Depressed tolerance to fluorocarbon-simulated ischemia in failing myocardium due to impaired $[\text{Ca}^{2+}]_i$ modulation

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Depressed tolerance to fluorocarbon-simulated ischemia in failing myocardium due to impaired $[\text{Ca}^{2+}]_i$ modulation. Am J Physiol Heart Circ Physiol 278: H1446–H1456, 2000.—The aim of this study was to investigate the tolerance of failing myocardium from postinfarction rats to simulated ischemia. Myocardial infarction (MI) was induced by ligation of the left coronary artery in male Wistar rats. Isometric force and free intracellular $[\text{Ca}^{2+}]_i$ concentration ($[\text{Ca}^{2+}]_i$) were measured in isolated left ventricular papillary muscles from sham-operated and post-MI animals 6 wk after surgery. Ischemia was simulated by using fluorocarbon immersion with hypoxia. Results showed that mechanical performance was depressed during the period of hypoxia in physiological salt solution (44 ± 7% of baseline in sham vs. 30 ± 6% of baseline in MI, $P < 0.05$) or ischemia (16 ± 2% of baseline in sham vs. 9 ± 1% of baseline in MI, $P < 0.01$) accompanied by no corresponding decrease of peak $[\text{Ca}^{2+}]_i$. (hypoxia: 51 ± 8% of baseline in sham vs. 46 ± 7% of baseline in MI, $P = \text{NS}$; ischemia: 47 ± 5% of baseline in sham, 39 ± 7% of baseline in MI, $P = \text{NS}$). After reoxygenation, $[\text{Ca}^{2+}]_i$ rapidly returned to near preischemic basal levels, whereas developed tension in fluorocarbon remained significantly lower. This dissociation between peak $[\text{Ca}^{2+}]_i$ and isometric contractility was more pronounced in the failing myocardium from postinfarction rats. In conclusion, more severe impairment of $[\text{Ca}^{2+}]_i$, homeostasis in the failing myocardium from postinfarction rats increases susceptibility to ischemia-reperfusion injury.

postischemic depression of ventricular contractile function was first described in dogs by Heyndrickx et al. (22) and further elucidated as myocardial stunning by Braunwald and Kloner (10). Various mechanisms have been implicated in this phenomenon, including oxygen-derived free radical toxicity (8, 42), impaired myocardial energy production and use (18, 40), damage of extracellular collagen matrix (47), and the impairment of myocardial perfusion (44).

Recent investigations have shown that impaired intracellular $[\text{Ca}^{2+}]_i$ concentration ($[\text{Ca}^{2+}]_i$) modulation is a major primary factor in the pathogenesis of myocardial stunning (12, 24, 29, 33). $[\text{Ca}^{2+}]_i$ increased after 20 min of global ischemia in isolated perfused hearts and remained transiently elevated during early reflow (24, 33). When isolated ferret hearts subjected to 15 min of normothermic ischemia are reperfused with solutions containing low concentrations of $\text{Ca}^{2+}$, the postischemic contractile abnormalities are significantly attenuated (29). Exposure of isolated ferret hearts to a transient $\text{Ca}^{2+}$ overload in the absence of ischemia produced mechanical and metabolic abnormalities similar to myocardial stunning (26). However, our previous studies demonstrated a gradual decrease of systolic $[\text{Ca}^{2+}]_i$ that occurs simultaneously with the onset of contractile depression during hypoxia (5, 24, 32). Reoxygenation of ventricular muscle resulted in a rapid recovery and overshoot in $[\text{Ca}^{2+}]_i$, but a corresponding recovery in developed tension did not occur. Relative to the normal heart, the effects of hypoxia and ischemia on $[\text{Ca}^{2+}]_i$ and contractility in failing myocardium are an even more complex process. Reported data from our laboratory demonstrated that contractile dysfunction in failing myocardium is associated with alterations of $[\text{Ca}^{2+}]_i$ homeostasis (19, 43). Clinical and experimental data indicate an impaired tolerance to ischemia in hypertrophied hearts (34, 37). This can be particularly important to patients undergoing ischemic cardiac arrest and reperfusion during cardiac surgery as well as to those receiving thrombolytic therapy or coronary angioplasty for acute myocardial infarction (MI). The putative reasons are biochemical or other metabolic alterations (37, 46) and/or excitation-contraction uncoupling (13) during ischemia in hypertrophied and failing ventricular myocardium.

To date, there have been no attempts to assess contractile dysfunction as an independent factor affecting the tolerance of the heart to ischemia. Previous studies performed by our laboratory (31, 35) and others (38, 39) demonstrated a marked impairment of left ventricular function by gross observation and hemodynamic measurement in animals with MI. Thus large MI induced by permanent ligation of the left anterior descending coronary artery could be used as an appropriate model of failing myocardium.

In view of the importance of establishing the cellular mechanism of posts ischemic stunning, we used the previously reported ischemia model with fluorocarbon
immersion (5) in failing papillary muscles from postinfarction rats to test the hypothesis that failing myocardium exhibits an enhanced sensitivity to ischemia.

METHODS

Experiments were performed in male Wistar rats (Taconic, Germantown, NY) initially weighing 250–300 g (age: 10–12 wk), housed individually under climate-controlled conditions with a 12:12-h light/dark cycle, with free access to a standard rat chow and tap water ad libitum. MI was induced by left anterior coronary artery ligation with a modified technique as previously described (35, 39). Rats were anesthetized with pentobarbital sodium (60 mg/kg) by intraperitoneal injection. Animals were intubated and ventilated with carbogen (a mixture of 95% O2-5% CO2) by using a small animal ventilator (Harvard Apparatus, South Natick, MA). The left coronary artery was ligated by using 6-0 surgical silk. MI was verified by observing blanching of the myocardium distal to the ligation and changes in the surface electrocardiogram recording. After recovery of spontaneous respiratory efforts, each animal was extubated and subsequently observed until it fully recovered from anesthesia. The sham-operated rats underwent an identical surgery without ligation of the coronary artery. The studies were conducted under the guidelines of the American Physiological Society “Guiding Principles for Research Involving Animals and Human Beings.”

Isometric muscle performance. Six weeks after surgery, the rats were euthanized while under deep anesthesia with pentobarbital sodium. The heart was rapidly excised and placed in a dissecting chamber containing modified Krebs-Henseleit solution with the following composition (in mM): 120 NaCl, 5.9 KCl, 5.5 dextrose, 2.5 NaHCO3, 1.2 MgCl2, and 1.0 CaCl2 (pH 7.4), bubbled with carbogen at room temperature. The noninfarcted left ventricular papillary muscle, which is characterized as a relatively hypertrophied muscle due to the compensatory effect of the remaining viable myocardium, was carefully dissected and then fixed to a muscle holder with a spring clip. The tendinous end of the muscle was vertically connected to a strain-gauge tension transducer (model MBI 341, Senstec, Columbus, OH) with a silk thread. The muscle was then mounted in a 50-ml tissue transducer (model MBI 341, Sensotec, Columbus, OH) with a spring clip. The tendinous end of the muscle was carefully stretched to the length at which maximal tension developed (Lmax). The following isometric contraction parameters were recorded from each muscle at this maximal length: developed tension (DT, tension produced by the stimulated muscle), time to peak tension (TPT, time from the beginning of the contraction to peak tension), and time to 90% relaxation (R90, time from peak tension to 90% of relaxation). Subsequently, the loading procedure for aequorin was performed (see below). At the end of the experiment, the muscles were blotted and weighed. The cross-sectional area was determined from muscle weight and length by assuming a uniform cross section and a specific gravity of 1.05.

Aequorin light signal measurement. Aequorin (Dr. John Blinks, Friday Harbor Lab, Friday Harbor, WA) was loaded into the nonstimulated muscle prepara- tion by the microinjection technique (25). The preparation was briefly raised from the organ bath, and a 1–2 µM aequorin solution (2 mg/ml) was injected under the epimysium at the base of the muscle with a short-shank, low-resistance glass micropipette. After an equilibration period of 90–120 min, stimulation was restarted at 0.33 Hz. The aequorin light signal was detected with a photomultiplier tube (PM2BB, Thorn EMI Electron Tubes, Rockaway, NJ) and converted into a voltage signal. Analog signals from the isometric force transducer and electronic photometer were recorded with a chart-strip recorder (model 56-1X 40-006158, Gould Instrument Systems, Cleveland, OH). To improve the signal-to-noise ratio, 64–128 steady-state light signals and isometric twitches were averaged with a digital oscilloscope (model 4094, Nicolet Instrument, Madison, WI) for quantitative measurement. Parameters derived from the light signals included the amplitude of the light transient, time to peak light (TPL), and time from peak to 90% fall in peak light (RL90). The free [Ca2+]i was estimated by normalizing the recorded light signal during isometric twitches (L) by the maximal amount of light emitted after the lysis of the muscle membranes (Lmax) at the end of the experiment with a 5% solution of the detergent, Triton X-100, in phosphate-free physiological salt solution containing 50 mM Ca2+. The normalized light signal was then converted to [Ca2+]i using an in vitro calibration curve as previously reported (24).

Experimental protocol. After the equilibration period and measurement of baseline parameters, isolated papillary muscles were exposed to a 20-min period of hypoxia (95% N2-5% CO2) followed by 20 min of reoxygenation (95% O2-5% CO2). After the hypoxiareoxygenation cycle in physiological salt solution, oxygenated fluorocarbon [Fluorinert (FC-47), 3M, St. Paul, MN] was substituted and equilibrated for a 20-min period. The cycle of 20 min hypoxia and 20 min reoxygenation was subsequently repeated in muscle preparations with fluorocarbon immersion. Finally, the bath solution was switched back to a physiological salt solution.

Statistical analysis. All values are given as means ± SE. Data were evaluated by ANOVA with repeated measures. Differences between individual groups were compared by using the Student’s t-test and considered significant at P < 0.05.

RESULTS

General characteristics of animals. A total of 16 successfully operated rats made up the surgical group. Eight rats had undergone a permanent ligation of the coronary artery and eight rats had received a sham operation. Six weeks after the operation, the left ventricular weight, left ventricular weight-to-body weight ratio, and muscle cross-sectional area from postinfarction rats were significantly increased compared with sham-operated animals (Table 1). At baseline, the isolated noninfarcted papillary muscles were exposed to 20-min periods of hypoxia (95% N2-5% CO2) followed by 20 min of reoxygenation (95% O2-5% CO2). After the hypoxiareoxygenation cycle in physiological salt solution, oxygenated fluorocarbon [Fluorinert (FC-47), 3M, St. Paul, MN] was substituted and equilibrated for a 20-min period. The cycle of 20 min hypoxia and 20 min reoxygenation was subsequently repeated in muscle preparations with fluorocarbon immersion. Finally, the bath solution was switched back to a physiological salt solution.

Statistical analysis. All values are given as means ± SE. Data were evaluated by ANOVA with repeated measures. Differences between individual groups were compared by using the Student’s t-test and considered significant at P < 0.05.

Effect of hypoxia on rat myocardium. After measurement of baseline data, papillary muscle preparations were exposed to 20-min periods of hypoxia, and isomet-
isometric force showed a moderate decline associated with no corresponding decrease of peak \([Ca^{2+}]_i\) in both sham and MI groups (Figs. 1 and 2). The depressed isometric contractility was more pronounced in failing myocardium from postinfarction rats (44.0 ± 7.3% of baseline in sham-operated and 30.2 ± 6.4% of baseline in MI papillary muscles, \(P < 0.05\)), whereas the peak \([Ca^{2+}]_i\) showed no significant difference after 20 min of hypoxia in physiological salt solution (sham: 50.8 ± 7.8% of baseline; MI: 46.2 ± 6.9% of baseline; \(P = NS\)). Figures 2 and 3 are examples of isometric force recordings and intracellular \(Ca^{2+}\) transients in physiological salt solution with hypoxia-reoxygenation cycle. After 20 min of reoxygenation in physiological salt solution, mechanical performance recovered to near basal values and showed no significant changes between the sham and MI groups (96.2 ± 13.2% of baseline in the sham group vs. 90.8 ± 10.7% of baseline in the MI group, \(P = NS\)). The amplitude of the peak \([Ca^{2+}]_i\) recovered almost entirely to baseline value with no difference between the sham and the MI groups. No qualitative differences were found in the diastolic \([Ca^{2+}]_i\), or resting tension in the sham or the MI group during hypoxia or subsequent reoxygenation in physiological salt solution (data not shown).

Table 1. General characteristics of sham-operated and myocardial infarction rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
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<tr>
<td>BW, g</td>
<td>413.5 ± 20.3</td>
<td>382.6 ± 17.2</td>
</tr>
<tr>
<td>LVW, mg</td>
<td>736.2 ± 28.6</td>
<td>986.8 ± 23.6*</td>
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<tr>
<td>LVW/BW, mg/g</td>
<td>1.79 ± 0.16</td>
<td>2.35 ± 0.28*</td>
</tr>
<tr>
<td>CSA, mm²</td>
<td>0.74 ± 0.08</td>
<td>0.89 ± 0.11*</td>
</tr>
<tr>
<td>DT, mN/mm²</td>
<td>12.6 ± 1.5</td>
<td>11.5 ± 1.2</td>
</tr>
<tr>
<td>TPT, ms</td>
<td>105.1 ± 16.3</td>
<td>134.5 ± 19.1*</td>
</tr>
<tr>
<td>RT₉₀, ms</td>
<td>136.5 ± 18.2</td>
<td>178.8 ± 23.8*</td>
</tr>
<tr>
<td>Systolic ([Ca^{2+}]_i), µM</td>
<td>0.61 ± 0.06</td>
<td>0.59 ± 0.07</td>
</tr>
<tr>
<td>Diastolic ([Ca^{2+}]_i), µM</td>
<td>0.28 ± 0.03</td>
<td>0.34 ± 0.05*</td>
</tr>
<tr>
<td>TPL, ms</td>
<td>49.7 ± 7.6</td>
<td>62.3 ± 7.4*</td>
</tr>
<tr>
<td>RL₉₀, ms</td>
<td>76.6 ± 8.5</td>
<td>89.6 ± 7.9*</td>
</tr>
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Values are means ± SE; \(n = 8\) rats. Sham, sham-operated rat papillary muscles; MI, failing rat papillary muscles from 6 wk postinfarction; BW, body weight; LVW, left ventricular weight; LVW/BW, ratio of left ventricular weight/body weight; CSA, papillary muscle cross-sectional area; DT, developed tension; TPT, time to peak tension; RT₉₀, time from peak tension to 90% relaxation; \([Ca^{2+}]_i\), peak free intracellular \(Ca^{2+}\) concentration; TPL, time to peak light signal; RL₉₀, time from peak light to 90% decline. *\(P < 0.05\) MI vs. Sham.

Fig. 1. Developed tension (DT, A) and peak systolic intracellular \(Ca^{2+}\) concentration \((\text{[Ca}^{2+}\text{]})_i\) (B) in response to hypoxia-reoxygenation cycle in papillary muscles from sham-operated (Sham) and myocardial infarcted (MI) rats at 6 wk post-MI (\(n = 8\) each group) with physiological salt solution. *\(P < 0.05\), **\(P < 0.01\) vs. Baseline; #\(P < 0.05\) MI vs. Sham.
Fig. 2. Aequorin light signal and isometric contraction from representative rat papillary muscles of Sham (A) and MI rats (B) during hypoxia-reoxygenation cycle in physiological salt solution. Top trace: aequorin light signal; bottom trace: isometric contraction.

Fig. 3. Continuous strip-chart recordings of isometric force in isolated rat papillary muscles from Sham (A) and MI rats (B) during hypoxia-reoxygenation cycle in physiological salt solution.
The TPT and RT\textsubscript{90} were abbreviated in both groups after 20 min of hypoxia, although these values were prolonged in the MI group relative to the sham group (Fig. 4). However, the RL\textsubscript{90} was significantly prolonged compared with basal values after 20 min of hypoxia in both sham and MI groups. Prolongation of the RL\textsubscript{90} in the MI group remained more pronounced than that in the sham group (Fig. 4). After 5 min of reoxygenation, time courses of isometric force and $\text{Ca}^{2+}$ transients had recovered to baseline. No values of TPL in either group were different from the baseline, during hypoxia, or with subsequent reoxygenation.

Effect of ischemia on rat myocardium. After 20 min of reoxygenation in the physiological salt solution, muscle preparations were then exposed to 20-min periods of fluorocarbon continuously bubbled with oxygen. No qualitative differences in the light signal or isometric tension were observed with fluorocarbon in either sham-operated or failing rat myocardium compared with the physiological salt solution (Fig. 5). Ischemia was induced for 20 min by switching the salt solution to fluorocarbon bubbled with a mixture of 95% $\text{N}_2$-5% $\text{CO}_2$. A combination of reduced isometric force development and increased resting tension were found in both sham and MI muscle preparations; these effects were more pronounced in the failing rat myocardium (developed tension: 15.7 ± 2.4% of baseline in the sham group vs. 8.9 ± 1.5% of baseline in the MI group, $P < 0.01$; resting tension: 2.3 ± 0.4 mN/mm\textsuperscript{2} in the sham group vs. 5.6 ± 0.8 mN/mm\textsuperscript{2} in the MI group, $P < 0.01$). However, recovery of developed tension was much slower in fluorocarbon than in physiological salt solution with reoxygenation in both sham and MI groups. The post-ischemic depression of isometric contraction was accentuated in the failing muscle preparation compared with normal muscle preparations (Figs. 5–7).

Systolic $\text{[Ca}^{2+}\text{]}_{i}$ decreased to 46.8 ± 8.2% of baseline in the sham group ($P < 0.01$ vs. baseline) and 39.4 ± 7.5% of baseline in the MI group ($P < 0.01$ vs. baseline) after 20 min of ischemia, but no significant change was observed between sham and MI groups. The decreased peak $\text{[Ca}^{2+}\text{]}_{i}$ was rapidly reversed by reoxygenation with fluorocarbon in both sham and MI groups (Figs. 5 and 6). Diastolic $\text{[Ca}^{2+}\text{]}_{i}$ showed quantitative increases after 20 min of hypoxia in fluorocarbon immersion in both the sham and MI groups (from 0.28 ± 0.03 to 0.31 ± 0.04 µM in the sham group, $P < 0.05$; and from 0.34 ± 0.05 to 0.38 ± 0.06 µM in the MI group, $P < 0.01$). Reoxygenation of hypoxic papillary muscles resulted in a rapid recovery of resting tension, whereas the diastolic $\text{[Ca}^{2+}\text{]}_{i}$ remained higher than in the basal condition and was more pronounced in the MI group (sham: 0.30 ± 0.03 µM; MI: 0.36 ± 0.06 µM, $P < 0.01$).

Fig. 4. Time intervals of isometric contraction (A) and $\text{[Ca}^{2+}\text{]}_{i}$ transient (B) at baseline and during hypoxia-reoxygenation cycle in physiological salt solution with papillary muscles from Sham and MI rats (6 wk post-MI; n = 8 each group). TPT, time to peak tension; RT\textsubscript{90}, time from peak tension to 90% relaxation; TPL, time to peak light; RL\textsubscript{90}, time from peak light to 90% decline. *$P < 0.05$, **$P < 0.01$ vs. baseline; #$P < 0.05$ MI vs. Sham.
The parameters of time courses of mechanical performance and Ca\(^{2+}\) transients showed a mild prolongation but no significant change in fluorocarbon compared with that in physiological salt solution with oxygenation. After 20 min of ischemia, TPT and peak RT\(_{90}\) were significantly abbreviated in both sham and MI groups. In contrast, the TPT and the RL\(_{90}\) were significantly prolonged after 20 min of ischemia and were greater in the MI group (Fig. 8). With reoxygenation, the TPT and RT\(_{90}\) recovered promptly to preischemic values, whereas the RL\(_{90}\) recovered more slowly. By 20 min of reoxygenation, the parameters of time courses of isometric performance and Ca\(^{2+}\) transient, except RL\(_{90}\), returned to preischemic levels. The prolonged RL\(_{90}\) was greater in the MI group compared with the sham group (Fig. 8). After the muscle preparations were switched back to physiological salt solution instead of fluorocarbon, no significant differences were found compared with baseline conditions in physiological salt solution with regard to developed tension, [Ca\(^{2+}\)] availability, or time courses in mechanical contractility and Ca\(^{2+}\) transient.

**DISCUSSION**

Fluorocarbon is a biologically and chemically inert liquid in which CO\(_2\) and O\(_2\) are highly diffusible (4). Because of the nonpolar structure of the fluorocarbon, however, substrates that are utilized by the muscle preparation may become exhausted because they are not replenished and metabolites accumulate because egress from the interstitial space is limited. Thus almost complete restriction of extracellular fluid surrounding the preparation with fluorocarbon in papillary muscles has been successfully used in our laboratory (5) as an appropriate model to simulate ischemia.

After 20 min of hypoxia in either physiological salt or fluorocarbon solution in the present study, the exaggerated reduction of developed tension relative to peak systolic [Ca\(^{2+}\)] was more pronounced in papillary muscles from MI rats with failure. Recovery of isometric force after 20 min of reoxygenation remained significantly lower in these rat papillary muscles compared with the sham-operated muscle preparations, although peak [Ca\(^{2+}\)] returned to nearly equivalent levels. The mechanism of this enhanced sensitivity to hypoxia/ischemia-induced contractile dysfunction in postinfarction failing rat myocardium may be primarily associated with impaired [Ca\(^{2+}\)] modulation. Abnormal [Ca\(^{2+}\)] handling has been suggested as a major cause of contractile dysfunction in failing the human (19) and rat myocardium (43). In particular, elevation of the diastolic [Ca\(^{2+}\)] may generate temporal and spatial...
inhomogeneities of \([\text{Ca}^{2+}]\), which, in turn, increase diastolic tone and ventricular dysfunction. \(\text{Ca}^{2+}\) release and uptake by the sarcoplasmic reticulum (SR) essentially determine the amplitude and time course of the \(\text{Ca}^{2+}\) transient as well as the incidence of diastolic \(\text{Ca}^{2+}\) oscillations (1, 45). A large number of experimental studies have indicated that the SR from failing myocardium exhibits a reduced \(\text{Ca}^{2+}\) transport capacity (21, 41). Krause and co-workers (28) found that a decrease in the ability to transport \(\text{Ca}^{2+}\), concomitant with a reduction in the activity of the associated \(\text{Ca}^{2+}-\text{Mg}^{2+}\) ATPase activity, resulted in the contractile dysfunction of the stunned myocardium. Slower removal of \(\text{Ca}^{2+}\) from the myofibrils would be expected to result in delayed relaxation and abnormal diastolic properties after ischemia, which had been confirmed by our present finding. The failing myocardium from postinfarction rats, by virtue of inherent differences in \(\text{Ca}^{2+}\) regulation that result in a diminished capacity to resequester \(\text{Ca}^{2+}\), may be more vulnerable to diastolic \(\text{Ca}^{2+}\) overload induced by ischemia. In the present study, diastolic \([\text{Ca}^{2+}]\) was elevated in both sham-operated and failing papillary muscles after 20 min of ischemia and remained higher after 20 min of reoxygenation, but the elevated level was more pronounced in failing myocardium from postinfarction rats. In contrast, more depression of isometric force was found in failing myocardium from postinfarction rats after 20 min of simulated ischemia, with no recovery after 20 min of reoxygenation. The isometric twitch was abbreviated with hypoxia in both physiological salt solution and fluorocarbon immersion, whereas after simulated ischemia the \(\text{Ca}^{2+}\) transient was prolonged especially with regard to the decline of the \(\text{Ca}^{2+}\) transient. The effects of reoxygenation on the \(\text{Ca}^{2+}\) transient and isometric tension were also in opposite directions; i.e., the isometric force was remarkably prolonged by reoxygenation, whereas the decline of the \(\text{Ca}^{2+}\) transient remained prolonged compared with baseline conditions. This persistent delay in the decline from peak light could result from an impaired function of the SR \(\text{Ca}^{2+}\) pump leading to a protracted reuptake of \(\text{Ca}^{2+}\) from the cytosol and/or a deficient SR \(\text{Ca}^{2+}\) release. Thus the deterioration of the failing myocardium with ischemia appears to be primarily due to abnormal \([\text{Ca}^{2+}]\) modulation resulting from SR dysfunction.
In addition to impairment of [Ca$^{2+}$]i homeostasis, accumulation of metabolites during fluorocarbon-simulated ischemia may contribute in part to myocardial stunning by indirectly affecting [Ca$^{2+}$]i handling. In the present study, infarcted rats 6 wk after permanent coronary ligation developed hypertrophy of noninfarcted left ventricular papillary muscles as defined by muscle cross-sectional area. After 20 min of immersion with hypoxia in fluorocarbon, more anoxic products, e.g., lactic acid, should be released from hypertrophied noninfarcted papillary muscles from postinfarction rats. After 20 min of reoxygenation in failing myocardium with fluorocarbon immersion, accumulated metabolites were retained because egress of metabolic products from the interstitial space was limited (4, 11), although we do not have direct data that indicate the cellular concentrations of metabolites. When fluorocarbon was finally replaced by oxygenated physiological salt solution, accumulated metabolites were retained because egress of metabolic products from the interstitial space was limited (4, 11), although we do not have direct data that indicate the cellular concentrations of metabolites. When fluorocarbon was finally replaced by oxygenated physiological salt solution, the isometric force rapidly recovered to prehypoxic values. Bing et al. (5) found the concentration of lactate increased in muscle preparations maintained in fluorocarbon relative to physiological salt solution during hypoxia. Gwathmey and Hajjar (20) reported that accumulated metabolites inhibit SR Ca$^{2+}$-ATPase. Increased lactic acid with simulated ischemia (2, 5) caused a profound reduction of tension and an increase in the diastolic [Ca$^{2+}$], and duration of the Ca$^{2+}$ transients in isolated ventricular muscles. This evolving metabolic acidosis is believed to directly displace Ca$^{2+}$ from intracellular binding sites, including troponin C, thereby reducing actomyosin ATPase activation and force generation per unit change in Ca$^{2+}$ (6). Accumulation of intracellular P, during ischemia has been also implicated as a contributor to postischemic contractile failure (30). Gao and co-workers (16) demonstrated that both maximal Ca$^{2+}$-activated force and the Ca$^{2+}$ sensitivity of the myofilaments are reduced in stunned cardiac muscle. In addition to structural modification of the myofilaments (23), elevated intracellular Mg$^{2+}$ in stunned myocardium (36) is known to be an important factor affecting the Ca$^{2+}$ sensitivity of the myofilaments (17). There is also abundant evidence implicating oxygen free radicals in the pathogenesis of myocardial stunning (7). In addition to their effects on [Ca$^{2+}$], homeostasis, oxygen free radicals may attribute to the decrease of Ca$^{2+}$ sensitivity in skinned cardiac muscles by increasing oxidized glutathione (15). However, the effect of lactate or other metabolites on [Ca$^{2+}$] remains to be fully defined.

Previous studies have been performed under a variety of conditions that mimic some aspects of ischemia, e.g., anoxia (3, 9). In contrast to our findings, i.e., postischemic stunning recovered promptly when fluorocarbon was replaced by physiological salt solution, Bolli et al. (9) showed the ventricular contractile function in canine experiments with remains depressed up to 24 h after reperfusion. As a different experimental model, it is problematical to compare the myocardial stunning in quantitative terms due to the variability inherent in the experiment preparations. From the analysis of individual dogs by Bolli et al. (9), a sensitive coupling exists between ischemic performance and postischemic function in the low flow range, whereby small changes in flow are associated with large changes in the subsequent recovery of function. Heart rate, systolic blood pressure, and thickening fraction during ischemia are also related to myocardial function after reperfusion. The dissimilarity between the results of Bolli et al. (9) and those of the present study may reflect the differences in the experimental animal species (canine vs. rat), the study condition (in vivo vs. in vitro), and parameters studied (regional heart function vs. isomet-
Fig. 8. Effects of simulated ischemia with FC on time intervals of isometric contraction (A) and intracellular light transient (B) in papillary muscles from Sham and MI rats (n = 8 each group). PSS, value in physiological salt solution with oxygenation. *P < 0.05 vs. PSS and FC; #P < 0.05 MI vs. Sham.
ric force of papillary muscle). However, in these other experimental models, perfusate flow continues so that the consequences of accumulation of ions and products of metabolism are not usually observed. In Langendorff perfused heart ischemia models (27), coronary vascular collapse with ischemia could be part of the reasons for an associated decline of mechanical contractility and Ca^{2+} availability. Both the regional and the global models are susceptible to the superimposed effects of coronary vascular turgor (14), which may be potentiated by postischemic edema (8). Prompt recovery of mechanical performance during reoxygenation subsequently takes place when fluorocarbon is replaced by physiological salt solution, i.e., to fully wash out anoxic metabolic products, which is consistent with the concept that metabolites have an important effect on the postischemic stunning. To avoid these multiple factors in the intact heart, experiments using isolated papillary muscle in the present study are of interest because it is possible to simplify the analysis of the effects.

Clinical implication. Stunning has been shown to occur following acute MI in which recanalization of the occluded coronary artery occurs spontaneously due to pharmacological thrombolysis or acute coronary angioplasty. Abnormal [Ca^{2+}]_i handling plays a major role in the pathophysiology and pathogenesis of myocardial stunning. The present finding should provide not only a conceptual framework for further investigation of the tolerance to ischemia-reperfusion injury with contractile dysfunction but also a rationale for developing clinically applicable interventions designed to prevent postischemic myocardial stunning.

We conclude that failing myocardium from postinfarction rats exhibits a potential to develop mechanical dysfunction during fluorocarbon-simulated ischemia. That depressed tolerance to ischemia in failing myocardium with more depression of isometric force during hypoxia-reoxygenation sequence with fluorocarbon immersion are primarily related to severe impairment of the modulation of myocardial stunning. Metabolite retention appears to be a primary factor in the modulation of myocardial stunning.

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