Altered phosphorylation and calcium sensitivity of cardiac myofibrillar proteins during sepsis

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Altered phosphorylation and calcium sensitivity of cardiac myofibrillar proteins during sepsis. Am J Physiol Regulatory Integrative Comp Physiol 281: R408–R416, 2001.—Altered phosphorylation and Ca2+ sensitivity of cardiac myofibrillar proteins during different phases of sepsis were investigated. Sepsis was induced by cecal ligation and puncture (CLP). The results show that phosphorylation of troponin I (TnI) was increased by 268% during the early phase (9 h after CLP) but decreased by 46% during the late phase (18 h after CLP) of sepsis. Phosphorylation of C protein was increased by 76% during the early phase but decreased by 41% during the late phase of sepsis. Phosphorylation of myosin light chain-2 (MLC-2) remained unaltered during the early phase but was decreased by 38% during the late phase of sepsis. Phosphorylation of TnT was unaffected during the progression of sepsis. The increases in the phosphorylation of TnI and C protein during early sepsis were associated with the decrease in the Ca2+ sensitivity of myofilaments and the increases in myocardial changes in tension development (+dP/dt max) and cAMP level. The decreases in the phosphorylation of TnI and C protein during late sepsis coincided with the declines in the activities of myofibrillar ATPase, Ca2+ sensitivity of myofilaments, myocardial +dP/dt max, and cAMP content. The increases and the decreases in the phosphorylation of TnI and C protein, +dP/dt max, and the tissue cAMP level were sensitive to isoproterenol stimulation and propranolol inhibition. These findings suggest that alterations in the phosphorylation of myofibrillar proteins, such as TnI, C protein, and MLC-2, and changes in the activities and the Ca2+ sensitivity of myofibrillar ATPase may contribute to the altered cardiac function during the progression of sepsis. Furthermore, the sepsis-induced alterations in the phosphorylation and Ca2+ sensitivity of cardiac myofibrillar proteins were mediated via a β-adrenergic receptor pathway.

MYOFIBRILLAR PROTEINS COMPRISE three major classes of proteins, namely contractile proteins (myosin and actin), regulatory proteins (troponin and troponin complex), and structural proteins (C protein, α-actinin, etc.). Interactions among these major classes of myofibrillar proteins determine the contractile properties of the heart (22, 27, 35). Contractile proteins convert the chemical energy of ATP hydrolysis into mechanical work. Regulatory proteins regulate the actin-myosin cross-bridge attachment. Structural proteins provide some mechanical linkage and stability properties to the contractile and regulatory proteins (22, 35). Protein phosphorylation has been recognized as an important mechanism for regulating cellular function and metabolism. Recent advances in the studies of cardiovascular pathophysiology have indicated that changes in the phosphorylation states of myofibrillar proteins underlie cardiac dysfunction under various disease conditions including ischemia, hypertrophy, and heart failure (3, 15, 16, 18, 19, 22, 30). Myocardial function was significantly altered in patients and in animal models during sepsis (21, 26, 32). Altered myocardial function during sepsis involves complex regulatory mechanisms. Although changes in myofibrillar proteins have been implicated to be an etiologic factor contributing to cardiac dysfunction in endotoxin and septic shock, the observations were confined to the histological examination of the myofilaments (17, 31) and the measurement of myofibrillar Mg2+-ATPase activities (4, 8, 21).

Because the phosphorylation and the Ca2+ sensitivity of myofibrillar proteins play an important role in the regulation of cardiac function, the present study was undertaken to correlate sepsis-induced alterations in the phosphorylation and Ca2+ sensitivity of the three major classes of myofibrillar proteins with changes in cardiac contractility during different phases of sepsis.

MATERIALS AND METHODS

Animal model. All animal experiments in this study were performed with the approval of the Animal Care Committee of Saint Louis University School of Medicine and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing from 275 to 300 g were used. All animals were fasted overnight with free access to water. Sepsis was in-
duced by cecal ligation and puncture (CLP), as described by Wichterman et al. (34) with minor modification. While the rats were under anesthesia with halothane, a laparotomy was performed, and the cecum was ligated just distally to the ileoceleal valve to avoid any intestinal obstruction and punctured twice with an 18-gauge needle. The cecum was then returned to the peritoneal cavity, and the abdomen was closed in two layers. Control rats were sham operated (a laparotomy was performed, and the cecum was manipulated but neither ligated nor punctured). All animals were resuscitated subcutaneously with 4 ml/100 g body wt of normal saline at the completion of surgery and also at 7 h postsurgery. Early and late sepsis refers to those animals killed at 9 and 18 h, respectively, after CLP. The mortality rates were 0% for control, 3% for early sepsis, and 29% for late sepsis.

Preparation of cardiac myofibrils and phosphorylation of myofibrillar proteins. Hearts removed from control or septic rats under urethane anesthesia were retrogradely perfused by the Langendorff technique under constant pressure (80 cm H2O) at 37°C with Krebs-Henseleit buffer (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 2.5 CaCl2, 0.1 KH2PO4, pH 7.4, 25 NaHCO3, and 10 glucose) for 10 min (first perfusion). The perfusion circuit was then switched to a recirculating system containing the same buffer in the presence of [32P]orthophosphoric acid (2.0 μCi/ml) and proceeded for 30 min (second perfusion). The second perfusion allowed 32P incorporation into cellular ATP, which acts as the phosphate donor for protein phosphorylation (23). At the end of the second perfusion, the hearts were perfused for 5 min with nonradioactive buffer to wash out radioactivity (third perfusion). When the effects of isoproterenol or propranolol were studied in some experiments, atropine (0.1 μM) and prazosin (0.1 μM) were present in the perfusion media to block muscarinic and α1-adrenergic receptors, respectively. All of the perfusion solutions were saturated with 95% O2-5% CO2. At the end of the third perfusion, hearts were freeze-clamped with aluminum clamps precooled in liquid nitrogen, and the samples were then used for the isolation of cardiac myofibrils.

Cardiac myofibrils were prepared according to the method described by Solaro et al. (29) with modification. All solutions contained leupeptin (1 μg/ml), pepstatin A (1 μg/ml), aprotinin (2 μg/ml), and phenylmethylsulfonyl fluoride (0.2 mM) as protease inhibitors, and NaF (50 mM), EDTA (5 mM), and KH2PO4 as phosphatase inhibitors. The frozen myocardium were pulverized under liquid nitrogen and homogenized with a Teflon-glass homogenizer three times for 30 s in 15 ml of buffer A [0.3 M sucrose, 30 mM KH2PO4, pH 7.0, and 1.0 mM dithiothreitol (DTT)]. The homogenates were centrifuged at 3,500 g for 10 min. The pellets were suspended in 20 ml of buffer B (0.1 M KH2PO4, pH 7.0, and 1 mM DTT), rehomogenized, and centrifuged at 3,500 g for 10 min. This procedure was repeated twice. The resulting pellets were then suspended in buffer C containing 1% (vol/vol) Triton X-100 and centrifuged at 3,500 g for 10 min. The pellets were washed three times as described above with buffer C (60 mM KCl, 30 mM imidazole, pH 7.0, 2 mM MgCl2, and 1 mM EDTA). The pellets were resuspended in buffer C, stored in −70°C, and then used as cardiac myofibril preparation.

Cardiac myofibril preparations were subjected to SDS-PAGE (5–20% acrylamide gradient gels) for the separation of myofibrillar proteins. The phosphorylated myofibrillar proteins were identified by autoradiography as described previously (5, 32). Myocardial ATP content and the specific radioactivity of tissue [γ-32P]ATP were determined as described earlier (32). 32P incorporation into myofibrillar proteins was quantified by counting the radioactivity of the dried gel tracks cut into 3-mm slices (5, 32).

Assay of myofibrillar ATPase activity. The activities of myofibrillar ATPase were measured as described by Resink et al. (32). To determine myofibrillar Mg2+-ATPase activity, the reaction mixture contained 30 mM KCl, 5 mM NaCl, 30 mM imidazole, pH 7.0, 3 mM MgCl2, 1 mM DTT, various concentrations of free Ca2+, and cardiac myofibril preparation (150 μg protein) in a final volume of 1 ml. The free Ca2+ concentrations were calculated using an EGTA-CaCl2-buffering system as described by Fabiato (6).

Measurement of cardiac contractility. Hearts obtained from control and septic rats were perfused with nonradioactive buffer by the same protocol as described above. A saline-filled latex balloon was inserted into the left ventricle through the left atrium and connected to a pressure transducer (Statham P23 DB) for the measurement of left ventricular pressure. The left ventricular end-diastolic pressure was adjusted at 6 mmHg at the beginning of the experiments. The maximal rate of increase (+dP/dtmax) and decrease (−dP/dtmax) of left ventricular pressure development was monitored on a multichannel polygraph (Grass Instruments model 79D). At the end of each experiment, the heart was freeze-clamped, stored at −70°C, and then used for the assay of myofibrillar ATPase activity.

Other assays and statistical analysis. For measurement of heart Ca2+ content, 100 mg of frozen tissue sample from nonradioactive perfused hearts were homogenized at 4°C in a buffer containing 50 mM Tris, pH 7.5, and 4 mM EGTA. The homogenates were heated for 10 min in a boiling water bath. After centrifugation, the Ca2+ content in the supernatant was determined by radioimmunoassay with a commercial [3H]Ca2+ assay kit (Amersham). Na+-K+-ATPase, Ca2+- ATPase, and azide-sensitive ATPase activities were measured according to the method of Lowry et al. (14). Results are expressed as means ± SE. Statistical analysis of the data was performed by using one-way ANOVA followed by Student-Newman-Keuls test. P < 0.05 was considered statistically significant.

Materials. [32P] labeled orthophosphoric acid and [γ-32P] ATP were purchased from ICN Biomedicals (Costa Mesa, CA). Na2ATP, isoproterenol, prazosin, propranolol, atropine, leupeptin, pepstatin, A, aprotinin, Triton X-100, and molecular weight standard proteins were purchased from Sigma Chemical (St. Louis, MO). Other chemicals and reagents were of analytic grade.

RESULTS

Table 1 depicts marker enzyme activities in cardiac homogenates and myofibrils isolated from control and...
septic rat hearts. The yield of myofibrils was similar among control, early, and late septic groups. 

**Table 1.** Marker enzyme activities in cardiac homogenates and myofibrils isolated from control and septic rat hearts

<table>
<thead>
<tr>
<th>Yield of Myofibrils, mg/g wet wt</th>
<th>Na(^+)-K(^+)-ATPase, (\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{h}^{-1})</th>
<th>Ca(^{2+})-ATPase, (\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{h}^{-1})</th>
<th>Azide-Sensitive ATPase, (\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{h}^{-1})</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>20.5 ± 1.5</td>
<td>1.06 ± 0.28</td>
<td>4.32 ± 0.24</td>
</tr>
<tr>
<td>Early sepsis</td>
<td>18.9 ± 1.4</td>
<td>1.13 ± 0.17</td>
<td>4.54 ± 0.37</td>
</tr>
<tr>
<td>Late sepsis</td>
<td>19.1 ± 1.5</td>
<td>0.96 ± 0.28</td>
<td>4.16 ± 0.20</td>
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</table>

Values are means ± SE. Number of experiments was 6 for each group.

Figure 1 depicts a representative experiment of the autoradiography from SDS-PAGE of the myofibrillar proteins labeled with \(^{32}\)P in the control and septic groups. Perfusion of intact beating hearts with \(^{32}\)P-labeled solution shows basal \(^{32}\)P incorporation into numerous proteins, including C protein (140 kDa), troponin T (TnT) (39 kDa), TnI (27 kDa), and myosin light chain-2 (MLC-2) (19 kDa). When hearts were perfused in the presence of isoproterenol, phosphorylation of TnI and C protein was enhanced, whereas the phosphorylation of TnT and MLC-2 remained unaffected in all three experimental groups. When hearts were perfused in the presence of propranolol, the phosphorylation of TnI and C protein was diminished. These results are in agreement with previous findings that TnI and C protein were responsive, whereas TnT and MLC-2 were unresponsive to \(\beta\)-adrenergic stimulation and inhibition (2, 16, 30).

Figure 2 shows quantitative analysis of \(^{32}\)P incorporation into TnI and C protein in control, early, and late septic rat hearts. Phosphorylation of TnI was increased by 268% \((P < 0.01); Fig. 2A, column 3 vs. 1\) during the early hyperdynamic phase, whereas it was decreased by 46% \((P < 0.05); Fig. 2A, column 6 vs. 1\) during the late hypodynamic phase of sepsis. Phosphorylation of C protein was increased by 76% \((P < 0.01); Fig. 2B, column 3 vs. 1\) during the early phase, whereas it was decreased by 41% \((P < 0.05); Fig. 2B, column 6 vs. 1\) during the late phase of sepsis. Phosphorylation of TnI was significantly stimulated by isoproterenol within each respective group \(Fig. 2A, column 2 vs. 1, column 4 vs. 3, and column 7 vs. 6\). The isoproterenol-stimulated phosphorylation of TnI was increased by 30% \((P < 0.01); Fig. 2A, column 4 vs. 2\) during early sepsis, whereas it was decreased by 50% \((P < 0.01)\) during late sepsis \(Fig. 2A, column 7 vs. 2\). Similar results were
obtained for phosphorylation of C protein in regard to isoproterenol stimulation. It is noteworthy that the observed increases in the phosphorylation of TnI and C protein during early sepsis were completely blocked by propranolol (Fig. 2A, comparison among columns 5, 3, and 1; Fig. 2B, comparison among columns 5, 3, and 1). These findings indicate that phosphorylation of TnI and C protein underwent a biphasic change, i.e., an increase during the early phase followed by a decrease during the late phase of sepsis. Furthermore, the sepsis-induced alterations in the phosphorylation of TnI and C protein were mediated through a \(\beta\)-adrenergic receptor pathway.

Figure 3 depicts quantitative analysis of \(^{32}\)P incorporation into TnT and MLC-2 in the rat hearts during the progression of sepsis. As shown in Fig. 3A, phosphorylation of TnT remained relatively unchanged during the early and late phases of sepsis (comparison among columns 1, 3, and 6). Isoproterenol and propranolol had no effect on the phosphorylation of TnT (comparison among columns 1–7). As shown in Fig. 3B, phosphorylation of MLC-2 was relatively unchanged during early sepsis, but it was reduced by 38% \((P < 0.05; \text{column 6 vs. 1})\) during late sepsis. Neither isoproterenol nor propranolol affected phosphorylation of MLC-2 (comparison among columns 1–7). These data indicate that phosphorylation of TnT remained unaffected during the progression of sepsis. Although the phosphorylation of MLC-2 was intact during the early phase, it was significantly decreased during the late phase of sepsis. Furthermore, \(\beta\)-adrenergic stimulation or inhibition had no effect on the phosphorylation of TnT and MLC-2.

Figure 4 shows the effect of different \(Ca^{2+}\) concentrations, and Table 2 depicts the effects of \(\beta\)-adrenergic agonist and antagonist on the activities of myofibrillar \(Mg^{2+}\)-ATPase in the control, early sepsis, and late sepsis. A sigmoid relationship between myofibrillar \(Mg^{2+}\)-ATPase activities and \(Ca^{2+}\) concentrations was observed both in the control and septic groups. During
These data indicate that myofibrillar Mg\textsuperscript{2+}-ATPase activities were inhibited by 19–36% (P < 0.05) at lower Ca\textsuperscript{2+} concentrations (<10\textsuperscript{-6} M) but were relatively unchanged at high Ca\textsuperscript{2+} concentrations (>10\textsuperscript{-6} M). During the late phase of sepsis, myofibrillar Mg\textsuperscript{2+}-ATPase activities were inhibited at low and high Ca\textsuperscript{2+} concentrations (10\textsuperscript{-8} to 2.5 x 10\textsuperscript{-5} M). Analysis of data using Eadie-Hofstee plots reveals that the V\textsubscript{max} for Ca\textsuperscript{2+} was not significantly affected during the early phase, but it was decreased by 21% (P < 0.05) during the late phase of sepsis. The K\textsubscript{m} value for Ca\textsuperscript{2+} was increased by 48% (P < 0.05) and 109% (P < 0.01) during the early and late phases, respectively, of sepsis (Table 2). The V\textsubscript{max} for Ca\textsuperscript{2+} for myofibrillar Mg\textsuperscript{2+}-ATPase was unaffected by isoproterenol. The K\textsubscript{m} values for Ca\textsuperscript{2+} were increased (+39%, P < 0.05) in the control, decreased (−54%, P < 0.01) in the late sepsis, but unaffected in the early sepsis by isoproterenol. These data indicate that myofibrillar Mg\textsuperscript{2+}-ATPase was impaired during the early and late phases of sepsis, and the impairment was associated with a decrease in the Ca\textsuperscript{2+} sensitivity of the myoflament.

Figure 5 depicts the effects of sepsis on the myosin Ca\textsuperscript{2+}-ATPase and K\textsuperscript{+}-EDTA-ATPase activities. Myosin Ca\textsuperscript{2+}-ATPase activity was unchanged during early sepsis but was decreased by 16% (P < 0.05) during late sepsis (Fig. 5A). Myosin K\textsuperscript{+}-EDTA-ATPase activity was unaffected during the early phase but was reduced by 24% (P < 0.05) during the late phase of sepsis (Fig. 5B). Isoproterenol and propranolol had no effect on regulating myosin ATPase activities (data not shown). These results indicate that myosin Ca\textsuperscript{2+}-ATPase and K\textsuperscript{+}-EDTA-ATPase activities were relatively unchanged during the early phase, but they were impaired during the late phase of sepsis. The impairment in myosin Ca\textsuperscript{2+}-ATPase and K\textsuperscript{+}-EDTA-ATPase was not associated with β-adrenergic mediation.

Figure 6 depicts changes of cardiac contractile parameters during the progression of sepsis. Myocardial +dP/dt\textsubscript{max} was increased by 19% (P < 0.05; Fig. 6A, column 3 vs. 1) during the early phase of sepsis, whereas it was reduced by 40% (P < 0.01; Fig. 6A, column 6 vs. 1) during the late phase of sepsis. The −dP/dt\textsubscript{max} remained relatively unchanged (Fig. 6B, column 3 vs. 1) during early sepsis, but it was decreased by 47% (P < 0.01; Fig. 6B, column 6 vs. 1) during late sepsis. The +dP/dt\textsubscript{max} was stimulated significantly by isoproterenol within each respective group (Fig. 6A, column 2 vs. 1, column 4 vs. 3, and column 7 vs. 6). There were no differences in the isoproterenol-stimulated +dP/dt\textsubscript{max} among control, early sepsis, and late sepsis groups (Fig. 6A, columns 2, 4, and 7). The −dP/dt\textsubscript{max} was significantly increased by isoproterenol within each respective group (Fig. 6B, column 2 vs. 1, column 4 vs. 3, and column 7 vs. 6). The isoproterenol-stimulated −dP/dt\textsubscript{max} was increased by 16% (P < 0.05) (Fig. 2A, column 4 vs. 2) during early sepsis, whereas it was decreased by 24% (P < 0.01) during late sepsis (Fig. 2A, column 7 vs. 2). The observed increases in the −dP/dt\textsubscript{max} during early sepsis were completely blocked by propranolol (Fig. 6A, comparison among columns 5, 3, and 1). These data indicated that during the early hyperdynamic phase of sepsis, cardiac contraction was increased, whereas relaxation remained unaffected. During the late hypodynamic phase of sepsis, contraction and relaxation were both impaired. Furthermore, the sepsis-induced alterations in myocardial contraction and relaxation were mediated through a β-adrenergic receptor pathway.

Figure 7 shows alterations in cardiac cAMP levels during the early and late phases of sepsis. Tissue cAMP level was increased by 34% (P < 0.01; column 3 vs. 1) during early sepsis, whereas it was decreased by 24% (P < 0.01; column 6 vs. 1) during late sepsis. Isoproterenol significantly increased cAMP levels within each respective group (column 2 vs. 1, column 4 vs. 3, and column 7 vs. 6). The isoproterenol-stimulated

Table 2. Effect of sepsis on myofibrillar Mg\textsuperscript{2+}-ATPase activity and influence of β-adrenergic agonist and antagonist in rat hearts

<table>
<thead>
<tr>
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<th>Control</th>
<th>Isoproterenol</th>
<th>Early Sepsis</th>
<th>Late Sepsis</th>
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<tr>
<td>Myofibrillar Mg\textsuperscript{2+}-ATPase</td>
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<tr>
<td>$V_{max}$, nmol·mg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>244 ± 13</td>
<td>236 ± 11</td>
<td>220 ± 14</td>
<td>216 ± 7</td>
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<tr>
<td>$K_{m}$, µM</td>
<td>0.23 ± 0.02</td>
<td>0.32 ± 0.04\textsuperscript{a}</td>
<td>0.34 ± 0.06\textsuperscript{b}</td>
<td>0.31 ± 0.03</td>
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Values are means ± SE. *P < 0.05 and †P < 0.01 vs. control; ‡P < 0.01 vs. late sepsis. Number of experiments was 8 for each group.
cAMP level was increased by 17% ($P < 0.05$) (column 4 vs. 2) during early sepsis but was decreased by 19% ($P < 0.05$) (column 7 vs. 2) during late sepsis. The observed increase in tissue cAMP level during early sepsis was completely blocked by propranolol (comparison among columns 5, 3, and 1). These results indicate that myocardial cAMP content underwent a biphasic change during the progression of sepsis, i.e., an increase during the early phase and a decrease during the late phase of sepsis. Furthermore, the sepsis-induced alterations in myocardial cAMP level were mediated through a $\beta$-adrenergic receptor pathway.

**DISCUSSION**

Data presented in the present study demonstrate that phosphorylation of TnI and C protein underwent a biphasic change during the progression of sepsis, i.e., an increase during the early phase followed by a decrease during the late phase of sepsis. The increases in the phosphorylation of TnI and C protein during early sepsis were associated with the decrease in the Ca$^{2+}$ sensitivity of myofilaments and the increases in myocardial +dP/dt$_{max}$ and cAMP level. The decreases in the phosphorylation of TnI and C protein during late sepsis coincided with the declines in the activities of myofibrillar ATPase, Ca$^{2+}$ sensitivity of myofilaments, myocardial $\pm$dP/dt$_{max}$, and cAMP content. The increases and decreases in the phosphorylation of TnI and C protein, $\pm$dP/dt$_{max}$, and tissue cAMP level were sensitive to isoproterenol stimulation and propranolol inhibition. These findings suggest that alterations in the phosphorylation of myofibrillar proteins such as TnI, C protein, and MLC-2, and changes in the activities and the Ca$^{2+}$ sensitivity of myofibrillar ATPase may contribute to the altered cardiac function during the progression of sepsis. Furthermore, the altered phosphorylation and Ca$^{2+}$ sensitivity of cardiac myofibrillar proteins during the progression of sepsis were mediated through a $\beta$-adrenergic receptor pathway.

**Fig. 5.** Changes in the activities of myosin Ca$^{2+}$-ATPase (A) and myosin K$^+$-EDTA-ATPase (B) during the progression of sepsis. Myosin Ca$^{2+}$-ATPase and myosin K$^+$-EDTA-ATPase activities were measured as described in MATERIALS AND METHODS. The number of experiments was 8 for each group. $^*P < 0.05$ compared with control.

**Fig. 6.** Effect of sepsis on cardiac contractile parameters in perfused hearts isolated from control and septic rats. A: changes in the maximal rate of increase of left ventricular pressure development (+dP/dt$_{max}$). B: changes in the maximal rate of decrease of left ventricular pressure (−dP/dt$_{max}$). The number of experiments was 8 for each group. $^*P < 0.05$ and $^**P < 0.01$ compared with control; $^1P < 0.05$ and $^11P < 0.01$ compared with early sepsis; $^†P < 0.01$ compared with late sepsis. $^+And^-$ as in Fig. 1.
Cardiac TnI acts as a molecular switch during the contraction and relaxation cycle (22, 28). During the diastole in which intracellular Ca\(^{2+}\) concentration is below the threshold for binding to TnC, TnI binds to actin-tropomyosin (actin-Tm) and inhibits interaction between myosin and actin through steroc blocking of myosin-binding sites on actin and by inhibiting a kinetic step in the myofibrillar ATP hydrolysis cycle from the actin-myosin complex (11, 30). During the systole, a rise in intracellular Ca\(^{2+}\) concentration and subsequent binding of Ca\(^{2+}\) to TnC result in an increased affinity of TnC for the ATPase-inhibiting region of TnI and eventually functional detachment of TnI from actin. This allows TnI to move away from actin-Tm and ultimately leads to activation of myofibrillar Mg\(^{2+}\)-ATPase and muscle contraction (28). Studies on myofibrillar protein phosphorylation have indicated that phosphorylation of TnI exerts an allosteric effect on Ca\(^{2+}\) binding to TnC, decreasing the sensitivity of TnC for Ca\(^{2+}\), and thus increasing the amount of Ca\(^{2+}\) that is required to bind to TnC to produce contraction (22). In so doing, TnI phosphorylation facilitates the rate of myocardial relaxation (30). Because TnI phosphorylation plays an important role in regulating cardiac function, our finding that TnI phosphorylation was increased during early but decreased during the late phases of sepsis suggests that TnI does not play a role in regulating the altered cardiac function during the progression of sepsis.

Myosin molecule is an asymmetric hexamer, consisting of a pair of heavy chains and two pairs of nonidentical light chains (24). In cardiac muscle myosin, two types of light chains are found, one with a molecular mass of 27 kDa and termed MLC-1 (essential light chain) and the other with a molecular mass of 19 kDa and referred to as MLC-2 (regulatory light chain) or phosphorylated light chain (22). The myosin molecule is generally divided into motor domain (the globular head) and tail domain (the rod). These two domains function independently in that the head retains the motor or enzymatic activity and the tail retains the ability to self-assemble into filament-like structures (24). The binding sites for actin, ATP, and the two types of light chains are located in the myosin head. Phosphorylation of MLC-2 correlates with increases in the \(V_{max}\) of myofibrillar Mg\(^{2+}\)-ATPase and myosin...
Ca$_{2+}$-ATPase (23). In an intact heart, phosphorylation of MLC-2 has been reported to correlate with increased maximum tension or dP/dt values (19, 27). In the present study, we found that MLC-2 phosphorylation was reduced during the late phase of sepsis, and this reduction was associated with decreases in the activities of myofibrillar Mg$^{2+}$-ATPase, myosin Ca$_{2+}$-ATPase, K$^+$-EDTA-ATPase, and $\pm$dp/dt$_{max}$. These data indicate that the reduction in the phosphorylation of MLC-2 contributes to the depressed cardiac function as observed during the late phase of sepsis. Furthermore, it is noteworthy that myosin K$^+$-EDTA-ATPase activity has been used as an index for the integrity of S1 and S2 thiol groups of the enzyme (25). Our finding that the observed decrease in the myosin Ca$_{2+}$-ATPase activity coupled with the decrease in K$^+$-EDTA-ATPase activity suggests that S1 and S2 thiol groups of the enzyme may be compromised during the late phase of sepsis.

The mechanism responsible for the alterations in the phosphorylation of TnI, C protein, and MLC-2 during the progression of sepsis is not completely understood. Cardiac TnI and C protein are excellent substrates for protein kinase A (10, 27), whereas MLC-2 is phosphorylated by a specific enzyme, myosin light chain kinase (2). Physiologically, TnI and C protein phosphorylation is regulated by $\beta$-adrenergic receptor stimulation (30). The observed alterations in the phosphorylation of TnI and C protein during the progression of sepsis are most likely due to the altered expression of $\beta$-adrenergic receptors. This contention is supported by our previous findings that $\beta$-adrenergic receptors were overexpressed during the early phase but underexpressed during the late phase of sepsis (32). In current studies, phosphorylation of TnI and C protein was responsive to isoproterenol stimulation and propranolol inhibition within each respective experimental group including control, early sepsis, and late sepsis. The $\beta$-adrenergon-stimulated phosphorylation of TnI and C protein remained significantly increased during the early phase but decreased during the late phase of sepsis. The altered phosphorylation of TnI and C protein with $\beta$-adrenergon stimulation also correlated with changes in tissue levels of cAMP. These data reinforce the contention that the altered TnI and C protein phosphorylation as observed during the progression of sepsis is mediated by changes in the expression of $\beta$-adrenergic receptors. The mechanism responsible for the reduced phosphorylation of MLC-2 during the late phase of sepsis may involve structural disruption of myofilaments as reported previously (17, 31) and changes in myosin light chain kinase activity. Further investigation is required to clarify the exact mechanism leading to the altered phosphorylation of MLC-2 during sepsis.

Perspectives

The data presented in this study indicate that alterations in the phosphorylation of myofilibril proteins such as TnI, C protein, and MLC-2, and changes in the activity and the Ca$_{2+}$ sensitivity of myofilibril ATPase were associated with altered cardiac contractility during different phases of sepsis. Furthermore, the sepsis-induced alterations in the phosphorylation and Ca$_{2+}$ sensitivity of cardiac myofilibril proteins were mediated via a $\beta$-adrenergic receptor pathway. These findings may have a therapeutic implication for the management of septic patients.

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