Degradation of rat cardiac troponin I during ischemia independent of reperfusion

Brian S. Palmer, Paul F. Klawitter, Peter J. Reiser, and Mark G. Angelos

Departments of Emergency Medicine and Oral Biology, Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio 43210

Submitted 23 February 2004; accepted in final form 5 May 2004

Palmer, Brian S., Paul F. Klawitter, Peter J. Reiser, and Mark G. Angelos. Degradation of rat cardiac troponin I during ischemia independent of reperfusion. Am J Physiol Heart Circ Physiol 287: H1269–H1275, 2004. First published May 13, 2004; 10.1152/ajpheart.00149.2004.—Cardiac troponin I (cTnI) degradation has been noted in the stunned myocardium of rodents after ischemia and reperfusion and is one proposed mechanism for the decreased left ventricular (LV) contractility in posts ischemic hearts. cTnI degradation has been best described after reperfusion of the ischemic myocardium. The effect of ischemia, independent of reperfusion, on cTnI breakdown has not been well characterized. We tested the hypothesis that progressive cTnI degradation occurs with increasing durations of ischemia and that this ischemia-based degradation is, in part, oxidant mediated. Isolated perfused rat hearts underwent global ischemia of 15, 20, or 25 min with and without reperfusion. A second series of hearts was treated with the antioxidants tiron (10 mM) and N-acetylcysteine (4 mM) before 20 min of global ischemia without reperfusion. cTnI degradation was measured using a cTnI-specific antibody and Western blot analyses. A progressive increase in cTnI degradation was seen with increasing duration of ischemia (no reperfusion), which correlated with the return of LV developed pressure during reperfusion. The extent of cTnI degradation was increased in hearts pretreated with antioxidants, although the qualitative degradation pattern was not altered. We conclude that a time-dependent cTnI breakdown occurs during global ischemia that is independent of reperfusion. cTnI breakdown during ischemia is further increased in the presence of antioxidants, suggesting ROS generated during ischemia may play a cTnI protective role.

Stunning

After relatively short durations of global ischemia (≤20 min), reperfusion results in left ventricular (LV) dysfunction, termed myocardial stunning (2, 3). The clinical significance of this phenomenon is the reversible nature of this process, in contrast to the irreversible myocardial dysfunction that occurs with necrosis or apoptosis. It is surmised that the degradation of cardiac troponin I (cTnI) by Ca2+-dependent proteases results in decreased maximal force and a relative insensitivity to calcium (14). However, conflicting results in different species have led to a lack of consensus regarding the significance of cTnI degradation. cTnI breakdown occurs in the rat myocardium (8, 9, 17, 23) but not in large animals in which stunning has been induced (15, 16, 21, 22). There have been reports claiming that cTnI degradation is no different from controls (13), that degradation occurs only during reperfusion (9), and that degradation occurs during both ischemic and reperfusion intervals (9, 23, 27). Model-specific differences, including differences in the species studied and the duration of the ischemic insult, as well as the time point for probing degradation of cTnI (during ischemia or during reperfusion), may contribute in part to the current controversy concerning cTnI degradation and possible causal links to depression of LV function during reperfusion.

A recent report (4) suggests that elevated end-diastolic pressure, independent of ischemia, causes the same cTnI breakdown pattern as ischemia and reperfusion in the rat heart. Because prolonged duration of ischemia and reperfusion typically leads to increases in diastolic pressure, they suggested that cTnI may be tied to ischemia and reperfusion only to the degree that they lead to increased diastolic pressure. In this study, we sought to isolate the effects of ischemia on cTnI breakdown independent of reperfusion effects. We chose durations of ischemia that typically have been associated with stunning in perfused rat heart in previous studies. We hypothesized that progressive cTnI degradation occurs with increasing durations of ischemia. On the basis of earlier studies in rat myocardium that show that ROS exposure simulates ischemia-induced stunned myocardium (18) and results in cTnI degradation (28), we hypothesized that this ischemia-based cTnI degradation is in part oxidant mediated. To test these hypotheses, we utilized the previously well-characterized isolated perfused rat heart model in which significant cTnI has been noted after ischemia and reperfusion. Our findings suggest an ischemia-dependent cTnI degradation, which is independent of reperfusion, yet predictive of return of contractile function during reperfusion, and is increased during global ischemia in the presence of antioxidants.

METHODS

Langendorff preparation. Male Sprague-Dawley rats, weighing between 320 and 450 g (Harlan; Indianapolis, IN), were used in accordance with guidelines of the National Institutes of Health (NIH Pub. No. 85-23, Revised 1996) and the approval of the Ohio State University Laboratory Animal Care and Use Committee. Rats were anesthetized with intraperitoneal pentobarbital sodium (50–65 mg/kg). After surgical tracheostomy, rats were mechanically ventilated (Harvard Apparatus; South Natick, MA) with room air to provide adequate ventilation. The right superficial jugular vein was isolated, and 1,000 U/kg heparin was administered. After rapid cannulation of the aorta, retrograde coronary perfusion at 85 mmHg with Krebs-Henseleit buffer (1.25 mM CaCl2, 5.5 mM glucose, 112 mM NaCl, 25 mM NaHCO3, 5 KCl mM, 1.2 mM MgSO4, 1 mM K2PO4, and 0.2 mM octanoic acid, bubbled with 95% O2-5% CO2; pH 7.4) was initiated in situ before excision. While the heart was on a glass column...
heat exchanger, perfusion was maintained at 85 mmHg with warmed (37.4°C) perfusate, and hearts were immersed in a water-jacketed, temperature (37.4°C)-controlled glass chamber to ensure normothermia throughout the perfusion protocol. A fluid-filled latex balloon was inserted into the LV and adjusted to an end-diastolic pressure of 5 mmHg during a baseline equilibration period. All hearts were paced at 300 beats/min. Pacing was continued for 3 min into ischemia and resumed 3 min after reperfusion was started. Isovolumic LV pressure was continuously sampled using Digi-Med electronics (Micro-Med; Lexington, KY).

Ischemia-reperfusion protocols. Hearts were initially block randomized to six different experimental groups: three “ischemia-only” groups were subjected to global ischemia durations of 15, 20, and 25 min without reperfusion, and three groups received the same durations of ischemia with 30 min of reperfusion. Nonischemic control hearts were perfused for 20 min to identify the degree of cTnI breakdown before the onset of ischemia, and a second group of nonischemic controls were perfused for 55 min to determine the effects of perfusion independent of ischemia. All hearts were initially perfused for 10 min to allow for equilibration, followed by a 10-min baseline period before the induction of ischemia. To determine the sequence of cTnI breakdown during reperfusion, additional experiments were performed consisting of three hearts subjected to 30 min of global ischemia followed by 30 min of reperfusion, and three hearts subjected to 30 min of ischemic for 20 min and subjected to 1, 5, or 30 min of reperfusion. Also, two additional groups of hearts were subjected to either 30 min of ischemia only or 30 min of ischemia and 30 min of reperfusion to directly measure the amount of intact cTnI remaining after prolonged ischemia and reperfusion.

In a separate series of experiments that was designed to determine antioxidant effects on cTnI degradation during ischemia without reperfusion, hearts were perfused before ischemia with buffer containing tiron (10 mM), a superoxide scavenger (5, 26) as well as a scavenger of hydroxyl radical (1). Control hearts received only oxygenated Krebs-Henseleit buffer. All antioxidant and control hearts were subjected to 20 min of warm (37°C) ischemia without reperfusion.

At the end of each protocol, hearts were freeze clamped in liquid nitrogen and lyophilized for 24 h before LV tissue was extracted for Western blot analysis.

Western blot and densitometric analysis. LV tissue was prepared for one-dimensional gel electrophoresis as previously described (26). Protein bands were probed with 8I-7, a cTnI monoclonal antibody (diluted 1:1,000, Spectral Diagnostics; Toronto, Ontario, Canada) that is highly specific for rat cTnI (17). An alkaline phosphatase-conjugated secondary antibody (Promega, Madison, WI) was used for color development. Proper loading conditions necessary to quantitate both nondegraded cTnI as well as cTnI degradation products were determined as follows: 1–10 μl of a single representative sample were loaded on a 10-lane gel. After color development after incubation with the primary antibody 8I-7, densitometric analysis using software (Scion) was used to quantitate protein amounts for cTnI and observe the degradation bands under each of the 10 different loads. Quantitative densitometric measurements of cTnI and cTnI degradation bands were made within an established linear range. To determine the linear range for the cTnI degradation products as well as the cTnI band, a calibration plot was run with protein loading ranging from 0.7 to 7.3 μg/lane. cTnI degradation bands were noted to be in the linear range with a loading of ~2–4 μg/lane, whereas the linear range for the cTnI band was under 2 μg/lane. Four micrograms per lane loading conditions were used to quantitatively assess cTnI degradation products. The same controls were replicated on each gel, and densitometric measurements were normalized to the control on each gel to allow comparison of samples run on different gels. Molecular mass markers were used to approximate the cTnI degradation product size (Amer sham Biosciences; Piscataway, NJ; and Bio-Rad; Hercules CA).

Data analysis. Statistical comparisons of the cTnI degradation intensities for the three ischemia-only groups were made with n = 6 hearts/group, in all groups and for the antioxidant-loaded hearts (n = 5–8/group). Analysis was done using ANOVA with a post hoc Tukey test. P < 0.05 was considered statistically significant. The densitometric amounts of degradation for bands 3, 4, and 5 only were summed within each ischemia-only group, yielding an estimate of the extent of increase in overall cTnI degradation. In three other groups of hearts subjected to the same durations of ischemia and then reperfused for 30 min, group averages for developed pressure were determined. Linear regression analysis was performed by pairing group averages of developed pressure in the three reperfused groups with group averages of cTnI degradation determined in the three ischemia-only groups. All results are expressed as group means ± SE.

RESULTS

Control nonischemic hearts perfused for 55 min (Fig. 1A) and 20 min (Fig. 1B) failed to show any appreciable cTnI breakdown in contrast to ischemia. After 15–25 min of global ischemia without any reperfusion, five degradation bands were reproducibly identified on Western blots of LV homogenate after 15, 20, and 25 min of ischemia only (Fig. 1B). These degradation bands range in size from just greater than 21.5 kDa to just less than 14.4 kDa. Densitometric analysis of each of these five bands showed a progressive increase in the density of the 18.4- and 14.4-kDa cTnI degradation bands with an increasing duration of ischemia (Fig. 1C). During reperfusion, under normal loading conditions, this five-band cTnI degradation pattern was rapidly lost. The degradation bands present at the end of 20 min of ischemia were visible 1 min into reperfusion but nearly gone by 5 min of reperfusion (Fig. 2A). Only one of the 18.4-kDa bands was clearly identifiable after 30 min of reperfusion under normal loading conditions. By overloading the gel (loading 29.1 μg/lane), the second 18.4- and 14.4-kDa bands were identified during reperfusion, albeit greatly reduced in intensity (Fig. 2B). At 30 min of reperfusion, the intensity of all five bands seen at the end of ischemia was significantly reduced (Fig. 2B). The rapid alteration in the pattern and density of cTnI degradation bands with early reperfusion highlights the striking change in cTnI breakdown pattern observed during ischemia with ischemia and 30 min of reperfusion. The five-band cTnI degradation pattern observed during ischemia was substantially reduced during reperfusion and was only seen with significant increases in loading conditions (Fig. 3). Differences in cTnI breakdown during ischemia relative to reperfusion were further suggested by the preponderance of cTnI loss occurring during ischemia relative to reperfusion. After 30 min of ischemia, there was an ~25% decline in total cTnI with no significant additional change noted after 30 min of reperfusion (Fig. 4) with the 8I-7 antibody.

The degree of cTnI breakdown during ischemia was highly correlated with the degree of LV contractile dysfunction exhibited during reperfusion. LV developed pressure at the end of the 30-min reperfusion period showed a strong inverse correlation with the degree of cTnI degradation at the end of ischemia (Fig. 5). A strong correlation was seen between the individual cTnI degradation bands 3, 4, and 5 (but not bands 1 and 2) and LV developed pressure during reperfusion. The correlation between degradation bands at the end of ischemia
and LV developed pressure during reperfusion had a $R^2$ value of 0.90 for band 3, $R^2$ value of 0.996 for band 4, and $R^2$ value of 0.996 for band 5. This correlation of cTnI degradation and LV function was not found after hearts were reperfused.

Degradation of cTnI breakdown at the end of 20 min of ischemia (without reperfusion) was increased in hearts pretreated with the antioxidants tiron and NAC compared with 20-min ischemia control (no antioxidant) hearts (Fig. 6, A and B).

DISCUSSION

We report here the time-dependent nature of cTnI degradation during ischemia, which is independent of both reperfusion and elevations in LV end-diastolic pressure (4, 17). We also note the correlation of cTnI breakdown during ischemia and LV contractile function during reperfusion. This correlation is lost once reperfusion occurs, because a different pattern of cTnI breakdown (or possibly washout) is apparent compared with the ischemic process. Furthermore, the degradation of cTnI during ischemia appears to increase in the presence of antioxidants. The cTnI changes reported in this study were noted after relatively short periods of ischemia, ≤30 min, without reperfusion. In a correlative study (19), we noted no significant increases in creatine kinase release in hearts reperfused after periods of global ischemia of 15 and 20 min. This lack of necrosis after these durations of ischemia is consistent with previous studies in the perfused mouse (25) and rat heart (7).

This study highlights the distinctive differences in the pattern of cTnI degradation during ischemia versus reperfusion. Previous studies have noted that rat cTnI degradation increases with increasing duration of ischemia (17). However, a critical difference in our study, relative to others, is that our results were obtained after ischemia without reperfusion, whereas most studies characterizing cTnI breakdown have looked after reperfusion of the ischemic myocardium. cTnI breakdown has been well characterized after reperfusion in the isolated perfused rat heart. McDonough et al. (17) noted after mild ischemia conditions with reperfusion (15-min ischemia/45-min reperfusion) that 48% of cTnI was degraded to a 22-kDa cTnI degradation product and three high-molecular-mass complexes corresponding to ~55, 66, and 75 kDa. The 22-kDa fragment was analyzed by mass spectrometry and identified as a single sequence of 193 residues (cTnI 1–193). The three high-molecular-mass complexes were all found to be between 30 and 40 kDa. With more severe ischemia and reperfusion (60-min ischemia/45-min reperfusion), the 22-kDa fragment was further degraded to two additional products with molecular

and LV developed pressure during reperfusion had a $R^2$ value of 0.90 for band 3, $R^2$ value of 0.996 for band 4, and $R^2$ value of 0.996 for band 5. This correlation of cTnI degradation and LV function was not found after hearts were reperfused.

Degradation of cTnI breakdown at the end of 20 min of ischemia (without reperfusion) was increased in hearts pretreated with the antioxidants tiron and NAC compared with 20-min ischemia control (no antioxidant) hearts (Fig. 6, A and B).

DISCUSSION

We report here the time-dependent nature of cTnI degradation during ischemia, which is independent of both reperfusion and elevations in LV end-diastolic pressure (4, 17). We also note the correlation of cTnI breakdown during ischemia and LV contractile function during reperfusion. This correlation is lost once reperfusion occurs, because a different pattern of cTnI breakdown (or possibly washout) is apparent compared with the ischemic process. Furthermore, the degradation of cTnI during ischemia appears to increase in the presence of antioxidants. The cTnI changes reported in this study were noted after relatively short periods of ischemia, ≤30 min, without reperfusion. In a correlative study (19), we noted no significant increases in creatine kinase release in hearts reperfused after periods of global ischemia of 15 and 20 min. This lack of necrosis after these durations of ischemia is consistent with previous studies in the perfused mouse (25) and rat heart (7).

This study highlights the distinctive differences in the pattern of cTnI degradation during ischemia versus reperfusion. Previous studies have noted that rat cTnI degradation increases with increasing duration of ischemia (17). However, a critical difference in our study, relative to others, is that our results were obtained after ischemia without reperfusion, whereas most studies characterizing cTnI breakdown have looked after reperfusion of the ischemic myocardium. cTnI breakdown has been well characterized after reperfusion in the isolated perfused rat heart. McDonough et al. (17) noted after mild ischemia conditions with reperfusion (15-min ischemia/45-min reperfusion) that 48% of cTnI was degraded to a 22-kDa cTnI degradation product and three high-molecular-mass complexes corresponding to ~55, 66, and 75 kDa. The 22-kDa fragment was analyzed by mass spectrometry and identified as a single sequence of 193 residues (cTnI 1–193). The three high-molecular-mass complexes were all found to be between 30 and 40 kDa. With more severe ischemia and reperfusion (60-min ischemia/45-min reperfusion), the 22-kDa fragment was further degraded to two additional products with molecular
masses of 16 and 15 kDa. These fragments were identified at the end of reperfusion using the same monoclonal antibody we used (8I-7 MAb). With molecular mass markers, we identified similar size fragments corresponding to our band 5 during ischemia, but also additional bands, which are readily seen during ischemia but are rapidly lost during reperfusion.

Using a similar duration of ischemia (20 min), Gao et al. (9) noted a band of molecular mass ~26 kDa different from the native cTnI (~31 kDa) after 20 min of ischemia and 20 min of reperfusion with Western blot analysis. They noted a linear increase in protein band density using a loading of 5–15 μg protein/lane, which is similar to our loading conditions. Using a variety of antibodies (excluding 8I-7 MAb), they did not visualize any degradation bands for troponin T, tropomyosin, actin, myosin light chain-1, or myosin light chain-2. This was in contrast to cTnI, where degradation bands were noted in the stunned myocardium. However, with the use of different antibodies and models, early changes in contractile proteins during ischemia have been noted. Hein et al. (11), using ischemic cardiomyopathic human heart tissue and monoclonal antibodies against various contractile proteins, noted changes in the localization pattern of myosin, actin, tropomyosin, and tropinin T as early as 10 min after the onset of ischemia with complete disruption after 20 min of ischemia. Changes in tubulin were noted after 10 min of ischemia with complete disruption at 120 min. Desmin and myomesin showed changes at 30–40 min, with complete disruption at 90–120 min. Of note in this study was the use of diseased hearts and the observation that ischemic damage occurred earlier in the contractile proteins than the cytoskeleton and subcellular organelles (11).

In another study, Van Eyk et al. (23), using three different cTnI antibodies (excluding 8I-7 MAb), reported a single cTnI degradation product after 15-min ischemia/45-min reperfusion or 60-min ischemia (23). After 60 min of ischemia with 45 min of reperfusion, at least two degradation products were noted. This duration of ischemia is much longer than our ischemia durations and results in necrosis and enzyme release, as opposed to ischemia of ~20 min.

Fig. 2. A: normal loaded (5.9 μg/lane) Western blot showing the characteristic 5-band ischemia-only degradation pattern for single representative samples from the 15’ I, 20’ I, and 25’ I groups and the subsequent rapid washout of ischemia-only bands, recorded in 3 additional hearts freeze clamped at 1, 5, and 30 min of reperfusion (rep) after 20 min of global ischemia. B: overloaded (29.1 μg/lane) Western blot showing the same samples and lane assignments as for A. Under overloaded conditions, degradation bands are shown at the end of 15, 20, and 25 min of ischemia and compared with the degradation patterns seen after 1, 5, and 30 min of reperfusion after 20 min of ischemia. With the onset of reperfusion, there is a rapid change in the pattern and density of cTnI degradation products, so that by 5 min into reperfusion the ischemia-only pattern of cTnI degradation is nearly gone.

Fig. 3. Degradation products noted at the end of 30 min of ischemia and 30 min of reperfusion under increasing loading conditions. Three different protein loads (7, 37, and 74 μg/lane) were used for both samples to identify residual cTnI degradation products. The rapid loss of ischemia-only degradation bands by the end of reperfusion is demonstrated. Residual cTnI degradation products are only seen under higher loading conditions.

Fig. 4. Densitometric results showing relative amounts of intact (non-degraded) left ventricular (LV) cTnI in control hearts, hearts made globally ischemic for 30 min, and hearts globally ischemic for 30 min followed by reperfusion for 30 min. cTnI decreased after 30 min of ischemia to 75% of control levels (*P = 0.035) but was unchanged after 30 min of reperfusion compared with the end of ischemia.
cTnI DEGRADATION DURING ISCHEMIA INDEPENDENT OF REPERFUSION

Recently, Feng et al. (4) noted cTnI degradation similar to ischemia and reperfusion with elevated preload and no ischemia in the isolated perfused rat heart using the same 8I-7 monoclonal antibody that we used and a bovine TnI monoclonal antibody (clone C5). Both of these cTnI antibodies have been shown to cross-react in rats (17). Their findings suggest that the pattern of cTnI breakdown may be generated independent of ischemia and reflect the ischemia-reperfusion-induced increased end-diastolic pressure. Our data suggest that ischemia, independent of elevated LV diastolic pressure, is a direct determinant of cTnI breakdown. We observed significant cTnI breakdown during periods of ischemia too short (15 and 20 min) to induce contracture and before reperfusion, with its associated rise in LV end-diastolic pressure.

Because cTnI is directly involved in the regulation of crossbridge cycling, degradation of cTnI has been an attractive mechanism to account for the apparent decreased Ca²⁺ sensitivity and depressed contractile function seen in stunned myocardium. Despite studies in the rat myocardium showing significant cTnI breakdown with stunning (8, 9, 17, 23), other studies have failed to find troponin degradation in stunned myocardium in canine and swine models of regional myocardial ischemia (14, 16, 21, 22). A recent study (20) utilizing the isolated perfused rabbit heart also failed to show cTnI degradation except after prolonged ischemia. In support of cTnI degradation as a mechanism of myocardial stunning, a transgenic mouse model expressing one of the main cTnI degradative products, cTnI 1–193, reproduces the phenotype of myocardial stunning (18). Studies utilizing a cTnI gene knockout model in the mouse further support the importance of cTnI in the normal contractile state. In this knockout model, skeletal cTnI partially compensates for the absence of cTnI but then declines over time, leading to impaired cardiomyocyte relaxation, a reduction in calcium sensitivity, acute heart failure, and death (12).

In contrast to models of stunning, more severe periods of ischemia are clearly associated with cTnI breakdown and loss. Fishbein et al. (6) used antibodies to cTnI and cTnT in myocardial tissue from multiple species (dogs, pigs, and rats) subjected to periods of ischemia ranging from 30 min to 6 h. They noted significant cTnI and cTnT tissue loss in eventual areas of myocardial necrosis and no loss of staining in non-necrotic myocardium. This severity of ischemia differs from models of myocardial stunning (such as ours) in which ischemia is not of sufficient duration to result in necrosis. It is important to note that there appears to be significant species differences in cTnI breakdown in response to ischemia and reperfusion. Our study was performed in rats, a species in which cTnI breakdown has been well described in contrast to large animal models. Thus one must be cautious in extrapolating our results from the rat model to larger animals and humans.

Previous studies have not found a correlation between cTnI breakdown and the return of LV function during reperfusion

Fig. 5. Graph showing linear regressions between LV developed pressure (LVDP) after 15, 20, and 25 min of global ischemia and cTnI degradation after the same durations of global ischemia. Linear regression analysis was performed on LVDP vs. band 3 (inverted triangles), band 4 (triangles), band 5 (squares), and the sum of densitometric results for bands 3–5 (circles). Regression lines are shown for hearts subjected to 15 min of global ischemia (open symbols), 20 min ischemia (closed symbols), and 25 min of ischemia (bold open symbols). Bars display means of global ischemia (open symbols), 20 min ischemia (closed symbols), and 25 min ischemia (bold open symbols). Bars display means ± SE for the respective average developed pressures shown (the SEs for cTnI degradation are smaller than the respective symbol sizes used) (n = 6/group). For the sum of bands 3–5, the regression line is LVDP = 104 − 0.03(cTnI degradation) with an R² = 0.98.

Fig. 6. A: Western blot showing cTnI degradation in hearts pretreated with the antioxidants tiron and N-acetylcysteine (NAC) compared with controls (no antioxidants). All hearts were freeze clamped at the end of 20 min of ischemia before reperfusion. Note the increased density of the degradation bands in the antioxidant-treated groups (*P < 0.05). Likewise, the percentage of total cTnI degradation (sum of bands 1–5) is greater in the tiron- and NAC-treated groups compared with controls (*P < 0.05).
conclude that the degradative processes differ during global cTnI breakdown pattern with the onset of reperfusion, we cTnI breakdown. On the basis of the rapid alteration of the elevated end-diastolic pressure, is an important determinant of ischemia for ischemic durations between 15 and 25 min. dent of reperfusion, and correlates with the duration of global ischemia before reperfusion) may have occurred during global ischemia before reperfusion) may have important cardioprotective signaling functions, which include cTnI preservation that may be attenuated or lost in the presence of antioxidants.

Oxygen free radicals are thought to be a primary mechanism of myocardial stunning; however, the interaction of oxygen free radicals and contractile proteins is unclear (2). Exposure of rabbit cardiac trabeculae to hydroxyl radicals results in cTnI degradation similar to ischemia (28). In the present study, pretreatment of hearts with the antioxidants tiron and NAC increased cTnI breakdown during ischemia. This suggests that oxygen free radicals generated during ischemia (but not reperfusion) may inhibit cTnI breakdown during ischemia through an as-yet-undefined mechanism and thereby add a cTnI protective benefit. ROS production during global ischemia is much less than the large burst of ROS generation seen in the first few minutes of reperfusion (29). A cardioprotective role of low levels of ROS production during ischemia is consistent with a proposed role for low levels of oxidants acting as important signaling molecules in preconditioning with its subsequent cardioprotection (24).

The importance of oxygen free radicals generated during global ischemia and their role in ischemic cTnI degradation is not resolved and requires further investigation. Future studies utilizing the mouse cardiac cTnI knockout model to better understand the role of oxygen free radicals on cTnI degradation may be helpful. Oxidant production in low levels (such as may occur during global ischemia before reperfusion) may have important cardioprotective signaling functions, which include cTnI preservation that may be attenuated or lost in the presence of antioxidants.

In summary, our results suggest that substantial cTnI degradation occurs during global myocardial ischemia, is independent of reperfusion, and correlates with the duration of global ischemia for ischemic durations between 15 and 25 min. Unlike changes observed during reperfusion, the extent of cTnI degradation correlates with postischemic LV dysfunction, suggesting that ischemia itself, independent of reperfusion or elevated end-diastolic pressure, is an important determinant of cTnI breakdown. On the basis of the rapid alteration of the cTnI breakdown pattern with the onset of reperfusion, we conclude that the degradative processes differ during global ischemia and reperfusion. The role of oxygen free radicals relative to cTnI breakdown may have divergent roles during ischemia and during reperfusion.

ACKNOWLEDGMENTS

Present address of P. Klawitter: Dept. of Emergency Medicine, State Univ. of New York Upstate Medical Center, Syracuse, NY 13210.

GRANTS

This study was supported by the American Heart Association-Ohio Affiliate (to M. G. Angelos), the Emergency Medicine Foundation (Cardiac arrest survival award to M. G. Angelos), and National Heart, Lung, and Blood Institute Fellowship Grant 1F32HL-10216 (to P. F. Klawitter).

REFERENCES


