Defibrillation depresses heart sarcoplasmic reticulum calcium pump: a mechanism of postshock dysfunction

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Jones, Douglas L., and Njanoor Narayanan. Defibrillation depresses heart sarcoplasmic reticulum calcium pump: a mechanism of postshock dysfunction. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H98–H105, 1998.—Presently, the only therapy for ventricular fibrillation is delivery of high-voltage shocks. Despite "successful defibrillation," patients may have poor cardiac contractility, the mechanisms of which are unknown. Intracellular Ca^{2+} handling by the sarcoplasmic reticulum (SR) plays a major role in contractility. We tested the hypothesis that defibrillation shocks interfere with Ca^{2+} transport function of cardiac SR. Rats anesthetized with pentobarbital sodium had bilateral electrodes implanted subcutaneously for transthoracic shocks. A series of 10 shocks, 10 s apart, at 0–250 V was delivered from a trapezoidal defibrillator. The hearts were rapidly removed, SR-enriched membrane vesicles were isolated, and ATP-dependent Ca^{2+} uptake and Ca^{2+}-stimulated ATP hydrolysis were determined. There was a marked, shock-related decline in Ca^{2+} uptake, whereas adenosinetriphosphatase activity remained unaltered. The polypeptide compositions were similar in control and shocked SR. In Langendorff hearts, shocks also decreased contractility and slowed relaxation. These data indicate that shocks with current densities similar to defibrillation depress Ca^{2+}-pumping function of cardiac SR because of uncoupling of ATP hydrolysis and Ca^{2+} transport. Shock-induced impairment of Ca^{2+} pump function may underlie postshock myocardial dysfunction.

calcium pump activity; arrhythmia; fibrillation

HIGH-VOLTAGE DEFIBRILLATION shock remains the only reliable therapy available to salvage the life of a patient experiencing ventricular fibrillation. Unfortunately, the efficacy of defibrillation shocks is inconsistent both between individuals and even within the same individual between bouts of fibrillation (11). Although some mechanisms contributing to this variation are understood, such as poor electrode contact with high electrode impedance and improper electrode placement, other factors leading to variation and prolonged delay to return of sinus rhythm and protracted poor or slow recovery of hemodynamic function postshock are not readily explained. Return to sinus rhythm has been reported to occur <50% in out-of-hospital cardiac defibrillation (32). Clinically there are several instances of documented poor hemodynamics and depressed contractile function after defibrillation shocks. Various mechanisms have been suggested to contribute to this depression, including electrolyte shifts, ischemia, electro poration of the sarcolemma, postischemic myocardial stunning, as well as contributions from a variety of dysfunctions already present before the fibrillation bout.

In experimental models, electroporation has clearly been shown to occur in the sarcolemma (11); however, recovery of membrane integrity is rapid (seconds) and cannot readily explain protracted depression lasting ≥10 min (15). Also, protracted alterations are seen in action potential characteristics (16) that are not modified by agents that affect sarcolemmal Ca^{2+} handling, such as propranolol and verapamil (15). In the mammalian heart, 65–80% of the beat-to-beat Ca^{2+} transient occurs via the sarcoplasmic reticulum (SR) (2). However, no studies have specifically examined the effects of defibrillation shocks on Ca^{2+} handling by the SR. The objective of the present study was to test the hypothesis that defibrillation shocks interfere with Ca^{2+} transport function of cardiac SR.

METHODS

Animals. Male Wistar rats (Charles River, Mississauga, Canada) weighing 250–300 g were housed in the animal quarters on a 12:12-h light-dark cycle and an ad libitum food and water regime. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care, and the animal use protocol was approved by the University of Western Ontario Animal Care Committee. On the day of death, the rats were anesthetized with pentobarbital sodium (Somnitol, 60 mg/kg; Canada Packers, Hamilton, Canada) and 1-cm incisions were made on the left and right chest for subcutaneous insertion of two 0.8-cm diameter gold-plated circular electrodes (Grass Instruments, Quincy, MA) just beneath the skin on the chest wall.

A separate group of 10 rats were anesthetized, and indwelling femoral cannulas were implanted to monitor blood pressure throughout shock delivery and postshock; 6 similarly anesthetized rats served as controls. Cannulas were connected via pressure transducers (COBE, Bramalea, Canada) to a BioPac System Digital monitor (model MP100) and a personal computer to continuously monitor blood pressure. Blood pressure data from one shocked rat was not analyzed because of technical recording difficulties.

In a third group of anesthetized rats, the hearts were rapidly removed and placed on a Langendorff apparatus perfused with Tyrode solution at 37°C and pH 7.4 for delivery of shocks and measurements of contractility and relaxation using ultrasound crystals. The 2.45-mm crystals were fixed on the long axis of the heart, from the apex to the groove at the anterior junction of the ventricles and the aortic root, using Vetbond glue (3M Animal Care Products, St. Paul, MN). A second pair of crystals was similarly attached to the mid left and right ventricular free walls. The crystals were attached to a Digital Sonomicrometer (Sonometrics, London, Canada), the output from which was passed to the BioPac system, which allowed on-line monitoring and off-line calculation of maximum and minimum distances and half-relaxation times. For each recording interval, 10 sequential contraction-relaxation measurements were obtained and averaged for each animal.

Shock delivery. Two rats were connected in series to a trapezoidal defibrillator (model 2376 or 2394, Medtronic, Mississauga, Canada). Pairs were randomized to receive 10 shocks separated by 10 s at stored voltages of 0, 50, 100, 150, or 250 stored volts. Shocks were trapezoidal monophasic pulses of 5-ms duration from the 50-μF capacitor bank. Stored voltages and paired animals were selected to provide peak current per gram of heart for the rat heart, which approximated those of subthreshold and successful defibrillation shocks in patients and large animal studies (10, 11) and previous studies with shocks delivered to papillary muscle (17).

In pilot experiments in Langendorff hearts, it was determined that a 125-Ω series resistor was necessary to reduce the peak current to approximate the current when the paired animals received transthoracic 150-V shocks. Therefore, data are only presented for the Langendorff hearts with the 125-Ω series resistor. For shock delivery, two disk electrodes were glued to 5-cm-long wooden dowels taped to the opposite arms of a tube clamp on a retort stand. Because the positioning of the electrodes impeded the movement of the heart slightly, a baseline recording measurement of contractility was taken before the clamp and shock electrodes were positioned in contact with the heart with the disks orthogonal to both pairs of ultrasound crystals. The electrodes were removed after delivery of the last shock. At the end of the 15-min recording interval, the hearts were removed for histological examination.

Isolation of SR-enriched membrane vesicles. Immediately after the last shock, or ~1 min after electrode placement for control rats receiving no shocks, the hearts were rapidly removed, cleaned of major vessels and atria, and then washed in ice-cold 10 mM $NaHCO_3$ (pH 6.8) buffer to remove blood, and the ventricular tissue was used for the isolation of SR membranes as described previously (28). Briefly, the tissue was minced and homogenized in 6 vols (based on ventricular weight) of ice-cold buffer (10 mM $NaHCO_3$, pH 6.8) using a Brinkman Instruments Polytron homogenizer (3 bursts of 15-s duration with 30-s intervals, speed setting 8). The homogenate was centrifuged at 1,000 *g* for 10 min at 4°C. The supernatant was decanted and kept in an ice slurry. The pellet was resuspended in four times the ventricular weight of ice-cold buffer and then centrifuged at 1,000 *g* for 10 min at 4°C. The supernatant was decanted and combined with the first supernatant, and the pellet was discarded. The combined supernatant was centrifuged at 8,000 *g* for 20 min at 4°C. The supernatant was decanted, and the pellet was discarded. Solid KCl (44 mg/ml) was added to the supernatant (final concn 0.6 M), swirled to dissolve, and left on ice for 25 min and then centrifuged at 40,000 *g* for 60 min at 4°C. The supernatant was discarded, and the pellet containing SR-enriched membrane vesicles was resuspended in a 10 mM tris(hydroxymethyl)aminomethane (Tris)-maleate-100 mM KCl buffer (pH 6.8) to give a protein concentration of ~3 mg/ml. Protein was determined using the method of Lowry et al. (18) using bovine serum albumin as a standard.

Preparation of heart homogenates. In addition to SR, homogenates of ventricular myocardium from control and shocked rats were also used in some of the experiments. For this, the homogenates were prepared by homogenizing the ventricles in 10 vols (based on ventricular weight) of 10 mM Tris-maleate-100 mM KCl buffer (pH 6.8) using a Polytron PT-10 homogenizer (3 bursts of 15-s duration with 30-s interval, speed setting 8), returning the beaker to the ice between bursts. In the second experiment, in which the blood pressure was recorded, hearts were removed 15 min after the last shock, or 20 min after surgery for nonshocked control rats, for preparation of homogenates.

Determination of Ca^{2+} uptake and Ca^{2+}-ATPase activities. ATP-dependent, oxalate-facilitated Ca^{2+} uptake by SR and cardiac homogenates was determined using a Millipore filtration technique as detailed elsewhere (20). The standard incubation medium (total volume 1 ml) contained (in mM) 50 Tris-maleate (pH 6.8), 5 $MgCl_2$, 2.5 ATP, 120 KCl, 5 potassium oxalate, 5 NaN_3, and 0.1 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), membrane or homogenate fraction (30 μg protein in SR, 80 μg protein in homogenate), and varying concentrations of $^{45}CaCl_2$ (8,000–10,000 counts·min^{-1}·nmol^{-1}). All assays were performed at 37°C. The Ca^{2+} uptake reaction was initiated by the addition of membrane fraction after preincubation of the rest of the assay components for 3 min. The free Ca^{2+} concentration in the assay medium was determined according to the computer program of Fabiato (4). The data on Ca^{2+} concentration dependence on Ca^{2+} uptake were analyzed using nonlinear regression curve fitting using the SigmaPlot program (Jandel Scientific). The data were fit to the equation

$$v = (V_{max}[Ca^{2+}]^{n_H})/(K_{0.5}^{n_H} + [Ca^{2+}]^{n_H})$$

where v is the measured mean Ca^{2+} uptake activity at a given Ca^{2+} concentration ($[Ca^{2+}]$); V_{max} is the maximum activity achieved; $K_{0.5}$ is the $[Ca^{2+}]$ giving one-half V_{max}, and n_H is the Hill coefficient.

The incubation medium used for the assay of Ca^{2+}-adenosinetriphosphatase (ATPase) was identical to that described for Ca^{2+} uptake except that [γ-^{32}P]ATP was used instead of nonradioactive ATP and nonradioactive $CaCl_2$ was used instead of $^{45}CaCl_2$. To determine the basal ATPase (Mg^{2+}-ATPase) activity, assays were also carried out in the absence of Ca^{2+} and in the presence of 0.2 mM EGTA. The incubations were carried out at 37°C for 3 min, and the reaction was stopped by the addition of 1 ml 12% trichloroacetic acid-2 mM KH_2PO_4. Next, 0.1 ml each of 25 mM ATP and 0.1% bovine serum albumin were added to the tubes. The tubes were centrifuged (3,000 revolutions/min, 10 min) and the ^{32}P released from [γ-^{32}P]ATP was extracted and quantified as described by Sulakhe and Drummond (27). The basal ATPase activity was subtracted from the enzyme activity measured in the presence of Ca^{2+} to obtain Ca^{2+}-ATPase activity.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein composition of cardiac SR isolated from control and shocked rats was determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as previously described (20).

Data analysis. The results are presented as means ± SE. Statistical significance was evaluated by Student's *t*-test. Regression lines were determined with nonlinear regression curve fitting using the SigmaPlot program. Blood pressure, contractility, and half-relaxation time were analyzed with analysis of variance (ANOVA) (treatment × subject design) and paired Student's *t*-test. A probability of <5% ($P < 0.05$) was taken as the level of significance.

RESULTS

Figure 1 shows the effect of varying shock intensities on Ca^{2+} uptake by SR measured at a saturating free Ca^{2+} (8.2 μM) in the assay. Increasing shock voltage depressed the Ca^{2+} uptake activity of SR membranes in a dose-dependent manner. A plateau appeared to occur between 100 and 250 V, and the largest change occurred between 50 and 100 V. Even at the lowest

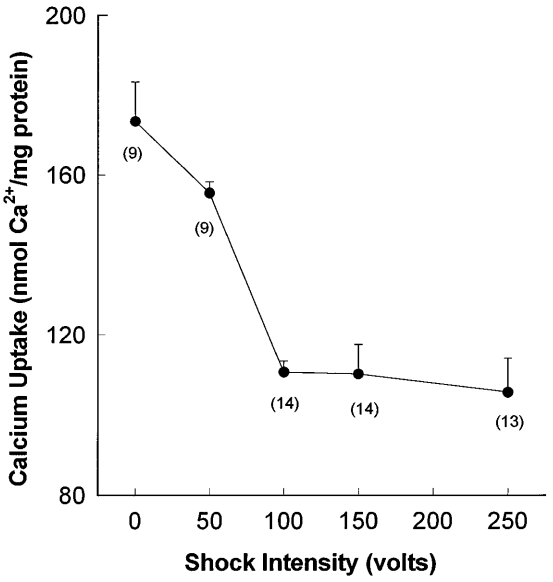

Fig. 1. Shock voltage-dependent reduction in Ca^{2+} uptake by cardiac sarcoplasmic reticulum (SR) vesicles. Initial free Ca^{2+} concentration in the assay medium was 8.2 μM. Each value is significantly below that of control (0 V). Values at 100, 150, and 250 V were not statistically different from each other. Symbols are means; bars are SE. Number of animals is given in parentheses.

voltage of 50 V, the decrease (~10%) was statistically significant.

Figure 2 shows the time course of Ca^{2+} uptake by SR derived from control rats and rats receiving 150-V shocks. The rate of Ca^{2+} uptake, measured in the presence of 8.2 μM free Ca^{2+} in the assay medium, was significantly lower (~33%) in SR from the shocked rats compared with that from the control rats (Fig. 2*A*). The diminished rates of Ca^{2+} uptake by SR from shocked rats persisted when the Ca^{2+} uptake assay medium was supplemented with ruthenium red (50 μM), an SR Ca^{2+}-release channel blocker (Refs. 3, 19; Fig. 2*B*). Therefore, the observed depression in Ca^{2+} uptake activity of SR from the shocked rats does not appear to be caused by an increase in the rate of Ca^{2+} release from the SR. Ruthenium red augmented the rates of Ca^{2+} uptake by SR from both control and shocked rats to a similar extent (~40%). Thus the drug was equally effective in blocking Ca^{2+} release from SR derived from control and shocked rats.

Figure 3 shows the effects of varying Ca^{2+} concentrations on the Ca^{2+} uptake activities of SR from control rats and rats receiving 150-V shocks. When the free Ca^{2+} concentration in the assay medium was varied from 0.237 to 8.241 μM, SR from shocked rats showed significantly lower rates (56 to 61%; $P < 0.05$) of Ca^{2+} uptake than the membranes from control rats at all Ca^{2+} concentrations tested. The kinetic parameters of Ca^{2+} uptake derived from these data indicated that the V_{max} of Ca^{2+} uptake by SR, but not the $K_{0.5}$ for Ca^{2+}, was altered in the shocked rats [V_{max} (nmol $Ca^{2+} \cdot mg^{-1} \cdot min^{-1}$): control, 66.3 ± 1.3; shocked, 40.1 ± 1.4; $K_{0.5}$ for Ca^{2+} (μM): control, 1.14 ± 0.05; shocked, 1.04 ± 0.07; n_H: control, 2.0 ± 0.1; shocked, 2.0 ± 0.2].

To exclude the possibility that differences in the relative purity of SR membranes isolated from control and shocked rats contributed to the observed difference in the Ca^{2+} uptake activities of these membranes, additional experiments were performed using unfractionated cardiac muscle homogenates from control and shocked (150 V) rats. In these experiments, Ca^{2+} uptake activities were measured at a saturating free Ca^{2+} concentration (8.2 μM) using cardiac tissue homogenates as well as SR isolated from the same hearts (control and shocked). The results showed significantly reduced Ca^{2+} uptake activity in both homogenates and SR from shocked compared with control rats (Fig. 4).

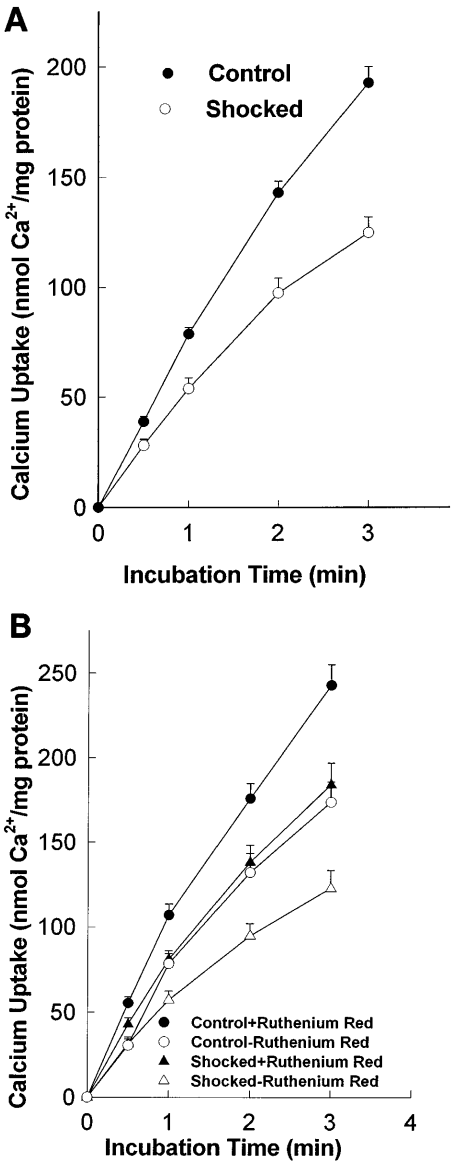

Fig. 2. *A*: time course of Ca^{2+} uptake by SR from control and shocked (150 V) rats. Rate of Ca^{2+} uptake is lower in shocked compared with control SR. Values of shocked rats were significantly ($P < 0.01$) lower than corresponding values of control rats. *B*: comparison of rates of Ca^{2+} uptake by cardiac SR from control and shocked (150 V) rats in absence and presence of Ca^{2+}-release channel blocker ruthenium red (50 μM). Initial free Ca^{2+} concentration in assay medium was 8.2 μM; $n = 8$ animals for each group.

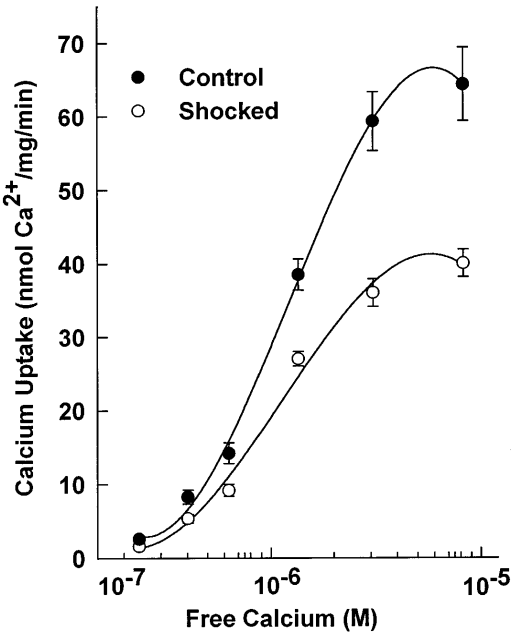

Fig. 3. Effect of varying Ca^{2+} concentration on Ca^{2+} uptake by cardiac SR from control and shocked (150 V) rats. Values are significantly lower in SR from shocked rats at all concentrations ($P < 0.01$). Curves fit by nonlinear regression yielded $r^2 = 0.998$ for control and $r^2 = 0.991$ for shocked (150 V) rats; $n = 8$ animals for each group.

The magnitude of shock-induced depression of Ca^{2+} uptake activity was similar in SR (31%) and homogenate (36%) fractions from shocked compared with control rats (Fig. 4). The data from these experiments also showed that the relative enrichment in the Ca^{2+} uptake activity of SR compared with homogenate was similar (~9-fold) in the case of control and shocked rats. Thus it is unlikely that differences in the relative purity of SR preparations from control and shocked rats contributed to the observed differences in Ca^{2+} uptake activity. In further support of this, the polypeptide composition of SR (determined by SDS-polyacrylamide gel electrophoresis) derived from the hearts of control and shocked (150 V) rats was found to be essentially similar (Fig. 5). Scanning and quantification of the electropherograms did not reveal any significant difference in the amount of protein in individual peptide bands including the ~105-kDa band representing Ca^{2+}-ATPase. The 97.4-kDa peptide band, which presumably represents phosphorylase, was, however, diminished in the SR from shocked rats.

In additional experiments, we examined whether the observed shock-induced depression in the Ca^{2+} uptake activity of SR was also accompanied by concomitant impairment in the energy transduction function of the Ca^{2+}-pumping ATPase. In these experiments, ATP-dependent Ca^{2+} uptake and Ca^{2+}-stimulated ATP hydrolysis were determined using the same SR preparations

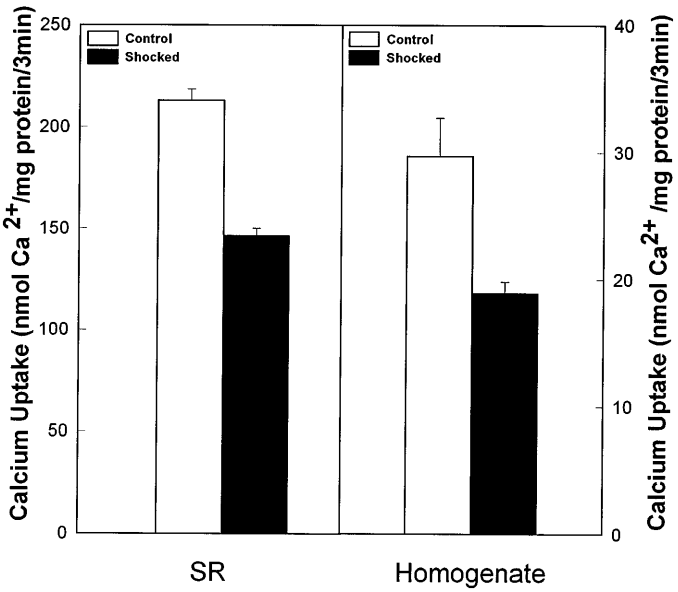

Fig. 4. Comparison of Ca^{2+} uptake by cardiac homogenates and SR from control and shocked (150 V) rats. Depression of Ca^{2+} uptake by homogenate was 36% and SR was 31%. Initial free Ca^{2+} concentration in assay medium was 8.2 μM; $n = 6$ animals for each group.

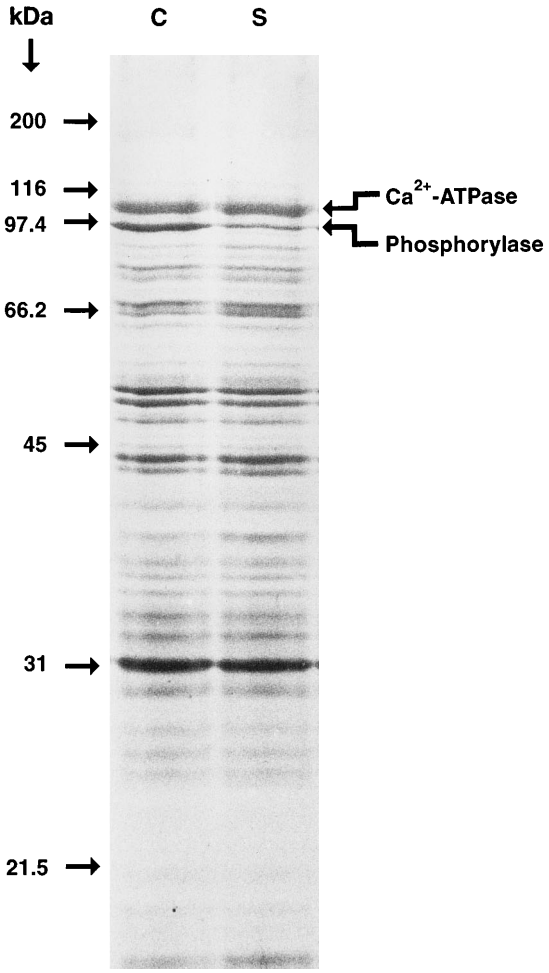

Fig. 5. Comparison of polypeptide profiles of cardiac SR isolated from control and shocked (150 V) rats. Polypeptide composition and relative intensity of individual peptide bands are similar in control and shocked SR except for diminished content of phosphorylase (97.4-kDa band) in shocked SR. Protein profiles similar to those shown were obtained in 3 additional experiments using separate SR preparations. Numbers on *left* indicate molecular mass.

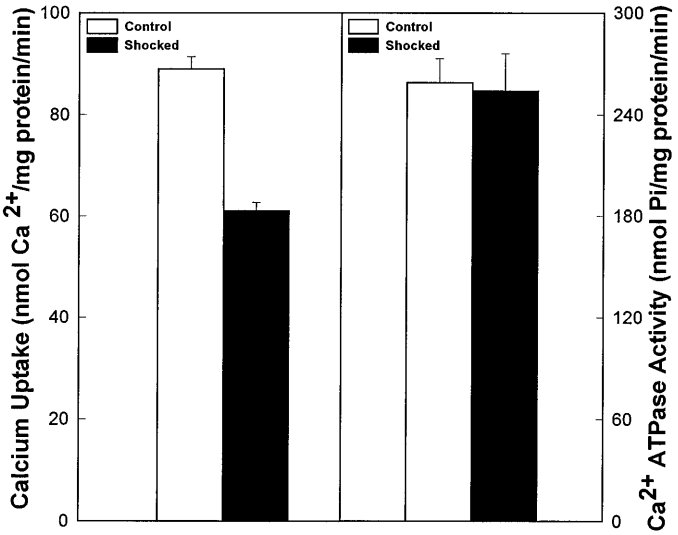

Fig. 6. Comparison of ATP-dependent Ca^{2+} uptake (*left*) and Ca^{2+}-stimulated ATP hydrolysis (*right*) by cardiac SR from control and shocked (150 V) hearts. Initial free Ca^{2+} concentration in assay medium was 8.2 μM. Decrease in Ca^{2+} uptake activity of SR from shocked (150 V) compared with control rats is significant, whereas there was no difference in Ca^{2+}-stimulated ATP hydrolysis.

isolated from control and shocked (150 V) rats. The results showed that the Ca^{2+}-uptake activity but not the Ca^{2+}-stimulated ATP hydrolysis was depressed in SR from shocked rats (Fig. 6). The shock-induced depression in Ca^{2+} uptake appeared to parallel cardiac function abnormalities as demonstrated by the transient reduction in blood pressure monitored during shock delivery and postshock. Systolic, mean, and diastolic pressures were all depressed significantly at 30 and 60 s postshock. There was a rebound at 5–10 min postshock (P = not significant), with eight of nine rats having pressures above baseline values at 5 min. All values had returned to baseline preshock values ~15 min postshock (Table 1). Uptake of Ca^{2+} by homogenates derived from the ventricular myocardium of rats killed 15 min after shock delivery averaged 13.3 ± 2.6 nmol·μg protein^{-1}·min^{-1} (n = 6), which did not differ from uptake values of similarly operated, nonshocked control rats (15.6 ± 3.1 nmol·μg protein^{-1}·min^{-1}, n = 5).

To determine the effects of shocks on contractile function in the absence of hormonal and/or autonomic influence, shocks were also delivered to hearts supported on the Langendorff apparatus, with contractility/relaxation measured with ultrasound crystals (Fig. 7).

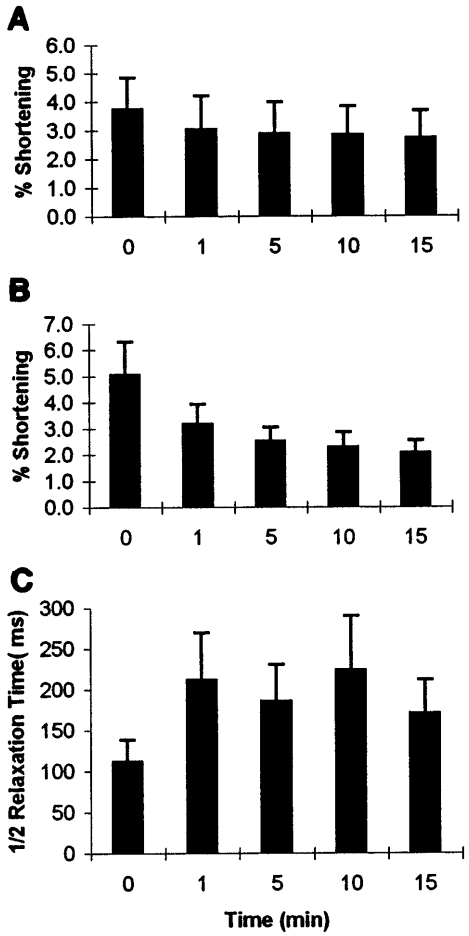

Fig. 7. Effects of 150-V shocks on contractility and relaxation of Langendorff isolated hearts. *A*: average percentage long-axis shortening after shocks. There was a trend that approached but was not statistically significant (F = 1.424, P < 0.27). *B*: average percentage short-axis shortening after shocks (F = 4.477, P < 0.01). *C*: sets of individual half-relaxation times after shocks (P < 0.05 for control and 1st minute only). 0, Preshock recording; 1, 5, 10, and 15, times from last shock delivery.

There was a significant reduction in shortening of the distance between the two chambers (short axis, P < 0.01) and a similar trend in the shortening in the long axis, which did not reach significance (P < 0.27). The half-relaxation time increased in all six hearts, on average approximately doubled at 1 min postshock (211.5 ± 58.4 ms) from baseline (112 ± 26.3 ms, P < 0.05). However, there was considerable variability between hearts in the subsequent responses at 5, 10, and

Table 1. *Blood pressure response to 150-V transthoracic shocks in anesthetized rats*

	Preshock Baseline	Postshock				
		30 s	60 s	5 min	10 min	15 min
Systolic	99.8 ± 4.6	89.3 ± 3.9	92.7 ± 4.4	104.7 ± 6.0	104.0 ± 6.0	98.8 ± 4.4
Diastolic	66.1 ± 3.3	54.5 ± 5.6	57.4 ± 5.9	69.1 ± 3.2	71.2 ± 4.0	64.2 ± 4.1
Mean	79.1 ± 3.3	67.6 ± 4.7	70.6 ± 5.1	82.9 ± 3.5	84.5 ± 4.9	99.8 ± 4.6

Values (in mmHg) are means ± SE; n = 9 rats. Systolic, diastolic, and mean blood pressure values were monitored throughout shock delivery and postshock as described in METHODS. There was a significant effect of shocks as determined by analysis of variance (treatment × subject design) for all blood pressure parameters (P < 0.01). All values for all animals remained below preshock (baseline) values at 30 s (P < 0.01) and past 60 s (P < 0.05) postshock, with rebound at 5 and 10 min followed by return to baseline 15 min postshock.

15 min postshock, with all six hearts returning towards baseline by 15 min (Fig. 7*C*).

DISCUSSION

The present results demonstrate that the ATP-energized Ca^{2+} uptake activity of cardiac SR isolated from rats receiving defibrillation shocks is significantly depressed (35–40%) compared with controls. The shock-induced depression in Ca^{2+} uptake activity of SR was voltage dependent but was not accompanied by alterations in the Ca^{2+}-ATPase content of the SR, Ca^{2+}-stimulated ATP hydrolysis, or the apparent affinity of the Ca^{2+}-ATPase for Ca^{2+}. Thus the shock-induced depression in Ca^{2+} uptake activity of the SR is apparently caused by impaired efficiency of coupling ATP hydrolysis to Ca^{2+} transport. The mechanistic basis of this shock-induced uncoupling of ATP hydrolysis and Ca^{2+} transport is unclear at present. Altered Ca^{2+} transport activities without parallel alterations in ATPase activities have been encountered in a number of instances, indicating that the intrinsic ATP-hydrolyzing activity of the transport system is not the sole determinant of the ion transport function. For example, *1*) binding of anti-ATPase antibodies to SR membranes has been found to cause marked inhibition of Ca^{2+} transport without altering ATPase activity and passive permeability to Ca^{2+} of the SR membranes (29). *2*) Diminished Ca^{2+} transport rates and enhanced ATPase activities have been observed in reconstituted "Ca^{2+} pump" vesicles of SR compared with original membranes. The decline in Ca^{2+} transport rates could not be accounted for by altered Ca^{2+} permeability of the reconstituted vesicles (23, 24). *3*) Tryptic cleavage at a specific region of the ATPase (26) or a single amino acid substitution ($Tyr^{763} \rightarrow Gly$) in the primary structure of the ATPase (1) was found to abolish Ca^{2+} transport function while ATP hydrolysis still remained functional. *4*) Aging was found to be accompanied by a decline in the Ca^{2+} uptake but not the Ca^{2+}-stimulated ATPase activity of SR from rat cardiac (20, 21) and slow-twitch skeletal muscles (22).

In the present study, effective blockade of the ryanodine receptor/Ca^{2+}-release channel by ruthenium red (3, 19) did not overcome the shock-induced depression in Ca^{2+} uptake activity of SR. Therefore, it is unlikely that a shock-induced enhancement in Ca^{2+} release through the Ca^{2+}-release channel contributes to the observed depression in the Ca^{2+} uptake activity of SR. Injury to the cardiac sarcolemmal membrane (electroporation) after defibrillation shocks has been reported (13). Whether defibrillation shocks induce any electroporation of the SR is not known. Myocardial functional deficits that may occur as a result of electroporation of the sarcolemma are short lived, and recovery rapidly occurs within seconds (12, 13). The defibrillation-induced impairment in SR function reported here, apparently, persists for minutes.

It is possible that shock-induced depression in the Ca^{2+}-sequestering activity of SR may contribute to the depression in cardiac function observed in some patients after defibrillation shocks (10). The uptake and release of Ca^{2+} by the SR is central to the maintenance of a normal excitation-contraction-relaxation cycle of the heart (2, 5, 14). The ATP-energized Ca^{2+} uptake activity of isolated SR vesicles is widely accepted as the manifestation of the Ca^{2+}-sequestering (Ca^{2+} pump) function of these membranes in vivo (5, 9). Thus the depressed Ca^{2+} sequestration by SR membranes would be expected to reflect a similar depression in vivo. Such diminution in SR function in vivo would lead to prolongation of cardiac muscle relaxation and thus impaired mechanical restitution. Furthermore, with depressed Ca^{2+} uptake into the SR compartment, there would be diminished release of Ca^{2+} for subsequent contractions and this would compromise cardiac contractile force.

When 150-V shocks were delivered to rats that had indwelling femoral arterial cannulas, all blood pressure measures were reduced immediately postshock ($P < 0.01$). These transient blood pressure responses remained below baseline for all animals beyond 60 s, had rebounded by 5–10 min, and subsequently returned to baseline by 15 min. This rebound is consistent with the previously demonstrated autonomic activation postshock (30), which would also alter sarcolemmal Ca^{2+} transients postshock (2, 10, 14, 30). The return to baseline corresponded to a time when the Ca^{2+} uptake activity of heart homogenates also did not differ from those of similarly operated control nonshocked rats. This time course of 10–15 min for recovery is similar to our previous finding of depressant electrophysiological effects after similar intensity shocks delivered to isolated papillary muscles (16, 17).

When shocks were delivered directly to the hearts on the Langendorff apparatus, they resulted in an immediate prolongation of relaxation. There was a disparity between the magnitude and duration of effects on contractility and relaxation and, indeed, between contractility measured in the two different axes. Some of these differences could be due to differences in effects of the shocks on the SR and sarcolemma, as well as the current density profiles through the different regions of the myocardium. There were also differences between the time course of effects seen on the Langendorff heart and the whole animal or isolated papillary muscle. One possible factor contributing to these differences may be the relative distribution of current delivered directly to the heart in the Langendorff hearts versus that which was shunted either through the tissue bath containing the papillary muscle or through the rest of the body in the whole animal transthoracic shock study. In the Langendorff heart, it was not possible to match resistance, peak current, peak voltage, and total energy delivery to that of the whole animal, and these differences could explain some of the differences in results. Determining the relative contributions of these factors requires consideration of current distribution, heart geometry, and electrode configurations (30), which is beyond the scope of the present investigation. On the other hand, it is noteworthy that the postshock restitution response of the Langendorff hearts demonstrated considerably more variability than the contractility responses as a group and even in the same heart.

Variability in postshock cardiac function is one characteristic also clearly evident in patients after defibrillation shock (10, 11).

It is possible that this depression in Ca^{2+} uptake contributes to the postshock myocardial dysfunction observed in some patients after a defibrillation shock (10). Why this might differ from patient to patient is unclear. Age-associated diminution in general cardiac performance is well known, and there is an age-associated decline in SR Ca^{2+} pump activity (6, 20), which might contribute to poor cardiac performance postshock and inability to pace the heart (16). However, there does not appear to be a good correlation between patient characteristics and defibrillation success or postshock recovery (10, 11).

Additional factors that may contribute to poor cardiac performance postshock include the magnitude and characteristics of the delivered defibrillation energy. A relationship between energy delivery and myocardial damage has been demonstrated previously (7, 25, 30), which is consistent with the shock intensity-related depression of SR Ca^{2+} pump function observed in the present study. Another factor that may contribute to depressed function is alteration in the phosphorylation status of Ca^{2+} cycling proteins at the level of the SR (5, 8, 14, 33) and/or myofilaments (31). At present, we have not investigated this possibility. Also, the observed decrease in SR-associated phosphorylase suggests that there may be other subcellular alterations not yet determined that may contribute to postshock cardiac dysfunction. These remain to be determined.

In conclusion, we have demonstrated that trapezoidal direct current shocks, with parameters similar to defibrillation shocks, elicit protracted and dose-dependent depression of Ca^{2+}-sequestering activity of cardiac SR. The impairment of SR Ca^{2+} pump function was caused by an uncoupling of ATP hydrolysis from Ca^{2+} transport. This shock-induced impairment of Ca^{2+} pump function is suggested to contribute to postshock myocardial dysfunction, consistent with the shock-induced reduction in contractility and relaxation.

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