Protein phosphatase 2A-mediated cross-talk between p38 MAPK and ERK in apoptosis of cardiac myocytes

Qinghang Liu and Polly A. Hofmann

Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee 38163

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Mounting evidence suggests a cross-talk between the p38 MAPK pathway and ERK or JNK pathways in a variety of eukaryotic cells. Recently, p38 MAPK-mediated inhibition of ERK activity was reported in the regulation of low-density lipoprotein-receptor expression (31). A growth-suppressing role of p38 MAPK by antagonism of the mitogenic ERK pathway has also been reported in carcinoma cells (2, 12). Furthermore, activation of p38 MAPK inhibits ERK and MAPK/ERK kinase (MEK) in NIH 3T3 cells (37). These observations led us to hypothesize that a cross-talk mechanism exists in ventricular myocytes whereby p38 MAPK inhibits the oxidative stress-induced ERK pathway. Cross-talk between p38 MAPK and ERK pathways, which have opposing effects in cell apoptosis, would represent a novel mechanism for the regulation of oxidative stress-induced apoptosis.

The cellular mechanism underlying cross-talk between p38 MAPK and ERK pathways remains unknown. However, it is clear that regulation of MAPKs involves a dynamic interplay between kinases and phosphatases. ERKs are activated by phosphorylation on both conserved threonine and tyrosine residues and are inactivated upon dephosphorylation by dual-specificity phosphatases, tyrosine phosphatases, and serine-threonine phosphatases (4, 11, 15, 27, 41). Serine-threonine protein phosphatase 2A (PP2A) can dephosphorylate MEK and ERK-family kinases in vitro (4, 5, 14, 15, 27, 41). Moreover, inhibition of PP2A leads to activation of MEK and ERK (17, 29). However, the precise role of PP2A in the regulation of the ERK signaling pathway in vivo has yet to be determined. We wanted to determine whether PP2A associates with and modulates components of the ERK pathway in ventricular myocytes.

Recent studies (37) demonstrate that activation of p38 MAPK by arsenite blocks the activation of ERK via a serine-threonine phosphatase in NIH 3T3 fibroblasts. It was also reported (6) that p38 MAPK, through PP2A, regulates activation of the JNK pathway in human neutrophils. In addition, we previously reported (22) that PP2A is modulated by a p38 MAPK-dependent pathway in adult ventricular myocytes. Therefore, we hypothesized that p38 MAPK inhibits ERK signaling through a PP2A-mediated mechanism in ventricular myocytes during oxidative stress.

Findings from the present study demonstrate that p38 MAPK activation does exert inhibitory effects on ERK signaling in ventricular myocytes. We present evidence that PP2A physically associates with the ERK signaling components ERK and MEK. In addition, ERK-associated PP2A activity is increased by H2O2 stimulation through a p38 MAPK-dependent mechanism that allows for interaction of two opposing MAPK pathways during oxidative stress.

Address for reprint requests and other correspondence: P. A. Hofmann, Dept. of Physiology, Univ. of Tennessee Health Science Center, 884 Union Ave., Memphis, TN 38163 (E-mail: phofmann@physiol1.utmem.edu).

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pathway. Oxidative stress-induced apoptosis in ventricular myocytes was also decreased through inhibition of p38 MAPK or PP2A and was increased through inhibition of the ERK pathway. Therefore, our data support a role for PP2A downstream of p38 MAPK in the negative regulation of the ERK pathway. We conclude that the cross-talk between p38 MAPK and ERK through a PP2A-mediated mechanism provides a novel pathway for the regulation of apoptosis in ventricular myocytes.

MATERIALS AND METHODS

Isolation of adult rat ventricular myocytes. All animals were handled in accordance with the guidelines of the Animal Care and Use Committee at the University of Tennessee Health Science Center. Ventricular myocytes were enzymatically isolated from adult Wistar rats (body wt, 200–250 g) as previously described (22).

Western analysis of MAPKs. Activations of ERK and p38 MAPK were assessed by Western blotting with antibodies that specifically recognize the phosphorylated, active forms of these kinases. The phosphorylation states of ERK and p38 MAPK were determined using an anti-phospho-ERK and an anti-phospho-p38 MAPK antibody (catalog no. 9101 and 9211, New England Biolabs; Beverly, MA). Total p38 MAPK and ERK protein values were determined with an anti-ERK (catalog no. sc-93, Santa Cruz Biotechnology; Santa Cruz, CA) and an anti-p38 MAPK antibody (catalog no. 9212, New England Biolabs). The immunoblots were visualized using enhanced chemiluminescence (New England Biolabs). Densitometry was performed on scanned images using NIH Image 1.6 software, and values were normalized for corresponding controls in each experiment.

Immunoprecipitation. Immunoprecipitation was performed as previously described with some modifications (8). Ventricular myocytes were lysed, and the supernatant was precleared with protein A/G agarose and rabbit/mouse IgG. Immunoprecipitations were performed with either an anti-ERK (catalog no. sc-93, Santa Cruz Biotechnology), anti-PP2A (catalog no. 05-421, Upstate Biotechnology; Lake Placid, NY), anti-MEK (catalog no. 06-269, Upstate Biotechnology), or rabbit/mouse IgG and subsequent immunoblotting with an anti-PP2A, anti-ERK, or anti-MEK antibody.

PP2A phosphatase-activity assay. Ventricular myocytes were pretreated with 5 μmol/l SB-203580 or vehicle for 1 h and then 100 μmol/l H2O2 for 15 min. Immunoprecipitation was performed using an anti-ERK or anti-PP2A antibody as described above. PP2A enzymatic activity in the immunocomplex was assayed according to the manufacturer’s protocol (V2460, Promega; Madison, WI). The reaction was initiated by the addition of 3 μl of 1 mmol/l phosphopeptide substrate and was incubated at 37°C for 10 min. The reaction was terminated by addition of 50 μl of molybdate dye-additive mixture. Absorbance of the supernatant was measured at 630 nm. Phosphatase activity was calculated using a phosphate standard curve. PP2A activity was defined as the activity that was inhibited by incubation of the immunoprecipitates with 5 mmol/l okadaic acid (OA) for 30 min.

Determination of cell apoptosis by cell death-detection ELISA. Isolated adult ventricular myocytes were incubated according to the protocol of Zhou et al. (42) with some modifications. Ventricular myocytes were pretreated with 5 μmol/l SB-203580, 10 μmol/l PD-98059, 10 μmol/l OA, 1 μmol/l fostriecin (Fos), or vehicle for 1 h followed by treatment with 100 μmol/l H2O2 for 6 h. Cell extracts were then prepared according to the manufacturer’s protocol, and histone-associated DNA fragments in the cytosolic fraction of the cell lysates were measured spectrophotometrically at 405 nm (kit no. 1774425, Boehringer-Mannheim; Indianapolis, IN).

Evaluation of caspase-3 cleavage by Western blot analysis. Cells were pretreated as indicated and exposed to vehicle or 100 μmol/l H2O2 for 6 h. They were then washed twice with cold Dulbecco’s phosphate-buffered saline buffer and lysed in ice-cold CHAPS cell extract buffer that contained 50 mmol/l HEPES (pH 7.4), 2 mmol/l EDTA, 1 mmol/l DTT, 0.1% of 3-(3-cholamidopropyl)-dimethylammonio)-1-propanesulfonate (CHAPS), 0.1% Triton X-100, 20 μg/ml leupeptin, 10 μg/ml pepstatin A, 10 μg/ml aprotinin, and 1 mmol/l PMSF. After 30 min of incubation on ice, the lysate was centrifuged at 10,000 g at 4°C for 10 min. The supernatant was used to determine uncleaved and cleaved caspase-3 values. Western blotting was performed with an anti-caspase-3 antibody (catalog no. sc-7148, Santa Cruz Biotechnology) and horseradish peroxidase-conjugated secondary antibody (1:16,000 dilution; A0545, Sigma). Caspase-3-reactive bands were visualized with enhanced chemiluminescence.

Statistical analysis. All data were analyzed by two-way ANOVA and the appropriate post hoc test. All values are expressed as means ± SE. P < 0.05 was chosen to indicate statistical significance.

RESULTS

Inhibition of p38 MAPK or PP2A enhances H2O2-stimulated ERK activation in ventricular myocytes. The effects of oxidative stress on the MAPK signaling pathway were determined in adult rat ventricular myocytes. Time courses of p38 MAPK and ERK activation in ventricular myocytes are shown in Fig. 1. A rapid p38 MAPK phosphorylation was observed after H2O2 exposure with a maximum response observed at 15 min that remained elevated for at least 60 min (Fig. 1A). Transient ERK phosphorylation was stimulated by H2O2 with a maximum response observed at 5 min and a return to basal level after 30 min (Fig. 1B). No significant effect on JNK phosphorylation was observed after 15 min of H2O2 exposure with a relative JNK phosphorylation of 1.14 ± 0.08 (n = 3) compared with control.

To establish whether ERK activation is modulated by p38 MAPK, ERK phosphorylation was measured in ventricular myocytes pretreated with the p38 MAPK inhibitor SB-203580 and subsequently stimulated with H2O2. H2O2-induced ERK phosphorylation was significantly increased by SB-203580 (Fig. 2, A and B), which suggests that p38 MAPK exerts an inhibitory effect on H2O2-stimulated ERK activation. Increased ERK phosphorylation was also observed in ventricular myocytes pretreated with SB-203580 in the absence of H2O2 but to a lesser extent than in H2O2-stimulated myocytes. Total ERK levels were not affected by H2O2 stimulation or p38 MAPK inhibition. Additional studies using the p38 MAPK inhibitor SB-239063 confirmed that H2O2-induced ERK phosphorylation was increased by p38 MAPK inhibition (Fig. 2C). In addition, the inactive analog SB-202474 had no effect on ERK phosphorylation.

To determine whether p38 MAPK regulates H2O2-induced ERK activation via PP2A, we examined the effects of the PP2A inhibitor OA on H2O2-induced ERK activation. OA augmented ERK phosphorylation in H2O2-stimulated cells and also increased ERK phosphorylation in non-H2O2-stimulated cells (Fig. 2). Similar effects were obtained when another PP2A inhibitor, Fos (1 μM), was used (data not shown).

The ability of the ERK pathway to modulate p38 MAPK was assessed by 10.220.32.246 on April 1, 2017 http://ajpheart.physiology.org/ Downloaded from .
activate p38 MAPK in cardiac myocytes was confirmed by Western blotting with phospho-p38 MAPK antibodies (Fig. 4A). We then tested the hypothesis that p38 MAPK activation with sodium arsenite can attenuate H$_2$O$_2$-induced ERK activation in ventricular myocytes (Fig. 4B). Sodium arsenite decreased both basal and H$_2$O$_2$-stimulated ERK phosphorylation. This inhibitory effect of sodium arsenite was reversed upon pretreatment of myocytes with the p38 MAPK inhibitor SB-203580 or the PP2A inhibitor OA before sodium arsenite exposure.

**PP2A physically associates with ERK and MEK in ventricular myocytes.** To evaluate the possible intracellular interactions of PP2A with components of the ERK signaling pathway in ventricular myocytes, lysates of ventricular myocytes were immunoprecipitated with anti-PP2A, anti-ERK, or anti-MEK antibodies, and the associated proteins were detected by Western blotting with relevant antibodies. PP2A was readily detectable in the ERK immunoprecipitates (Fig. 5A). Consistent with that, ERK was precipitated by an antibody to PP2A (Fig. 5B). The interaction between ERK and PP2A was specific, as IgG did not precipitate ERK or PP2A. We then examined the possible association of PP2A and MEK, a kinase upstream of...
of the protein phosphatase activity associated with ERK. H₂O₂ induces p38 MAPK-dependent pathway. To investigate whether H₂O₂ selectively inhibits PP2A in vitro, PP2A activity was measured in ERK immunoprecipitates with 5 nmol/l OA, a concentration that over other protein phosphatase activities. Once ERK was immunoprecipitated from both control and H₂O₂-treated cells (Fig. 7A). The association of PP2A with ERK was increased upon H₂O₂ treatment (Fig. 7B). Moreover, inhibition of p38 MAPK by SB-203580 attenuated H₂O₂-induced DNA fragmentation. In contrast, when cells were pretreated with the MEK inhibitor PD-98059 (10 μmol/l), H₂O₂-induced DNA fragmentation was further increased. PD-98059 significantly increased DNA fragmentation in H₂O₂-stimulated cells (Fig. 7C). No significant changes in association of PP2A with p38 MAPK were observed upon H₂O₂ treatment (data not shown).

To determine whether ERK-associated PP2A was enzymatically active and whether PP2A activity could be modulated by H₂O₂, PP2A activity in the ERK immunoprecipitates was assayed (Fig. 7C). The assay uses a synthetic phosphorylated peptide substrate selective for PP2A and an assay buffer without Ca²⁺ and Mg²⁺ to favor the measurement of PP2A activity. Incubation of ERK immunoprecipitates with 5 nmol/l OA, a concentration that selectively inhibits PP2A in vitro, inhibited 96.1 ± 2.4% (n = 3) of the protein phosphatase activity associated with ERK. H₂O₂ treatment of ventricular myocytes for 15 min led to an approximately twofold increase in ERK-associated PP2A activity (Fig. 7C). Furthermore, pretreatment of cells with the p38 MAPK inhibitor SB-203580 reversed H₂O₂-stimulated PP2A activity. Whole cell PP2A activity was not affected by H₂O₂ treatment. Whole cell PP2A activity values were 4.71 ± 0.27 in vehicle-
treated cells vs. 4.94 ± 0.38 pmol phosphate·min⁻¹·μg protein⁻¹ in H₂O₂-stimulated cells (n = 4). In addition, PP2A activity associated in PP2A immunoprecipitates was not altered by H₂O₂, with 16.29 ± 0.64 for vehicle-treated cells and 16.68 ± 0.95 pmol phosphate·min⁻¹·μg protein⁻¹ for H₂O₂-treated cells (n = 3). These observations suggest a specific ERK-associated PP2A activation by H₂O₂ in ventricular myocytes.

Inhibition of p38 MAPK or PP2A reduces H₂O₂-induced apoptosis. To determine whether the cross-talk between p38 MAPK and ERK via PP2A could influence H₂O₂-induced apoptosis, histone-associated DNA fragments in the cytoplasmic fraction were measured in adult cardiac ventricular myocytes pretreated with specific inhibitors before exposure to H₂O₂. There was an approximately fivefold increase in DNA fragments in the cytoplasmic fraction after H₂O₂ treatment (Fig. 8A). Pretreatment of ventricular myocytes with SB-203580 attenuated H₂O₂-induced DNA fragmentation. In contrast, when cells were pretreated with the MEK inhibitor PD-98059 (10 μmol/l), H₂O₂-induced DNA fragmentation was further increased. PD-98059 significantly increased DNA fragmentation in H₂O₂-stimulated cells (Fig. 7C). No significant changes in association of PP2A with p38 MAPK were observed upon H₂O₂ treatment (data not shown).

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mentation even in the absence of $H_2O_2$. Activation of p38 MAPK with sodium arsenite significantly stimulated DNA fragmentation in ventricular myocytes. This effect was inhibited by pretreatment with the p38 MAPK inhibitor SB-203580.

The effects of PP2A inhibition on $H_2O_2$-induced apoptosis were examined in adult ventricular myocytes. Pretreatment with either OA or Fos attenuated $H_2O_2$-induced DNA fragmentation (Fig. 8B). PP2A inhibition did not change the extent of spontaneous apoptosis in the absence of $H_2O_2$.

Capase-3 activation, monitored as the extent of caspase-3 cleavage, has been identified as a key effector step in cell apoptosis. As shown in Fig. 9, caspase-3 was detected as a 32-kDa band in unstimulated myocytes. The 32-kDa band decreased in intensity in response to $H_2O_2$ with an increase of a 17-kDa band observed. $H_2O_2$-induced conversion of uncleaved to cleaved active caspase-3 was markedly reduced by SB-203580 or OA.

**DISCUSSION**

Our data suggest that a cross-talk between p38 MAPK and ERK pathways via PP2A appears to exist in adult ventricular myocytes, and this novel pathway plays a role in the regulation of oxidative stress-induced apoptosis. We demonstrated in this study that in cardiac ventricular myocytes, 1) $H_2O_2$ leads to activation of both p38 MAPK and ERK; 2) inhibition of p38 MAPK enhances $H_2O_2$-stimulated ERK phosphorylation; 3) preactivation of p38 MAPK reduces $H_2O_2$-stimulated ERK phosphorylation via PP2A; 4) PP2A inhibition increases basal and $H_2O_2$-stimulated ERK phosphorylation; 5) PP2A coimmunoprecipitates with ERK and MEK in ventricular myocytes; 6) $H_2O_2$ increases ERK-associated PP2A enzymatic activity in a p38 MAPK-dependent fashion; and 7) $H_2O_2$-induced apoptosis was attenuated by inhibition of p38 MAPK and PP2A, whereas it was increased by MEK inhibition. Thus the cross-talk of proapoptotic p38 MAPK and antiapoptotic ERK pathways via PP2A provides a functional link between these two opposing signaling events in ventricular myocytes and represents a novel pathway to modulate apoptosis during oxidative stress (Fig. 10).

In the present study, $H_2O_2$ activated p38 MAPK and ERK in adult cardiac ventricular myocytes. Previous work has demon-
strated an inhibitory cross-talk between p38 MAPK and ERK signaling pathways in human hepatoma (31) and pancreatic cancer (12) cells. Here we provided evidence to demonstrate that p38 MAPK negatively regulates H2O2-induced ERK activation in cardiac ventricular myocytes. Ventricular myocytes were pretreated with 5 μmol/l SB-203580 (p38 MAPK inhibitor), 10 μmol/l PD-98059 (MEK inhibitor), or vehicle for 1 h before incubation with 100 μmol/l H2O2 or 100 μmol/l sodium arsenite for 6 h (A). Ventricular myocytes were pretreated with 10 nmol/l OA, 1 μmol/l fostriecin (Fos; PP2A inhibitor), or vehicle for 1 h followed by treatment with 100 μmol/l H2O2 for another 6 h (B). Cells were then lysed, and cytoplasmic histone-associated DNA fragments were determined by cell death ELISA. Data are from 4 independent experiments and are expressed relative to control values; *P < 0.05 compared with control (Con); #P < 0.05 compared with H2O2; †P < 0.05 compared with ARS.

Fig. 8. Inhibition of p38 MAPK (A) or PP2A (B) attenuated H2O2-induced DNA fragmentation in ventricular myocytes. Ventricular myocytes were pre-treated with 5 μmol/l SB-203580 (p38 MAPK inhibitor), 10 μmol/l PD-98059 (MEK inhibitor), or vehicle for 1 h before incubation with 100 μmol/l H2O2 or 100 μmol/l sodium arsenite for 6 h (A). Ventricular myocytes were pretreated with 10 nmol/l OA, 1 μmol/l fostriecin (Fos; PP2A inhibitor), or vehicle for 1 h followed by treatment with 100 μmol/l H2O2 for another 6 h (B). Cells were then lysed, and cytoplasmic histone-associated DNA fragments were determined by cell death ELISA. Data are from 4 independent experiments and are expressed relative to control values; *P < 0.05 compared with control (Con); #P < 0.05 compared with H2O2; †P < 0.05 compared with ARS.

Inhibition of p38 MAPK or PP2A (B) decreased H2O2-induced caspase-3 cleavage in ventricular myocytes. Ventricular myocytes were pretreated with 5 μmol/l SB-203580, 10 nmol/l OA, or vehicle for 1 h followed by exposure to 100 μmol/l H2O2 for 6 h. Uncleaved and cleaved caspase-3 in whole cell lysates were determined by Western blot analysis. Data are from 3 independent experiments and are normalized to control values; *P < 0.05 compared with control; #P < 0.05 compared with H2O2.

Fig. 9. Inhibition of p38 MAPK or PP2A decreased H2O2-induced caspase-3 cleavage in ventricular myocytes. Ventricular myocytes were pretreated with 5 μmol/l SB-203580, 10 nmol/l OA, or vehicle for 1 h followed by exposure to 100 μmol/l H2O2 for 6 h. Uncleaved and cleaved caspase-3 in whole cell lysates were determined by Western blot analysis. Data are from 3 independent experiments and are normalized to control values; *P < 0.05 compared with control; #P < 0.05 compared with H2O2.

Fig. 10. A proposed model of p38 MAPK-mediated PP2A inhibition of ERK activation. MEKK, MAPK/ERK kinase kinase; M KK3/6, MAPK kinase-3 and -6.
MAPK exerts a tonic inhibition on the ERK pathway under basal conditions. Our findings of a p38 MAPK-dependent inhibition of ERK phosphorylation in myocytes are consistent with the study of Westerman et al. (37), who demonstrated that specific activation of p38 MAPK by adenosine-derivato constitutively active MAPK kinase-3b inhibited ERK and MEK in NIH 3T3 fibroblasts. Furthermore, specific inhibition of p38 MAPK by a dominant-negative mutant of p38 MAPK or by SB-203580 was shown to activate the ERK pathway in Hep3 cancer cells (2).

Previous studies have shown that SB-203580 inhibits Raf in vitro but paradoxically stimulates cellular Raf activity (16). This phenomenon could be due to a negative-feedback mechanism in which Raf inhibits itself. However, activation of Raf by SB-203580, unlike the physiological stimulation of Raf by growth factors, failed to activate downstream ERK activity or cell proliferation (16). Thus it is unlikely that the effect of SB-203580 on ERK phosphorylation was due to its effect on Raf. In addition, we demonstrated that another highly selective p38 MAPK inhibitor, SB-239063, which does not inhibit Raf (33), also increased basal and H2O2-stimulated ERK phosphorylation (see Fig. 2C).

The cross-talk between p38 MAPK and ERK in cardiomyocytes appears to be one way, because PD-98059 and OA failed to influence H2O2-induced p38 MAPK activation (see Fig. 3). This observation is consistent with the study of Singh et al. (31), which demonstrates that modulation of endogenous ERK activity does not affect p38 MAPK activation in human hepatoma cells. Similar results have also been obtained by Aikawa et al. (3) to show that H2O2-induced p38 MAPK activation was not affected by PD-98059 in neonatal cardiomyocytes.

The signaling events responsible for the negative cross-talk between p38 MAPK and ERK pathways are unknown. A recent study (37) demonstrated that activation of p38 MAPK can block the ERK pathway at the level of MEK through activation of PP1/PP2A. It is also known (6) that p38 MAPK negatively regulates JNK activation via PP2A in human neutrophils. Furthermore, PP2A can dephosphorylate and inactivate MEK and ERK families of kinases in vitro (4, 5, 14, 15, 27, 41). Here we showed that PP2A inhibitors OA and Fos increased ERK phosphorylation in control and H2O2-stimulated cells. This effect was similar to that seen with a p38 MAPK inhibitor. It has been established that OA at a concentration ≤1 μM selectively inhibits PP2A activity with PP1 only marginally affected in intact cells (13, 23). Fos is a very specific PP2A inhibitor with IC50 values of 3.2 nM for PP2A and 131 μM for PP1. By using these two selective PP2A inhibitors that have different sites of action on PP2A (34), we demonstrated that negative regulation of ERK activation by p38 MAPK involves activation of PP2A or PP2A-like activity.

In the present study, PP2A was found to associate with ERK and MEK in adult ventricular myocytes. To our knowledge, this is the first evidence to demonstrate the intracellular interactions between PP2A and these two components of the ERK pathway. Association of PP2A with ERK was increased by approximately twofold upon H2O2 treatment. The H2O2-induced increase in PP2A-ERK association was reversed by inhibition of p38 MAPK. This strongly suggests that the p38 MAPK-induced decrease in ERK phosphorylation is mediated by PP2A. Recently a dynamic association of PP2A and ERK-2 upon β2-adrenergic receptor activation was demonstrated in keratinocytes (30). The mechanism by which p38 activates PP2A is unknown. Rapid activation of PP2A by H2O2 suggests that the most likely mechanism is p38 MAPK-mediated post-translational modification of PP2A subunits. PP2A activities can be regulated by posttranslational modifications on the catalytic and regulatory subunits (19, 39).

PP2A may act on the ERK signaling cascade at multiple levels (27). In vitro studies demonstrated that PP2A dephosphorylates and inactivates both ERK and MEK (4, 5, 14, 15, 27, 41). In contrast, Raf-1, the immediate upstream activator of MEK, is stimulated by PP2A (1). Inhibition of PP2A by pharmacological or transfection strategies either has no effect or inhibits Raf-1 and increases the activity of ERK and MEK in noncardiac cells (17, 29). These observations along with our coimmunoprecipitation data suggest that PP2A may negatively regulate ERK signaling cascades at the level of MEK and ERK.

The finding that PP2A associates with ERK and MEK in control nonstimulated cells suggests that PP2A plays a tonic regulatory role in ERK phosphorylation under physiological conditions. This idea is further supported by the observation that inhibition of PP2A increased basal phosphorylation of ERK. Thus PP2A association with ERK may lead to a basal rate of dephosphorylation that ensures only a transient phosphorylation and activation of ERK.

The functional consequences of cross-talk between p38 MAPK and ERK were investigated in a model of H2O2-induced apoptosis in adult ventricular myocytes. Inhibition of p38 MAPK reduced H2O2-induced apoptosis in adult ventricular myocytes. Activation of p38 MAPK significantly increased apoptosis in ventricular myocytes. This suggests a proapoptotic role of p38 MAPK in ventricular myocytes during oxidative stress. In contrast, inhibition of ERK activation enhanced H2O2-induced apoptosis. This suggests that ERK activation plays a role in protecting ventricular myocytes from apoptosis. Thus our data are consistent with findings reported in neonatal cardiomyocytes, where ERK protects cardiomyocytes from oxidative stress-induced apoptosis (3, 7, 40), and p38 MAPK promotes apoptosis (9, 18, 24–26, 40, 45). In the present study, PP2A inhibition also attenuated H2O2-induced DNA fragmentation and caspase-3 cleavage. Hence, we believe that oxidative stress leads to a dual activation of p38 MAPK-dependent apoptotic and ERK-dependent survival pathways with cross-talk between these pathways provided by PP2A.

Cardiac muscle predominantly expresses the α- and β-isofoms of p38 MAPK (20). It is known that overexpression of p38-α leads to induction of cardiomyocyte apoptosis, whereas overexpression of p38-β results in characteristic features of cardiac hypertrophy (36). From this we speculate that p38-α may be involved in the negative regulation of ERK activation and subsequent induction of apoptosis in ventricular myocytes. However, additional investigation is required to delineate whether and how different p38 isoforms may influence apoptosis in ventricular myocytes.

In summary, the present study demonstrates a cross-talk mechanism between p38 MAPK and ERK signaling pathways via PP2A in ventricular myocytes. This represents a novel cellular mechanism that allows for interaction of two opposing
MAPK pathways and fine modulation of apoptosis during oxidative stress.

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