Cardioprotection through a PKC-dependent decrease in myofilament ATPase

W. Glen Pyle, Yi Chen, and Polly A. Hofmann
Department of Physiology, University of Tennessee, Memphis, Tennessee 38163

Submitted 24 January 2003; accepted in final form 21 May 2003

PKC and myofibrillar Ca2+ were equally cardioprotective as observed that moderate decreases in myofibrillar ATPase could account for cardioprotection, we subjected hearts to decreases in Ca2+ effects of U50,488H (U50). To determine whether controls. PKC inhibitors abolished the cardioprotective effects of U50. To determine whether κ-opioid-PKC-dependent decreases in Ca2+-dependent actomyosin Mg2+-ATPase could account for cardioprotection, we subjected hearts to three separate actomyosin ATPase-lowering protocols. We observed that moderate decreases in myofibrillar ATPase were equally cardioprotective as κ-opioid receptor stimulation. Immunoblot analysis and confocal microscopy revealed a κ-opioid-induced increase in myofilament-associated PKC-ε, and myofibrillar Ca2+-independent PKC activity was increased after κ-opioid stimulation. This PKC-myofilament association led to an increase in troponin I and C-protein phosphorylation. Thus we propose PKC-ε activation and translocation to the myofilaments causes a decrease in actomyosin ATPase, which contributes to the κ-opioid receptor-dependent cardioprotective mechanism.

U50,488H: Western blot; confocal microscopy; protein kinase C activity; left ventricular developed pressure; κ-opioid

Cardioprotection is any physiological or therapeutic mechanism that contributes to the preservation of the heart by attenuating myocardial damage. When cardioprotective mechanisms are activated in advance of a potentially damaging condition, i.e., long duration ischemia, it is referred to as preconditioning. The observed cardioprotective effects of preconditioning include reduced tissue necrosis, improved contractile function, and decreased occurrence of posts ischemic dysrhythmias.

Although the protective effects of preconditioning are well known, the mechanism(s) responsible for this phenomenon has yet to be definitively identified. Murry et al. (22) noted that preconditioning slows the rate of ATP depletion during prolonged ischemia. Ex-
ment proteins, and cardioprotection. Thus the second specific aim of the present study was to investigate the role of PKC in both \(\kappa\)-opioid receptor-dependent cardioprotection and myofilament protein phosphorylation. Phenylephrine, an \(\alpha\)-adrenergic receptor agonist, was included in the present study to determine whether \(\kappa\)-opioid and \(\alpha\)-adrenergic receptor-dependent cardioprotection are mediated through a common intracellular mechanism that targets myofilament proteins.

In adult rat ventricular myocytes, four PKC isoforms have been identified: the classic calcium-dependent PKC-\(\alpha\); the novel calcium-independent PKC-\(\delta\) and -\(\epsilon\); and the atypical calcium-dependent PKC-\(\zeta\) (37). On activation various PKC isoforms translocate from the cytosol to target substrates in the plasma membrane, cytoskeleton, nuclei, or myofibrils in ventricular myocytes (37). Anchoring proteins proximal to target substrates, termed receptors for activated C-kinase (RACK), fixes specific isoforms of PKC near their target and facilitates interaction. We have previously reported that the application of exogenous PKC-\(\epsilon\) to cardiac myofilaments is sufficient to mimic \(\kappa\)-opioid and \(\alpha\)-adrenergic receptor-mediated reductions in Ca\(^{2+}\)-dependent actomyosin Mg\(^{2+}\)-ATPase activity (29). Thus our third aim was to determine whether \(\kappa\)-opioid and \(\alpha\)-adrenergic receptor-dependent cardioprotection increases the association of endogenous PKC-\(\epsilon\) with cardiac myofilaments. The anchoring of PKC-\(\epsilon\) to the myofilaments would fix activated PKC-\(\epsilon\) near its substrates and promote phosphorylation of the target protein(s).

**METHODS**

Langendorff-perfused hearts preparation. All animals were cared for in accordance with the guidelines of the Animal Care and Use Committee of the University of Tennessee, Memphis. Hearts were removed from female Wistar rats anesthetized by methoxyflurane (Metofane) inhalation. Isolated hearts were cannulated in ice-cold modified Krebs-Henseleit solution and mounted on a Langendorff perfusion apparatus. Modified Krebs-Henseleit solution contained (mM) 4.7 KCl, 118 NaCl, 1.2 MgSO\(_4\), 1.3 CaCl\(_2\), 25 NaHCO\(_3\), 11 glucose, 1.2 KH\(_2\)PO\(_4\), 0.05 EDTA, and 2 lactic acid (pH 7.4). Hearts were perfused with oxygenated (95% O\(_2\)-5% CO\(_2\)) at 37°C modified Krebs-Henseleit solution and placed in a 100-ml organ bath that contained oxygenated, 37°C modified Krebs-Henseleit solution. A pressure transducer (BLPR, World Precision Instruments; Sarasota, FL) was inserted through the left atrium into the left ventricle and pacing was initiated at 300 beats/min. Pacing voltage was set at twice the threshold value.

Ischemia-reperfusion was done as previously reported (30). Hearts were perfused with agonists, antagonists, or vehicle for 2 min, commencing 12 min before the start of global ischemia. Some hearts were perfused with modified Krebs-Henseleit solution containing inhibitors of second messengers for 15 min before and during 2 min of agonist treatment. This perfusion duration was sufficient to inhibit kinase-dependent changes in myofilament activation without altering baseline function (data not shown). A subsequent 10-min perfusion in the absence of all agonists-antagonists-inhibitors washed out these agents before ischemia. Some hearts were reperfused for the first 20 min under one of three separate conditions meant to decrease actomyosin Mg\(^{2+}\)-ATPase activity. The first group was reperfused with modified Krebs-Henseleit solution containing 5 mM 2,3-butanedione monoxime (BDM). This concentration of BDM has been shown to inhibit actomyosin Mg\(^{2+}\)-ATPase activity without affecting intracellular Ca\(^{2+}\) concentration (3). A second group of hearts was reperfused with modified Krebs-Henseleit solution oxygenated with 85% O\(_2\)-15% CO\(_2\) to induce acidosis. Increasing CO\(_2\) to 15% reduces intracellular pH from 7.0 to 6.7 (25). The final group of hearts, in which reperfusion was altered to decrease actomyosin Mg\(^{2+}\)-ATPase, underwent hypothermia (32°C) for the first 20 min after the end of ischemia.

Preischemic left ventricular developed pressure (LVDP) and left ventricular end-diastolic pressure (EDP) were determined by averaging values from the 8-min period immediately before the agonist-antagonist treatment. Postischemic pressures were determined by averaging values from the last 20 min of the 60 min of postischemic reperfusion. LVDP and EDP were stable during baseline perfusion and during the final 20 min of postischemic reperfusion for all groups. LVDP and EDP were altered by agonist-antagonist treatment but returned to baseline values before the onset of global ischemia.

Hearts were included in data analysis if they had a preischemic LVDP of 80–150 mmHg and EDP of 5–15 mmHg. Hearts were excluded from statistical analysis if they showed irreversible postischemic dysrhythmias after 20 min of reperfusion. Greater than 95% of all hearts satisfied these criteria.

**Myofibrillar ATPase measurement.** ATPase buffers with Ca\(^{2+}\) concentrations of pCa\(^{2+}\) 4.0 and 9.0 were used. pCa\(^{2+}\) 4.0 buffer contained (in mM) 23.5 KCl, 5 MgCl\(_2\), 3.2 ATP, 2 EGTA, 20 imidazole, and 2.2 CaCl\(_2\) (pH 7.0). The pCa\(^{2+}\) 9.0 buffer contained 26 mM KCl, 5.1 mM MgCl\(_2\), 3.2 mM ATP, 2 mM EGTA, 20 mM imidazole, and 4.9 \(\mu\)M CaCl\(_2\) (pH 7.0). For experiments in which pH and temperature were altered, solution composition was slightly modified to keep pCa\(^{2+}\) values constant at 4.0 and 9.0. Myofibrils (final concentration of 1 to 2 mg/ml total protein) containing regulated actin were isolated from untreated rat hearts (30) and added to ATPase buffers warmed to 37°C (control), except where noted. Some buffers were supplemented with 5 mM BDM or pH reduced from 7.0 to 6.7 (acidosis). In a third treatment group, actomyosin Mg\(^{2+}\)-ATPase activity was measured in buffers heated to 37°C. After 8 min of incubation, the reaction was quenched with 2 ml of 20% trichloroacetic acid. Inorganic phosphate levels were determined according to the method of Fiske and SubbaRow. Inorganic phosphate production was found to be linear with respect to time under conditions described above (data not shown).

**Ventricular ATP.** ATP was quantified using the luciferin-luciferase enzyme technique (20). Hearts were perfused as described under Langendorff-perfused heart preparation and were removed immediately after global ischemia (i.e., no reperfusion). The ventricles were cut from the hearts, quickly frozen in liquid nitrogen, and homogenized in ice-cold modified Krebs-Henseleit solution with a pestle and cold mortar. The homogenate was used to measure ventricular ATP with a luciferin-luciferase assay kit (Sigma; St. Louis, MO). The light produced by ATP plus luciferin was used to calculate unknown ATP concentrations of samples. A small amount of homogenized ventricle was used to determine protein concentration with a biuret assay. Ventricular ATP levels were expressed as nanomoles of ATP per milligram of protein in the homogenate.

**PKC immunohistochemistry.** Myocytes were prepared as described by Lester and Hofmann (20) and incubated for 5 min with agonists-antagonists. Cells were washed in a 1 mmol/l...
Ca$^{2+}$ Ringer solution, and the myofilament fraction was isolated according to a modified protocol described by Huang et al. (17). Briefly, myocytes were resuspended in buffer containing Triton X-100, sonicated, and centrifuged for 10 min at 800 g. The resulting pellet contained myofibrils and some nuclei (myofilibrillar-nuclei fraction).

For immunoblotting, samples were run on SDS-polyacrylamide gels using 5% acrylamide stacking gels and 7.5% acrylamide resolving gels. Gel proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Immunoblotting was carried out using a protocol modified from Lester and Hoffmann (20). PVDF membranes were incubated for 1 h with monoclonal antibodies for PKC-ε (1:1,000), α (1:1,000), or β (1:1,000) from Transduction Labs (Lexington, KY). Density of PKC bands was determined using NIH Image software. Band density of all treatments was calculated and normalized to control density. Protein concentrations were determined by biuret assay before gel loading to ensure equal sample loading of each lane.

For confocal microscopy and immunofluorescence, the isolated myofilibrillar-nuclei fraction was incubated with monoclonal antibodies to PKC isoenzyme-specific PKC-ε (1:50) at 4 °C to establish the extent of change in actomyosin ATPase activity in the presence of 1 mmol/l CaCl$_2$. PKC activity was determined by subtracting PKC activity measured in the presence of 1 mmol/l EGTA from PKC activity.

RESULTS

Effects of pharmacological preconditioning and PKC inhibition on postischemic recovery. Preischemic LVDP or EDP between all groups before any intervention was not statistically different. After global ischemia and reperfusion, mean postischemic LVDP of control hearts was reduced to ~40% of preischemic LVDP (Fig. 1). Treatment with the α-opioid receptor agonist U50,488H and activation of α-adrenergic receptors with phenylephrine plus propranolol led hearts to retain ~75% of their preischemic LVDP (Fig. 1). This is a significant increase in postischemic LVDP compared with controls. EDP was also seen to improve as evidenced by the reduction in postischemic EDP. For example, postischemic EDP of untreated hearts was 59.4 ± 5.7 mmHg, whereas postischemic EDP of U50,488H pretreated hearts was 36.4 ± 3.8 mmHg.

The receptor-independent PKC activator PMA (20, 26) increased postischemic LVDP recovery compared with the control group, whereas hearts treated with α-PMA, the inactive form of PMA, had a similar recovery to control hearts (Fig. 1). The PKC inhibitor chelerythrine chloride significantly attenuated the postischemic LVDP recovery of both U50,488H and phenylephrine plus propranolol-treated hearts. The PKC inhibitor bisindolylmaleimide also significantly attenuated postischemic LVDP recovery of U50,488H-treated hearts. Pretreatment of hearts with chelerythrine chloride alone had no effect on mean postischemic LVDP compared with control postischemic LVDP.

Improved postischemic recovery with decreased actomyosin Mg$^{2+}$-ATPase activity. α-Opioid receptor activation and exogenously added PKC-ε both reduce actomyosin Mg$^{2+}$-ATPase by 20% compared with controls (29, 30). In the current study we compared myofilibrillar actomyosin Mg$^{2+}$-ATPase activity in the presence of 1) 5 mM BDM, 2) in buffers of pH 6.7, or 3) at 37°C to establish the extent of change in actomyosin Mg$^{2+}$-ATPase activity compared with ATPase activity.
measured at 32°C, pH 7.0 (control). Maximum Ca\textsuperscript{2+}- dependent actomyosin Mg\textsuperscript{2+}-ATPase activity was reduced by an average of 12% with BDM treatment and 8% under acidic conditions (Fig. 2). Increasing buffer temperature to 37°C increased maximum Ca\textsuperscript{2+}-dependent actomyosin Mg\textsuperscript{2+}-ATPase activity by 36% over control (32°C).

Ventricular pressures were measured in hearts that underwent ischemia followed by a 20-min reperfusion with Krebs-Henseleit solution modified with 1) 5 mM BDM, 2) gased with 15% CO\textsubscript{2} to induce acidosis, or 3) a 5°C reduction in temperature from 37°C to 32°C. Postischemic function, as determined by LVDP after 60 min reperfusion, was significantly improved in all three treatment groups compared with controls (Fig. 3).

Postischemic ATP. Whole heart ATP levels were measured at the end of 20 min global ischemia using a luciferin-luciferase assay. Perfusion of hearts with U50,488H or phenylephrine plus propranolol before global ischemia led to an increase in ATP concentration (Fig. 4). The PKC inhibitors chelerythrine chloride and bisindolylmaleimide abolished the U50,488H-dependent increase in ATP concentration.

PKC isoforms and activity associated with the myofilaments. The myofibrillar-nuclei fraction had an increase in PKC-\xi following both U50,488H and phenylephrine plus propranolol treatment of ventricular myocytes (Figs. 5 and 6). U50,488H treatment also led to an increase in PKC-\xib associated with the myofibril-
nuclei fraction, whereas phenylephrine plus propranolol had no effect on PKC-δ translocation to the myofibril-nuclei (Fig. 5). There were no significant changes in PKC-α or PKC-ζ in the myofibril-nuclei fraction with either agonist as determined by Western blot analysis. An increase in PKC-ε associated with the myofibrils after treatment with U50,488H or phenylephrine plus propranolol was also seen using confocal microscopy (Fig. 6). PKC-δ was found to associate with both the myofibrils and nuclei before and after treatment with U50,488H or phenylephrine. As such, agonist-induced increases in PKC-δ to the myofibrils were difficult to establish with this technique.

PKC activity associated with the myofibrillar fraction from untreated and treated ventricular myocytes was determined (Fig. 7). Ca\(^{2+}\)-independent myofibrillar-PKC activity from cells pretreated with U50,488H or phenylephrine plus propranolol was significantly increased by \(\sim 50\%\) compared with untreated control cells. Ca\(^{2+}\)-dependent myofibrillar PKC activity was unchanged with either U50,488H or phenylephrine plus propranolol treatment compared with control myocytes.

**Myofilament protein phosphorylation.** The \(\kappa\)-opioid receptor agonist U50,488H increased phosphorylation of C-protein, TnI, and LC\(_{2}\) (Fig. 8). Treatment with chelerythrine chloride plus U50,488H blocked the U50,488H-induced increase in TnI and C-protein phosphorylation (Table 1). Chelerythrine chloride did not affect U50,488H-dependent phosphorylation of LC\(_{2}\).

**DISCUSSION**

The present study provides evidence that cardioprotection could occur through a sequence of events consisting of an increase in myofilament-associated PKC, posttranslational modification of myofilament proteins, decreased myofilament ATP consumption, and improved postischemic cardiac function. To this end, we demonstrated an increased association of PKC-ε with the myofilaments following activation of cardioprotective \(\alpha\)-adrenergic or \(\kappa\)-opioid receptors. The increase in PKC-myofilament interaction resulted in the observed increase in Ca\(^{2+}\)-independent PKC activity associated with the myofibrillar-nuclei fraction. Moreover, \(\kappa\)-opioid receptor stimulation and PKC association-activation increased the phosphorylation of TnI and C-protein. The present study also demonstrates that \(\kappa\)-opioid and \(\alpha\)-adrenergic receptor-dependent cardioprotection is attenuated by PKC inhibitors. Finally, our data of the present and past studies (39) suggest reduction in actomyosin Mg\(^{2+}\)-ATPase activity during reperfusion accounts for the improved postischemic functional recovery. Thus this study is unique in that it provides evidence connecting increased myofilament-associated PKC, PKC-dependent phosphorylation of myofilament proteins, reduced actomyosin Mg\(^{2+}\)-ATPase activity, and postischemic cardioprotection.

We have previously shown that preischemic \(\kappa\)-opioid or \(\alpha\)-adrenergic receptor activation improves postischemic LVDP by \(\sim 30\%\) over untreated control hearts (30). The \(\kappa\)-opioid receptor antagonist norbinaltorphimine reversed the cardioprotective effects of U50,488H and supports the involvement of \(\kappa\)-opioid receptor-specific activation in this effect. The results of the present study are consistent with our previous findings and those of Banerjee et al. (5) who have reported an improvement in postischemic myocardial function with preischemic \(\alpha\)-adrenergic receptor activation. Activation of \(\kappa\)-opioid receptors with exogenous administration of U50,488H has been shown to increase cellular viability and twitch amplitude after 5 min of severe metabolic inhibition in isolated cardiomycocytes (42), a finding that is also consistent with our results. Schultz and colleagues have demonstrated ischemic preconditioning reduces myocardial infarct size (34) in the rat heart by activating \(\delta\)-opioid but not \(\kappa\)-opioid receptors (33). This suggests endogenous \(\kappa\)-opioid peptides are not involved in ischemic preconditioning but does not...
preclude a cardioprotective role for the activation of k-opioid receptors.

During and after myocardial ischemia diastolic calcium levels are increased. This, in turn, increases EDP. k-Opioid receptor activation, PMA, acidosis, and hypothermia all produced significant reductions in the postischemic increase of EDP, without affecting preischemic EDP. The reduction in postischemic EDP is likely due to the maintenance of calcium homeostasis and a reduction in cytosolic calcium accumulation. Preischemic administration of BDM also caused a trend to decrease the postischemic increase in EDP compared with postischemic control EDP, although this did not reach statistical significance. The inability of BDM to significantly attenuate the postischemic increase in EDP may be due to the large variance in postischemic EDP observed in this group. Alternatively, BDM is a known chemical phosphatase that dephosphorylates proteins such as C-protein and LC2 (40). It is conceivable that

Fig. 6. Confocal microscopy of isolated myofibrils immunostained with PKC-ε (A–D)- or PKC-δ (E–H)-specific antibodies. Isolated ventricular myocytes were untreated (A and E), treated with Phe + Pro (B and F), or U50 (C and G) and myofibrils isolated. Myofibrils were then immunostained with PKC-ε or PKC-δ. For D and H, the antibody was preincubated with PKC and then reacted with myofibrils to reveal nonspecific effects of immunostaining. Photos are representative of 3 isolations.

Fig. 7. Myofibrillar-nuclear PKC activity in cardiac myofibrils. Nonischemic isolated ventricular myocytes were treated with Phe + Pro (n = 5), U50 (n = 5), or were untreated controls (n = 5). Myofibrils-nuclei were isolated, and PKC activity was associated with the myofibrillar-nuclear fraction measured in the presence and absence of calcium using an exogenous substrate. Values are expressed as means ± SE. *P ≤ 0.05 vs. respective controls.
BDM-induced alterations in phosphorylation of myocardial proteins influences postischemic EDP.

Aitchison et al. (1) found that activation of κ-opioid receptors in Langendorff-perfused rat hearts exacerbates infarct size. These results are in contrast with our findings (present study and Ref. 29) as well as others (42, 43). One possible explanation for this may be the presence of κ-opioid receptor subtypes. Aitchison et al. (1) utilized bremazocine to activate κ-opioid receptors, whereas U50,488H was used in studies demonstrating a cardioprotective effect (30, 41, 42). Bremazocine is a κ-opioid receptor agonist with a preference for κ2-opioid receptors (32), whereas U50,488H interacts preferentially with κ1-opioid receptors (32). Thus the activation of κ2-opioid receptors may be cardiotoxic, whereas κ1-opioid receptor activation is cardioprotective.

The initiation of cardioprotection through the stimulation of membrane-bound receptors leads to the activation of numerous intracellular signaling pathways, which in turn induces a variety of changes in the myocardocyte. To determine the specific role of decreased cross-bridge cycling in protecting the myocardium against postischemic dysfunction, we reduced actomyosin Mg2+-ATPase activity through receptor-independent methods. Myofilament ATPase was reduced by reperfusing hearts with an acidic or hypothermic modified Krebs-Henseleit solution, or with 5 mM BDM. These methods are well known to decrease myofilament ATPase brought about a profound improvement in postischemic contractile function. Moreover, we previously demonstrated that the κ-opioid and α-adrenergic receptor-dependent decrease in actomyosin ATPase (30) leads to improved postischemic ATP content of the heart.

Although acidosis, hypothermia, and BDM all decrease myofilament ATP use by slowing cross-bridge cycling, other nonmyofilament effects may contribute to cardioprotection with these treatment protocols. However, it appears the nonmyofilament effects vary significantly among these three treatment groups. For example, BDM (36) and hypothermia (19) both inhibit ATP-sensitized K+ (K_ATP) channel opening, whereas acidosis (39, 43) has been shown to have variable effects on these channels. This is of interest because opening of mitochondrial K_ATP channels has been strongly linked to the cardioprotective effects of several preconditioning protocols. The attenuation of intracellular Ca2+ overload, another proposed cardioprotective mechanism, is also inconsistently affected by these treatments. Acidosis increases the systolic Ca2+ transient (2), whereas BDM (3) and moderate hypothermia (16) do not alter the Ca2+ transient in cardiac muscle. Again, the known effect shared by all these protocols is a reduction in actomyosin Mg2+-ATPase activity. Thus these studies, along with our observation that κ-opioid receptor activation also decreases actomyosin Mg2+-ATPase activity (30), suggests that depressed actomyosin ATPase is a significant mechanism used to reduce ischemic damage in hearts. However, we acknowledge that further research into possible shared effects of these treatment protocols is required before being able to definitively attribute reduced ATPase as the mechanism of cardioprotection.

In the present study, we establish that PKC activation induces an approximate 20% reduction in maximum Ca2+-activated actomyosin Mg2+-ATPase. PKC also increases myofilament Ca2+ sensitivity such that at submaximal Ca2+ concentration, an approximate...
5% increase in tension occurs (29). This increase in tension may increase ATP use to such an extent as to negate any ATPase “savings.” However, our results show that ATP levels from isolated, Langendorff-perfused hearts (i.e., at submaximal Ca\textsuperscript{2+} concentration) both in the absence of ischemia (29) and after ischemia (Fig. 4) are significantly higher than those of controls. Thus PKC-induced reduction in actomyosin ATPase does appear to contribute to a conservation of ATP in the isolated heart. Moreover, previous work has demonstrated a significant PKC-dependent decrease in myofilament ATPase also occurs at Ca\textsuperscript{2+} concentration as low as 3 μM (31). Given localized Ca\textsuperscript{2+} has been calculated to reach 10 μM in contracting myocytes (7), we are confident in stating reduced ATPase does occur in the in vivo beating heart following PKC activation, and this contributes to the observed increase in ATP levels.

Agents that mediate preconditioning through PKC often simultaneously activate multiple PKC isoforms that have multiple cellular targets. Several studies have suggested PKC-ε is both sufficient and necessary for the mediation of myocardial protection. Introduction of a homologous PKC-ε RACK octapeptide increases PKC-ε association with cross-striated structures and reduces postischemic cell death (12). A cardioprotective effect is also seen in transgenic mice overexpressing the homologous PKC-ε RACK (12). Furthermore, overexpression of PKC-ε in rabbit cardiomyocytes reduces ischemic damage (27). Gray et al. (15) have reported a PKC-ε-specific antagonist (epsilon V1–2 peptide) abolishes preconditioning in cultured cardiac myocytes. Our findings are consistent with the previously published work of others who have outlined a cardioprotective role for PKC-ε. However, the results of the current study are novel in that we show κ-opioid and α-adrenergic receptor-dependent cardioprotection leads to an increase in PKC-ε associated with the myofilaments. Furthermore, we show for the first time that the κ-opioid receptor agonist U50,488H increases TnI and C-protein phosphorylation through a PKC-dependent pathway. These findings support the hypothesis that cardioprotection involves an increase in myofilament-associated PKC and PKC-dependent posttranslational modification of myofilament proteins.

Although PKC has been shown to be a significant component in the intracellular signaling cascade mediating cardioprotection (this study, reviewed in Ref. 23), some studies have suggested that downstream targets of PKC, including mitogen-activated protein kinases, contribute to the beneficial effects of preconditioning (27). Our study confirms previous work supporting a protective role for PKC in the rat heart; however, it does not address the possible involvement of signaling proteins that may be activated or inactivated downstream of PKC.

A number of intracellular mechanisms have been proposed to explain receptor-agonist induced improvements in postischemic myocardial function (reviewed in Ref. 23). Our studies do not refute, nor substantiate, these theories. Our current and previous studies (30) suggest a slowing of actin-myosin cycling may reduce Ca\textsuperscript{2+}-dependent actomyosin Mg\textsuperscript{2+}-ATPase activity to improve postischemic function. Results from the present study also indicate that activation of κ-opioid or α-adrenergic receptors increases the activity of myofibrillar-associated PKC-ε. Increases in PKC-ε associated with the myofilaments increase the phosphorylation levels of several myofibrillar proteins and reduce actomyosin Mg\textsuperscript{2+}-ATPase activity. From these data we propose slowing of the cyclic interaction between actin and myosin reduces ATP consumption and provides more ATP for ATP-dependent ion channels and pumps. Continued functioning of ATP-dependent ion channels and pumps during and after ischemia would act to maintain Ca\textsuperscript{2+} homeostasis and thus decrease protein degradation brought about by Ca\textsuperscript{2+}-activated proteases and cellular contracture.

Present address of W. G. Pyle: Dept. of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

DISCLOSURES

This study was supported by the National Heart, Lung, and Blood Institute Grant HL-48839 and was done during the tenure of an Established Investigatorship (P. A. Hofmann) of the American Heart Association.

REFERENCES


