Effects of Hypoxia and Reoxygenation on Regular Contractions and Postrest Contraction in Rat Papillary Muscles

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SUMMARY

The effects of hypoxia and reoxygenation on regular contractions and postrest contractions (PRC) of papillary muscle of rats were studied. Isometric tension was measured during two cycles of hypoxia and reoxygenation. Trains of 80 externally continuous stimulations at 40/min were applied. PRCs were evoked by a stimulus train after a 60 sec resting interval.

After 90 min of hypoxia (the first hypoxia period), regular contractions and PRCs decreased to $5.6 \pm 2.0\%$ and $23.4 \pm 2.4\%$ of baseline values, respectively ($p < 0.001$; $n = 18$). After 90 min of reoxygenation, the recovery of the PRCs ($44.4 \pm 3.4\%$) was better than that of the regular contractions ($23.3 \pm 3.3\%$) ($p < 0.01$; $n = 18$). After 30 min of hypoxia (the second hypoxic period), regular contractions and PRCs decreased to $2.2 \pm 0.6\%$ and $13.6 \pm 1.6\%$ of baseline values, respectively ($p < 0.001$; $n = 18$). However, the recovery from the second hypoxic injury was not significant for either regular contractions or PRCs. The % diastolic tension, which was normalized to the baseline for regular contractions, increased to $113.2 \pm 6.9\%$ and $133.6 \pm 8.4\%$ at the end of the first and the second hypoxic periods, respectively. There was statistically significant correlation between the % diastolic tension and the % hypoxic injury of PRCs ($p < 0.002$; $n = 18$). There was no significant relationship between % diastolic tension and % hypoxic injury of regular contractions. There was no statistically significant correlation between % diastolic tension and % recovery from the hypoxic injury of either regular contractions or PRCs.

Since the PRCs are thought to be more dependent on calcium stored in the sarcoplasmic reticulum (SR) than regular contractions, SR function seems to be resistant to transient hypoxia. The marked increase in diastolic tension during repeated hypoxia may indicate SR dysfunction,

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Received for publication September 10, 1990.

Accepted October 9, 1990.

leading to irreversible myocardial damage. Thus, both hypoxia and reoxygenation may regulate the recovery from hypoxic dysfunction.

Additional Indexing Words:

Hypoxia Reoxygenation Postrest contraction (PRC) Papillary muscle Rat

A brief transient occlusion of a coronary artery does not result in myocyte necrosis. However, the contractile function of the viable myocardium is depressed for hours to days after restoration of blood flow. This phenomenon has come to be known as myocardial stunning.¹⁾ Despite its clinical importance, the physiological features of the stunned myocardium are not well characterized and the mechanisms underlying depressed function have not been resolved.

It is well known that there is substantial variation in the ratio of cardiac sarcoplasmic reticulum (SR) calcium release to transsarcolemmal calcium influx in different species.^{2)–4)} It has been suggested that the relative contribution of SR calcium release to tension development in rat ventricle is large.²⁾

The first potentiated contraction, elicited by restimulation after a rest period, has been called a postrest contraction (PRC).⁵⁾ It is now known that PRC is mostly dependent on the Ca^{2+} in SR.⁶⁾ In this study hypoxia and reoxygenation were repeated in rat papillary muscles and PRCs were measured as an assay of SR function. The purpose of this study was to clarify the contributions of the SR to hypoxic injury and the stunned myocardium, and to determine whether hypoxic dysfunction regulates reoxygenation dysfunction.

METHODS

Tissue preparation:

Male and female Wistar rats, weighing 250–300 g were used.⁶⁾ The heart was quickly removed under urethane anesthesia (100 mg/100 g body weight), and placed in normal physiologic saline containing NaCl (115.3 mM), KCl (2.5 mM), Na_2HPO_4 (1.55 mM), NaH_2PO_4 (1.55 mM), CaCl_2 (1.8 mM), and tubocurarine chloride (0.013 mM). Papillary muscles (1–2 mm in diameter) were removed from the right ventricles and mounted in a 2 ml chamber with one end fixed and the other end connected to an isometric tension transducer (NEC, model ME-4021) with surgical silk. Muscles were stretched to approximately 1.2 times their resting length.

Solution:

The control superfusate was a modified Tyrode's solution containing NaCl (136.9 mM), KCl (4.0 mM), CaCl₂ (1.8 mM), MgCl₂ (1.0 mM), NaH₂PO₄ (0.33 mM), Na₂HPO₄ (2.44 mM), glucose (5.5 mM). This solution had been prewarmed to 30°C and bubbled with 100% O₂ before entering the chamber. The hypoxic solution was prepared by bubbling the glucose-free Tyrode solution with 100% N₂. The pH was maintained at 7.4 in both solutions.

Stimulation and recording:

Muscles were stimulated by passing 5V pulses of 2 msec duration (Cardiac stimulator BC-02A, Fukuda Denshi); they were driven at 40/min for 60 min to obtain steady-state twitch tensions and isometric tension was recorded (Pen recorder VP-6513A, Panasonic Electronic).

Regular contractions and PRCs:

After an equilibration period of at least 60 min in the control solution, a train of 80 regular stimulations was delivered to the preparation, followed by a resting interval of 60 sec. The PRCs were then elicited by stimulations. Muscles remained mounted for 90 min in the hypoxic solution (the first hypoxic period) and were superfused with the reoxygenated control solution in the presence of glucose (the first reoxygenation period). The muscles

Fig. 1. Definition of % hypoxic injury to regular contractions or PRCs, % recovery from hypoxic injury of regular contractions or PRCs, and % diastolic tension.

Regular contractions: a, c, e; PRCs: b, d, f; Diastolic tension: g, h.

- 1) % Hypoxic injury to regular contractions (%): $(a - c)/a \cdot 100$
- 2) % Hypoxic injury to PRCs (%): $(b - d)/b \cdot 100$
- 3) % Recovery from hypoxic injury of regular contractions (%): $(e - c)/(a - c) \cdot 100$
- 4) % Recovery from hypoxic injury of PRCs (%): $(f - d)/(b - d) \cdot 100$
- 5) % Diastolic tension (%): $(g/a) \cdot 100, (h/a) \cdot 100$

were then superfused for 30 min with the hypoxic solution (the second hypoxic period), and were superfused a second time with the reoxygenated control solution in the presence of glucose (the second reoxygenation) in order to clarify the effects of hypoxia and reoxygenation on regular contractions and PRCs.

One μM noradrenaline was applied in another group of 5 muscle preparations after the first reoxygenation period (90 min) and in a third group of 4 muscle preparations after the second reoxygenation period (90 min) to assess whether the muscles can be utilized as a model of stunned myocardium.

Data analysis and measurement:

All data were analyzed statistically using Student's t-test for paired data. % Hypoxic injury and % recovery from hypoxic injury for regular contraction and PRC are defined in Fig. 1.

RESULTS

Representative recordings of regular contractions and PRCs during the first hypoxic and reoxygenation periods are shown in Fig. 2. Hypoxia induced a marked decrease in regular contractions, a moderate decrease in PRCs, and an increase in diastolic tension. Reoxygenation induced a moderate recovery of both regular contractions and PRCs, and a decrease in diastolic tension.

Noradrenaline (1 μM) was applied after the first reoxygenation period. Noradrenaline significantly augmented the magnitude of regular contractions from $37.4 \pm 11.9\%$ of baseline measurements to $53.0 \pm 16.9\%$ ($p < 0.05$; $n = 5$). However, noradrenaline did not affect the magnitude of regular contraction after the second reoxygenation period (from $10.1 \pm 2.2\%$ to $10.3 \pm 2.5\%$; $n = 4$, NS).

Fig. 2. Influences of hypoxia and glucose elimination for 90 min and reoxygenation for 90 min on regular contractions and PRCs. Recordings from one papillary muscle are shown.

The statistical analyses of relative changes in regular contractions, PRCs, and % diastolic tension during hypoxia and reoxygenation are shown in Fig. 3. At the end of the first hypoxic period regular contractions and PRCs decreased to $5.6 \pm 2.0\%$ ($n=18$) and $23.4 \pm 3.3\%$ ($n=18$) of baseline, respectively. At the end of the first reoxygenation period, regular contractions and PRCs recovered to $23.3 \pm 2.4\%$ ($n=18$) and $44.4 \pm 3.4\%$ ($n=18$) of baseline values, respectively. At the end of the second hypoxic period, regular contractions and PRCs decreased to $2.2 \pm 0.6\%$ ($n=18$) and $13.6 \pm 1.6\%$ ($n=18$) of baseline, respectively. At the end of the second reoxygenation period, though, they both recovered partially to $10.1 \pm 2.2\%$ ($n=8$) and $22.6 \pm 4.9\%$ ($n=8$) of baseline values, respectively. The % diastolic tension increased to $113.2 \pm 6.9\%$ ($n=18$) and $133.6 \pm 8.4\%$ ($n=8$) at the end of the first and the second hypoxic periods, respectively. It is noteworthy that only 8 papillary muscles were analyzed after the second hypoxic and reoxygenation periods; the other 10 muscles were used for protocols which were not presented in this paper.

There was a statistically significant correlation between the % diastolic tension and the % hypoxic injury of PRCs (first hypoxic period: $r=0.83$,

Fig. 3. The time course for the hypoxic and reoxygenation-induced changes in the magnitude of regular contractions—RC (●), PRCs (○), and % diastolic tension (▲). All points except for the second reoxygenation period represent the mean of 18 experiments. Data during the second reoxygenation period represent the mean of 8 experiments; the vertical bars indicate SE.

Fig. 4. The relationship between % diastolic tension and % hypoxic injury of regular contractions (a: the first hypoxic period; c: the second hypoxic period) or PRCs (b: the first hypoxic period; d: the second hypoxic period).

Fig. 5. The relationship between % diastolic tension and % recovery from hypoxic injury of regular contractions (a: the first hypoxic period; c: the second hypoxic period) or PRCs (b: the first hypoxic period; d: the second hypoxic period).

$p < 0.002$, $n = 18$; second hypoxic period: $r = 0.67$, $p < 0.002$, $n = 18$). A significant correlation was not observed between the % diastolic tension and changes in regular contractions (Fig. 4). There was no statistically significant correlation between % diastolic tension and % recovery from hypoxic injury for either PRCs or regular contractions (Fig. 5).

DISCUSSION

A rest period during the regular stimulation of isolated cardiac muscles causes a large transient augmentation of subsequent twitch tension,⁵⁾ termed a PRC. The Ca^{2+} content of the SR reflects both cytoplasmic Ca^{2+} and extracellular Ca^{2+} entry into the cell.^{7),8)} It is well known that methylxanthines inhibit both PRCs and post-extrasystolic potentiation.^{9),10)} Endoh et al¹¹⁾ reported that calcium channel blockers significantly decreased the strength of regular contractions, but that PRCs were not significantly changed. It is now known that PRCs are mostly dependent on the Ca^{2+} content of SR. In the present study, we used PRCs to assess the functional status of the SR.^{7),8),12)} A resting interval of 60 sec was chosen because the maximum PRC is obtained with a 60–90 sec resting interval at a basic cycle length of 1,500 msec.⁶⁾

In this study regular contractions were depressed more markedly than PRCs during both the first and second hypoxic periods. These pieces of evidence suggest that the integrity of SR Ca^{2+} reserves is resistant to hypoxia in rats, possibly reflecting the large calcium capacity of SR in these species.⁶⁾

The amplitude of the diastolic tension is due mainly to residual Ca^{2+} -myofilament interactions. These interactions reflect an increased intracellular Ca^{2+} content during diastole, which is usually regulated by the SR Ca^{2+} pump and sarcolemmal Na^{+} - Ca^{2+} exchange mechanism. A rapid decline in developed tension during hypoxia has been attributed to a substantial increase in inorganic phosphate¹³⁾ and a very large fall in pH. It is now known that acidosis¹⁴⁾ and free oxygen radicals¹⁵⁾ decrease the rate of Ca^{2+} accumulation by the SR. In fact, ischemia causes impairment of Ca^{2+} uptake by the SR.¹⁶⁾ In this experiment, there was a significant correlation between the % diastolic tension and the % hypoxic injury of PRCs, while there was no relationship between % diastolic tension and the % hypoxic injury of regular contractions (Fig. 4). These results imply that the increase in cytoplasmic calcium during hypoxia may be derived mainly from the SR but not extracellular calcium. In other words, % diastolic tension may represent the degree of inhibited SR function.

After the first reoxygenation period, the regular contractions were

severely depressed to 20–25% of the baseline, while PRCs were depressed to approximately 45% of the baseline values (Fig. 3). Noradrenaline significantly augmented the magnitude of regular contractions after the first reoxygenation period. However, noradrenaline did not affect the magnitude of regular contractions after the second reoxygenation period. It is well known that catecholamines and calcium infusion improve the function of stunned myocardium.¹⁷⁾ Thus, the first reoxygenation period is comparable to the stunned myocardium and the myocardium appears to be injured irreversibly after the second reoxygenation period.

Recently, Kusuoka et al^{18),19)} reported a paradoxical increase in Ca^{2+} transients in stunned myocardium. They suggested that contractile failure in stunned myocardium is due to a decrease in both the myofilament sensitivity to Ca^{2+} and maximal Ca^{2+} -activated force. It is presently unclear whether the myocardium is “stunned” during ischemia or during reperfusion, since recovery is impossible without reoxygenation. Since there is no good correlation between % diastolic tension and % recovery from hypoxic injury (Fig. 5), reperfusion injury may influence both regular contractions or PRCs. This implies that reoxygenation may induce SR dysfunction.¹⁵⁾ From these findings, we suggest that both hypoxia and reoxygenation are factors that produce stunned myocardium.

The present study suggests that stunned myocardium deteriorates further during a second cycle of hypoxia and reoxygenation. Thus, residual myocardial function may be further injured by restenosis and reperfusion *in vivo*. We suspect that viability of the ischemic myocardium may depend on residual SR function.

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