of steady-state contractions (p<0.01 at 120 min). During the second period of hypoxia, the contractions of both types decreased severely to the same degree (N.S. at 120 min). During the second period of reoxygenation, the recovery of potentiated contractions was better.
Fig. 3. Time course for the hypoxia-induced changes and reoxygenation-induced changes in the strength of the regular contractions (reg.: ●), potentiated contractions (P.C.: ○) and diastolic tension (rest.: -●-●-). Data points are mean ± SEM from 13 experiments. Analysis of variance was performed on 5 time points every 120 min. In analyzing data within each time point with $f_1 = 4$ and $f_2 = 60$, F ratios equal to or greater than 3.65 are significant at 1%. Results of Duncan's test in two adjacent time points shown. n.s. = not significant. ★★ P<0.001, ★ p<0.01, ☆ p<0.05; steady-state regular contraction vs. potentiated contraction.

than that of steady-state contractions (p<0.01 at 120 min), though the redevelopment in contractions of both types was very poor (19±5%, 14±4%).

Figures 4 and 5 show the correlation between the recovery of contraction after reoxygenation and the index representing hypoxia-induced depression such as % hypoxic depression and % diastolic tension. There were no significant correlations between the hypoxia-induced depression just before reoxygenation and the recovery at 120 min of reoxygenation. During the first 120 min of hypoxia, % depression of steady-state contractions and % depression of potentiated contractions were 73±4% and 76±4%, respectively. In the second 120 min of hypoxia, % depression of steady-state contractions and % depression of potentiated contractions were 97±1% and 97±1%, respectively. After the first episode of hypoxia, % recovery of steady-state contractions and potentiated contractions were 49±6% and 62±7%. After the additional episode of hypoxia, % recovery of steady-state contractions and potentiated contractions were 12±4% and 17±6%, respectively. Percent recovery of contractions was less than 20% in the
Fig. 4. Relationship between percent hypoxic depression of contraction just before reoxygenation and percent recovery of contraction at 120 min of reoxygenation. Closed symbols: steady-state contractions, open symbols: potentiated contractions, circles: the first hypoxia or reoxygenation, triangles: the second hypoxia or reoxygenation.

Fig. 5. Relationship between percent diastolic tension at hypoxia just before reoxygenation and percent recovery of contraction at 120 min of reoxygenation. Symbols shown are as those in Fig. 4.

preparations showing marked elevation of % diastolic tension of over 120%. (One of 13 preparations after the first reoxygenation period and 7 of 13 preparations after the second reoxygenation period showed % diastolic tension of over 120%.)
DISCUSSION

The prolonged contractile dysfunction, which persists for several hours to many days, observed following periods of myocardial ischemia in the absence of irreversible cellular injury has been termed "stunned myocardium". It is well known that postischemic low Ca²⁺ perfusion reduces reperfusion damage. However, it is unclear whether the stunned myocardium occurs during hypoxia or on reoxygenation since without reoxygenation no recovery is possible at all. From the finding that there was no good relation between the degree of recovery in contractile function at 120 min of reoxygenation and the degree of hypoxic depression, as shown in Fig. 4, it seems that hypoxia alone does not regulate the degree of recovery from hypoxia-induced contractile failure, which is in good agreement with our previous findings in rat papillary muscles.

It is well known that catecholamines and postextrasystolic beats improve the function of stunned myocardium. Ito et al demonstrated that postischemic myocardium retains a normal contractile reserve in response to calcium in a dose-dependent manner. It is now known that PESP depends upon the sarcoplasmic reticulum (SR) function. The potentiated contraction used in the present study, which is the maximum PESP, seems to be dependent on SR function since potentiated contractions are inhibited by caffeine. Schouten et al concluded that the maximum force in trabeculae of rat hearts is determined by the capacity of the SR, from the findings that high [Ca²⁺]₀, low [Na⁺]₀, K⁺ depolarization, and PESP lead to the same maximum force. We suppose that the mechanism of positive inotropic effect is the same for potentiated contraction and transient Ca²⁺ infusion during one cycle of contraction.

In the present study, the positive inotropic effect of a potentiated contraction is almost abolished and the elevation of diastolic tension is extremely marked in reoxygenation after the second hypoxic episode, though the redevelopment of potentiated contractions was better than that of steady-state contractions (p<0.01). From the findings of very poor recovery of contractions in the preparations showing marked elevation of % diastolic tension over 120% (Fig. 5), we may regard about half of the preparations (7 of 13) after the second period of hypoxia in the present study as irreversibly damaged myocardium. Most of the preparations after the first period of hypoxia are regarded as reversibly damaged myocardium since the elevation of % diastolic tension is not significant, the recovery of contractile function is good as shown in Fig. 3, and a high concentration of Ca²⁺ (7.2 mM CaCl₂) augments contractions (data not shown).
Neely et al\textsuperscript{13} reported that accumulation of by-products of glycolytic metabolism, especially H\textsuperscript{+}, NADH, and lactate, may play an important role in the pathogenesis of ischemic myocardial injury. It is well known that increasing H\textsuperscript{+} depresses not only Ca\textsuperscript{2+} loading of the cardiac SR and the rate of Ca\textsuperscript{2+} accumulation, but also the Ca\textsuperscript{2+}-induced release of Ca\textsuperscript{2+} from the SR\textsuperscript{14}. In fact, Lee et al\textsuperscript{15} reported that ischemia for 60 to 90 min causes impairment of Ca\textsuperscript{2+} uptake by the SR isolated from ischemic tissue. Both transsarcolemmal Ca\textsuperscript{2+} influx and SR Ca\textsuperscript{2+} release contribute to tension generation. It is possible then, that the SR function of the muscles after hypoxia, especially in the second intervention, is damaged from the findings of very poor recovery from hypoxia-induced contractile depression.

Leijendekker et al\textsuperscript{16} has shown that rigor development by lack of ATP is the most important contributor to diastolic tension generation. The marked elevation of diastolic tension was recognized at the second 120 min period of hypoxia. The second reoxygenation cannot reduce the elevated diastolic tension significantly, as shown in Fig. 3. We suppose that diastolic tension is one of the major determinants of the level achieved by a potentiated contraction in the severely hypoxic state.

In summary, the preparations after the first 120 min period of hypoxia can be used as a model of stunned myocardium and the preparations after the additional 120 min of hypoxia may be used as a model of irreversibly damaged myocardium. Hypoxia-induced dysfunction (stunned myocardium) is further deteriorated by a new episode of hypoxia, that is, the residual function of stunned myocardium may be further injured by restenosis in vivo. Potentiated contraction is abolished in myocardium following severe hypoxia. The marked elevation of diastolic tension is one of the major determinants of the level achieved by steady-state and potentiated contractions in the severely hypoxic state. The redevelopment of force following reoxygenation is not determined by the degree of hypoxia-induced contractile depression.

\textbf{REFERENCES}