

The Molecular Mechanism Underlying Morphine-Induced Akt Activation: Roles of Protein Phosphatases and Reactive Oxygen Species

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Abstract Although Akt is reported to play a role in morphine's cardioprotection, little is known about the mechanism underlying morphine-induced Akt activation. This study aimed to define the molecular mechanism underlying morphine-induced Akt activation and to determine if the mechanism contributes to the protective effect of morphine on ischemia/reperfusion injury. In cardiac H9c2 cells, morphine increased Akt phosphorylation at Ser⁴⁷³, indicating that morphine upregulates Akt activity. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a major regulator of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling, was not involved in the action of morphine on Akt activity. Morphine decreased the activity of PP2A, a major protein Ser/Thr phosphatase, and inhibition of PP2A with okadaic acid (OA) mimicked the effect of morphine on Akt activity. The effects of morphine on PP2A and Akt activities were inhibited by the reactive oxygen species (ROS) scavenger *N*-(2-mercapto-propionyl)glycine (MPG) and the

mitochondrial K_{ATP} channel closer 5-hydroxydecanoate (5HD). In support, morphine could produce ROS and this was reversed by 5HD. Finally, the cardioprotective effect of morphine on ischemia–reperfusion injury was mimicked by OA but was suppressed by 5HD or MPG, indicating that protein phosphatases and ROS are involved in morphine's protection. In conclusion, morphine upregulates Akt activity by inactivating protein Ser/Thr phosphatases via ROS, which may contribute to the cardioprotective effect of morphine.

Keywords Morphine · Akt · Protein phosphatase 2A · Protein Ser/Thr phosphatase · ROS

Introduction

It is well known that opioids induce cardioprotection against ischemia/reperfusion injury by triggering acute and delayed precondition in various experimental models [1]. The cardioprotective effects of opioids have been attributed to the activation of δ [2–4] or κ [5, 6] opioid receptors. In addition to protein kinase C (PKC) [7, 8], mitochondria K_{ATP} channels [4, 9], tyrosine kinase [10], and mitogen-activated protein kinase (MAPK) [11, 12], Akt has been shown to play a critical role in opioid-induced acute cardioprotection [13–17]. Although the activated Akt may mediate cardiac cell survival through downstream signals such as GSK-3 β , eNOS, and mTOR [18, 19], the mechanism by which opioids (as well as other cardioprotective ligands) activate Akt remains unknown.

Akt was originally cloned as an oncogenic protein and is critical for cell growth, transcription regulation, and cell survival [20]. Akt is activated downstream of phosphatidylinositol 3-kinase (PI3K) in response to

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stimulation of receptor tyrosine kinases and G-protein coupled receptors (GPCRs) [21]. Activation of PI3K leads to generation of phosphatidylinositol-3',4',5'-triphosphate (PIP₃), which in turn translocates Akt to plasma membrane where Akt is activated through phosphorylation of Thr308 and Ser473. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) negatively regulates the PI3K/Akt signaling by dephosphorylating PIP₃ to phosphatidylinositol-3',4'-triphosphate (PIP₂). Thus, down-regulation of PTEN activity by phosphorylation or oxidation results in Akt activation [22]. Akt activity is also negatively regulated by dephosphorylation of Thr308 and Ser473 sites by protein Ser/Thr phosphatases such as PP2A [23]. Accordingly, an inhibition of Ser/Thr phosphatases may lead to Akt activation.

The purpose of this study was to determine the molecular mechanism by which morphine regulates Akt activity. First, we tested if morphine activates Akt in cardiac H9c2 cells. Second, we examined if morphine activates Akt by inactivating PTEN. Third, we determined the role of PP2A in the action of Akt. Fourth, we investigated the potential mechanism by which morphine alters PP2A activity, focusing on the role of reactive oxygen species (ROS). Finally, we investigated the roles of PP2A and ROS in the cardioprotective effect of morphine.

Materials and Methods

Cell Culture

Rat heart tissue-derived H9c2 cardiac myoblast cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U penicillin/streptomycin at 37°C in a humidified 5% CO₂–95% air atmosphere.

Chemicals and Antibodies

Morphine, *N*-(2-mercaptopropionyl)glycine (MPG), 5-hydroxydecanoate (5HD), and okadaic acid (OA), wortmannin were purchased from Sigma Chemical (St. Louis, MO). AKTI and wortmannin were purchased from EMD Biosciences (La Jolla, CA). PP2A activity assay kit was purchased from Promega (Madison, WI). Antibodies were obtained from Cell Signaling Technology (Beverly, MA).

Western Blotting Analysis

Equal amount of protein lysates (whole cell or mitochondria or cytosol) was loaded and electrophoresed on

SDS–polyacrylamide gel and transfected to a PVDF membrane. Membranes were probed with primary antibodies. Each primary antibody binding was detected with a secondary antibody and visualized by the ECL method. Equal loading of samples were confirmed by reprobing membranes with anti-tubulin or anti-GAPDH antibody.

Identification of Reduced and Oxidized Forms of PTEN

The reduced and oxidized forms of PTEN were analyzed with Western blotting as previously described [24].

PP2A Activity Assay

PP2A activity was determined using Ser/Thr phosphatase assay kit (Promega) according to the manufacturer's instruction. Briefly, cell lysates (25 µl) were centrifuged at 15,000×*g* for 1 h after a short sonication. Endogenous-free phosphate was removed from the lysate supernatant with Sephadex G-25 resin spin column. Ser/Thr phosphate activity was measured colorimetrically by the capacity to dephosphorylate a synthetic –754 Da phosphopeptide through formation of molybdate. The total Ser/Thr phosphatase activity for each sample was expressed as a percentage of the value measured in cell lysate without PP2A substrates.

Measurement of Intracellular ROS

Cardiomyocytes cultured on a 24-well tissue culture plate were incubated with 20 µM dichlorodihydrofluorescein diacetate (H₂DCFDA) at 37°C for 20 min. After washing with phosphate buffered saline (PBS), the cells were incubated in standard tyrode solution including (mM) NaCl 140, KCl 6, MgCl₂ 1, HEPES 5, CaCl₂ 1.8, and glucose 5.8 (pH 7.4) for ROS measurements. DCF fluorescence was measured with a fluorescent plate reader. The fluorescence was excited at 480 nm and collected at 530 nm. The change in fluorescent intensity for each experimental group was expressed as a percentage of respective control value. Temperature was maintained at 37°C throughout the experiment.

Cell Viability Assay

The cell viability was assessed by propidium iodide (PI) fluorometry using a fluorescence reader (SpectraMax, Molecular Devices, Sunnyvale, CA). Fluorescence intensity was measured at excitation and emission wavelengths of 540 and 590 nm, respectively. Cells in 12-well plates coated with laminin were incubated in standard tyrode solution containing (mM) NaCl 140, KCl 6, MgCl₂ 1, CaCl₂ 1, HEPES 5, and glucose 5.8 (pH 7.4) for 2 h before

experiments. Background fluorescence intensity (B) was measured 20 min after addition of PI (30 μM). Cells were then subjected to 90 min simulated ischemia followed by 30 min of reperfusion (see experimental protocols). After 30 min of reperfusion, fluorescence intensity (R) was measured again. Experiments were terminated by addition of digitonin (300 μM). The final fluorescence intensity (F) was measured 20 min after addition of digitonin. The cell viability was calculated by the following formula: $100(F - R)/(F - B) \%$.

Experimental Protocols

To examine the effects of morphine on Akt, PTEN, and PP2A activities, cells were exposed to 0.1 μM morphine for 20 min. Inhibitors were applied 10 min before exposure to morphine. To test the effect of morphine on cardiac ischemia/reperfusion injury, cells were exposed to a simulated ischemia solution (glucose-free tyrode solution containing 10 mM 2-deoxy-D-glucose and 10 mM sodium dithionite) for 90 min followed by 30 min of reperfusion with the normal tyrode solution. Morphine (0.1 μM) was applied 20 min before the onset of ischemia.

Statistical Analysis

Data are expressed as mean \pm SEM and obtained from at least six experiments (n in figure legends). Statistical significance was determined using one-way ANOVA followed by Tukey's test. A value of $P < 0.05$ was considered as statistically significant.

Results

To test if morphine could increase Akt activity in H9c2 cells, we detected the phosphorylation status of Akt at Ser⁴⁷³. As shown in Fig. 1a, morphine increased Akt phosphorylation in a dose-dependent manner with the peak at 0.1 μM , indicating that morphine can activate Akt in H9c2 cells. However, this effect was lost at a higher concentration of 10 μM . The effect of morphine (0.1 μM) was reversed by the Akt inhibitor AKTI (10 μM).

To determine the potential role of PTEN in the action of morphine on Akt, we tested the effects of morphine on total PTEN protein levels and PTEN phosphorylation at Ser³⁸⁰/Thr^{382–383}. Figure 2a shows that morphine did not alter either the total or the phosphorylated PTEN. Further experiments with cells treated with H₂O₂ resulted in the appearance of the higher-mobility form of PTEN (the oxidized form) (Fig. 2b). In contrast, cells treated with morphine for 20 min did not show this form. Further

experiments showed that PTEN was not oxidized by morphine 5 or 10 min after the exposure to morphine (bottom, Fig. 2b). These results suggest that PTEN may not be involved in the action of morphine on Akt.

To test if morphine activates Akt by inhibiting Ser/Thr protein phosphatases, we examined the effect of morphine on the activity of PP2A, a major Ser/Thr phosphatase. As shown in Fig. 3a, morphine significantly inhibited PP2A activity, suggesting that morphine may activate Akt by inhibiting Ser/Thr protein phosphatases. To investigate the mechanism by which morphine inactivates Ser/Thr phosphatases, we tested if *N*-(2-mercapto-propionyl)glycine (MPG), a scavenger of ROS, could alter the inhibitory action of morphine on PP2A activity. The effect of morphine was inhibited by MPG (1 mM), suggesting that morphine may inhibit Ser/Thr phosphatases via ROS. The effect of morphine was further abrogated by the mitochondrial K_{ATP} channel closer 5HD (500 μM), whereas the mitochondrial K_{ATP} channel opener diazoxide (200 μM) mimicked the action of morphine to decrease PP2A activity, indicating that mitochondrial K_{ATP} channel opening accounts for the inhibitory effect of morphine on PP2A. In support, the protein Ser/Thr phosphatase inhibitor OA (20 nM) markedly increased Akt phosphorylation (Fig. 3b). These results suggest that morphine may activate Akt by inhibiting Ser/Thr phosphatases.

To confirm the roles of ROS and the mitochondrial K_{ATP} channels in the action of morphine, we examined if MPG and 5HD could inhibit the effect of morphine on Akt phosphorylation. As shown in Fig. 4a, morphine-induced Akt phosphorylation was inhibited by both MPG and 5HD. The mitochondrial K_{ATP} channel opener diazoxide increased Akt phosphorylation. Based on these observations, it is highly likely that morphine activates Akt by inhibiting Ser/Thr phosphatases via ROS. Since PKC plays a central role in morphine's protection, we tested if the PKC inhibitor chelerythrine (5 μM) could alter Akt phosphorylation by morphine. Figure 4b shows that the effect of morphine was not altered by chelerythrine, indicating that PKC does not contribute to morphine-induced Akt activation.

To further determine the role of ROS in the action of morphine, we tested if morphine produces ROS in H9c2 cells by measuring DCF fluorescence. Figure 5 shows that morphine significantly increased DCF fluorescence compared to the control, indicating that morphine can produce ROS in H9c2 cells. This effect of morphine was reversed by the mitochondrial K_{ATP} channel closer 5HD, suggesting that morphine generates ROS by opening mitochondrial K_{ATP} channels. The PI3K inhibitor wortmannin (0.1 μM) did not affect the effect of morphine on DCF fluorescence, implying that Akt may not act upstream of ROS generation in morphine's protection.

Fig. 1 a Western blot analysis of Akt phosphorylation (Ser⁴⁷³) in cardiac H9c2 cells. Morphine increased Akt phosphorylation in a dose-dependent manner ($n = 7$ each group). * $P < 0.05$ versus control (0 μM). **b** Western blot analysis of Akt phosphorylation (Ser⁴⁷³) in cardiac H9c2 cells. The effect of morphine on Akt phosphorylation was reversed by AKTI (10 μM , $n = 5$). * $P < 0.05$ versus control; # $P < 0.05$ versus morphine

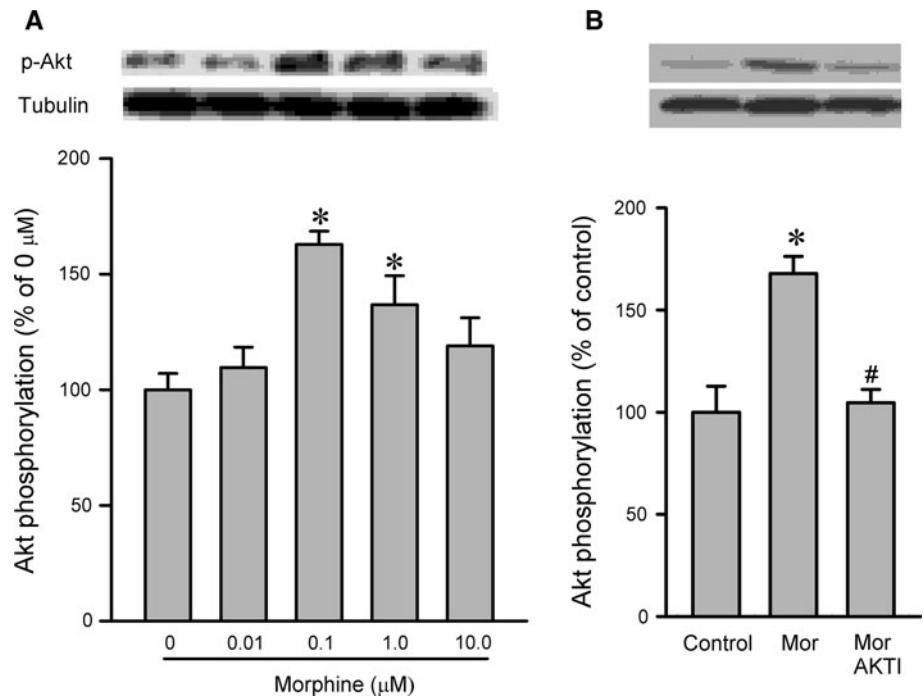
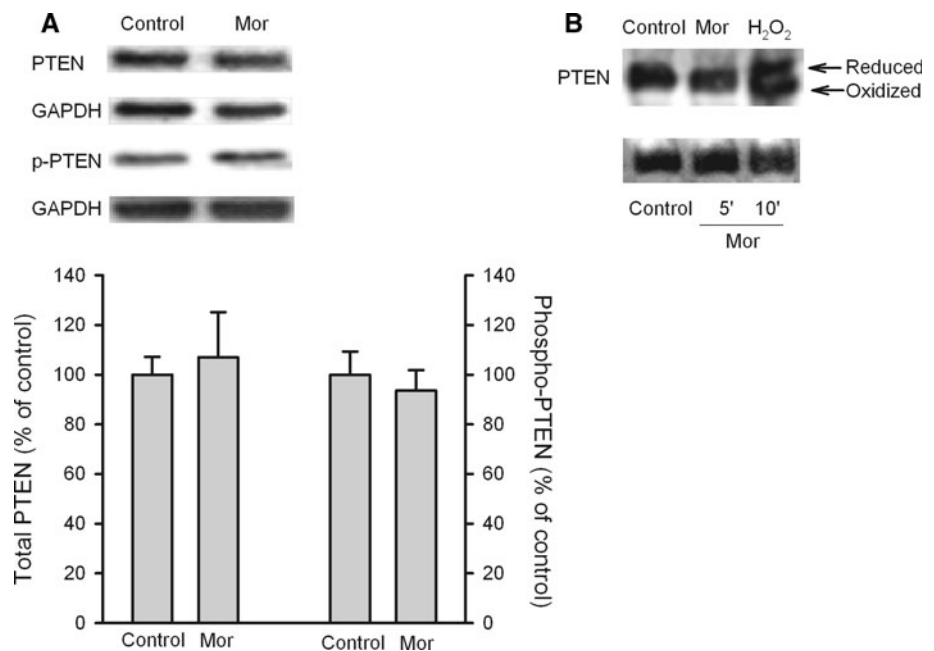


Fig. 2 a Western blot analysis of the total and phosphorylated PTEN (Ser³⁸⁰/Thr^{382–383}) levels in cardiac H9c2 cells. Neither the total PTEN protein level nor the phosphorylation of PTEN was altered by morphine (0.1 μM , $n = 6$ each group). **b** Identification of the reduced and oxidized forms of PTEN with Western blot. PTEN is oxidized by 500 μM H₂O₂, as indicated by the appearance of the higher-mobility form band (the oxidized form). The oxidized form was not seen in cells treated with morphine



Given the critical roles of ROS, Ser/Thr protein phosphatases, and the mitochondrial K_{ATP} channels in the action of morphine on Akt activity, these factors may contribute to the cardioprotective effect of morphine on ischemia/reperfusion injury. To assess the roles of these factors in morphine's cardioprotection, cardiac H9c2 cells were subjected to 90 min simulated ischemia followed by 30 min of reperfusion. As shown in Fig. 6a, compare to the control morphine (0.1 μM) dramatically increased cell viability. This effect of morphine was inhibited by 5HD,

whereas diazoxide increased cell viability, indicating an involvement of the mitochondrial K_{ATP} channels in morphine's protection. The ROS scavenger MPG also inhibited the protective effect of morphine, suggesting a role of ROS in morphine's protection. The protective effect of morphine was also reversed by wortmannin (0.1 μM). Further experiments showed that the protein Ser/Thr phosphatase inhibitor OA improved cell viability (Fig. 6b), supporting the role of the protein Ser/Thr phosphatases in the action of morphine. The effect of OA was reversed by wortmannin

Fig. 3 a Analysis of PP2A activity. Morphine (0.1 μM , $n = 6$) decreased PP2A activity in H9c2 cells compared to the control, an effect that was inhibited by MPG (1 mM) and 5HD (500 μM) ($n = 6$ in each group). Diazoxide (200 μM , $n = 6$) inhibited PP2A activity. * $P < 0.05$ versus control; # $P < 0.05$ versus morphine. **b** Western blot analysis of Akt phosphorylation (Ser⁴⁷³) in cardiac H9c2 cells. OA (20 nM, $n = 6$) increased Akt phosphorylation compared to the control ($n = 6$). * $P < 0.05$ versus control

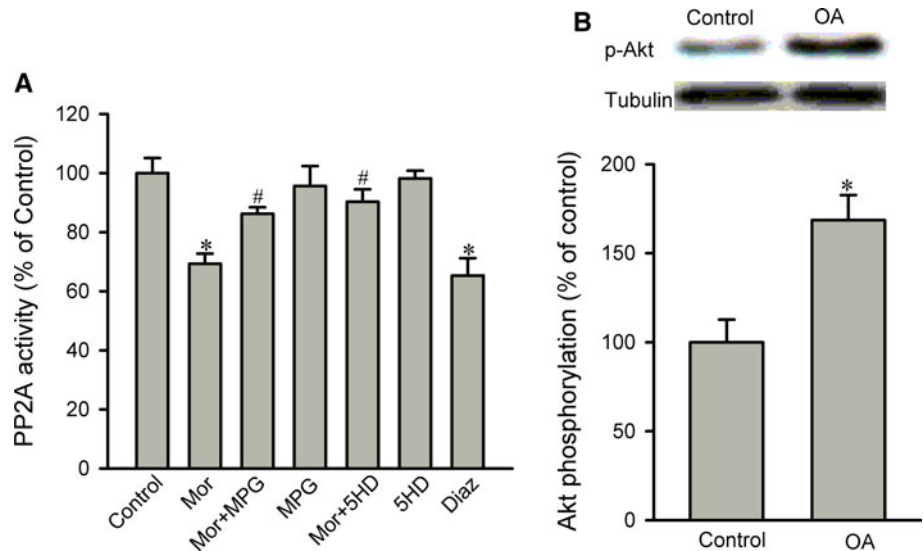
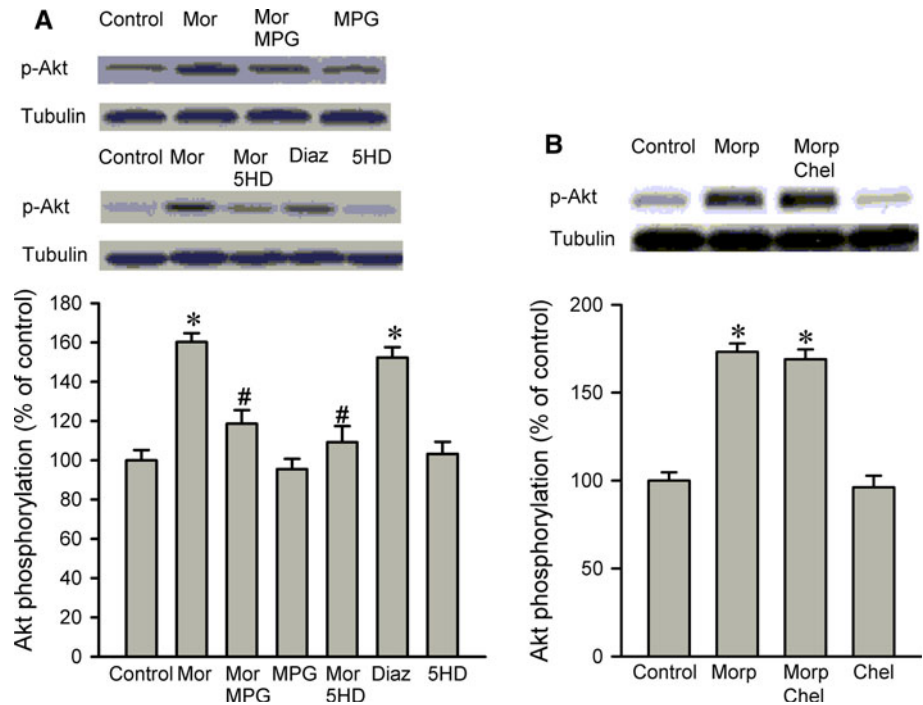


Fig. 4 a Western blot analysis of Akt phosphorylation (Ser⁴⁷³) in cardiac H9c2 cells. The effect of morphine ($n = 7$) on Akt phosphorylation was inhibited by MPG (300 μM , $n = 7$) and 5HD (500 μM , $n = 7$). Diazoxide (200 μM , $n = 7$) enhanced Akt phosphorylation. MPG or 5HD alone did not alter Akt phosphorylation. * $P < 0.05$ versus control; # $P < 0.05$ versus morphine. **b** Western blot analysis of Akt phosphorylation (Ser⁴⁷³) in cardiac H9c2 cells. The PKC inhibitor chelerythrine (5 μM , $n = 6$) did not alter the effect of morphine on Akt phosphorylation. * $P < 0.05$ versus control



but not by 5HD or MPG, implying that the mitochondrial K_{ATP} channel opening and ROS generation may occur upstream (but not downstream) of Ser/Thr protein phosphatases inhibition in the protective action of morphine.

Discussion

Akt plays an important role in cardioprotection against ischemia/reperfusion injury [21, 25]. Morphine protects the heart from ischemia/reperfusion injury triggering either

precondition [1] or postconditioning [26] mechanism, and Akt is proposed to mediate the protection [13, 16]. In this study, morphine at lower concentrations (0.1 and 1 μM) could activate Akt but the effect was lost at a higher concentration of 10 μM . This observation is consistent with the data of a previous study [9] in which the cardioprotective effect of morphine was seen at 0.1 and 1 μM but was lost at 10 μM . They proposed that stimulation of another opioid receptor (other than δ receptor) at the higher concentration of morphine antagonizes the protective effect mediated by δ receptor at low concentrations. Thus, it is

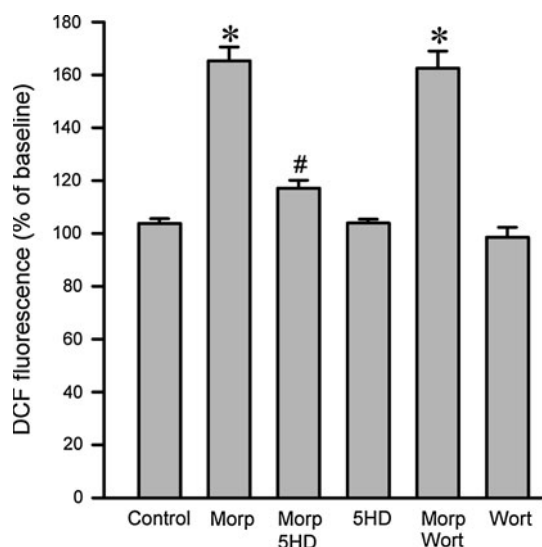


Fig. 5 Summarized data for DCF fluorescence intensity measured 20 min after exposure to morphine (0.1 μ M). Morphine ($n = 16$) increased DCF fluorescence intensity compared to the control ($n = 16$), which was reversed by 5HD (500 μ M, $n = 16$). 5HD alone ($n = 16$) did not alter the fluorescence intensity. The PI3K inhibitor wortmannin (0.1 μ M) did not alter the effect of morphine ($n = 14$). Wortmannin alone did not alter the fluorescence intensity. * $P < 0.05$ versus control; # $P < 0.05$ versus morphine

possible that in this study morphine at the concentration of 10 μ M may have also antagonized the Akt-activation action through the same mechanism.

Akt is activated downstream of PI3K in response to stimulation of membrane receptors [19, 21]. Thus, PI3K signaling is the major determinant of Akt activity. Under physiological conditions, the lipid and protein phosphatase PTEN negatively regulates the PI3K signaling by

dephosphorylating PIP3 generated by PI3K. Inhibition of PTEN may lead to cardioprotection against ischemia/reperfusion injury through the activation of the PI3K/Akt signaling pathway [22]. Indeed, it has been reported that loss of PTEN activity is responsible for induction of preconditioning in isolated rat hearts [27]. The importance of PTEN in cardioprotection was further demonstrated by a recent study showing that the loss of the cardioprotective effect of atorvastatin was associated with an increase in PTEN levels in the heart [28]. Thus, we sought to test if morphine activates Akt through inactivation of PTEN. Although the regulatory mechanism for PTEN activity remains unclear, phosphorylation and oxidation may deactivate PTEN [29]. In this study, we found that morphine did not alter PTEN phosphorylation levels, indicating that morphine may not inactivate PTEN through phosphorylation. Since PTEN is a constitutively active phosphatase, its activity also depends on its cellular level [22]. In this study, morphine did not alter cellular PTEN protein levels. Thus, it is unlikely that morphine upregulated Akt activity by regulating PTEN protein levels in this study. PTEN can also be oxidized and inactivated by ROS [24]. Since morphine induces preconditioning by producing ROS in the heart [30], it is worthy to test that morphine can oxidize PTEN and inhibit PTEN. However, our data showed that morphine did not oxidize PTEN. Therefore, it is tenable to propose that PTEN may not play a role in morphine-induced Akt activation in cardiac cells. Future studies directly measuring PTEN activity in the presence of morphine will give us a clearer answer.

Akt is activated by PDK-dependent phosphorylation of Ser⁴⁷³ and Thr³⁰⁸ residues [31]. Dephosphorylation of the

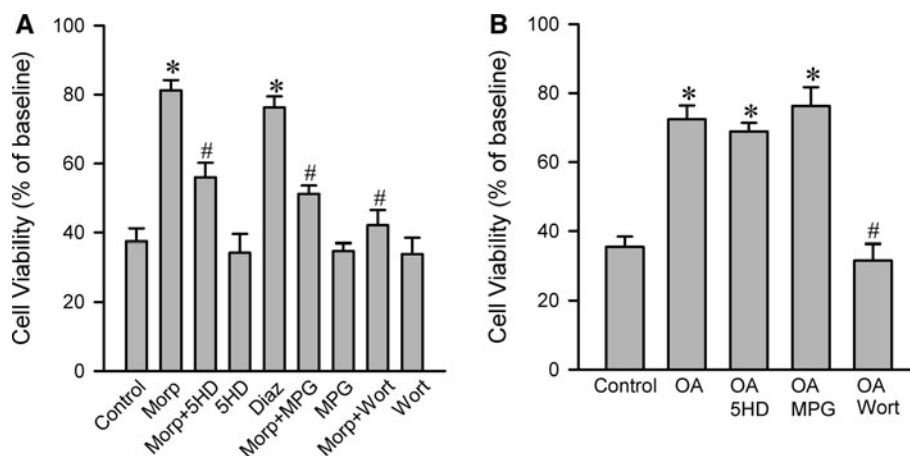


Fig. 6 a Cell viability assay in H9c2 cells subjected to 90 min simulated ischemia followed by 30 min of reperfusion. Morphine (0.1 μ M, $n = 6$) increased cell viability compared to the control ($n = 6$), an effect that was prevented by MPG (300 μ M, $n = 6$), 5HD (500 μ M, $n = 6$), and wortmannin (0.1 μ M, $n = 6$). Diazoxide increased cell viability (200 μ M, $n = 6$). * $P < 0.05$ versus control;

$P < 0.05$ versus morphine. **b** Cell viability assay in H9c2 cells subjected to 90 min simulated ischemia followed by 30 min of reperfusion. OA (20 nM, $n = 6$) increased cell viability and this was not altered by either 5-HD (500 μ M, $n = 6$) or MPG (300 μ M, $n = 6$). The effect of OA was reversed by wortmannin (0.1 μ M, $n = 6$). * $P < 0.05$ versus control; # $P < 0.05$ versus OA

two residues by Ser/Thr protein phosphatases can inactivate Akt [23, 32]. The major Ser/Thr protein phosphatases in mammalian cells are PP1, PP2A, and PP2B [33]. In this study, we tested if morphine upregulates Akt activity through inhibition of Ser/Thr protein phosphatases by measuring PP2A activity. We found that morphine decreased PP2A activity, suggesting that morphine may increase Akt activity by inactivating protein Ser/Thr phosphatases. We further demonstrated that inhibition of Ser/Thr protein phosphatases with OA also mimicked the action of morphine to activate Akt and to prevent ischemia/reperfusion injury, reinforcing the critical role of PP2A in morphine-induced Akt activation. The protective effect of PP2A inhibition was also demonstrated by a previous study in which fostriecin, an inhibitor of PP2A, protected rabbit hearts from ischemia/reperfusion injury by reducing infarct size, although PP2A is not involved in the mechanism of ischemic preconditioning [34]. Similarly, the Ser/Thr phosphatase inhibitors OA and calyculin A were also reported to protect cardiomyocytes from metabolic inhibition [35]. Based on these data, we propose that the activation of Akt by morphine could in major part be attributed to the ability of morphine to prevent dephosphorylation of the active form of Akt through inactivation of protein Ser/Thr phosphatases. A recent study by Lee et al. [36] has also demonstrated that exogenous zinc activates Akt through inhibition of protein Ser/Thr phosphatases and protects cardiac mitochondria from oxidative stress. Thus, chemicals that selectively and reversely inhibit protein Ser/Thr phosphatases are promising for the treatment of the patients with acute myocardial infarction.

Our data show that ROS are involved in the mechanism by which morphine inhibits protein Ser/Thr phosphatases. It has been reported that cadmium-induced ROS inhibit PP2A in PC12 cells [37]. Treatment of human T cells with H₂O₂ can also inactivate PP2A [38]. Thus, it is possible that ROS play a role in the inhibitory action of morphine on protein Ser/Thr phosphatases, since morphine protects the heart from ischemia/reperfusion injury by producing ROS in rabbit hearts [30]. In this study, the inhibitory effect of morphine on PP2A activity was partially but significantly blocked by the ROS scavenger MPG. Moreover, the effect of morphine on Akt phosphorylation was inhibited by MPG and the cardioprotective effect of morphine was inhibited by MPG. These observations clearly indicate that morphine activates Akt by inactivating PP2A via ROS, although the detailed mechanism through which ROS suppress PP2A activity remains explored. Our data also showed that the cardioprotective effect of OA was not altered by MPG, supporting that ROS act upstream but not downstream of Ser/Thr protein phosphatases inactivation in the protective effect of morphine. It is well known that ROS play an important role in the mechanism of ischemic

preconditioning presumably through activation of downstream pro-survival kinases such as ERK, Akt, and GSK-3 β [39]. However, little is known about the mechanism by which ROS activate these kinases. Since the activities of all these kinases are negatively regulated by protein phosphatases, the current finding that ROS inactivate PP2A may also shed light on the mechanism underlying ischemic preconditioning.

The PKC has been reported to play a critical role in opioid-induced cardioprotection [8, 40]. To examine the role of PKC in morphine-induced Akt activation, we tested the effect of the PKC inhibitor chelerythrine on Akt phosphorylation and found that chelerythrine did not alter the morphine's action of Akt activity. Thus, PKC may not be involved in ROS-induced Akt phosphorylation in this study, although PKC activation occurs downstream of ROS generation by mitochondrial K_{ATP} channel opening in the preconditioning mechanism [39, 41]. This finding indicates that PKC activation may occur downstream of Akt activation or is parallel to Akt activation in the action of morphine.

Ischemic preconditioning produces ROS by opening mitochondrial K_{ATP} channels and protects the heart from ischemia/reperfusion injury [42]. Acetylcholine and bradykinin also induce preconditioning by producing ROS through opening of mitochondrial K_{ATP} channels [43, 44]. Thus, it is conceivable that the opening of mitochondrial K_{ATP} channels is a critical step to produce ROS in the preconditioning mechanism. An earlier study demonstrated that morphine preconditions chick embryonic ventricular myocytes by producing ROS through mitochondrial K_{ATP} channels [45]. Similarly, Cohen et al. [30] documented that the anti-infarct effect of morphine in isolated rabbit hearts was blocked by MPG and 5HD, suggesting that the importance of ROS and mitochondrial K_{ATP} channels in morphine's cardioprotection. In this study, morphine was able to rapidly produce ROS in cardiac H9c2 cells and this was inhibited by 5HD, demonstrating that morphine produces ROS by opening mitochondrial K_{ATP} channels. Based on this finding together with the observation that morphine regulates Akt and PP2A activities, it is clear that opening of mitochondrial K_{ATP} channels by morphine leads to the generation of ROS, which in turn activates Akt via PP2A. However, it has been proposed that Akt activation may occur upstream of ROS generation in the triggering mechanism of preconditioning [39]. In this study, the ROS-producing effect of morphine was not altered by wortmannin, suggesting that Akt activation may not occur upstream of ROS generation, at least in H9c2 cells. The observation that the protective effect of OA was not affected by 5HD further authenticates our conclusion.

In summary (Fig. 7), morphine activates Akt by inhibiting Ser/Thr phosphatases via ROS, which may serve as an

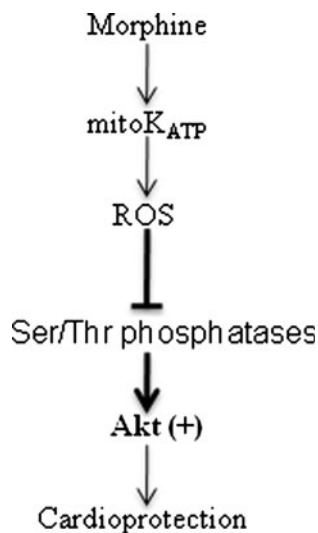


Fig. 7 Molecular mechanism by which morphine activates Akt

important mechanism whereby morphine protects cardiac cells from ischemia/reperfusion injury. The important regulator of the PI3K/Akt signaling PTEN appears not to be involved in the action of morphine. It is, therefore, reasonable to propose that chemicals reversely and selectively inhibiting Ser/Thr phosphatases may have great potentials to treat patients with acute myocardial infarction.

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