Acute p38 MAPK activation decreases force development in ventricular myocytes

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Chen, Yi, Ravi Rajashree, Qinghang Liu, and Polly Hofmann. Acute p38 MAPK activation decreases force development in ventricular myocytes. Am J Physiol Heart Circ Physiol 285: H2578–H2586, 2003.—Evidence suggests that p38 mitogen-activated protein kinase (MAPK) activation influences cardiac function on an acute basis. The characterization and mechanisms by which this occurs were investigated in the present study. Adult rat ventricular myocytes treated with 1 mM arsenite for 30 min had a 16-fold increase in p38 MAPK phosphorylation that was attenuated by SB-203580 (a p38 MAPK inhibitor). Extracellular signal-regulated protein kinase (ERK) and c-Jun NH2-terminal kinase (JNK) were also minimally activated, but this activation was not sensitive to SB-203580. In addition, arsenite caused a p38 MAPK-independent translocation/activation of protein phosphatase 2a (PP2a) and decrease in phosphorylation of myosin light chain 2 (LC2). Arsenite-p38 MAPK activation led to translocation of heat shock protein 27 but not αB-crystallin to the myofilaments. Using isolated cardiomyocytes, we determined that arsenite reduces isometric tension without a change in Ca2+ sensitivity of tension via p38 MAPK and lowers myofilament actomyosin Mg2+-ATPase activity in a p38 MAPK-independent manner. Thus arsenite reduces isometric tension through p38 MAPK phosphorylation of proteins that decreases myocardial force development.

Mitogen-activated protein kinase (MAPK) signal transduction pathways, which are an integral part of a cell’s stress response, work through changes in gene transcription and protein expression. However, a growing number of studies also suggest there are posttranslational effects of MAPK activation on cell and tissue functions. Specifically, the 38-kDa MAPK (p38 MAPK) has been implicated in causing posttranslational modifications and immediate translocation of cytosolic proteins to cytoskeletal elements and other nonnuclear domains. For example, p38 MAPK-dependent phosphorylation of heat shock protein 27 (HSP-27) triggers reorganization of F-actin in vascular endothelial cells (18). This in turn may modulate cell permeability and migration. In the heart, p38 MAPK activation causes an immediate decrease in developed pressure (25), and p38 MAPK has been implicated in both improving and exacerbating ischemia-reperfusion damage observed directly after ischemia (1, 37). The present study focuses on understanding if and how p38 MAPK activation influences cardiac function over the short term. p38 MAPK is activated by a number of pharmacological and pathological stimuli in the heart. β-Adrenergic (26) and adenosine (17) stimulation, reactive oxygen species (8), pressure overload (15), and ischemia and reperfusion (1, 37) all increase p38 MAPK activation. p38 MAPK activation increases or causes no change in intracellular Ca2+ (25, 26), phosphorylates small HSPs such as HSP-27 (8) and αB-crystallin (21), and activates p70 S6 kinase (38) acutely in hearts. It is unclear in what way the early events in p38 MAPK activation influence the cardiac force-producing machinery. It has been noted that the negative inotropic effects elicited by p38 MAPK activation can occur without changes in L-type Ca2+ currents or Ca2+ transients (25). Thus p38 MAPK may act by reducing myofilament Ca2+ sensitivity of force production (25). p38 MAPK may affect myofilament function via phosphorylation of αB-crystallin. Phosphorylation of αB-crystallin and HSP-27 causes translocation of these proteins to the Z band and may influence sarcomere stabilization (3, 16, 22). However, direct evidence of changes in myofilament function due to p38 MAPK is not available. Thus the specific goal of the present study was to determine the acute effect and mechanism by which p38 MAPK may alter myocardial contractile characteristics.

Arsenite, which is trivalent arsenic, is a commonly used tool to study the effects of p38 MAPK activation. The predominant effect of arsenite is a strong and sustained activation of p38 MAPK (21, 38). Thus arsenite-induced activation of p38 MAPK was used in the present study to examine the role of p38 MAPK on cardiac contractile function.

Materials and Methods

All procedures for this study were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Isolation of cardiac myocytes. Adult ventricular myocytes were collagenase-isolated according to the method of Lester...
et al. (23). Isolated cells were suspended in oxygenated Ringer solution that contained 1.3 mM CaCl₂, 0.1% bovine serum albumin, 118 mM NaCl, 4.8 mM KCl, 1.2 mM MgCl₂, 2 mM KH₂PO₄, 25 mM HEPES (pH 7.4), 5 mM pyruvate, 11 mM glucose, and 1 mM insulin. A 70% yield of viable cells was typical with isolations containing <50% of rod-shaped cells discarded.

**Cell treatment protocol.** Isolated myocytes were preincubated with 5 or 25 μM SB-203580, 10 μM SP-600125, or DMSO (vehicle) for 1 h at room temperature. SB-203580 is an inhibitor of p38 MAPK activation, whereas SP-600125 is known to inhibit c-Jun NH₂-terminal kinase (JNK). Sodium arsenite (1 mM final concentration) or saline (vehicle) was then added to the cells for 30 min or as otherwise indicated.

**Immunoblotting of MAPKs.** Treated cells were pelleted and resuspended in ice-cold digitonin solution, which contained 0.05% digitonin, 20 mM Tris·HCl (pH 7.4), 10 mM EGTA, and 5 mM EDTA (31). Digitonin treatment was for 5 min on ice with gentle trituration. Cells were then centrifuged at 12,500 g for 5 min at 0°C. The resulting supernatant, which contained the cytosolic fraction, was combined with urea sample buffer for electrophoresis. Western blot procedures were carried out according to our previously published methods (24). An increase in phospho-MAPK in the cytosolic fraction is one measure of the second-messenger state of myocytes are exposed to before functional measurements are made (see below).

Antibodies used to identify proteins in the MAPK pathway included the dual-phosphorylated (hence activated) form of p38 MAPK and total p38 MAPK, antibodies to phosphorylated JNK and total JNK, and antibodies to phosphorylated extracellular signal-regulated protein kinase (ERK) and total ERK. Antibodies were purchased from Santa Cruz Biotechnology and Cell Signaling Technology [catalog nos. SC-474, SC-6254, and SC-93 (1:500 dilution); CS-9101S, CS-9211, and CS-9212 (1:1,000 dilution)].

**Translocation of aB-crystallin, HSP-27, and protein phosphatase 2a.** For aB-crystallin studies, cells were pelleted and lysed with digitonin (see above) to obtain the cytosolic fraction. The postdigitonin pellet was used as the particulate fraction without further processing. Additional aB-crystallin localization studies were conducted using the extraction and fractionation procedures of Boelen et al. (4) and the Triton X-100-based preparation procedure used for determination of isometric tension. Results (not shown) were identical to those shown for the digitonin fractionation procedure. A positive control for aB-crystallin translocation was obtained by incubating cells at 45°C for 60 min.

For protein phosphatase 2a (PP2a) studies, cells were pelleted and lysed with digitonin to obtain the cytosolic fraction. The postdigitonin pellet was further processed by treatment with a 1% Triton X-100 solution that contained 10 mM HEPES (pH 7.4), 10 mM KCl, 1 mM DTT, 1 mM EDTA, 1.5 mM MgCl₂, and 25% glycerol. After 30 min of tritonization and centrifugation at 12,500 g, the supernatant contained the solubilized PP2a of the particulate fraction and was used for Western blot analysis. The pellet obtained after tritonization centrifugation contained no remaining immunoactive PP2a (data not shown). All fractions used for determination of PP2a localization were incubated in 0.1 N NaOH for 30 min in 30°C (5) before Western blot analysis. This alkalization fully demethylates PP2a and addresses concerns about the influence of methylation on immunoreactivity (5, 14). After NaOH treatment, samples were neutralized with HCl.

For HSP-27 studies, the Triton X-100-insoluble particulate fraction was used. After 30 min of tritonization and centrifugation at 12,500 g, the supernatant was removed, and the pellet that contained nonsolubilized HSP-27 was used in Western blot analysis.

**Western blot procedures utilized anti-aB-crystallin (1:2,000 dilution; catalog no. 238702, Calbiochem), anti-PP2a catalytic subunit antibodies (1:1,000 dilution; catalog no. 05-421, Upstate Biotechnology), and anti-HSP-27 (1:250 dilution; catalog no. SC-1049, Santa Cruz Biotechnology).**

**32P autoradiography of cardiac myofilament proteins.** Autoradiography using treated cells was carried out using procedures identical to those described in our previous publications (24, 33). Radioactive myocytes were not processed further because the added protocol would have increased technician exposure time. Myofilament phosphorylation patterns assessed with 32P autoradiography are similar in receptor-activated, membrane-intact cells and cells treated with 0.3% Triton X-100 for 5 min (20).

**Isometric tension as a function of Ca²⁺ concentration.** Treated cells were pelleted and resuspended in a saline solution (pCa 9.0) that contained 0.3% Triton X-100. After 5 min of tritonization, the demembranated cells were washed and placed on ice for use up to 24 h later. Skinned cardiomyocytes are used to control intracellular Ca²⁺ and remove the effects of possible changes in Ca²⁺ handling brought about by the treatment protocols. Mechanical measurements of myocytes were carried out using procedures and equipment identical to those described by Lester et al. (23) and Pyle et al. (33). In brief, myocytes were attached via glue (Great Stuff Foam, Insta-Foam; Marietta, GA) -coated glass micropipets to a piezoelectric translator and force transducer. Myocytes were observed and photographed to assess sarcomere length and sarcomere length uniformity. Cells that maintained a constant and uniform sarcomere length between 1.8 and 2.2 μM in a solution with pCa 4.5 and pH 7.0 were used. A pCa-pCa relationship was obtained by initially measuring force during maximal activation (pCa 4.5, pH 7.0), then measuring force during contractions at randomly chosen submaximal pCa and pH values, and then again at pCa 4.5 and pH 7.0 to assess any decline in cell performance. Maximum active tension was calculated as the difference between force generated in a pCa 4.5 solution and the passive tension measurement obtained in a pCa 0.0 solution (20). Only those cells that retained >75% of their maximum active tension from the first to the last contraction were included in the data analysis. Maximum tension, Ca²⁺ sensitivity of tension [pCa at which 50% tension is observed (pC₅₀)], and degree of cooperative activation (as judged by the steepness of the tension-pCa relationship) were assessed from the tension-pCa relationship. The maximum tension per cross-sectional area was calculated assuming a cell cross-sectional area with a cell depth that was 60% of the cell width (13). Composition of the pCa solutions can be found in the work of Lester et al. (23).

Ca²⁺-dependent actomyosin ATPase. Myofibrils from treated myocytes were prepared, and Ca²⁺ -activated actinomyosin ATPase values were determined according to our previously published methods (33). In brief, the rate of release of P was measured under the following conditions (in mM): 25 KCl, 5 MgCl₂, 3.15 NaATP, 20 imidazole, pH 7.0, 2 EGTA, and varying CaCl₂. The assay was performed using 1 mg/ml myofibrils. The reaction was terminated after 2 min at 32°C with the addition of 20% trichloroacetic acid. P, was determined using the method of Fiske and Subbarow. Protein concentrations were determined by the Lowry method.
RESULTS

Arsenite exposure induced a 16-fold increase in p38 MAPK phosphorylation in rat ventricular myocytes over a 30-min period compared with controls (Figs. 1 and 2). This effect was attenuated by pretreatment of the cells with the p38 MAPK inhibitor SB-203580 (Figs. 1–3). Arsenite also caused a transient phosphorylation of ERK at 5 and 15 min and an increase in the phosphorylation of JNK at 30 min (see Figs. 1 and 2); however, these effects on JNK and ERK were not significantly altered by pretreatment with 25 μM SB-203580 (data not shown).

The ability of the heat shock protein αB-crystallin to localize to specific proteins is one index of its activation. For example, incubating cells at 45°C for 45 min caused a redistribution of αB-crystallin from the cytosol to the particulate-myofilament fraction (Fig. 4). However, αB-crystallin translocation did not occur after exposure to 1 mM arsenite for 30 min at 25 or 37°C (Fig. 4). To insure that cell fractionation using digitonin did not influence the results (Fig. 4), the experiments at 25°C were repeated using two different Triton X-100-based lysis procedures (see MATERIALS AND METHODS). The use of Triton X-100 in the lysis buffer yielded

Statistics. A one- or two-way ANOVA was conducted and followed by a Tukey test. Values are expressed as means ± SE; P < 0.05 was selected to indicate significance.
similar results to those shown in Fig. 4 and confirmed a lack of effect by arsenite on αB-crystallin translocation in ventricular myocytes.

The ability of the HSP to localize to specific proteins is an index of its activation. Arsenite exposure caused an increased HSP-27 in the particulate-myofilament fraction (Fig. 5) compared with cells not treated with arsenite (control). SB-203580 (5 μM) blocked the increase in HSP-27 that was associated with the particulate-myofilament fraction compared with controls. SB-203580 treatment by itself caused a 28 ± 14% (mean ± SE; n = 4 myocytes) increase in HSP-27 relative to untreated cells that was most likely due to inhibition of the basal activity of p38 MAPK. Coomassie blue staining for protein on similarly loaded SDS gels demonstrated that all samples contained an equal concentration of protein (data not shown).

![Fig. 3](image1.jpg)

**Fig. 3.** Ratios of cumulative phosphorylated p38 MAPK to p38 MAPK in cytosolic fractions of ventricular myocytes exposed to 30 min of arsenite (Ars), 60-min pretreatment with the p38 MAPK inhibitor SB-203580 (SB; 25 μM), SB + Ars, or vehicle (Con). Data are means ± SE of results from 7 cell isolations; *P < 0.05 compared with Con or SB alone; †P < 0.05 compared with Ars.

![Fig. 4](image2.jpg)

**Fig. 4.** Typical Western blots (A) and cumulative data (B) of localization of heat shock protein 27 (HSP-27) with arsenite exposure in the presence and absence of 5 μM SB-203580. Ventricular myocytes were exposed to SB-203580 or vehicle (untreated) for 1 h before being exposed to 1 mM arsenite or vehicle (control) for 30 min, and the Triton X-100-insoluble particulate-myofilament fraction was isolated. Cumulative data are relative to the appropriate control (untreated or SB-203580 treated) and are presented as means ± SE of results from 4 cell isolations; *P < 0.05 compared with control, untreated cells.

![Fig. 5](image3.jpg)

**Fig. 5.** Typical Western blots (A) and cumulative data (B) of localization of heat shock protein 27 (HSP-27) with arsenite exposure in the presence and absence of 5 μM SB-203580. Ventricular myocytes were exposed to SB-203580 or vehicle (untreated) for 1 h before being exposed to 1 mM arsenite or vehicle (control) for 30 min, and the Triton X-100-insoluble particulate-myofilament fraction was isolated. Cumulative data are relative to the appropriate control (untreated or SB-203580 treated) and are presented as means ± SE of results from 4 cell isolations; *P < 0.05 compared with control, untreated cells.
Arsenite exposure caused translocation of the PP2a catalytic subunit to the particulate fraction (Fig. 6). Translocation of PP2a to specific subcellular domains has been associated with increased localized activity of PP2a (5, 14). Arsenite-dependent PP2a translocation was not attenuated by pretreatment of the cells with the p38 MAPK inhibitor SB-203580. SB-203580 treatment alone did not cause PP2a translocation. The relative particulate PP2a density with SB-203580 treatment in the absence of arsenite was 0.86 ± 0.08, value normalized to control density (mean ± SE; n = 5 myocytes).

$^{32}$P autoradiography demonstrated that phosphorylation of myosin light chain 2 (LC2) was decreased by short-term arsenite treatment of ventricular myocytes (Fig. 7). This decrease was not blocked by the p38 MAPK inhibitor SB-203580. Neither troponin I phosphorylation (Fig. 7) nor other myocardial proteins (data not shown) exhibited any change in basal phosphorylation levels with arsenite exposure.

The relationships between isometric tension as a function of Ca$^{2+}$ concentration at pH values of 7.0 and 6.6 were established in myocytes that had been treated with arsenite or vehicle and subsequently skinned. Arsenite caused a reduction in active tension at pH 7.0 from pCa 5.8 to 4.5 (Fig. 8A). Maximum isometric tension, pCa 4.5 at pH 7.0, decreased by ~25% with short-term arsenite exposure, and this decrease was blocked by pretreatment with the p38 MAPK inhibitor SB-203580 (Fig. 9A). The arsenite-induced decrease in isometric tension was not blocked by inhibition of JNK using SP-600125 (Fig. 9B). Assessment of the passive tension in a solution with pCa 9.0 and pH 7.0 revealed that there was no difference between control (n = 26 myocytes) and arsenite (n = 29 myocytes)-treated cells (0.27 ± 0.04 vs. 0.28 ± 0.03 g/mm$^2$, respectively). Normalization of tension to the maximum value established that the Ca$^{2+}$ sensitivity of tension did not change with arsenite treatment compared with vehicle treatment of the cells (see Fig. 8B). Neither the pCa$_{50}$ nor the slope (Hill coefficient) of the tension-pCa relationships were significantly influenced by arsenite exposure. At pH 7.0, the pCa$_{50}$ values were 5.72 ± 0.03 for control and 5.67 ± 0.05 for arsenite-pretreated cells. At pH 6.6, the pCa$_{50}$ value was 5.18 ± 0.04 for control and 5.15 ± 0.02 for arsenite-pretreated cells.

Mean Ca$^{2+}$-dependent actomyosin Mg$^{2+}$-ATPase activity values for myofilaments isolated from cells obtained after 30 min of vehicle or arsenite (1 mM) exposure were 452.8 ± 26.3 and 365.8 ± 25.5 nmol P$_i$·min$^{-1}$·mg$^{-1}$, respectively (Fig. 10). Thus arsenite significantly lowered myofilament mean Ca$^{2+}$-dependent actomyosin Mg$^{2+}$-ATPase activity. Pretreatment of cells with the p38 MAPK inhibitor SB-203580 did not block the ability of arsenite to decrease Ca$^{2+}$-dependent actomyosin Mg$^{2+}$-ATPase activity.

**DISCUSSION**

Adult ventricular myocytes exposed to arsenite for 30 min have an increased phosphorylation/activation of p38 MAPK, which in turn causes a decrease in the tension-generating abilities of the myofilament proteins without changing the myofilament Ca$^{2+}$ sensitivity of tension. This effect does not involve αB-crystallin localization to the sarcomeres but does increase localization of HSP-27 to the myofibril-containing fraction.
Through a p38 MAPK-independent manner, arsenite also increases PP2a activity that is associated with the myofilaments and decreases LC2 phosphorylation. Consistent with past studies (7, 30), we determined that decreased LC2 phosphorylation decreases Ca\(^{2+}\)/H\(_{11001}\)-dependent actomyosin Mg\(^{2+}\)/H\(_{11001}\)-ATPase activity.

A 10-min exposure to 0.5 mM arsenite leads to a 16-fold increase in the phosphorylation of p38 MAPK with no change in ERK phosphorylation in cultured neonatal cardiac myocytes (21). A 30-min exposure to 1 mM arsenite was shown to not affect JNK or ERK activation but to increase p38 MAPK activity five- to sixfold in adult cardiac myocytes (38). The present study confirmed the effects of arsenite to strongly activate p38 MAPK in ventricular myocytes and demonstrated modest increases in ERK and JNK that were not significantly reduced by SB-203580. SB-203580 greatly attenuated the arsenite-induced phosphorylation of p38 MAPK. Why SB-203580 blocks phosphorylation of p38 MAPK in some (present study and Refs. 19, 28, 39) but not all (29, 35) studies is a matter of controversy. We speculate this may be due to an SB-203580-induced conformational change in p38 MAPK that inhibits phosphorylation at one of the two phosphorylation sites. Thus a decrease in phospho-p38 MAPK level is seen only on SB-203580 exposure when a given monophospho-p38 MAPK antibody is used as the reporter. Nonetheless, it has been clearly demonstrated that SB-203580 inhibits the activity of p38 MAPK (11).

Liao et al. (25) demonstrated that a 3.4-fold increase in phosphorylation of p38 MAPK in adult rat ventricular myocytes leads to a 45% reduction in the extent of cell shortening. This effect was seen without changes in the Ca\(^{2+}\) transients, troponin I phosphorylation, or intracellular pH value. Liao and colleagues hypothesized that the myofilaments have a decreased responsiveness to Ca\(^{2+}\) that results in a decrease in cell shortening (25). The present study demonstrates that arsenite-induced p38 MAPK activation does not reduce the Ca\(^{2+}\) sensitivity of tension. This conclusion was reached using solutions at pH values of both 7.0 and 6.6. Acidosis is helpful to accentuate any differences in the Ca\(^{2+}\) sensitivity of myofilament tension between

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**Fig. 8.** Cumulative plot of absolute (A) or relative (B) tension as a function of pCa at pH values of 7.0 and 6.6 for ventricular myocytes that were exposed to arsenite or vehicle (control) and skinned. Absolute tension values are presented for data obtained at pH 7.0; data are normalized to cell cross-sectional area. Relative tension values were normalized to maximum active force produced at pCa 4.5 and pH 7.0 or 6.6. Each group represents the mean ± SE of results from 5 cells.

**Fig. 9.** Cumulative maximum active tension produced by ventricular myocytes treated with arsenite, SB-203580 (25 μM), SB-203580 + arsenite, or vehicle (Con) (A); or arsenite, SP-600125 (SP), SP-600125 + arsenite, or vehicle (B). After treatment, myocytes were skinned and attached to a force transducer, and maximum active tension was determined (see MATERIALS AND METHODS). Tension was normalized to the cross-sectional area (CSA) of the cells. Each group represents the mean ± SE of results from 15–29 individual cells; *P < 0.05 compared with either Con or SP.
Arsenite did cause a decrease in tension generation at all levels of activating Ca$^{2+}$. We believe this effect is mediated by p38 MAPK based on three observations. First, arsenite induced a 16-fold increase in phosphorylation of p38 MAPK and moderate increases in phosphorylation of ERK and JNK. SB-203580 exposure decreased the phosphorylation of p38 MAPK only. Second, the arsenite-induced decrease in tension is attenuated by the p38 MAPK inhibitor SB-203580. Third, there is a lack of effect of the JNK inhibitor SP-600125 on the arsenite-induced decrease in tension. Although our studies showed no effect of 25 μM SB-203580 on JNK, others have shown that SB-203580 at 25 μM can inhibit both p38 MAPK and JNK (9). As such, the JNK inhibitor SP-600125 was utilized to confirm the lack of involvement of JNK in the arsenite-induced decrease in tension.

The mechanism by which short-term arsenite exposure decreases tension in ventricular myocytes is not clear. We hypothesized that interaction of αB-crystallin with the myofilament scaffolding proteins might be involved. This hypothesis was based on the observations that arsenite induces phosphorylation of αB-crystallin (21), ischemia induces αB-crystallin phosphorylation and translocation to the Z line of myofibrils (16), and αB-crystallin binds to myofilament proteins such as titin and desmin (3, 16). However, our data suggest that it is unlikely that αB-crystallin is involved in the observed decrease in myofilament force, because arsenite-induced p38 MAPK activation did not cause αB-crystallin translocation to the myofilaments at 25 or 37°C. A second possibility we investigated was that HSP-27 is the link between p38 MAPK activation and myofilaments. Previous studies have demonstrated that p38 MAPK activation phosphorylates and causes the translocation of HSP-27 to the Z line of the sarcomeres (8, 34). In the present study, arsenite-p38 MAPK activation caused a translocation of HSP-27 to the myofibril-containing fraction of the ventricular myocytes. Blocking p38 MAPK activation with 5 μM SB-203580 blocked HSP-27 translocation.

The specific myofilament protein that is responsible for the p38 MAPK-dependent decrease in force is unknown. HSP-25 and -27 specifically bind to vinculin and α-actinin (12). Vinculin is located in costameres and intercalated disks; it links the cell membrane to Z bands/thin filaments. This allows for the distribution of force throughout and between myocytes. In our preparations, the cell membrane that contained vinculin was removed by detergent treatment before any mechanical measurements were made. Thus it seems unlikely that vinculin was involved in explaining our observations. α-Actinin is a sarcomeric protein associated with the Z line and is involved in linking the thin filaments to one another. This may allow force to be transmitted or distributed between sarcomeres. Others have shown that caspase-3 will cleave α-actinin, along with a number of other proteins, and decrease Ca$^{2+}$-activated force production of cardiomyocytes (10). These data taken together suggest that α-actinin is a reasonable candidate for mediating the decrease in force brought about by the proposed arsenite-p38 MAPK-HSP27 pathway. However, it is also worth noting that a decrease in force with no change in Ca$^{2+}$ sensitivity of tension is also observed in studies in which the intermediate filament desmin is genetically knocked out (2). Additional studies are required to establish how p38 MAPK decreases force production.

Our studies demonstrate that arsenite also causes a translocation of PP2a in ventricular myocytes but that p38 MAPK is probably not involved. Additional studies are needed to delineate the pathway from arsenite to PP2a activation. One consequence of PP2a activation appears to be LC2 phosphorylation. This fits with a recent study that demonstrates that PP2a can readily dephosphorylate one of the regulator factors of LC2 phosphatase and thereby increase its activity and lead to the dephosphorylation of LC2 in smooth muscle (37). A known functional effect of LC2 phosphorylation is an increase in actomyosin ATPase activity. Taken together, these studies support an arsenite-PP2a-LC2 pathway of action that is separate from p38 MAPK-HSP27 signaling. We speculate that Ca$^{2+}$-induced PKC activation may be key to the ability of arsenite to activate PP2a in cardiomyocytes, because arsenite has been shown to activate PKC in epidermal JB6 cells (6), and PKC can alter PP2a activity (reviewed in Ref. 36).

It should be noted that a recent study using cultured adult rat cardiomyocytes with adenoviral transfection of a constitutively active kinase upstream of p38 MAPK demonstrated a p38 MAPK-dependent decrease in maximum velocity of cell shortening (25). These changes were attenuated by coexpression of a p38 MAPK dominant-negative mutant (25). Maximum ve-
locity of cell shortening depends on Ca\(^{2+}\) transients and the Ca\(^{2+}\)-dependent actomyosin Mg\(^{2+}\)-ATPase activity. Thus our observation that p38 MAPK inhibition does not attenuate arsenite-induced decreases in actomyosin ATPase activity appears to be at odds with the observation of a p38 MAPK-dependent decrease in the velocity of cell shortening (25). Clearly there are a number of significant experimental differences between the two studies that could account for the disparate results.

The use of arsenite, a known metabolic inhibitor, in the present study raises concerns over the specificity of the effects observed. However, this concern is minimized by the experimental design and data obtained. First, the present experiments exposed quiescent cells to arsenite at 25°C for 30 min. ATP use is minimal under these conditions and an energy deficit is unlikely. Subsequent to arsenite exposure, cells were skinned and washed of all arsenite, and ATP was provided from an exogenous source. Second, some measures of myocyte function were normal after arsenite treatment. For example, the tension-pCa relationship was not significantly different between arsenite- and vehicle-treated groups. Finally, the ability of the p38 MAPK inhibitor to block the arsenite-induced decrease in maximum tension and HSP-27 translocation argues against a nonspecific, cytotoxic effect of arsenite being the cause of the present observations.

Typically, p38 MAPK activation has been associated with changes in transcriptional factors with functional changes occurring on the time scale of days to weeks. The goal of the present study was to begin to uncover whether and how p38 MAPK can influence cardiac contractile function on an acute basis. We found that phosphorylation of p38 MAPK is associated with an immediate depression in force-producing abilities. Our data is consistent with the hypothesis that this is due to HSP-27-induced changes at the myofilaments and modification of sarcomeric scaffolding proteins such as \(\alpha\)-actinin. In addition, use of arsenite uncovered a pathway new to our understanding in cardiac myocytes that involves translocation of PP2a and LC2 dephosphorylation.

DISCLOSURES

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