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Glucocorticoids Improve Calcium Cycling in Cardiac Myocytes After Cardiopulmonary Bypass

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Background. **Glucocorticoids can reduce myocardial dysfunction associated with ischemia and reperfusion injury following cardiopulmonary bypass (CPB) and circulatory arrest. The hypothesis was that maintenance of cardiac function after CPB with methylprednisolone therapy results, in part, from preservation of myocyte calcium cycling.**

Methods. **Piglets (5–7kg) underwent CPB and 120min of hypothermic circulatory arrest with (CPB-GC) or without (CPB) methylprednisolone (30 mg kg^{-1}) administered 6h before and at CPB. Controls (No-CPB) did not undergo CPB or receive glucocorticoids ($n = 6$ per treatment). Myocardial function was monitored *in vivo* for 120min after CPB. Calcium cycling was analyzed using rapid line-scan confocal microscopy in isolated, fluo-3-AM-loaded cardiac myocytes. Phospholamban phosphorylation and sarco(endo)plasmic reticulum calcium-ATPase (SERCA2a) protein levels were determined by immunoblotting of myocardium collected 120min after CPB. Calpain activation in myocardium was measured by fluorometric assay.**

Results. **Preload recruitable stroke work *in vivo* 120min after reperfusion decreased from baseline in CPB (47.4 ± 12 *versus* 26.4 ± 8.3 slope of the regression line, $P < 0.05$), but was not different in CPB-GC (41 ± 8.1 *versus* 37.6 ± 2.2, $P = 0.7$). In myocytes isolated from piglets, total calcium transient time remained unaltered in CPB-GC (368 ± 52.5 ms) compared with controls (434.5 ± 35.3 ms; $P = 0.07$), but was prolonged in CPB myocytes (632 ± 83.4 ms; $P < 0.01$). Calcium transient amplitude was blunted in myocytes from CPB (757 ± 168nM) compared with controls (1127 ± 126nM, $P < 0.05$) but was maintained in CPB-GC (1021 ± 155nM, $P > 0.05$). Activation of calpain after CPB was reduced with glucocorticoids. Phospholamban phosphorylation and SERCA2a protein levels in myocardium were decreased in CPB compared with No-CPB and CPB-GC ($P < 0.05$).**

Conclusions. **The glucocorticoid-mediated improvement in myocardial function after CPB might be due, in part, to prevention of calpain activation and maintenance of cardiac myocyte calcium cycling.**

Key Words: **ischemia/reperfusion; cardiopulmonary bypass; circulatory arrest; physiology/pathophysiology; neonate; calcium; calcium cycling; SERCA2a; phospholamban.**

INTRODUCTION

Cardiopulmonary bypass (CPB) employed for repair of congenital heart disease can have significant and detrimental effects on neonatal and infant myocardium [1–3]. Glucocorticoids have become accepted therapy aimed at decreasing cardiac dysfunction associated with CPB and ischemic arrest. Several studies have shown that preoperative and/or intraoperative glucocorticoid administration can attenuate myocardial and pulmonary dysfunction in children undergoing CPB during heart surgery [4–7].

Calcium influx during reperfusion of ischemic tissues is an important mediator of reperfusion injury, activating inflammatory, proteolytic, and cell death pathways [8, 9]. The immature cardiac myocyte might also be more sensitive to calcium-mediated injury, as the calcium cycling mechanisms are not the same as in adult hearts [10, 11]. The calcium influx during ischemia

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and reperfusion activates calcium-dependent proteases, such as calpains. Calpain activation in the myocardium with ischemia and reperfusion is implicated in the degradation of cytoskeletal proteins [12], contractile proteins, such as troponin I [13], and calcium cycling proteins, such as sarco(endo)plasmic reticulum (SR) calcium-ATPase 2a (SERCA2a), and phospholamban (PLB) [14, 15].

The calcium transport system within the cardiac myocyte depends upon SERCA for rapid removal of calcium from the cytosol during relaxation and controls the amount of calcium available for release from the SR for subsequent contraction [16, 17]. Phospholamban regulates SERCA2a activity in the myocardium by inhibiting the calcium pump. Phosphorylation of PLB by cAMP-dependent protein kinase on serine 16 and calcium/calmodulin-dependent protein kinase II on threonine 17 relieves SERCA inhibition leading to increased calcium transport. Increased SERCA2a protein levels and activity result in improved myocardial contractility and calcium cycling after myocardial ischemia and reperfusion [18, 19].

Previous studies by our research group have shown that glucocorticoid therapy prior to and during CPB and deep hypothermic circulatory arrest (DHCA) is associated with maintained myocardial function in neonatal piglets [20, 21]. The mechanism underlying the improvement after CPB is associated with maintenance of calpastatin, the endogenous inhibitor of calpain, and reduced calpain activity within the myocardium [20]. In the present study, we hypothesized that maintenance of calcium dynamics within the cardiac myocyte is a potential mechanism for the beneficial effects of glucocorticoid therapy on the immature myocardium after CPB and circulatory arrest.

MATERIAL AND METHODS

Cardiopulmonary Bypass

All animals received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animals Resources and published by the National Institutes of Health (NIH publication no. 86-23, revised 1996). The Institutional Animal Care and Use Committee at Cincinnati Children's Hospital Research Foundation also approved the protocol.

An established model of CPB with DHCA has been utilized extensively by our laboratory to elucidate mechanisms of myocardial and pulmonary dysfunction and to develop therapies to attenuate this injury [20–24]. The model results in sufficient injury in untreated animals to maintain greater than 90% survival but allows for determination of the mechanisms of injury and investigation of therapeutic interventions. Crossbred piglets weighing 5–7 kg were anesthetized, mechanically ventilated, and subjected to CPB and DHCA as previously described [20, 21, 24]. Pentobarbital infusion (20 mg $kg^{-1}\cdot h$–^{1}), intermittent fentanyl citrate (10 mcg$\cdot kg^{-1}\cdot h$–^{1}), and pancuronium bromide (0.1 mg $kg^{-1}\cdot h^{-1}$) were used with doses sufficient to maintain deep general anesthesia. Pressure catheters (Millar Instruments, Houston, TX) were placed in the pulmonary artery and in the right and left ventricles (RV and LV). Six piezoelectric crystals were placed in the myocardium at the anterior, posterior, base, and apex of the LV and at the widest point of RV and LV free walls. The distances between crystals on three axes were measured by sonomicrometry. Sonolab data collection and Cardiosoft analysis software (Sonometrics, ON, Canada) monitored cardiac function. Pressure-volume loops generated during preload reduction by transient vena caval occlusion allowed for measures of cardiac contractility relatively independent of heart rate and load. Left ventricular preload recruitable stroke work (PRSW), the time constant of isovolumic relaxation (Tau), maximal elastance (Emax), and end diastolic pressure-volume relationship (EDPVR) were calculated. Baseline measurements of cardiac functions were taken after a 30-min equilibration period.

Animals were administered heparin and placed on CPB with cannulation *via* the carotid artery and right atrial appendage. The CPB prime consisted of 800 mL direct-drawn whole porcine blood (Animal Biotech Industries, Danboro, PA). Hematocrit on CPB was maintained at 25% to 30% and calcium at 0.6–0.8 mg$\cdot L^{-1}$ with a flow rate of 100 mL$\cdot kg^{-1}\cdot min^{-1}$. Once on CPB, animals were cooled to a rectal temperature of 18°C over approximately 40 min. The bypass circuit was then turned off and the animal packed in ice. The heart was protected with topical cold saline and ice. Circulatory arrest was maintained for 120 min. Cardiopulmonary bypass was reinstituted, and the animals were warmed to 38°C over 45 min on CPB. Piglets were removed from CPB and maintained under anesthesia for 120 min. Left ventricular tissue was collected after 120 min of reperfusion for isolation of cardiac myocytes and for snap freezing for later analysis.

Animals were randomly divided into the experimental groups:

(1) No-CPB, ($n = 7$), heart tissue taken immediately after sternotomy to be used as controls.
(2) CPB, animals undergo CPB with no treatment ($n = 6$), and
(3) CPB-GC, methylprednisolone (30 mg$\cdot kg^{-1}$, intravenously) was administered 6 h before CPB in addition to an intra-operative dose of 30 mg$\cdot kg^{-1}$ in the pump prime (CPB-GC, $n = 6$). The methylprednisolone dose was the same as that used clinically at our institution and the two-dose regimen was determined to improve clinical outcomes in pediatric heart surgery patients compared with the single intraoperative dose [5]. In addition, the same dosing schedule has previously provided cardioprotection in this neonatal piglet model [21, 24]. The primary investigators responsible for surgery and care of the animal during the experiment were blinded to the treatment.

Myocyte Isolation and Fluo-3-AM loading

Left ventricular myocardial sections were excised from piglets either immediately after sternotomy (No-CPB) or at 120 min post-bypass in the CPB groups. Heart sections were cannulated onto a modified Langendorff perfusion apparatus as previously described [25, 26]. Heart sections were perfused in low calcium minimal essential medium (S-MEM, Joklick modified medium; Invitrogen, Carlsbad, CA) for 5 min at 37 °C, then switched to S-MEM containing 0.7 mg mL^{-1} collagenase D (Boehringer-Mannheim) for 7–15 min. Both solutions were gassed with 95% oxygen and 5% carbon dioxide and adjusted to pH 7.4. Dissociated cells were loaded with the calcium indicator, fluo-3-AM (Invitrogen), with a final concentration of 2 mmol$\cdot L^{-1}$ in S-MEM containing 2% horse-serum. Cells were washed twice and de-esterified in S-MEM.

Calcium Transient Recordings

The fluo-3-AM-loaded myocytes were attached to a laminin-coated glass slide in a flow-through chamber and bathed in modified Tyrode's solution ((g$\cdot L^{-1}$) 8 sodium chloride, 0.2 potassium

chloride, 0.1 magnesium chloride, 0.05 sodium dihydrogen phosphate, 1 sodium bicarbonate, 1 d-glucose, 1 mmol·L^{-1} calcium chloride, pH 7.4). Calcium transients of fluo-3-AM-loaded myocytes were measured by rapid line-scan confocal microscopy using a ×40/1.40 oil objective from 15–20 different cells from each animal. Image analysis is performed using Simple PCI software (Silicon Graphics, Mountain View, CA). Line-scans were performed at 100 Hz. Myocytes were field-stimulated using parallel platinum wires by 7 ms pulses 20% above threshold. Calcium transients are recorded following steady state pacing at 1 Hz, and line-scan images are acquired at 0.5 Hz.

Measurement of Intracellular Calcium Transients

Intracellular calcium concentrations were determined relative to calcium calibration curves generated by confocal microscopy. Following acquisition of calcium transient data, 100 mL aliquots of fluo-3-AM-loaded myocytes from each batch of cells were used for calcium measurements. Cells are placed in separate conical vials containing a known concentration of 0, 700, 1000, or 1200 nmol·L^{-1} calcium using the Calcium Calibration Kit Concentrate (Invitrogen). The Kd value for calcium-ethylene glycol tetra-acetic acid is adjusted according to pH, ionic strength, and temperature to arrive at appropriate standard concentrations. In addition, each vial contains (mmol·L^{-1}) 10 calcimycin (Invitrogen), 50 2,3 butane-dione-monoxime, 0.2 dinitrophenol, 140 sodium chloride, 2 potassium chloride, 0.5 magnesium chloride, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.25 sodium dihydrogenphosphate, 5.6 glucose, 10 sodium pyruvate, 5 L-carnitine, and 5 mg·L^{-1} insulin. Calcium standardizations were performed using caffeine-treated functionally-inactivated cardiomyocytes. The myocytes were allowed to equilibrate in each calcium concentration, transferred to slides, and scanned by confocal microscopy. Fluo-3-AM fluorescence was measured by pixel intensity. Live cell calcium transient recordings were normalized to corresponding calibration curves.

Protein Immunoblotting

LV free wall tissues collected at 120 min after CPB and DHCA were homogenized in 10 mmol·L^{-1} 3-[N-morpholino] propane sulfonic acid buffer with protease and phosphatase inhibitors and stored at –80°C until used. Western blots were performed with 30 mcg total proteins separated on 4%–12% acrylamide bis-tris gradient gels (Invitrogen) by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Some membranes were immunoblotted with antibodies for total PLB and phosphorylation site-specific antibodies for PLB serine 16 and threonine 17 (Fluorescience Ltd., Leeds, United Kingdom). Secondary antibodies were alkaline phosphatase-conjugated goat anti-rabbit or anti-mouse IgG. Proteins were visualized with a chemiluminescent detection system according to the manufacturer's instructions (Invitrogen). Protein levels are reported as the percent phosphorylated of total PLB in each lane. Other immunoblots were incubated with anti-SERCA2a antibodies (Abcam, Cambridge, MA) or anti-calpastatin domain 1 antibodies (Millipore, Billerica, MA). Secondary antibodies were alkaline phosphatase-conjugated mouse IgG or chicken IgY. Immunoblots were also incubated with anti-glyceraldehyde 3 phosphate dehydrogenase [(GAPDH), Abcam] for normalization of the blots. Calpastatin and SERCA2a data are presented as the ratio of the densitometry of the target proteins to GAPDH.

Calpain Activity Assay

Calpain activity was measured in LV homogenates from tissue collected 120 min after CPB and DHCA with a commercial kit according to the manufacturer's instructions (Calbiochem, San Diego, CA). The fluorometric assay measures total calpain 1 and 2 activity with an assay range of 63–1000 ng·mL^{-1}.

Statistical Analysis of Data

Repeated-measures analysis of variance was used to analyze serial data over time and *post hoc* comparisons made by Fisher's *post hoc* least significant difference test were used, when appropriate, to evaluate differences between individual time points within treatment groups. Comparisons between treatments were made by analysis of variance with a P value ≤ 0.05 considered significant. Personnel blinded to the treatment group status conducted analyses using Statview 4.01 software (Abacus Concepts, Berkeley, CA). Data are reported as means ± standard deviations.

RESULTS

Cardiac Function

Control animals subjected to CPB and DHCA without glucocorticoid treatment exhibited myocardial dysfunction. Representative pressure-volume loops from subsequent animals demonstrate significant differences in maximal elastance and stroke work (Fig. 1). In CPB animals there was impaired systolic and diastolic function after CPB and DHCA demonstrated by reduced PRSW and Emax, along with increased τ and EDPVR (Table 1). In contrast, there were no changes from baseline measurements in these cardiac parameters in glucocorticoid-treated animals. Myocardial contractility was significantly better in CPB-GC compared with CPB animals after 120 min of reperfusion following CPB and DHCA.

Calcium Cycling

Altered calcium dynamics were measured by rapid line-scan microscopy with images obtained from individual myocytes isolated from animals in the *in vivo* treatment groups, No-CPB, CPB, and CPB-GC (Fig. 2). The total calcium transient time was significantly increased after CPB compared with No-CPB (632 ± 83 msec *versus* 434 ± 35 msec, $P < 0.01$), while treatment with glucocorticoids prevented the lengthening of calcium transient time (368 ± 52 ms, Fig 3). Both calcium transient amplitude and transient time to peak were unchanged from No-CPB in glucocorticoid-treated animals while untreated animals undergoing CPB and DHCA had decreased transient amplitude and extended time to peak, (amplitude (nM): No-CPB = 1127 ± 26, CPB-GC = 1021 ± 155, CPB = 757 ± 109, $P < 0.05$ CPB *versus* No-CPB and CPB-GC), and (time to peak (ms): No-CPB = 54 ± 16, CPB-GC = 57 ± 14, CPB = 109 ± 43, $P < 0.05$ CPB *versus* No-CPB and CPB-GC).

Calcium Cycling Proteins

Phosphorylation of LV phospholamban at serine-16 was decreased after CPB ($P = 0.003$, Fig. 4). Although serine-16 phosphorylation levels with glucocorticoid treatment was lower than in No-CPB controls

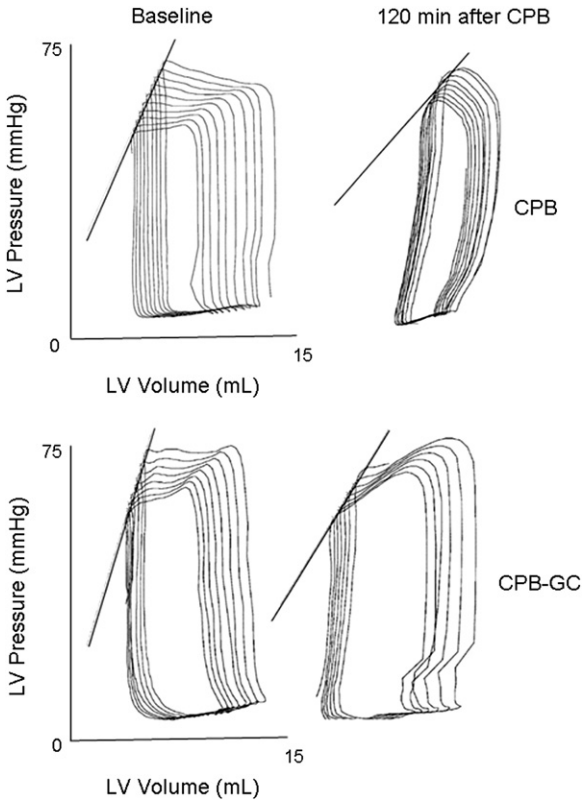

FIG. 1. Representative pressure-volume loops demonstrating significant narrowing of the width (decreased stroke volume) and a decrease in maximal elastance demonstrated by decreased slope of the regression line in untreated hearts following cardiopulmonary bypass. In the glucocorticoid-treated group, the stroke volume and slope of the regression line were maintained similar to baseline, indicating a preserved inotropic state. Data values are in Table 1. CPB = cardiopulmonary bypass; GC = glucocorticoids.

TABLE 1

Left Ventricular Function

	Baseline	120 in Reperfusion
Preload recruitable stroke work (slope of regression line)		
CPB	47.4 ± 12.3	26.4 ± 8.3[a]
CPB-GC	41.0 ± 8.1	37.6 ± 2.2[b]
Maximal elastance (slope of regression line)		
CPB	18.9 ± 2.1	9.6 ± 2.9[a]
CPB-GC	24.4 ± 6.2	16.8 ± 2.8[b]
Time constant of isovolumic relaxation (msec)		
CPB	59.7 ± 7.6	93.7 ± 8.0[a]
CPB-GC	42.7 ± 3.2	58.3 ± 17.2[b]
End diastolic pressure volume relationship		
CPB	0.10 ± 0.05	0.46 ± 0.18[a]
CPB-GC	0.09 ± 0.04	0.26 ± 0.10[a,b]

CPB = cardiopulmonary bypass; GC = glucocorticoids.

Comparisons over time are made by repeated measures of ANOVA and *post hoc* Fishers' PLSD, where appropriate. Comparisons between treatments are made by unpaired Student's *t*-test. Data are means ± standard deviations.

[a] $P < 0.05$ *versus* baseline.

[b] $P < 0.05$ *versus* CPB at same time point.

(0.76 ± 0.08 *versus* 1.02 ± 0.12 ratio of densitometry of phosphoprotein to total phospholamban in each lane, $P = 0.04$), levels were higher than phosphorylation levels in the CPB group (0.44 ± 0.12, $P = 0.02$ *versus* CPB-GC). Phosphorylated phospholamban at threonine-17 was also decreased after CPB (0.16 ± 0.05 ratio of densitometry of phosphoprotein to total phospholamban, $P = 0.02$) compared with No-CPB (1 ± 0.42). Again phosphorylation levels were lower in CPB-GC (0.46 ± 0.17) compared with No-CPB but were much higher than in the CPB group ($P = 0.04$). The ratio of the densitometry of SERCA2a protein to GAPDH in the LV decreased 120 min after CPB but was maintained with glucocorticoids at control levels (CPB = 0.38 ± 0.03 *versus* CPB-GC = 0.48 ± 0.06, $P = 0.05$; No-CPB = 0.46 ± 0.07, Fig. 5).

Calpastatin Protein and Calpain Activity Levels

Myocardial calpastatin protein levels in LV collected 120 min after CPB (ratio of densitometry of target protein to GAPDH) were reduced (0.22 ± 0.02) compared with LV tissue from CPB-GC (0.31 ± 0.03, $P = 0.05$, Fig. 6). Glucocorticoids maintained calpastatin levels similar to control levels (0.31 ± 0.02) and was higher than in CPB animals ($P = 0.04$). Calpain activity (relative fluorescent units·mg protein^{-1}·min^{-1}) in LV tissue increased 120 min after CPB and DHCA (253 ± 28) compared with No-CPB (209 ± 26). Glucocorticoid therapy prevented the activation of calpain in the LV after CPB (204 ± 33, $P = 0.8$ *versus* No-CPB and $P = 0.03$ *versus* CPB).

DISCUSSION

These data provide a mechanism by which glucocorticoids might preserve myocardial function in neonatal myocytes subjected to CPB and DHCA. Although the benefits of high dose steroids in conjunction with DHCA remains controversial, especially in regards to cerebral protection [27, 28], there is evidence that glucocorticoids are cardioprotective after CPB and DHCA in animal models [20, 21, 23, 24, 29]. The pre- and intraoperative doses used in this study have also been shown to decrease postoperative inflammatory markers in pediatric cardiac surgery patients [5]. The load-independent measures used to assess ventricular function using pressure-volume relationships reaffirm the ventricular dysfunction associated with CPB and DHCA and the benefit of glucocorticoid therapy detected in our previous studies [20, 21, 24]. The ability to associate these measures of myocardial contractility in an intact heart with calcium cycling in myocytes harvested from

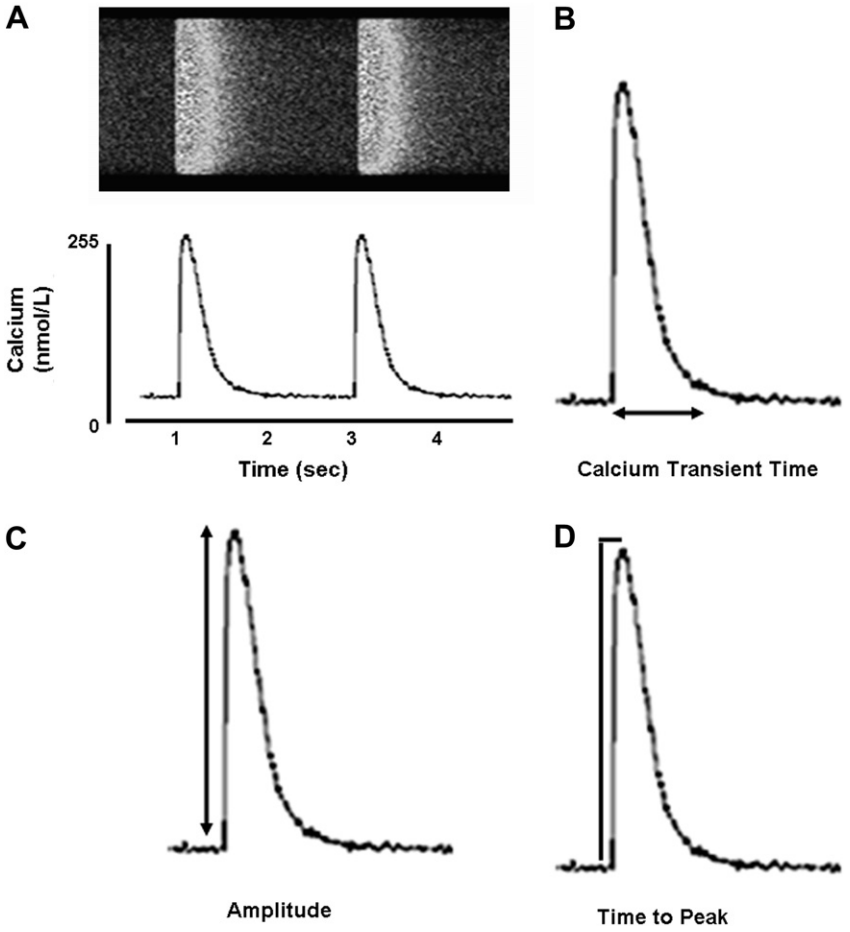

FIG. 2. Determination of calcium transient characteristics. (A) (Top) Representative confocal microscopy line-scan imaging from electrically-paced cardiomyocytes (0.5 Hz). (Below) Calcium transient profile derived from line-scan image. (B) Calcium transient time determined by elapsed time from onset of calcium transient to return to baseline. (C) Calcium concentration amplitude determined by the difference between diastolic and systolic calcium concentrations. (D) Time to peak determined by elapsed time from diastolic to peak systolic calcium concentration.

the same hearts is a unique feature of this study. For example, PRSW, a measure of the systolic function of the heart, and Tau, a measure of the rate of relaxation and diastolic function, were unchanged from controls in glucocorticoid-treated animals, as was calcium transient amplitude and time of transient decline. In contrast, untreated animals had decreased PRSW and longer Tau in the intact heart that was associated with a drop in calcium transient amplitude and extended calcium transient time in myocytes isolated from untreated hearts after undergoing CPB. The rate of relaxation in the intact heart is reflective of the intracellular calcium transient decline [30]. Global ischemia reduces the rate of relaxation in the myocyte with subsequent diastolic dysfunction and is closely related to intracellular calcium homeostasis [30, 31]. In addition, diastolic stiffness, evident in the increased EDPVR after CPB and DHCA in this study, can be ascribed to incomplete diastolic clearance of calcium, resulting in persistent tension in the ventricles [30].

Regulation of cytosolic calcium occurs through extracellular calcium transport by membrane proteins, such as L-type calcium channels, and intracellular cycling through the SR, mediated by SERCA uptake and ryanodine SR-release channels [32]. Specifically, calcium re-uptake from the cytosol into the SR depends upon the expression level of the SERCA calcium pump and the pump's affinity for calcium, which is mediated by the phosphorylation state of PLB [17]. Phospholamban in the phosphorylated state increases the activity of SERCA and uptake of calcium into the sarcoplasmic reticulum [17, 33]. Phospholamban serine-16 and threonine-17 are phosphorylated by protein kinase A and calcium/calmodulin-dependent protein kinase, respectively. Phosphorylation at either amino acid, separately or in tandem, is sufficient to increase cardiac relaxation rates *in vivo* [33].

Levels of SERCA2a protein in the myocardium are linked to ventricular function with reduced SERCA2a expression correlating with heart failure. The

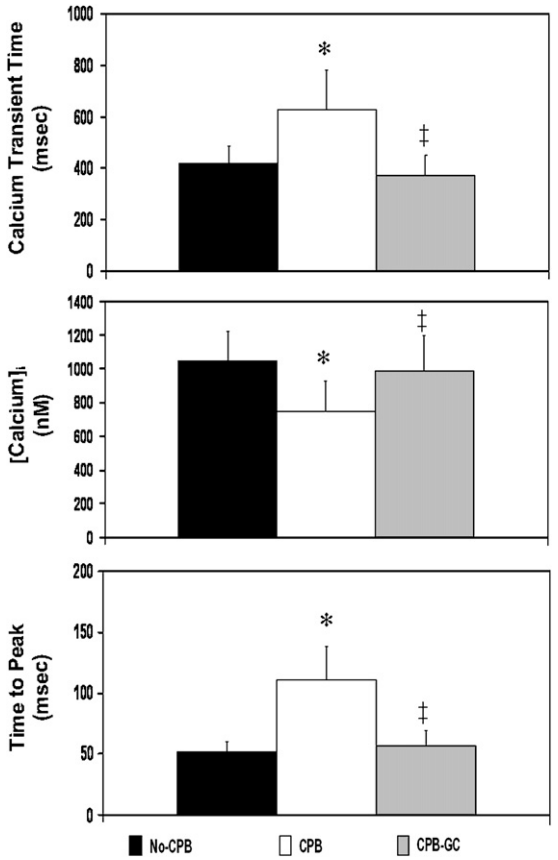

FIG. 3. Calcium transient characteristics. (Top) Total calcium transient time for No-CPB, CPB, and CPB-GC. (Center) Calcium transient amplitude. (Bottom) Time to peak. $^{*}P < 0.05$ *versus* no-CPB; $^{\ddagger}P < 0.05$ *versus* CPB. CPB = cardiopulmonary bypass, GC = glucocorticoids.

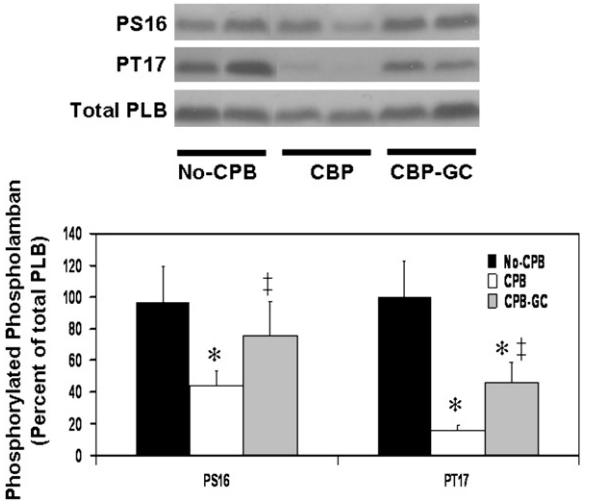

FIG. 4. Immunoblot analysis of phosphorylated and total phospholamban in LV. (Upper) Representative immunoblots of phosphorylation of phospholamban (PLB) at serine-16 (PS16) and threonine-17 (PT17) and total PLB in LV collected at 120 min after cardiopulmonary bypass (CPB). (Lower) Graph of immunoblot data expressed as the percent of total PLB that was detected by the phospho-specific antibodies for PLB PS 16 and PT17. $^{*}P < 0.05$ *versus* No-CPB; $^{\ddagger}P < 0.05$ *versus* CPB. GC = glucocorticoids.

decreased SERCA2a levels and dephosphorylation of PLB after CPB and DHCA in this study agree with other models of ischemia and reperfusion including Langendorff-perfused hearts [30] and isolated cardiac myocytes [31]. The increase in dephosphorylated PLB at both serine-16 and threonine-17 after CPB and DHCA might be responsible, at least in part, for the decline in calcium transient amplitude and the increase in transient time. In addition, the maintenance of PLB in the phosphorylated state with glucocorticoid therapy is reflected in the prevention of the decline *in vitro* calcium transients and the *in vivo* ventricular function associated with CPB and DHCA.

Our prior findings of calpastatin preservation and a reduction in calpain activity with glucocorticoid therapy prior to and during CPB and DHCA may help to elucidate the mechanisms underlying the beneficial effects of glucocorticoids [20, 21, 24]. Calpain, which in addition to directly degrading cytoskeletal and contractile proteins, can degrade calcium transport proteins, as SERCA2a [14, 15], and calcium channels [34]. French and colleagues demonstrated that ischemia and reperfusion induced calpain activation that resulted in SERCA2a degradation in rats. In addition, administration of a calpain inhibitor protected against myocardial SERCA2a degradation and maintained cardiac function after ischemia and reperfusion [14]. The maintenance of SERCA2a content with glucocorticoid therapy in this study might be, in

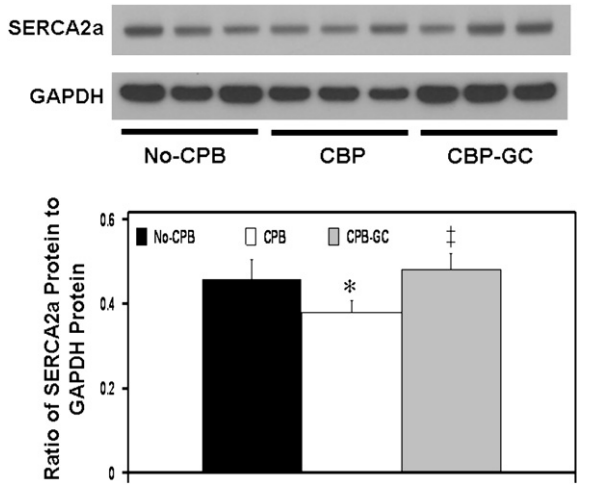

FIG. 5. Immunoblot analysis of sarco(endo)plasmic reticulum calcium ATPase in LV. (Upper) Representative immunoblots of sarco(endo)plasmic reticulum calcium ATPase 2a (SERCA2a) and glyceraldehyde phosphate dehydrogenase (GAPDH) in LV collected at 120 min after cardiopulmonary bypass (CPB). (Lower) Graph of immunoblot data expressed as the ratio of SERCa2a protein to GAPDH densitometry. $^{*}P < 0.05$ *versus* No-CPB; $^{\ddagger}P < 0.05$ *versus* CPB. GC = glucocorticoids.

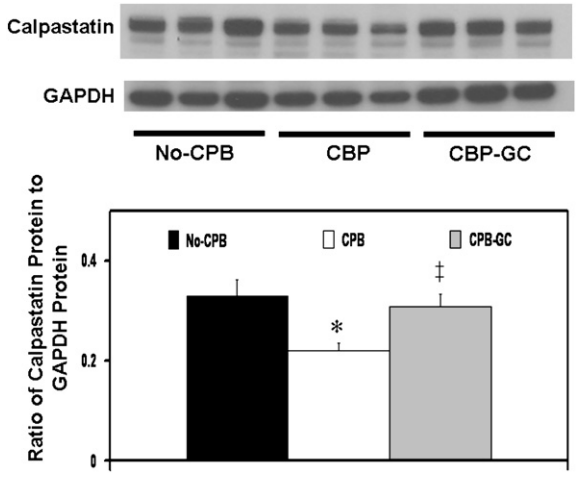

FIG. 6. Immunoblot analysis of calpastatin in LV. (Upper) Representative immunoblots of calpastatin and glyceraldehyde phosphate dehydrogenase (GAPDH) in LV collected at 120 min after cardiopulmonary bypass (CPB). (Lower) Graph of immunoblot data expressed as the ratio of calpastatin protein to GAPDH densitometry. $^{*}P < 0.05$ *versus* No-CPB; $^{\ddagger}P < 0.05$ *versus* CPB. GC = glucocorticoids.

part, a result of the prevention of calpain activation after CPB and DHCA.

In addition to effects on SR proteins, calpastatin, the endogenous inhibitor of calpains, might also have a direct effect on maintaining L-type calcium channel function. The L-domain of calpastatin can prevent L-type calcium channel run-down [35] and reprime channels [36, 37] by interacting with the calmodulin binding site of the L-type Cav1.2 channel [38]. Furthermore, the effects of calpastatin on calcium channel activity are independent of any calpain inhibitory actions [35]. Hence, glucocorticoid-mediated preservation of calpastatin after ischemia and reperfusion might also directly improve calcium dynamics in cardiac myocytes.

One limitation of this study is that we have examined only the SR calcium re-uptake regulatory proteins. Further studies will investigate calcium release from the SR and the contribution of extracellular calcium through ion channel activation.

In summary, these data demonstrated that glucocorticoids preserve intracellular calcium cycling proteins in isolated cardiac myocytes. Although the mechanisms by which glucocorticoids maintain calcium handling are not entirely clear, glucocorticoids can reduce ischemia and reperfusion-induced calpain activation and subsequent inactivation of calcium transport proteins. This study provides further evidence of the multiple and diverse mechanisms by which glucocorticoids might protect the myocardium, and continues to support the clinical use of glucocorticoids to minimize ischemia and reperfusion injury.

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