Changes in excitation-contraction coupling in an isolated ventricular myocyte model of cardiac stunning

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Louch, William E., Gregory R. Ferrier, and Susan E. Howlett. Changes in excitation-contraction coupling in an isolated ventricular myocyte model of cardiac stunning. Am J Physiol Heart Circ Physiol 283: H800–H810, 2002—To investigate cardiac stunning, we recorded intracellular [Ca\(^{2+}\)], contractions, and electrical activity in isolated guinea pig ventricular myocytes exposed to simulated ischemia and reperfusion. After equilibration, ischemia was simulated by exposing myocytes to hypoxia, acidosis, hyperkalemia, hypercapnia, lactate accumulation, and substrate deprivation for 30 min at 37°C. Reperfusion was simulated by exposure to Tyrode solution. Field-stimulated myocytes exhibited stunning upon reperfusion. By 10 min of reperfusion, contraction amplitude decreased to 43.0 ± 5.5% of preischemic values (n = 15, P < 0.05), although action potential configuration and sarcoplasmic reticulum Ca\(^{2+}\) stores, assessed with caffeine, were normal. Diastolic [Ca\(^{2+}\)] and Ca\(^{2+}\) transients (fura 2) were also normal in stunned myocytes. In voltage-clamped cells, peak L-type Ca\(^{2+}\) current was reduced to 47.4 ± 4.5% of preischemic values at 10 min of reperfusion (n = 21, P < 0.05). Contractions elicited by Ca\(^{2+}\)-induced Ca\(^{2+}\) release and the voltage-sensitive release mechanism were both depressed in reperfusion. Our observations suggest that stunning is associated with reduced L-type Ca\(^{2+}\) current but that alterations in Ca\(^{2+}\) homeostasis and release are not directly responsible for stunning.

In the heart, ischemia and reperfusion markedly change the coupling of excitation to contraction [excitation-contraction (E-C) coupling]. Ischemia causes abbreviation of action potentials and a decline in resting membrane potential (RMP) (13, 23). In addition, contractile activity is markedly decreased (26). With reperfusion, electrical activity may recover fully, although a transient period of arrhythmias may occur early in reperfusion (34). However, contractile activity may show a prolonged period of depression that lasts well beyond electrical recovery (19; for a review, see Ref. 11). This prolonged contractile dysfunction is called myocardial stunning (6).

Most studies of myocardial stunning have been conducted in situ hearts or in isolated perfused hearts. These investigations largely focused on factors that reduce or exacerbate myocardial stunning (for a review, see Ref. 5). Less is known about the cellular changes that underlie myocardial stunning. However, some studies have described changes in expression and function of proteins involved in E-C coupling. Decreases in the amounts of different proteins that are important in sequestration and release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) also have been reported. For example, SR Ca\(^{2+}\)-ATPase, which is responsible for SR uptake of Ca\(^{2+}\), has been reported to be decreased in stunned hearts (32, 36). The density of SR Ca\(^{2+}\) release channels (ryanodine receptors) also has been reported to be reduced in stunning (21, 37, 39, cf 38). In addition, phosphorylation of both SR Ca\(^{2+}\)-ATPase and ryanodine receptors by Ca\(^{2+}\)/calmodulin-dependent protein kinase may be decreased in stunning (32). Other studies indicate that decreased myofilament Ca\(^{2+}\) sensitivity plays an important role in contractile depression in stunning (17, 20, 31). This decrease in Ca\(^{2+}\) sensitivity may reflect proteolysis of troponin I in stunning (18). Together, these observations suggest that changes in SR Ca\(^{2+}\) uptake, SR Ca\(^{2+}\) release, and myofilament Ca\(^{2+}\) sensitivity might all contribute to contractile depression in stunning. Furthermore, decreased density of L-type Ca\(^{2+}\) channels and reduced capacity of the sarcoplasmal Na\(^{+}/Ca\(^{2+}\) exchanger have been observed in ischemia (3, 10, 33, 35). These findings suggest that transsarcomemmal Ca\(^{2+}\) fluxes also may be modified by ischemia and could contribute to stunning in reperfusion.

Although changes in many proteins that are central to E-C coupling have been reported, the consequences of these changes to stunning at the cellular level are not clear. The overall goals of the present study were to establish a single cell model of stunning in ventricular myocytes and to evaluate changes in E-C coupling that contribute to stunning in this model. In the present study, we adapted an isolated myocyte model of simulated ischemia and reperfusion, developed previously by us (8, 9), to investigate cellular changes in stunning. This model permits simultaneous measurements of transmembrane voltage, ion currents, and cell short-
ening. In addition, cytosolic Ca\(^{2+}\) levels and transients also can be measured in this model with Ca\(^{2+}\)-sensitive fluorescent dyes.

The specific objectives of this study were as follows: 1) to determine whether stunning can be elicited in our isolated myocyte model of ischemia and reperfusion; 2) to determine the possible roles of RMP, action potential configuration, and L-type Ca\(^{2+}\) current \((I_{Ca,L})\) in stunning in this model; 3) to compare changes in the efficacy of different mechanisms for E-C coupling in stunning; and 4) to determine whether stunning is related to changes in the magnitude of Ca\(^{2+}\) transients or Ca\(^{2+}\) release from SR stores in this model.

**METHODS**

**Cell isolation.** Experiments were conducted on isolated ventricular myocytes from guinea pigs and performed in accordance with the guidelines published by the Canadian Council on Animal Care. Male guinea pigs (325–375 g, Charles River) were anesthetized with pentobarbital sodium (160 mg/kg with 3.3 IU/g ip heparin). The aorta was cannulated in situ, and the heart was removed and perfused for 7–8 min at 10–12 ml/min with oxygenated (100% O\(_2\), 37°C) Ca\(^{2+}\)-free solution of the following composition (in mM): 120 NaCl, 3.8 KCl, 1.2 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 10 HEPES, and 11 glucose (pH 7.4 with NaOH). Collagenase A (25 mg/50 ml buffer, Boehringer-Mannheim) and protease (4.8 mg/50 ml, Sigma type XIV) were then included in the perfusate for 5 min. The ventricles were then minced in a buffer with the following composition (in mM): 80 KOH, 50 glutamic acid, 30 KCl, 30 KH\(_2\)PO\(_4\), 20 taurine, 10 HEPES, 10 glucose, 3 MgSO\(_4\), and 0.5 EGTA (pH 7.4 with KOH). Myocytes were placed in a 0.75-ml chamber on the stage of an inverted microscope. After 5–10 min, they were superfused at 3 ml/min at 37°C with Tyrode solution containing (in mM) 129 NaCl, 20 NaHCO\(_3\), 0.9 NaH\(_2\)PO\(_4\), 4 KCl, 0.5 MgSO\(_4\), 2.5 CaCl\(_2\), and 5.5 glucose (pH 7.4) gassed with 95% O\(_2\)-5% CO\(_2\). Mean contraction amplitude also was decreased during ischemia \((C)\). RMP and APD both recovered to preischemic levels by late reperfusion, when significant contractile depression (stunning) was observed. *Significant difference from control, \(P < 0.05\); \(n = 21\) myocytes from 21 hearts.

**General methods.** Myocytes were superfused for 10 min with Tyrode solution and then for 30 min with a solution mimicking the specific conditions of myocardial ischemia, including hypoxia, hypercapnia, hyperkalemia, acidosis, lactate accumulation, and substrate deprivation (14, 16). This “ischemic solution” had the following composition (in mM): 123 NaCl, 6 NaHCO\(_3\), 0.9 NaH\(_2\)PO\(_4\), 8 KCl, 0.5 MgSO\(_4\), 2.5
CaCl₂, and 20 Na-lactate, gassed with 90% N₂-10% CO₂ (pH 6.8). A 90% N₂-10% CO₂ gas phase was layered over the experimental chamber during simulated ischemia. Reperfusion was simulated by return to Tyrode solution and removal of the gas phase. Cells were exposed to only one cycle of ischemia and reperfusion.

**Measurement of action potentials and ionic currents.** Cells were impaled with high-resistance electrodes (18–25 MΩ) to minimize dialysis and avoid buffering intracellular Ca²⁺ levels. Electrodes were filled with 2.7 M KCl, and a 2.7 M KCl-agar bridge was used as a bath ground. Action potentials were recorded with conventional microelectrode techniques. Currents were recorded with discontinuous single electrode voltage-clamp techniques (sample rate 8–10 kHz). Recordings were made with an Axoclamp 2B amplifier (Axon Instruments).

The recording mode was alternated between conventional and voltage clamp. At 5-min intervals, action potentials (averages of 5 initiated at 2.8 Hz) were recorded for measurement of resting and action potentials. Action potentials were initiated by 3.5-ms current pulses delivered through the recording electrodes. Action potential duration (APD) was measured at 80% repolarization with respect to the action potential amplitude.

Between action potential recordings, cells were voltage clamped. Cells were held at −80 mV. All test steps were preceded by trains of ten 200-ms conditioning pulses to 0 mV to provide a consistent history of activation. Two voltage-clamp protocols were used. In one protocol, conditioning pulses were followed by a 500-ms repolarization to −52.5 mV to inactivate sodium current and then two sequential 250-ms test steps to −40 and 0 mV. Both voltage-clamp protocols were run once every 5 min during the experiment. pCLAMP 6.0 software (Axon Instruments) was used to generate stimulation and voltage-clamp protocols and to acquire and analyze data.

Contraction amplitudes were measured with respect to a reference point immediately before the onset of cell shortening. Thus contractions measured with this method are presented as positive deflections.

**Fluorescence measurements.** Intracellular Ca²⁺ was measured by whole cell photometry (DeltaRam, Photon Technology International). Myocytes were loaded with fura 2 by incubating them with the acetoxymethyl ester (0.1 µM) for 20 min at room temperature. The ratio of emission at 510 nm, during alternate excitation at 340 nm and 380 nm, was used to determine intracellular Ca²⁺ concentrations ([Ca²⁺]ᵢ). The fluorescence ratio was converted to [Ca²⁺] with a calibration curve determined in vitro experimentally at pH 7.2. The calibration curve was determined in the same experimental chamber and with the same optical path as used for data collection. Because intracellular pH has been reported to recover to preischemic levels rapidly upon reperfusion (22, 24), the calibration curve determined at pH 7.2 is appropriate for measurement of intracellular Ca²⁺ in reperfusion, which is the focus of this study. However, it should be noted that use of this calibration curve may have resulted in a slight underestimate in the measurements of intracellular Ca²⁺ made during the ischemic period. This may have occurred because the pH of the ischemic solution was 6.8, which has

![Fig. 3. Changes in membrane currents and contractions in voltage-clamped myocytes exposed to simulated ischemia and reperfusion. The voltage-clamp protocol (inset) was used to elicit L-type Ca²⁺ current (I_{Ca,L}; A) and Ca²⁺-induced Ca²⁺ release (CICR) contractions (B). Representative recordings show that peak I_{Ca,L} was reduced during ischemia and did not recover in reperfusion. CICR contractions were decreased in ischemia, recovered in early reperfusion, and were reduced in late reperfusion.](https://example.com/fig3.png)
been shown experimentally to change the dissociation constant for fura 2 from $-0.14$ to 0.18 $\mu$M (30). Fluorescence was recorded and measured with Felix software (version 1.4, Photon Technology International). The $\text{Ca}^{2+}$ transients were measured relative to diastolic $[\text{Ca}^{2+}]$ (background subtracted).

**Estimation of SR Ca$^{2+}$ stores.** Amplitudes of caffeine-elicted $\text{Ca}^{2+}$ transients were used as a measure of SR $\text{Ca}^{2+}$ content during ischemia and reperfusion. In these experiments, cells were impaled and exposed to control conditions for 10 min, ischemia for 30 min, and reperfusion for 40 min. A second group of cells were impaled, but not exposed to ischemic conditions, and served as time controls. In both groups, cells were stimulated with current pulses delivered through the microelectrode at 2 Hz. At 10, 40, 55, 70, and 80 min during the experiment, stimulation was briefly interrupted, and 10 mM caffeine was applied to cells for 1 s at 37°C with a rapid solution changer. Changes in solution with this device were triggered by the voltage-clamp protocol and were complete within 300 ms (27).

$\text{Ca}^{2+}$ transients and cell length in field-stimulated myocytes. In some experiments, myocytes were field stimulated continuously at 2 Hz through a pair of platinum electrodes. Myocytes were exposed to 30 min of simulated ischemia and 40 min of reperfusion. Parallel time controls were performed. Changes in cell length and fura 2 $[\text{Ca}^{2+}]$, were measured in separate experiments. Diastolic cell length and $[\text{Ca}^{2+}]$, were measured at a point immediately before responses. Systolic cell length and $[\text{Ca}^{2+}]$, were measured at peak values during responses. Cell shortening and $\text{Ca}^{2+}$ transients were calculated as the difference between diastolic and systolic measurements. Cell length and $[\text{Ca}^{2+}]$, were recorded at 5-min intervals throughout the experiment except for the first 5 min of reperfusion, when recordings were made every minute. Three responses were averaged for each recording period.

**Data analyses.** Differences between experimental groups and time controls were tested for statistical significance with a two-way repeated-measures ANOVA. All other data were analyzed relative to preischemic values using a one-way repeated-measures ANOVA. Post hoc comparisons were made with a Bonferroni test. Statistical analyses were performed with Sigmastat (version 2.0, Jandel). Data are presented as means ± SE. The value of $n$ represents the number of myocytes sampled. In most cases, each myocyte came from a different heart. Occasionally more than one myocyte from the same heart was utilized, therefore the numbers of myocytes and hearts are indicated in the figures for each data set. Because most myocytes were from different hearts, the use of multiple samples was not incorporated in the statistical analysis.

**RESULTS**

**Effects of ischemia and reperfusion on action potentials and contractions.** Figure 1 shows representative recordings of action potentials and contractions at selected times during an experiment. Action potentials abbreviated and cell membranes depolarized during ischemia (Fig. 1A). Ischemia also caused a marked reduction in the amplitude of contraction (Fig. 1B). Action potential configuration recovered rapidly upon reperfusion (Fig. 1A). Amplitudes of contractions increased in early reperfusion but decreased with continued reperfusion (Fig. 1B).

Figure 2 shows mean data describing changes in APD, RMP, and amplitudes of contraction during ischemia and reperfusion. APD abbreviated in ischemia and recovered slowly in reperfusion (Fig. 2A). In addition, cells depolarized in ischemia, but membrane potential recovered rapidly in reperfusion (Fig. 2B). Both RMP and APD returned to preischemic levels by late reperfusion. Contractions decreased significantly during ischemia. Upon reperfusion, contractions initially recovered to preischemic values but then decreased again in late reperfusion (Fig. 2C).

**Effects of ischemia and reperfusion in voltage-clamped myocytes.** Similar experiments were conducted in voltage-clamped myocytes to eliminate effects mediated by changes in action potential configuration. The voltage-clamp protocol used in these experiments is shown in Fig. 3, inset. Representative currents and contractions, recorded at selected times during ischemia and reperfusion, are shown in Fig. 3, A and B. Peak inward current declined in ischemia and showed little recovery in reperfusion (Fig. 3A). Contractions also were depressed by ischemic conditions but, in contrast to current, exhibited rapid recovery early in reperfusion, followed by sustained depression later in reperfusion (Fig. 3B).

Figure 4 shows mean measurements of currents and contractions. Myocytes exhibited a gradual decrease in $I_{\text{Ca,L}}$ during ischemia, with little recovery in reperfu-

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**Fig. 4.** Stunned myocytes exhibited reduced $I_{\text{Ca,L}}$ and recovery of steady-state current ($I_{SS}$). Mean $I_{\text{Ca,L}}$ amplitude was gradually reduced during ischemia and did not recover in reperfusion (A). However, outward $I_{SS}$ increased during ischemia and recovered to control levels in late reperfusion (B). As was observed for contractions elicited by action potentials, contractions elicited by CICR (C) were reduced during ischemia and recovered rapidly in early reperfusion. In late reperfusion, myocytes exhibited significant stunning. **Significant difference from control, $P < 0.05$; $n = 21$ myocytes from 21 hearts.**
We also measured the net current at the end of the activation step ($I_{SS}$) to determine whether ischemia was accompanied by a change in $I_{SS}$ at $t=0$. Figure 4A shows that $I_{SS}$ became significantly more outward during ischemia and that this declined early in reperfusion. Contractions were significantly depressed during ischemia but showed a rapid return to preischemic levels in early reperfusion (Fig. 4C). Recovery of contractions in early reperfusion was transient, and contraction amplitudes became significantly decreased in later reperfusion (Fig. 4C).

Contractions initiated by the voltage-sensitive release mechanism (VSRM) and CICR were similarly depressed in late reperfusion. The two-step voltage protocol (A) was used to separately elicit VSRM and CICR contractions. VSRM contractions were elicited by a voltage step to $-40$ mV and were associated with a small inward current. $I_{Ca,L}$ and CICR contractions were observed in response to the voltage step to $0$ mV. VSRM and $I_{Ca,L}$ contraction amplitudes were similarly affected by ischemia and reperfusion (B and C, respectively). Contractions initiated by both mechanisms were depressed compared with control levels during ischemia and late reperfusion.

**Effects of ischemia and reperfusion on the magnitude of SR Ca$^{2+}$ stores.** Figure 6A shows Ca$^{2+}$ transients recorded from a myocyte stimulated at $2$ Hz in normal

Fig. 6. Caffeine-elicited Ca$^{2+}$ transients were normal in stunned myocytes. A: representative recording of intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) in a myocyte exposed to $10$ mM caffeine. A $1$-s caffeine application was interpolated in a $2$-Hz stimulus train as indicated. The magnitude of caffeine-elicited Ca$^{2+}$ transients was not altered from values at $10$ min in time control experiments or during ischemia and reperfusion (B). Data are presented as a percentage of preischemic values (ischemia-reperfusion, $n=13$ myocytes from 12 hearts; time controls, $n=6$ myocytes from 6 hearts).
Tyrode solution. Each stimulus elicited a rapid transient rise in \([Ca^{2+}]\). Stimulation was interrupted briefly and the superfusate was rapidly switched for 1 s to one containing 10 mM caffeine. Caffeine application elicited a \(Ca^{2+}\) transient, which was taken as a measure of SR \(Ca^{2+}\) stores. After caffeine application, \(Ca^{2+}\) transients elicited by stimulation were temporarily reduced in magnitude. Caffeine-elicited \(Ca^{2+}\) transients were measured in cells exposed to ischemia and reperfusion and in cells that were not exposed to ischemia and therefore served as time controls (Fig. 6B). Surprisingly, the magnitudes of caffeine-induced transients did not change significantly in response to ischemia and reperfusion compared with time controls (Fig. 6B). These data indicate that the decrease in contraction observed late in reperfusion (Figs. 2, 4, and 5) cannot be attributed to a decline in SR stores of \(Ca^{2+}\).

Relation between cytosolic \(Ca^{2+}\) concentration and cell length in myocytes exposed to ischemia and reperfusion. To determine whether changes in cell length in ischemia and reperfusion are caused by corresponding changes in cytosolic \(Ca^{2+}\), we compared \(Ca^{2+}\) levels and cell shortening in field-stimulated myocytes. Figure 7 shows representative recordings of \(Ca^{2+}\) concentration and cell length in time controls in which myocytes were not exposed to ischemia. Diastolic \([Ca^{2+}]\) appeared to increase slightly during these experiments. There was no visible deterioration in the quality of recordings during the duration of the experiment.

\(Ca^{2+}\) transients (shaded region, Fig. 8A) and a gradual increase in diastolic \(Ca^{2+}\). By 60 min, diastolic \([Ca^{2+}]\), was significantly different from values at 10 min. Mean data for cell length are shown in Fig. 8B. Myocytes exhibited a slight reduction in both diastolic length and contraction magnitude (shaded region) with time. These data show that relatively stable recordings of \(Ca^{2+}\) concentrations and cell shortening could be measured for up to 80 min in field-stimulated time controls.

Figure 9 shows representative recordings of \(Ca^{2+}\) concentration and cell length from field-stimulated myocytes exposed to ischemia and reperfusion. During ischemia, contraction was markedly depressed (Fig. 9B). In contrast, the magnitude of \(Ca^{2+}\) transients changed little (Fig. 9A), although diastolic \(Ca^{2+}\) showed a slight increase in ischemia. Early reperfusion caused marked increases in the magnitudes of both contraction and \(Ca^{2+}\) transients beyond preischemic levels. Although \(Ca^{2+}\) transients were increased, diastolic \(Ca^{2+}\) levels actually declined. With continued reperfusion, \(Ca^{2+}\) transients recovered toward control levels, whereas contractions became depressed relative to preischemic levels.

Figure 10 shows mean measurements of diastolic and systolic \(Ca^{2+}\) concentration and cell length recorded throughout ischemia and reperfusion (A and B, respectively). Shaded areas represent the amplitudes of \(Ca^{2+}\) transients and contractions, which are considered quantitatively and are described in Fig. 12. During ischemia, myocytes exhibited a significant increase in diastolic \(Ca^{2+}\) concentration relative to preischemic
Upon reperfusion, diastolic length rapidly and significantly decreased and then recovered partially during the first 10 min of reperfusion. No further recovery occurred after the first 10 min.

Figure 12 compares the magnitudes of Ca\(^{2+}\) transients and contractions in cells exposed to ischemia and reperfusion with corresponding values from time controls. Ca\(^{2+}\) transients were only significantly different from time controls in early reperfusion, where there was an abrupt but transient increase (Fig. 12A). Figure 12B shows mean values for contraction during ischemia and reperfusion. During ischemia, cell shortening was significantly depressed relative to time controls. In early reperfusion, contraction showed a marked increase above contraction in time controls. This significant but brief increase in contraction was followed by sustained contractile depression.

DISCUSSION

The goals of the present study were to establish a model of stunning in isolated guinea pig ventricular myocytes and to evaluate changes in E-C coupling that contribute to stunning in this model. Our experiments demonstrate that, although action potential configuration and membrane potential recovered fully, myocytes in this model exhibited stunning. Furthermore, stunning persisted even when changes in action potentials in ischemia and early reperfusion were eliminated with voltage clamp. Contractile depression in reperfusion was evident regardless of whether contractions were initiated by CICR or the VSRM and occurred without changes in SR Ca\(^{2+}\) stores. In this model, reperfusion resulted in an initial brief rebound in cell contraction before stunning developed. This brief rebound in amplitude of contractions was accompanied by a parallel rebound in Ca\(^{2+}\) transients in early reperfusion. In contrast, stunning was not accompanied by a parallel change in Ca\(^{2+}\) transients in late reperfusion.

To investigate stunning in ventricular myocytes, we adapted a previously developed model of simulated ischemia and reperfusion (8). As shown by the time controls in the present study, this model exhibits stable Ca\(^{2+}\) transients and contractions up to 80 min of recording time. This stability allowed sufficient time for a complete cycle of ischemia and reperfusion. Our study clearly demonstrates that postischemic contractile depression occurs in this model and provides a model in which stunning can be correlated with possible changes in electrophysiology and Ca\(^{2+}\) transients. An additional advantage of this model is that contractile responses and Ca\(^{2+}\) transients can be elicited by action potentials or by voltage-clamp protocols. With the use of this model, we were able to investigate possible roles of RMP, action potential configuration, and I\(_{Ca,L}\) in stunning.

Ischemia and reperfusion caused marked changes in electrical activity. During ischemia, we observed decreased RMPs and abbreviation of action potentials, as reported in previous studies in single and multicellular preparations (8, 13, 23). In voltage-clamp experiments,
these changes were correlated with an increase in steady-state outward current and a decline in $I_{Ca,L}$. This decrease in $I_{Ca,L}$ may reflect a reduction in the density of L-type Ca$^{2+}$ channels reported by others in ligand binding studies (3, 35). Changes in resting potentials, action potential configuration, and steady-state outward current recovered in early reperfusion, and therefore likely did not play a role in stunning. In contrast, depression of $I_{Ca,L}$ persisted into late reperfusion. This reduction in magnitude of $I_{Ca,L}$ might be expected to contribute to stunning, because Ca$^{2+}$ released through CICR is proportional to the magnitude of $I_{Ca,L}$ (4). In addition, $I_{Ca,L}$ is believed to contribute to maintenance of SR stores of Ca$^{2+}$ (12). Thus inhibition of $I_{Ca,L}$ in late reperfusion might inhibit CICR both by decreasing this trigger for Ca$^{2+}$ release and by decreasing SR Ca$^{2+}$ stores available for release.

Because $I_{Ca,L}$ was observed to be depressed, and because previous studies have reported changes in proteins important for sequestration and release of SR Ca$^{2+}$ in stunned tissues (21, 32, 36, 37, 39), we assessed SR Ca$^{2+}$ stores in the present study. Interestingly, Ca$^{2+}$ stores remained constant through the cycle of ischemia and reperfusion. Thus stunning could not be attributed to a decrease in SR Ca$^{2+}$ stores in the present study. Furthermore, we did not observe depression of Ca$^{2+}$ transients in field-stimulated cells in reperfusion, which indicates that stunning could not be attributed to decreased Ca$^{2+}$ release. Normal Ca$^{2+}$ transients also have been reported in intact stunned myocardium (7, 24, 29). These results indicate that changes in Ca$^{2+}$-ATPase, ryanodine receptor density, or phosphorylation did not alter either SR Ca$^{2+}$ stores or Ca$^{2+}$ transients in the present model. Nevertheless, it is possible that compensatory changes in SR load and release may have obscured such changes (12). It also is possible that these protein structures are not normally limiting to SR function in unloaded myocytes or that changes in these proteins are not required for stunning to occur.

It is not clear how normal Ca$^{2+}$ transients are maintained during depression of $I_{Ca,L}$ in stunning. There are several possibilities. For example, 1) the gain of CICR (SR Ca$^{2+}$ released/$I_{Ca,L}$) might be increased,
and/or 2) elevation of intracellular Na\(^+\) levels in ischemia and reperfusion might shift the equilibrium of Na\(^+\)/Ca\(^{2+}\) exchange and thereby reduce loss of released Ca\(^{2+}\) through the sarcolemma. The latter mechanism might increase the recirculating fraction of SR Ca\(^{2+}\). Evidence for one or both of these possibilities will require further investigation. It also is possible that CICR might be depressed in stunning, but this is compensated by continued operation of the VSRM. This possibility was tested by comparing effects of stunning on CICR and the VSRM (15). Contractions initiated separately by these two mechanisms showed parallel decreases in amplitude during ischemia and late reperfusion. Thus stunning was not mediated by selective depression of either mechanism of E-C coupling.

Our investigations with caffeine-induced contractions indicated that ischemia and late reperfusion were not accompanied by significant changes in SR Ca\(^{2+}\) stores. Interestingly, a marked rebound in the amplitudes of Ca\(^{2+}\) transients and contractions was observed in early reperfusion. Thus in early reperfusion the brief overshoot in contraction is likely caused by an increase in the magnitude of the Ca\(^{2+}\) transients. The mechanism for this rebound is not clear. Because Ca\(^{2+}\) overload is believed to be an important event in ischemia and early reperfusion (5), one may postulate that SR stores might be elevated temporarily during early reperfusion. Because it was not practical to assess SR Ca\(^{2+}\) stores with caffeine at very short intervals, we could not determine whether SR stores were

Fig. 10. Stunning was not associated with obvious changes in Ca\(^{2+}\) transients. A: mean measurements of \([\text{Ca}^{2+}]_i\). Ischemia was associated with increased diastolic \([\text{Ca}^{2+}]_i\); and no obvious change in the magnitude of Ca\(^{2+}\) transients (shaded region). In early reperfusion, mean diastolic \([\text{Ca}^{2+}]_i\) partially recovered but increased gradually with continued reperfusion. Ca\(^{2+}\) transients were slightly reduced in late reperfusion. B: mean measurements of cell length. During ischemia, myocytes exhibited a marked reduction in the magnitude of cell shortening (shaded region) that was associated with a significant decrease in systolic length. With reperfusion, both diastolic and systolic cell length were decreased, and the magnitude of contractions was briefly increased. Contractions were reduced in late reperfusion, and diastolic cell length remained below control levels. *Significant difference from mean preischemic values, \(P < 0.05\); \(n = 15\) myocytes from 11 hearts for Ca\(^{2+}\) transients; \(n = 15\) myocytes from 10 hearts for cell length.

Fig. 11. In late reperfusion, diastolic \([\text{Ca}^{2+}]_i\), was normal, but resting cell length was depressed. Mean measurements of diastolic \([\text{Ca}^{2+}]_i\); and cell length are shown in A and B, respectively, for time controls and myocytes exposed to simulated ischemia and reperfusion. Data are normalized to mean preischemic values. Diastolic \([\text{Ca}^{2+}]_i\) was significantly elevated during ischemia but was unchanged from time control values in reperfusion. Diastolic length was slightly increased during ischemia and dramatically reduced in early reperfusion. With continued reperfusion, diastolic length partially recovered and was not significantly different from time controls after 60 min. *Significant difference from time controls, \(P < 0.05\). For the ischemia-reperfusion data, \(n = 15\) myocytes from 11 hearts for Ca\(^{2+}\) transients; \(n = 15\) myocytes from 10 hearts for cell length. For the time controls, \(n = 5\) myocytes from 4 hearts for Ca\(^{2+}\) transients; \(n = 12\) myocytes from 10 hearts for cell length.
marked depression of stimulated contractions, despite the presence of normal Ca\textsuperscript{2+} transients. Dissociation between the magnitudes of contractions and Ca\textsuperscript{2+} transients also was observed in reperfusion when stunning developed. Contractions remained depressed despite the persistence of Ca\textsuperscript{2+} transients with normal amplitudes. This observation closely resembles changes seen in intact hearts in stunning, and thus our results support the idea that contractile depression in stunned myocytes is largely a function of depressed myofilament Ca\textsuperscript{2+} sensitivity (17, 20, 31). Interestingly, diastolic cell length shortened greatly in early reperfusion and did not recover fully within the duration of our experiments. Corresponding changes in diastolic pressure have been reported for perfused hearts, where diastolic pressure remained elevated in early reperfusion and later in stunning (17). In the present experiments, incomplete relaxation of the myocytes was not accompanied by elevation of diastolic Ca\textsuperscript{2+}, which returned rapidly to control levels during reperfusion. Extrinsic effects such as coronary vascular dilatation also can be excluded in this isolated cell model; however, it is possible that changes in intrinsic factors such as myofilaments might contribute. At the present time, the mechanism for this diastolic dysfunction is not clear.

A previous study (28) examined the effects of exposure of isolated rat ventricular myocytes for 90 min to acidosis, substrate deprivation, and hypoxia. Contractile depression was studied by this combination of conditions. However, unlike the present study, Ca\textsuperscript{2+} transients were depressed during ischemia, and no overshoot in amplitudes of contractions or transients was observed early in reperfusion. Although this study did not address stunning, it did report a prolonged decrease in amplitude of contraction with return to control conditions (28). Whether this defect in contraction is analogous to stunning in the present model is not clear.

In the present study, we utilized an isolated guinea pig ventricular myocyte model of ischemia and reperfusion, in which cells exposed to hypoxia, hypercapnia, hyperkalemia, acidosis, lactate accumulation, and substrate deprivation exhibit stunning. Our previous studies with this model have shown that myocytes exposed to these conditions show contractile and electrophysiological changes that mimic those observed in ischemic myocardium in vivo (8, 9). The present study demonstrates that this model also mimics changes in contractions and Ca\textsuperscript{2+} transients reported to accompany stunning in intact hearts (7, 24, 29). Thus our study provides a reproducible model that can be useful in studies of cellular changes occurring in stunning.

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