Burn trauma alters calcium transporter protein expression in the heart

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BURN TRAUMA PRODUCES SEVERE cardiac contractile dysfunction (15, 18, 30). Because calcium handling by the cardiomyocyte is critical for normal cardiac function, alterations in calcium homeostasis may contribute to the cardiac deficits observed after burn injury. We have shown that burn trauma increases cardiomyocyte intracellular calcium levels, but the mechanism for this calcium accumulation has not been fully explored. We have shown further that pharmacological agents that specifically target subcellular regulation of calcium handling 1) restored cardiomyocyte calcium homeostasis, 2) reduced cardiac injury, and 3) improved cardiac performance (15, 30). In this regard, dantrolene, which alters calcium mobilization from the sarcoplasmic reticulum (SR), diltiazem, a calcium slow channel blocker, and amiloride, which alters the sodium/hydrogen exchanger, prevented burn-related myocardium calcium accumulation and provided cardioprotection (15, 30). In disease states such as ischemia-reperfusion, heart failure, and sepsis, myocardial contractile depression is paralleled by alterations in myocardial calcium transporter gene expression (5, 21, 24, 26, 28, 31). Changes in calcium transporter expression have not been examined in burn trauma; in the present study, we hypothesized that cardiomyocyte calcium overload after burn trauma is related to altered expression of essential calcium transporters that participate primarily in calcium removal from the myocyte cytosol.

Calcium entry into the cardiomyocytes during myocardial contraction is regulated by calcium transporters, which include the SR ryanodine receptor (SR calcium release channel), the sarcolemmal L-type calcium channel, and the sodium/calcium exchanger (5). Calcium entry via the L-type calcium channel provides the trigger for SR calcium release channel, which, in turn, provides calcium for myofilament activation. Although reverse-mode sodium/calcium exchange is thought to occur in the initial phase of the action potential, the calcium influx via this exchanger is not sufficient to activate calcium release from the SR in the normal rat myocardium (6). However, changes in sodium/calcium exchanger expression and/or activity have been shown to occur in ischemia-reperfusion and heart failure and may also occur after traumatic injury such as burn trauma (24).

The calcium transporters responsible for the removal of systolic calcium from the cytosol during myocardial relaxation include the SR calcium-ATPase (SERCA), sodium/calcium exchanger, and sarcolemmal calcium-ATPase. Bers and colleagues (3) showed that in rat myocardium the SERCA removes 92% of the activator calcium from the cytosol whereas the remaining calcium was extruded by the sarcolemmal sodium/calcium exchanger and calcium-ATPase. With respect to the sarcolemmal calcium transport mechanisms, the sodium/calcium exchanger removes 7% and the calcium-ATPase removes 1–2% of the systolic calcium (3, 19). Thus the bulk of calcium is removed from the cytosol by the SR, and impaired SR calcium uptake in pathological states such as sepsis has been linked to myocardial contractility abnormalities (31).

In the present study, we focused on burn-related alterations in calcium transporter proteins involved in the removal of calcium from the cardiomyocyte cytosol. We showed that burn injury increased protein expression of the SERCA, whereas burn injury increased the expression of the sodium-potassium-ATPase, transiently elevated sodium/calcium exchanger expression, and decreased L-type calcium channel expression. Alterations in myocardial calcium transporter protein expres-
sion occurred early in the postburn period and were followed by a progressive increase in cardiomyocyte calcium levels. These data suggest that alterations in calcium and sodium transport mechanisms play a significant role in cardiomyocyte accumulation of calcium and sodium observed after burn trauma.

METHODS AND MATERIALS

Experimental animals. Adult Sprague-Dawley rats (Harlan Laboratories, Houston, TX) weighing 325–360 g were used throughout the study. Animals were allowed 5–6 days to accclimate to their surroundings, and commercial rat chow and tap water were available throughout the experimental protocol. All work described herein was approved by The University of Texas Southwestern Medical Center Institutional Animal Care and Research Advisory Committee and was performed according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals as published by the American Physiological Society.

Burn procedure. Rats were deeply anesthetized with methoxyflurane and secured in a constructed template device as described previously (30). The skin exposed through the template was immersed in 100°C water for 12 s on each side to produce full-thickness cutaneous burns over 40% total body surface area. This burn technique produces complete destruction of the underlying neural tissue. After immersion, the rats were immediately dried and each animal was placed in an individual cage. All burned animals received standard fluid resuscitation consisting of 4 ml·kg⁻¹·%burn⁻¹ lactated Ringer solution with one-half of this calculated volume given intra-peritoneally immediately after completion of the burn injury and the remaining volume given 8 h postburn. Hearts were collected at 1, 2, 4, 8, and 24 h after injury (n = 3 at each time point). Animals not given burn injury served as appropriate controls (n = 4).

Cardiomyocyte isolation. To isolate cardiomyocytes, rats received an intraperitoneal injection of heparin (2,000 units) 20–30 min before death. The rats were decapitated, hearts were harvested and placed in a petri dish containing room-temperature heart medium [113 mM NaCl, 4.7 mM KCl, 0.6 mM KH₂PO₄, 0.6 mM NaH₂PO₄, 1.2 mM MgSO₄, 12 mM NaHCO₃, 10 mM KHCO₃, 20 mM d-glucose, 0.5× MEM amino acids (50×, GIBCO/BRL 11130-051), 10 mM HEPES, 30 mM taurine, 2.0 mM carnitine, and 2.0 mM creatine], which was bubbled constantly with 95% O₂–5% CO₂ for 15 min and the pH adjusted to 7.1 with 1 M NaOH. The solution was then filter sterilized and stored at 4°C until use. At the final concentration of calcium, the cardiomyocyte cell number was calculated and myocyte viability was determined.

Cardiomyocyte calcium and sodium measurement. Myocytes loading with fura-2-AM was accomplished over 45 min, whereas myocyte loading with sodium-binding benzofurazan isophthalate was accomplished over 1 h at room temperature in the dark. Myocytes were then suspended in 1.0 mM calcium containing MEM and washed to remove extracellular dye; myocytes were placed on a glass slide on the stage of a Nikon inverted microscope. The microscope was interfaced with Grooney optics for epi-illumination, a triocular head, phase optics, and ×30 phase-contrast objective and mechanical stage. Excitation illumination source (300 W compact xenon arc illuminator) was equipped with a power supply. In addition, this InCyt Im 2 fluorescence imaging system (Intracellular Imaging, Cincinnati, OH) included an imaging workstation and Intel Pentium Pro200 MHz-based personal computer. The computer-controlled filter changer was used for spectral emission discrimination between the 340 and 380-nm wavelength emissions. Images were captured by a monochrome charge-coupled device camera equipped with a television relay lens. InCyt Im2 image software allowed measurement of intracellular calcium and sodium concentrations from the ratio of the two fluorescent signals generated from the two excitation wavelengths (340 nm/380 nm); background was removed by the InCyt IM2 software. The calibration procedure included measuring fluorescence ratio with buffers containing different concentrations of either calcium or sodium. At each wavelength, the fluorescence emissions were collected for 1-min intervals and the time between data collection was 1–2 min. Because quiescent or noncontracting myocytes were used in these studies, the calcium levels measured reflect diastolic levels.

Calculation of the indicator fluorescence. A number of equations have been developed to convert fluorescence intensities or ratios into calcium concentrations. These equations include fluorescence response of the fura 2-AM indicator to different calcium concentrations as determined by the maximum and minimum fluorescence and include the dissociation constant of the indicator (K₀). Specifically, fluorescence measures are calculated according to the following protocol using fura 2-loaded cells and a ratio of fluorescence that is measured at 340- and 380-nm excitation. At the conclusion of the experiment, cells are incubated in a calcium-free solution containing the calcium ionophore A-23187, 10 μM; the solution also contains excess EGTA (10 mM, as a calcium chelator). The excitation wavelength of 340 and 380 nm and an emission of 510 nm are used. Measurements are begun in the EGTA/A-23187-containing solution, and fluorescence of the calcium-free indicator at 380-nm excitation (F₃₈₀) as well as fluorescence of the calcium-free indicator at 340-nm excitation (F₃₄₀) are determined. Rₘₜₐₓ is calculated as Rₘₜₐₓ = F₃₄₀/F₃₈₀ and is the ratio of fluorescence of a calcium-free solution measured at wavelengths of 340 and 380 nm. The extracellular solution is then changed to one containing calcium at a concentration of 10 mM plus 10 μM A-23187. A sharp decrease of fluorescence at 380-nm excitation is paralleled by an increase of fluorescence at 340 nm. After signal intensity has stabilized (usually 10 min), fluorescence of the calcium-bound form of the indicator at 380-nm excitation (F₃₈₀) is measured. These parameters are then used to calculate Rₘₜₐₓ from the equation Rₘₜₐₓ = F₃₄₀/F₃₈₀. Thus Rₘₜₐₓ is the fluorescence of maximal or saturated calcium level measured at wavelengths of 340 and 380 nm. Calcium concentrations ([Ca²⁺]) are then calculated using the equation for fura 2 (see Ref. 9a) where...
was then transferred to nitrocellulose. After being blocked overnight in 5% nonfat milk and probed for 1 h with either calcium-ATPase (Millipore, Bedford, MA). The membranes were blocked overnight in 10% denaturing gel was used to separate myocardial protein, which was then transferred to polyvinylidene difluoride membranes and a 7.5% gel for the L-type calcium channel immunoblots. The antibodies recognize the COOH-terminal domain of the cardiac isoform.

Western blot analysis. Western blots were performed using rat heart tissue (30 μg) harvested at several times after burn injury (or control). Briefly, frozen rat hearts were homogenized in ice-cold lysis buffer (0.5 g tissue/ml). The composition of the lysis buffer was 10 mM HEPES, pH 7.4, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT, 1 mM PMSF, and one Mini Complete Protease Inhibitor tablet per 10 ml of compete buffer (Roche Biochemicals, Mannheim, Germany). The homogenized samples were incubated on ice for 30 min and centrifuged at 10,000 g for 10 min at 4°C. Protein concentration was determined by the Bradford assay, using BSA for the standard curve (Bio-Rad Protein Assay Reagents, Hercules, CA).

Antibodies directed against the following calcium and sodium transporters were used: calcium-ATPase, L-type calcium channel (Affinity Bioreagents, Golden, CO), sodium/calcium exchanger (Chemicon, Temecula, CA), sodium/potassium-ATPase, and the ryanodine receptor (Upstate Biotechnology, Lake Placid, NY). The calcium-ATPase antibody is directed against the muscle-specific SERCA2 isofrom. The L-type calcium channel antibody recognizes the α2-subunit of this channel that is associated with the dihydropyridine-binding α1-subunit. The sodium/potassium-ATPase antibody recognizes the α1-subunit, and the ryanodine receptor antibody recognizes the COOH-terminal domain of the cardiac isoform.

After protein determination (Bio-Rad), the protein samples were separated on a 10% SDS-polyacrylamide gel for the SERCA Western blots and a 7.5% gel for the L-type calcium channel immunoblots. The protein was then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked overnight in 5% nonfat milk and probed for 1 h with either calcium-ATPase (1:300) or L-type calcium channel (1:250) antibodies. For the sodium/calcium exchanger Western blots, the membranes were blocked overnight in 3% BSA-1% milk and probed with the exchanger antibody (1:200) for 1 h. For the sodium/potassium-ATPase Western blots, a 10% denaturing gel was used to separate myocardial protein, which was then transferred to nitrocellulose. After being blocked overnight in 3% nonfat milk, the membrane was probed with sodium/potassium-ATPase antibody (0.075 μg/ml). For the ryanodine immunoblots, a 5% gel was used to separate the myocardial protein, which was transferred to nitrocellulose (Bio-Rad) overnight at 85 V by using a 4°C continuous cooling transfer apparatus. The blot was blocked in 3% milk and the ryanodine receptor antibody was added (1:600). The blots were washed (20 mM Tris, 135 mM NaCl, 0.1% Tween, pH 7.6) three times after the primary antibody incubation and then incubated for 1 h with the appropriate secondary antibody (1:2,000, Promega, Madison, WI). After the blots were washed, secondary antibody was added (1:2,000, Promega). After three washes, the bound antibodies were visualized by enhanced chemiluminescence. To control for variations in signal intensities between blots, the same control sample was used on each blot for normalization. Also, three separate immunoblots were used to determine expression of the protein of interest in each sample.

Quantification of the single band density was determined by using Quantity One software (Bio-Rad, version 4.4.0, build 36). Briefly, the radiographic film was scanned with a Scantek 7400c (Hewlett-Packard, Palo Alto, CA) and converted into a TIFF file (8-bit gray scale). Densitometry was performed by outlining the selected bands with the volume rectangle tool initially set on the control band of interest. Band density was expressed as arbitrary units per square millimeter.

Statistical analysis. All values are expressed as means ± SE. ANOVA was used to assess an overall difference among the groups for each of the variables. Levene’s test for equality of variance was used to suggest the multiple comparison procedure to be used. If equality of variance among the four groups was suggested, multiple comparison procedures were performed (Bonferonni); if inequality of variance was suggested by Levene’s test, Tamhane multiple comparisons (which do not assume equal variance in each group) were performed. Probability values < 0.05 were considered statistically significant (analysis was performed with SPSS, Windows, version 7.5.1).

RESULTS

Before specific mechanisms underlying cardiomyocyte accumulation of calcium after burn trauma were examined, rats were killed at several time points postburn (1, 2, 4, 8, 12, and 24 h), cardiomyocytes were isolated and loaded with fura 2-AM, and the time course of cardiomyocyte accumulation was examined. As shown in Fig. 1, a significant burn-related rise in cardiomyocyte calcium levels was evident 8 h postburn, and this myocyte loading of calcium increased progressively over the first 24 h postburn. We elected to examine the protein expression of various cardiac calcium transporters before the time of calcium accumulation by cardiomyocytes. We first focused on examining calcium transporters in the SR shown to regulate calcium removal from the cytosol, because calcium handling by the SR has been shown to be critical for normal cardiac function in the rat. Furthermore, our previous finding (that pharmacological strategies specifically targeting SR calcium handling ablated burn-related cardiomyocyte calcium overload) suggested that burn-related alterations in SR calcium transporters contribute to calcium dyshomeostasis (30). Data from the present study confirmed that burn trauma reduced cardiac SERCA protein expression as early as 1 h postburn, and this altered protein expression, measured by Western blot analysis, persisted through 24 h postburn (Fig. 2).

Because SERCA protein expression was altered by burn trauma, we next determined whether burn-mediated changes in the expression of the other SR calcium handling protein, the ryanodine receptor, might also occur. As measured by Western

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[K_\text{a}] = \frac{[R - R_{\text{max}}]}{R_{\text{max}} - R} \frac{[F_{380}]}{[F_{380}]}
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\(K_a\) has been determined to be 135 nM at 22°C and 224 nM at 37°C (Molecular Probes). The final step of the calibration procedure examines the contribution of autofluorescence and examines the intensity of fluorescence of unloaded cardiomyocytes with the fluorescence of cells loaded with indicator. Alternatively, we have used manganese to quench the fluorescence of fura 2-AM to further determine the contribution of autofluorescence under these experimental conditions.

To accomplish this, the extracellular solution is changed to one containing A-23187 (10 μM) and 2 μM of MnCl₂.
blot analysis, ryanodine receptor expression was significantly decreased 1–2 h postburn with the protein levels of this calcium transport protein returning to normal by 24 h postburn (Fig. 3). In addition to burn-mediated changes in the expression of SERCA, we examined the contribution of the two main sarcolemmal calcium transport mechanisms to postburn myocardial calcium accumulation. L-type calcium channel expression was transiently decreased 1 and 2 h postburn (Fig. 4) but returned toward baseline values 24 h postburn. Sodium/calcium exchanger protein expression is shown in Fig. 5. Expression of the sodium/calcium exchanger after burn injury was biphasic with expression of this exchanger significantly increased 2 h postburn but significantly decreased at 24 h postinjury. Because calcium and sodium levels are closely linked by the sodium/calcium exchanger and the expression of this transporter were altered by burn trauma, we then determined whether cardiomyocyte sodium was altered by burn injury. A significant increase in cardiomyocyte sodium was observed 4 h through 24 h postburn (Fig. 6), confirming that a burn-mediated rise in cardiomyocyte intracellular sodium preceded myocardial calcium loading. We therefore examined the effects of burn trauma on myocardial sodium-potassium-ATPase expression. Increased protein levels of this transporter were observed 2–24 h postburn (Fig. 7), likely occurring in response to the rise in intracellular sodium.

**DISCUSSION**

Data from the present study confirm that burn trauma promotes cardiomyocyte calcium loading. In addition, burn-mediated changes in the expression of several critical cardiac calcium transporters preceded the increase in cardiomyocyte calcium, providing one mechanism of calcium overload in burn trauma. Although several previous studies have shown that myocardial disease states produce calcium overload and altered transporter expression (2, 9, 10, 26), this is the first study confirming that these cellular events also occur after major burn trauma.

Increases in cardiomyocyte calcium have been linked to myocardial injury and dysfunction in cardiac disease states such as ischemia-reperfusion and heart failure (22, 26), as well as burn trauma and sepsis (15, 27). The deleterious effects of a rise in cellular calcium have been attributed to calcium-related activation of various nucleases, proteases, and phospholipases, resulting in loss of chromatin, protein, and/or membrane structure (8).
In the heart, calcium plays an essential role in cardiac contraction and relaxation. The primary calcium transporters that produce a rise in intracellular calcium during contraction are the sarcolemmal L-type calcium channel and the SR ryanodine receptor. The voltage-sensitive L-type calcium channels are activated by cardiac cell membrane depolarization with the resulting influx of calcium triggering the release of calcium from the SR through the ryanodine receptor (5). The L-type calcium channel is inactivated by increases in ryanodine receptor-derived cytosolic calcium, which serves as a protective mechanism to limit calcium overload under pathological conditions (1, 23).

In this present study, we observed a transient decrease in L-type calcium channel expression 1 and 2 h after burn trauma. Our earlier studies have shown that calcium channel blockers decreased burn-mediated calcium and sodium accumulation by cardiomyocytes and provided limited cardioprotection; however, inhibitors of either the SR calcium release channel or sodium/calcium exchanger were significantly more effective in modulating postburn myocyte calcium overload and in providing cardioprotection (15). It is possible that the protective effects of the L-type calcium channel inhibitors were related, in part, to their effects on SR calcium loading. Our data are consistent with previous reports describing a decrease in the L-type calcium channel activity during regional ischemia (22) and a substantial loss of the T-tubule network and a 50% decrease in density of the L-type calcium channels in a canine tachycardia-pacing model of heart failure (11). A reduction in calcium channel expression may initially limit calcium overload in the presence of diminished SERCA expression.

The calcium channel and the ryanodine receptor are located in the same microdomain of the cell (1), and the ryanodine receptor is gated by changes in intracellular calcium levels. Micromolar levels of calcium seen during L-type calcium channel activation stimulate the ryanodine receptor, whereas millimolar calcium levels inhibit the activity of this calcium transporter (13). Thus a decrease in the ryanodine receptor expression and/or activity may provide one mechanism for transporters involved in myocardial relaxation include the SR calcium-ATPase, the sodium/calcium exchanger, and the sarcolemmal calcium-ATPase (19). Because SERCA removes 92% of the contractile calcium from the cytosol in the rat heart (3, 9), this transporter is a critical determinant of calcium removal from the cytosol. The SR calcium-ATPase also regulates myocardial contraction because it replenishes SR calcium stores in preparation for the next contractile cycle (4). In light of these data, both contraction and relaxation in the rat heart are critically dependent on the function of SERCA.

Fig. 6. Time course of intracellular sodium accumulation ([Na⁺]/[H⁺]) by cardiomyocytes after burn trauma. Myocytes were prepared from 5–6 rats at each postburn time shown on the x-axis (2, 4, 8, 12, and 24 h). At least 20 myocytes from each rat were included for each time point. All values are means ± SE. *Significant difference from control at \( P < 0.05 \).

Fig. 7. Effects of burn trauma on myocardial sodium-potassium-ATPase protein expression as measured by Western blot (top) and densitometry (bottom). All values are means ± SE. *Significant difference from control at \( P < 0.05 \).
Burn trauma significantly reduced expression of rat cardiac SERCA protein in our study, a finding that is consistent with several reports describing decreased SERCA expression in myocardial disease. For example, Chossat et al. (7) showed that SERCA protein and mRNA levels were dramatically decreased in cardiac hypertrophy. Also, in a rat model of ischemia-reperfusion described by Temsah and colleagues (26), protein levels of SERCA were diminished by 70%, resembling the percent decrease in SERCA expression documented in our model of burn trauma. Although this present study confirms that burn trauma altered SERCA protein expression, we have described previously that severe burn injury decreases the maximal SR calcium uptake capacity (14), a finding consistent with a report by Wu et al. (31), who described decreased SERCA activity in rats with late-stage sepsis.

Decreased SERCA expression and/or activity in cardiac disease have directed interventional therapies to correct expression deficits. Del Monte et al. (9) described that adenoviral gene transfer of cardiac SERCA2a into failing rat hearts markedly improved myocardial calcium handling, contraction, and relaxation. Similarly, adenoviral gene transfer of the fast-twitch skeletal muscle SERCA1a to adult rat cardiomyocytes increased SR calcium loading and reduced diastolic intracellular calcium levels (7). Improved rates of both myocardial contraction and relaxation were described in mouse models with modest overexpression of SERCA2a (12). These data collectively suggest that sufficient SERCA expression is essential for the maintenance of cardiac function (7, 9, 20), and a reduction in SERCA protein levels promotes inadequate sequestration of cytosolic calcium, intracellular calcium overload, and myocardial contraction and relaxation deficits.

In addition to the SERCA, the sodium/calcium exchanger also removes calcium from the cardiomyocyte cytosol during relaxation. However, this calcium transport mechanism plays a minor role in myocyte calcium removal in the rat (3, 19). In the present study, burn trauma produced a biphasic sodium/calcium exchanger expression pattern. An initial increase in sodium/calcium exchanger protein levels may delay calcium overload in the presence of diminished SERCA expression (A in Fig. 8). However, this increased sodium/calcium exchanger expression may be responsible for burn-mediated sodium overload (B in Fig. 8), which was evident 4 h postburn. The protein levels of sodium/calcium exchanger fell 24 h postburn (C in Fig. 8), which may have limited further myocyte sodium accumulation. This proposed sequence of events is further supported by our present finding that cardiomyocyte sodium accumulation precedes calcium overload in burn trauma and is supported by data from our previous studies showing that the sodium/hydrogen and sodium/calcium exchange inhibitor amiloride decreased burn-mediated cardiomyocyte calcium and sodium overload and provided cardioprotection (30). Our data are also consistent with studies by Wang et al. (29), who described reduced sodium/calcium exchanger activity in late-stage sepsis.

Because calcium removal from the cytosol is dependent to some extent on myocyte sodium levels via the sodium/calcium exchanger, we determined whether burn trauma altered myocardial sodium-potassium-ATPase expression, contributing to early myocyte sodium overload. The sodium-potassium-ATPase exists as a heterodimer composed of a catalytic α-subunit and a β-subunit. Although there are three α-subunit isoforms in the human heart, the α1-subunit is the only isoform present in significant amounts in the rat heart (25). In the present study, burn injury increased cardiac expression of the α1-subunit of the sodium-potassium-ATPase (D on Fig. 8), which persisted 2–24 h after burn trauma. These data are consistent with reports using chronic models by both Kato et al. (16), who described that the α1- and β1-subunits were increased in late stage of heart failure in hamsters, and Sweadner et al. (25), who described that α1-subunit mRNA levels were elevated in hypertensive rat hearts. The increase in the α1-subunit of the sodium-potassium-ATPase observed in burn trauma may represent a compensatory mechanism to limit the progressive rise in intracellular sodium after injury (D in Fig. 8).

The alterations in calcium/sodium transporter protein levels likely represent changes in protein degradation and/or synthesis. The reduction in protein levels is most likely due to degradation of protein or muscle wasting, a widely documented phenomenon in the skeletal muscle of burn patients (2). Alterations in protein levels at the early time points may also reflect changes in mRNA stability. In fact, changes in SERCA, ryanodine receptor, and sodium/calcium exchanger mRNA levels occur within 1–2 h in ischemia-reperfusion and pressure overload models (17, 26). Elevated protein expression observed at 8 and 24 h postburn may reflect an increase in gene transcription.

Although elevated protein expression may increase calcium/sodium transporter activity because there would be more protein for ion transport, additional transporters may not be active. Likewise, a decrease in protein expression may reduce transporter activity, but the remaining transporters may exhibit

![Diagram of calcium transporters](http://jap.physiology.org/Downloaded/from http://jap.org)
increased activity to compensate for diminished expression. Thus alterations in protein expression do not always translate into changes in activity. Nevertheless, changes in the levels of sodium/calcium transporter protein expression have been correlated with alteration in transporter activity in ischemia-reperfusion and heart failure models (7, 16, 26). Furthermore, we have previously shown that sarcoplasmic reticular calcium uptake is diminished in burn trauma, and the present article shows that the expression of this transporter is also reduced (14).

In addition, several investigators have shown that altered expression of SERCA produces a similar change in SR calcium uptake (7, 9, 12). Because the magnitude of altered calcium/sodium transporter expression in the present study was similar to other studies in which changes in protein expression led to altered transporter activity (7, 9, 12), the changes in calcium/sodium transporter protein levels observed in burn trauma may be sufficient to alter activity.

In summary, our finding that burn trauma reduced expression of myocardial SR calcium-ATPase elucidates one mechanism by which major burn trauma may promote cellular calcium overload. These data, together with our previous finding that myocyte calcium accumulation precedes burned-reinnervation and relaxation defects, suggest that alterations in myocardial calcium homeostasis after burn trauma contribute to myocardial injury and dysfunction. Therefore, strategies designed to prevent alterations in SERCA expression would be expected to provide cardioprotection in burn injury and other cardiac disease states.

GRANTS

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REFERENCES