H$_2$O$_2$ activation of HSP25/27 protects desmin from calpain proteolysis in rat ventricular myocytes

Bradford C. Blunt, Aaron T. Creek, DeAnna C. Henderson, and Polly A. Hofmann

Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee

Submitted 15 March 2006; accepted in final form 17 March 2007

Blunt BC, Creek AT, Henderson DC, Hofmann PA. H$_2$O$_2$ activation of HSP25/27 protects desmin from calpain proteolysis in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 293: H1518–H1525, 2007. First published May 18, 2007; doi:10.1152/ajpheart.00269.2006.—Ischemia-reperfusion-induced Ca$^{2+}$ overload results in activation of calpain-1 in the heart. Calpain-dependent proteolysis contributes to myocardial dysfunction and cell death. Previously, preischemic treatment with low doses of H$_2$O$_2$ was shown to improve posts ischemic function and reduce myocardial infarct size. Our aim was to determine the mechanism by which H$_2$O$_2$ protects the heart. We hypothesized that H$_2$O$_2$ causes the activation of p38 MAPK which initiates translocation of heat shock protein 25/27 (HSP25/27) to the myofilament Z disk. We further hypothesized that HSP25/27 shields structural proteins, particularly desmin, from calpain-induced proteolysis. To address this hypothesis, we first determined that an ischemia-reperfusion-induced decrease in desmin content could be blocked by H$_2$O$_2$ pretreatment of hearts from rats. We next determined that ventricular myocytes that underwent Ca$^{2+}$ overload also demonstrated a calpain-dependent disruption of desmin that could be reduced by H$_2$O$_2$/p38 MAPK activation. Furthermore, myocytes acutely treated with H$_2$O$_2$ exhibited a decrease in cleavage of desmin upon exposure to exogenous calpain-1 compared with myocytes not pretreated with H$_2$O$_2$. The H$_2$O$_2$-induced attenuation of desmin degradation by calpain-1 was blocked by inhibition of p38 MAPK. In a final series of experiments, we demonstrated that cardiac myofilaments exposed to recombinant phosphorylated HSP27, but not nonphosphorylated HSP27, had a significant reduction in the calpain-dependent proteolysis of cytoskeletal proteins, such as desmin, from calpain-induced proteolysis. To address this hypothesis, we first determined that an ischemia-reperfusion-induced decrease in desmin content could be blocked by H$_2$O$_2$ pretreatment of hearts from rats. We next determined that ventricular myocytes that underwent Ca$^{2+}$ overload also demonstrated a calpain-dependent disruption of desmin that could be reduced by H$_2$O$_2$/p38 MAPK activation. Furthermore, myocytes acutely treated with H$_2$O$_2$ exhibited a decrease in cleavage of desmin upon exposure to exogenous calpain-1 compared with myocytes not pretreated with H$_2$O$_2$. The H$_2$O$_2$-induced attenuation of desmin degradation by calpain-1 was blocked by inhibition of p38 MAPK. In a final series of experiments, we demonstrated that cardiac myofilaments exposed to recombinant phosphorylated HSP27, but not nonphosphorylated HSP27, had a significant reduction in the calpain-dependent degradation of desmin compared with non-HSP27-treated myofilaments. These findings are consistent with the hypothesis that H$_2$O$_2$-induced activation of p38 MAPK and subsequent HSP25/27 translocation attenuates desmin degradation brought about by calpain-1 activation in ischemia-reperfused hearts.

ischemia-reperfusion; isolated heart; heat shock protein 25/27; p38 mitogen-activated protein kinase; actinin; troponin I

PROLONGED ISCHEMIA followed by reperfusion results in intracellular Ca$^{2+}$ overload and subsequent myocardial hypercontracture and dysfunction. The increase in intracellular Ca$^{2+}$ also leads to activation of the cysteine protease calpain, which degrades myocardial proteins. Both of the major isoforms of calpain require Ca$^{2+}$ for activation with $EC_{50}$ values of 2 M Ca$^{2+}$ for calpain-1 and 1 M Ca$^{2+}$ for calpain-2. Following Ca$^{2+}$-induced activation, calpain-1 has been shown to degrade α-actinin, desmin, α-fodrin, dystrophin, and troponin I (7, 16, 17, 21, 22, 25). α-Actinin and desmin are components of the Z disk. α-Actinin is responsible for cross-linking sarcomeric F-actin to the Z disk, whereas desmin is thought to help maintain lateral alignment of myofibrils and anchor the Z disk to the sarcolemma. Ischemia-reperfusion has been shown to decrease desmin content in whole hearts, and desmin proteolysis reduces maximal force production and Ca$^{2+}$ sensitivity in isolated cardiac myofilaments (12). Furthermore, the observed disruption to the Z disk and associated proteins following ischemia-reperfusion may damage the link between the sarcolemma and myofibrils and may contribute to the progressive increase in membrane permeability during reperfusion (1). Both α-fodrin and dystrophin associate directly with and stabilize the sarcolemmal membrane. Ischemia-reperfusion-induced disruption of dystrophin and degradation of α-fodrin correlate with increased reperfusion injury in the heart (7, 9). Thus it is thought that calpain-induced disruption of cytoskeletal proteins leads to increased membrane permeability and fragility which, in turn, may account for a rupture of the cell during reperfusion.

Studies into the role of reactive oxygen species and oxidative stress in ischemia-reperfusion injury have demonstrated that low doses of hydrogen peroxide (H$_2$O$_2$) are cardioprotective (19, 24). Sharma et al. (19) showed that acute preconditioning with 100 µM H$_2$O$_2$ before prolonged ischemia and reperfusion reduced lactate dehydrogenase and creatine kinase release into the coronary effluent and decreased infarct size in isolated hearts from rats. Similarly, Yaguchi et al. (24) observed improved recovery of posts ischemic left ventricular developed pressure and high-energy phosphate content with preischemic administration of 2 µM H$_2$O$_2$ in isolated hearts from rats. Low doses of H$_2$O$_2$ are known to activate p38 mitogen-activated protein kinase (p38 MAPK). Acute exposure to 30–100 µM H$_2$O$_2$ induces activation of p38 MAPK and leads to the phosphorylation of heat shock protein 25$^{\text{redox}}$/27$^{\text{human}}$ (HSP25/27) in ventricular myocytes (3, 5). Furthermore, p38 MAPK induces translocation of HSP25/27 from the cytosolic to the myofilament fraction in ventricular myocytes (2). Within myofilaments, HSP25/27 colocalizes with Z-disk proteins (10). Numerous studies have demonstrated that HSP25/27 can protect proteins from stress-induced damage. For example, overexpression of HSP27 protects from ischemia-reperfusion damage in both rat and canine myocardium (15, 23). These observations raise the possibility of a Z-disk protein(s) that is protected by H$_2$O$_2$/p38 MAPK activation.

Thus, for the present study, we hypothesized that the ischemia-reperfusion-induced activation of calpain and subsequent proteolysis of cytoskeletal proteins, such as desmin, can be reduced through localization of HSP25/27 to the Z disk. Preischemic treatment with H$_2$O$_2$ activates this protective path-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: P. Hofmann, Dept. of Physiology, Univ. of Tennessee Health Science Center, 894 Union Ave., Memphis, TN 38163 (e-mail: phofmann@physiol.utmem.edu).
way through p38 MAPK. Determining the mechanism(s) that H2O2 uses to protect the heart from ischemia-reperfusion will allow us to identify promising therapeutic targets for future research. Further, the findings of the present study provide background information for the consideration of clinical studies designed to determine whether activation of the H2O2 protective pathway might be of benefit when activated before procedures known to induce ischemia-reperfusion, i.e., coronary stent placement or thrombolysis.

**METHODS**

Isolated heart and ventricular myocyte preparations. All procedures were approved and carried out according to the guidelines set by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Female Wistar rats (196–205 g) were anesthetized with 65 mg/kg pentobarbital sodium. For studies done in whole hearts, the heart was excised and cannulated in ice-cold modified Krebs-Henseleit buffer consisting of (in mM) 118 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 11 glucose, 25 NaHCO3, and 1.3 CaCl2 (pH 7.4). Hearts were then mounted on a Langendorff apparatus and perfused with oxygenated Krebs-Henseleit buffer at 37°C. A transducer (BLPR, World Precision Instruments, Sarasota, FL) to be inserted into the left ventricle, and the balloon was inflated until a maximum developed pressure was observed with an end-diastolic pressure of 5–10 mmHg. Hearts were paced with electrodes at 300 beats/min. Isolated hearts underwent 5 min of paced equilibration and four periods of 5 min vehicle or 100 uM H2O2 perfusion followed by 5 min washout. Nonischemic hearts were harvested immediately after perfusion/washout, whereas ischemic-reperfused hearts were further subjected to 60 min of global ischemia and 30 min of reperfusion before harvesting. This protocol was identical to that of Matsumura et al. (16). Upon completion of reperfusion, the left ventricle was homogenized in a solution containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, 1% Triton X-100, and 1% protease inhibitor cocktail (Cat. No. P8340; Sigma, St. Louis, MO), and the homogenate was centrifuged at 1,000–2,000 g for 15 min. The pellet was then washed twice in a solution containing 60 mM KCl, 30 mM imidazole (pH 7.2), 2 mM MgCl2, and 1% protease inhibitor cocktail. The pellet was then resuspended in the wash solution, diluted with an equal volume of Laemmli sample buffer, and frozen for use in Western blot analysis. An analysis of desmin content was carried out with an equal volume of urea sample buffer and frozen for Western blot analysis of α-actinin, desmin, and troponin I.

To characterize the effects of exogenous calpain-1 on myofilament proteins, skinned myocytes were exposed to vehicle or calpain-1 for 3, 5, 10, and 15 min. Following calpain exposure, cells were centrifuged at 1,000 g for 1 min and the supernatants were diluted with an equal volume of urea sample buffer and frozen for Western blot analysis of α-actinin, desmin, and troponin I.

The extent and mechanism of H2O2-dependent protection from exogenous calpain were investigated using isolated ventricular myocytes with intact membranes that were (1) exposed to 0, 2, 30, and 100 uM H2O2 for 3 min; (2) exposed to 100 uM H2O2 for 0, 3, 5, and 15 min; or (3) treated with or without 5 uM SB-202190 for 30 min followed by treatment with or without 100 uM H2O2 for 3 min. Following drug treatment, membranes were removed as described, and skinned cells were incubated with calpain-1 for 5 min followed by centrifugation at 1,000–2,000 g for 1 min. The supernatants were diluted with an equal volume of urea sample buffer and frozen for Western blot analysis.

Exogenous phosphorylated HSP27 in myofilaments. Initially, we wished to determine whether phosphorylated HSP27 (pHSP27) and HSP27 associate to an equal extent with both normal and calpain-treated myofilaments. To do this, ventricular tissue was homogenized, sieved through a 250-µm polypropylene mesh, and incubated at pH 5.6 with or without calpain-1 for 5 min at room temperature. The reaction was stopped by an addition of 1 M calfstatin (Cat. No. 208902; EMD/Calbiochem). Samples were incubated with exogenous pHSP27, HSP27, or vehicle for 5 min and centrifuged at 5,000 g for 5 min. The pellet was then washed twice, diluted with urea sample buffer, and frozen for Western blot analysis.

To determine whether pHSP27 influences the extent of calpain-induced degradation of desmin, ventricular tissue homogenates were incubated with pHSP27, HSP27, MAPKAPK2 (MK2), or vehicle in a pH 5.6 solution with or without calpain-1 for 30 min at 30°C. Samples were diluted with an equal volume of urea sample buffer and frozen for Western blot analysis.

pHSP27 was prepared by incubating human recombinant HSP27 (Cat. No. 385891; EMD/Calbiochem) with constitutively active, human recombinant MK2 (Cat. No. 14–337; Upstate, Lake Placid, NY) at 30°C for 45 min in the presence of Mg2+ and ATP. Exogenous pHSP27 and appropriate controls (vehicle, HSP27, and MK2) were made before experimental protocols. For each identical ventricular homogenate aliquot, 2 µg HSP27 or HSP27 vehicle and 4.5 µg protein MK2 or MK2 vehicle were added.
Immunoblotting. Proteins were separated via sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and Western blot analysis was performed. Antibodies to desmin (Cat. No. D1033; Sigma), α-actin (Cat. No. A7811; Sigma), troponin-I (Cat. No. SC-8118; Santa Cruz Biotechnology, Santa Cruz, CA), HSP27 (Cat. No. SC-1049; Santa Cruz Biotechnology), and pHSP27-Ser82 (Cat. No. 07–646; Upstate) were used. Densitometry was carried out using NIH ImageJ software.

Statistical analysis. All values were reported as means ± SE. Statistical significance was assumed for \( P < 0.05 \). Data were analyzed by a standard analysis of variance followed by a Student \( t \)-test or, for those data normalized to a paired control, an ANOVA with complete block design followed by Fisher least significant difference post hoc test.

RESULTS

Calpain-induced degradation of purified desmin. Desmin was incubated with or without calpain-1 in the presence of \( \mathrm{Ca}^{2+} \) to identify the pattern of desmin degradation (Fig. 1). Full-length desmin has a predicted molecular mass of 52 kDa. In the absence of calpain, commercially purified chicken desmin had two distinct immunoreactive bands at 50 and 45 kDa. In the presence of calpain, there was a progressive loss of these higher molecular mass desmin immunoreactive proteins concomitant with the appearance of a band at ~32 kDa. Thus anti-desmin immunoreactive proteins at ~45 and ~32 kDa were designated desmin degradative products A and B, respectively.

Effect of ischemia-reperfusion and \( \mathrm{H}_2\mathrm{O}_2 \) on desmin content in isolated hearts. The effects of ischemia-reperfusion on desmin content of myofilaments from hearts pretreated with vehicle or \( \mathrm{H}_2\mathrm{O}_2 \) are shown in Fig. 2. The full-length desmin product was significantly decreased in myofilaments from the ischemia-reperfusion hearts compared with nonischemic hearts. There was no significant difference in the myofilament desmin content between nonischemic hearts and \( \mathrm{H}_2\mathrm{O}_2 \)-pretreated ischemic/reperfused hearts.

Effect of \( \mathrm{Ca}^{2+} \) overload on desmin in intact ventricular myocytes. The effects of \( \mathrm{Ca}^{2+} \) overload on desmin content in intact ventricular myocyte pretreated with vehicle, the calpain inhibitor MDL-28170, the p38 MAPK inhibitor SB-202190, and/or \( \mathrm{H}_2\mathrm{O}_2 \) are shown in Fig. 3. To perform these experiments, cells were pretreated with combinations of vehicle, 0.8 \( \mu \)M MDL-28170, 10 \( \mu \)M SB-202190, or 100 \( \mu \)M \( \mathrm{H}_2\mathrm{O}_2 \) and then challenged with a 6 mM extracellular \( \mathrm{Ca}^{2+} \) or maintained in a 1.25 mM \( \mathrm{Ca}^{2+} \) solution for 10 min. The full-length desmin product decreased with \( \mathrm{Ca}^{2+} \) challenge, and this decrease was attenuated by calpain inhibition. Acute \( \mathrm{H}_2\mathrm{O}_2 \) exposure by itself did not alter desmin content, but under \( \mathrm{Ca}^{2+} \) overload conditions, \( \mathrm{H}_2\mathrm{O}_2 \) blocked desmin loss. SB-202190 inhibition of p38 MAPK did not block \( \mathrm{Ca}^{2+} \)-induced proteolysis of desmin but did inhibit the ability of \( \mathrm{H}_2\mathrm{O}_2 \) to protect desmin.

To help establish that the \( \mathrm{Ca}^{2+} \) overload protocol with a 10-min exposure to an extracellular \( \mathrm{Ca}^{2+} \) of 6 mM was not irreversibly destroying ventricular myocytes, the percentage of rod-shaped myocytes (length-to-width ratio of 2:1 or greater) to total cell number was determined immediately after treatment. For myocytes treated with the vehicle solution over the 40-min pretreatment period but not challenged with high extracellular \( \mathrm{Ca}^{2+} \) (“Con” in Fig. 3), the rod-shaped to total cell percentage was 52 ± 4% in six isolations. For cells that were exposed to the vehicle solution and underwent the \( \mathrm{Ca}^{2+} \) overload protocol (“\( \mathrm{Ca}^{2+} \)in” in Fig. 3), the rod to total cell percentage was 44 ± 4% from these same isolations. These numbers were not significantly different. Furthermore, no significant difference was found in rod-shaped to total myocyte number between any of the drug pretreatment groups (Fig. 3) compared with the corresponding vehicle treatment group (data not shown).

Fig. 1. Representative Western blot illustrating calpain-1 degradation of desmin. Equal amounts of purified chicken desmin were incubated with (times of 10 and 60 sec) or without (time 0) calpain-1. A and B indicate desmin degradative products. Additional calpain-1 exposure causes disappearance of both A and B products (data not shown).

Fig. 2. Typical Western blot and cumulative analysis of desmin content in nonischemic (Non-Isch), vehicle-pretreated ischemic/reperfused (vehicle + Isch/Rep), and \( \mathrm{H}_2\mathrm{O}_2 \)-pretreated ischemic/reperfused (\( \mathrm{H}_2\mathrm{O}_2 + \) Isch/Rep) hearts. Isolated hearts were subjected to 4 times 5 min vehicle or 100 \( \mu \)M \( \mathrm{H}_2\mathrm{O}_2 \) followed by 5-min wash and then 60 min of global ischemia and 30 min of reperfusion. Left ventricular homogenates were examined by Western blot analysis for the full length desmin product (50 kDa). Densities from anti-desmin Western blots were normalized to the densities of 2 separate protein bands on silver-stained SDS-polyacrylamide gels. Values are expressed as means ± SE; \( n = 3 \) hearts/group. *\( P < 0.05 \) compared with nonischemic hearts.
Exogenous calpain-induced degradation of myofilament protein: effects of H₂O₂ and p38 MAPK inhibition.

Ventricular myocytes were skinned and exposed to low levels of exogenous calpain-1 in the presence of Ca²⁺/H₁₁₀₀₁. Calpain treatment resulted in myofilament proteins being cleaved and/or released as full-length products into the supernatant (Fig. 4). Calpain increased the desmin degradative product B in the myofilament supernatant at all time points (Fig. 4A). Troponin I, as the full-length product and a slightly lower molecular mass-cleaved product, was increased in the myofilament supernatant after 3, 5, and 10 min of calpain exposure (Fig. 4B). Troponin I was also present in the supernatant from vehicle-treated myofilaments. The origin of this troponin I is likely the large precursor pool of troponin I that is loosely associated with the myofilaments (14). Calpain also increased the α-actinin full-length protein released into the myofilament supernatant after 5, 10, and 15 min of exposure (Fig. 4C). A time-dependent increase in α-actinin and troponin I release and cleavage were also observed for myofilaments not exposed to exogenous calpain (Con in Fig. 4). These changes were not as great or as consistent as that seen with calpain addition. Activation of endogenous calpain, induced by the increase in bathing [Ca²⁺/H₁₁₀₀₁], could account for these effects.

Pretreatment of membrane intact ventricular myocytes with H₂O₂ reduced the appearance of cleaved desmin product into the supernatant of subsequently skinned and calpain-exposed cells (Fig. 5). Release of desmin degradative product B into the myofilament supernatant was significantly decreased in myocytes pretreated with 2, 30, or 100 μM H₂O₂ for 3 min compared with myocytes pretreated with vehicle (no H₂O₂; Fig. 5A). Release of desmin degradative product B into the myofilament supernatant was also significantly decreased in myocytes pretreated with 100 μM H₂O₂ for 3, 5, or 15 min compared with myocytes pretreated with vehicle (no H₂O₂; Fig. 5B).

The H₂O₂ attenuation of calpain-induced release of a cleaved desmin product (Fig. 5) was blocked by inhibition of p38 MAPK with SB-202190 (Fig. 6). To do these experiments, intact ventricular myocytes were treated for 30 min with 1 μM SB-202190, followed by exposure to 100 μM H₂O₂ or vehicle for 3 min. Cells were then skinned and exposed to calpain-1, and the post-calpain treatment supernatant was collected. H₂O₂-treated myocytes had an ~20% decrease in the desmin released into the supernatant, and this effect was blocked by SB-202190 pretreatment (Fig. 6).
Effects of exogenous pHSP27 on calpain-induced degradation of myofilament proteins. We phosphorylated human recombinant HSP27 with MK2 (Fig. 7). Rat cardiac myofilaments were then incubated with either pHSP27 or HSP27 and were washed, and the extent of pHSP27 or HSP27 remaining associated with the myofilaments was determined (Fig. 8). These studies were done to address the concern that differences in aggregation of pHSP versus HSP in solution might reduce binding to the myofilaments. However, we found that exogenous pHSP27 and HSP27 associate with myofilaments to an equal extent in the presence and absence of calpain exposure (Fig. 8).

The effect of exogenous HSP27 on calpain-induced degradation of desmin is shown in Fig. 9. Myofilaments were incubated with vehicle, MK2, HSP27, or pHSP27, and exogenous calpain-1 was added. The amount of desmin degradative product A was significantly decreased in the pHSP27 group compared with the vehicle group (Fig. 9). There were no significant differences between the vehicle, MK2, and HSP27 groups. The inability of MK2 to block desmin degradation, through phosphorylation of endogenous HSP25, was not unexpected since washed myofilaments contain very little HSP25 (see vehicle in Fig. 8). It should be noted that calpain-1 exposure time and temperature were increased in these experiments. The actual activity of the calpain lot used appeared to

Fig. 5. H2O2 concentration (A) and time-dependent (B) attenuation of the calpain-induced appearance of desmin degradative product B in the myofilament supernatant. A: myocytes were pretreated with vehicle (no H2O2) or with 2, 30, or 100 μM H2O2 for 3 min. Myofilaments were isolated, and all samples were exposed to calpain-1 for 5 min. B: myocytes were pretreated with vehicle (no H2O2) or with 100 μM H2O2 for 3, 5, or 15 min. Myofilaments were isolated, and all samples were exposed to calpain-1 for 5 min. Densities from anti-desmin Western blots were normalized to protein load based on the density of a calpain-resistant supernatant protein (~20 kDa) visualized on silver-stained SDS-polyacrylamide gels and normalized to the no H2O2 group response. Values are expressed as means ± SE; n = 3–6 myocyte isolations. *P < 0.05 compared with no H2O2.
be much lower than that of previous lots despite having the same specific activity as stated by the commercial supplier.

DISCUSSION

The present study demonstrated that ischemia-reperfusion-induced loss of desmin can be attenuated by H₂O₂ preconditioning in hearts. Ca²⁺ overload of isolated ventricular myocytes causes a similar disruption of desmin that is also reduced by H₂O₂. Desmin disruption due to Ca²⁺ overload occurs through calpain activation, and H₂O₂ protection of desmin requires p38 MAPK activation. Exposing skinned myocytes to exogenous calpain-1 also resulted in a degradation of desmin that was attenuated by pretreating the intact myocytes with H₂O₂. The protective effect of H₂O₂ on exogenous calpain-induced desmin degradation was blocked by an administration of the p38 MAPK inhibitor SB-202190. Finally, calpain-induced desmin degradation did not occur in myofilaments preincubated with recombinant pHSP27. These findings, in combination with work done by others, are consistent with the hypothesis that ischemia-reperfusion and subsequent Ca²⁺ overload result in activation of calpain-1 which leads to degradation of the myofilament structural protein desmin. Low doses of H₂O₂ have been shown to reduce postischemic dysfunction and area of infarct. In our studies, isolated hearts were perfused with 100 μM H₂O₂ before ischemia and reperfusion to examine the possibility that H₂O₂ may attenuate the loss of desmin. Desmin content of H₂O₂-treated ischemia-reperfused hearts was not significantly different than that of nonischemic hearts. Thus H₂O₂ attenuates desmin degradation due to ischemia-reperfusion in isolated hearts.

Disruption of cytoskeletal proteins such as desmin may contribute to or be a consequence of cell death induced by ischemia-reperfusion. In a study by Kido et al., ischemia-reperfusion-induced changes in dystrophin preceded a disruption of the cell membrane and necrosis. Desmin disruption has been demonstrated in ischemia-reperfused whole hearts with maintained Z-band structure and cell membranes. These findings, in combination with work done by others, are consistent with the hypothesis that ischemia-reperfusion and subsequent Ca²⁺ overload result in activation of calpain-1 which leads to degradation of the myofilament structural protein desmin. Low doses of H₂O₂ act, in part, by activating the p38 MAPK signaling pathway that results in phosphorylation of HSP25/27 by MK2. Once activated, pHSP25/27 is translocated from the cytosolic to the myofilament fraction where it shields desmin from calpain-induced degradation.

Hearts that underwent ischemia-reperfusion exhibited a significant decrease in myofilament desmin content compared with nonischemic hearts. This observation is in agreement with studies by others. Low doses of H₂O₂ have also been shown to reduce postischemic dysfunction and area of infarct. In our studies, isolated hearts were perfused with 100 μM H₂O₂ before ischemia and reperfusion to examine the possibility that H₂O₂ may attenuate the loss of desmin. Desmin content of H₂O₂-treated ischemia-reperfused hearts was not significantly different than that of nonischemic hearts. Thus H₂O₂ attenuates desmin degradation due to ischemia-reperfusion in isolated hearts.

Disruption of cytoskeletal proteins such as desmin may contribute to or be a consequence of cell death induced by ischemia-reperfusion. In a study by Kido et al., ischemia-reperfusion-induced changes in dystrophin preceded a disruption of the cell membrane and necrosis. Desmin disruption has been demonstrated in ischemia-reperfused whole hearts with maintained Z-band structure and cell membranes. These findings, in combination with work done by others, are consistent with the hypothesis that ischemia-reperfusion and subsequent Ca²⁺ overload result in activation of calpain-1 which leads to degradation of the myofilament structural protein desmin. Low doses of H₂O₂ act, in part, by activating the p38 MAPK signaling pathway that results in phosphorylation of HSP25/27 by MK2. Once activated, pHSP25/27 is translocated from the cytosolic to the myofilament fraction where it shields desmin from calpain-induced degradation.

Hearts that underwent ischemia-reperfusion exhibited a significant decrease in myofilament desmin content compared with nonischemic hearts. This observation is in agreement with studies by others. Low doses of H₂O₂ have also been shown to reduce postischemic dysfunction and area of infarct. In our studies, isolated hearts were perfused with 100 μM H₂O₂ before ischemia and reperfusion to examine the possibility that H₂O₂ may attenuate the loss of desmin. Desmin content of H₂O₂-treated ischemia-reperfused hearts was not significantly different than that of nonischemic hearts. Thus H₂O₂ attenuates desmin degradation due to ischemia-reperfusion in isolated hearts.

Disruption of cytoskeletal proteins such as desmin may contribute to or be a consequence of cell death induced by ischemia-reperfusion. In a study by Kido et al., ischemia-reperfusion-induced changes in dystrophin preceded a disruption of the cell membrane and necrosis. Desmin disruption has been demonstrated in ischemia-reperfused whole hearts with maintained Z-band structure and cell membranes. These findings, in combination with work done by others, are consistent with the hypothesis that ischemia-reperfusion and subsequent Ca²⁺ overload result in activation of calpain-1 which leads to degradation of the myofilament structural protein desmin. Low doses of H₂O₂ act, in part, by activating the p38 MAPK signaling pathway that results in phosphorylation of HSP25/27 by MK2. Once activated, pHSP25/27 is translocated from the cytosolic to the myofilament fraction where it shields desmin from calpain-induced degradation.

Hearts that underwent ischemia-reperfusion exhibited a significant decrease in myofilament desmin content compared with nonischemic hearts. This observation is in agreement with studies by others. Low doses of H₂O₂ have also been shown to reduce postischemic dysfunction and area of infarct. In our studies, isolated hearts were perfused with 100 μM H₂O₂ before ischemia and reperfusion to examine the possibility that H₂O₂ may attenuate the loss of desmin. Desmin content of H₂O₂-treated ischemia-reperfused hearts was not significantly different than that of nonischemic hearts. Thus H₂O₂ attenuates desmin degradation due to ischemia-reperfusion in isolated hearts.

Disruption of cytoskeletal proteins such as desmin may contribute to or be a consequence of cell death induced by ischemia-reperfusion. In a study by Kido et al., ischemia-reperfusion-induced changes in dystrophin preceded a disruption of the cell membrane and necrosis. Desmin disruption has been demonstrated in ischemia-reperfused whole hearts with maintained Z-band structure and cell membranes. These findings, in combination with work done by others, are consistent with the hypothesis that ischemia-reperfusion and subsequent Ca²⁺ overload result in activation of calpain-1 which leads to degradation of the myofilament structural protein desmin. Low doses of H₂O₂ act, in part, by activating the p38 MAPK signaling pathway that results in phosphorylation of HSP25/27 by MK2. Once activated, pHSP25/27 is translocated from the cytosolic to the myofilament fraction where it shields desmin from calpain-induced degradation.

Hearts that underwent ischemia-reperfusion exhibited a significant decrease in myofilament desmin content compared with nonischemic hearts. This observation is in agreement with studies by others. Low doses of H₂O₂ have also been shown to reduce postischemic dysfunction and area of infarct. In our studies, isolated hearts were perfused with 100 μM H₂O₂ before ischemia and reperfusion to examine the possibility that H₂O₂ may attenuate the loss of desmin. Desmin content of H₂O₂-treated ischemia-reperfused hearts was not significantly different than that of nonischemic hearts. Thus H₂O₂ attenuates desmin degradation due to ischemia-reperfusion in isolated hearts.

Disruption of cytoskeletal proteins such as desmin may contribute to or be a consequence of cell death induced by ischemia-reperfusion. In a study by Kido et al., ischemia-reperfusion-induced changes in dystrophin preceded a disruption of the cell membrane and necrosis. Desmin disruption has been demonstrated in ischemia-reperfused whole hearts with maintained Z-band structure and cell membranes. These findings, in combination with work done by others, are consistent with the hypothesis that ischemia-reperfusion and subsequent Ca²⁺ overload result in activation of calpain-1 which leads to degradation of the myofilament structural protein desmin. Low doses of H₂O₂ act, in part, by activating the p38 MAPK signaling pathway that results in phosphorylation of HSP25/27 by MK2. Once activated, pHSP25/27 is translocated from the cytosolic to the myofilament fraction where it shields desmin from calpain-induced degradation.

Hearts that underwent ischemia-reperfusion exhibited a significant decrease in myofilament desmin content compared with nonischemic hearts. This observation is in agreement with studies by others. Low doses of H₂O₂ have also been shown to reduce postischemic dysfunction and area of infarct. In our studies, isolated hearts were perfused with 100 μM H₂O₂ before ischemia and reperfusion to examine the possibility that H₂O₂ may attenuate the loss of desmin. Desmin content of H₂O₂-treated ischemia-reperfused hearts was not significantly different than that of nonischemic hearts. Thus H₂O₂ attenuates desmin degradation due to ischemia-reperfusion in isolated hearts.
data support the possibility that cytoskeletal proteins contribute to reperfusion injury rather than being a consequence of cell death. Furthermore, from our Ca\(^{2+}\)-preload studies, we established that desmin disruption due to an acute Ca\(^{2+}\) overload occurs without a decrease in the number of rod-shaped, membrane-intact ventricular myocytes. Thus the observed changes in desmin precede morphological changes consistent with irreversible Ca\(^{2+}\) overload, hypercontracture, and cell death.

Ca\(^{2+}\)-dependent activation of calpain-1, due to ischemia-reperfusion, has been shown to degrade cytoskeletal and regulatory myocardial proteins (16, 17, 21). In the present study, using intact cells, we demonstrated that endogenous calpain inhibition blocks desmin degradation due to Ca\(^{2+}\) overload (Fig. 3). Furthermore, using myofilaments exposed to exogenous calpain-1, we demonstrated a time-dependent increase in the appearance of immunoreactive desmin, α-actinin, and troponin I in the myofilament supernatant (Fig. 4). The calpain-dependent appearance of a lower molecular mass desmin product suggests that calpain-1 causes proteolysis and release of desmin into the supernatant. Calpain also causes an increased rate of release of a lower molecular mass tropinin I product along with the intact tropinin I compared with untreated controls. α-Actinin was only found as the full-length molecular mass in the supernatant of calpain-treated myofilaments, a finding consistent with work by Goll et al. (6). Thus, it is likely that calpain-1 cleaves a protein necessary for α-actinin to associate with the myofilaments.

Acute 100 μM H\(_2\)O\(_2\) exposure attenuates the disruption of desmin from intact cells and myofilaments (Figs. 3 and 4). One explanation for this effect is that H\(_2\)O\(_2\) reduces calpain activity. However, this seems unlikely given that myofilaments were washed free of H\(_2\)O\(_2\) before exogenous calpain exposure (Figs. 5 and 6), and myocytes pretreated with H\(_2\)O\(_2\) continued to have a calpain-dependent disruption of α-actinin or tropinin I in myofilaments (data not shown). Another explanation of the ability of H\(_2\)O\(_2\) to block desmin degradation is that it is able to activate a pathway that leads to the protection of specific protein(s). Consistent with this idea are our observations that the protective effect of H\(_2\)O\(_2\) on desmin disruption could be blocked by an administration of the p38 MAPK inhibitor SB-202190 (Figs. 3 and 6). This suggests that low doses of H\(_2\)O\(_2\) stimulate the p38 MAPK signaling pathway to limit the ability of calpain-1 to degrade desmin.

The mechanism by which p38 MAPK acts may involve a decrease in calpain activation or an effect on the target of calpain activation. However, past studies have linked p38 MAPK activation with an increase in in vivo calpain activity (8, 20). Thus it seems unlikely that p38 MAPK activation decreased exogenous calpain activity. Another possible mechanism of p38 MAPK action is through HSP25/27. Previous studies have demonstrated that stimulation of p38 MAPK results in the phosphorylation and translocation of HSP25/27 from the cytosolic to the Z-disk region of myofilaments (2, 3, 10, 11). Ischemic preconditioning has also been shown to induce a translocation of HSP25/27 in a p38 MAPK-dependent manner, and the activation of p38 MAPK-HSP25/27 correlates with an increase in postischemic myocardial function (18). Furthermore, overexpression of HSP27 has been shown to decrease lactate dehydrogenase release and to increase the number of ventricular myocytes that exclude trypan blue upon ischemic stress (15, 23). In the present study, cardiac myofilaments were incubated with recombinant HSP27 or pHSP27 before treatment with calpain-1 (Fig. 9). Exposure to exogenous pHSP27, but not HSP27, resulted in decreased calpain-induced desmin degradation. This lack of effect of the non-phosphorylated form of HSP27 was not due to a decrease in pHSP27 associated with the myofilaments compared with pHSP27 (Fig. 8). These data lend further support to the hypothesis that H\(_2\)O\(_2\) protection from ischemia-reperfusion damage is due, at least in part, to the p38 MAPK activation of HSP25/27 and the ability of pHSP25/27 to shield desmin from calpain-1 degradation in the heart.

GRANTS

This study was supported by the National Heart, Lung, and Blood Institute Training Fellowship HL-07641 (to B.C. Blunt) and Grant HL-48639 (to P. A. Hofmann) and American Heart Association Grant-In-Aid 0655584 (to P. A. Hofmann).

REFERENCES


