Cytosolic NADH redox and thiol oxidation regulate pulmonary arterial force through ERK MAP kinase

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Oeckler, Richard A., Elizabeth Arcuino, Mansoor Ahmad, Susan C. Olson, and Michael S. Wolin. Cytosolic NADH redox and thiol oxidation regulate pulmonary arterial force through ERK MAP kinase. Am J Physiol Lung Cell Mol Physiol 288: L1017–L1025, 2005.—An ERK MAP kinase-mediated contractile mechanism previously reported to be activated by peroxide and stretch in bovine coronary arteries is shown in this study to be present in endothelium-denuded bovine pulmonary arteries and subject to regulation by modulation of cytosolic NAD(H) redox through the lactate dehydrogenase reaction. Although our previous work identified an acute O2-dependent peroxide-mediated relaxation of bovine pulmonary arteries on exposure to lactate, a 30-min treatment with 10 mM lactate enhanced ERK phosphorylation and increased force generation to 30 mM KCl. Hypoxia inhibited these responses to lactate. Increases in ERK phosphorylation and the enhancement of force generation by lactate and stretch are attenuated in the presence of inhibitors of Nox oxidase (0.1 mM apocynin) or ERK activation (10 μM PD-98059) and by 0.1 mM ebselen. Additionally, incubation of pulmonary arteries with 10 mM pyruvate lowered basal levels of ERK phosphorylation, and it inhibited both the ERK phosphorylation and the enhancement in force generation to 30 mM KCl caused by stretch. Treatment of pulmonary arteries with the thiol oxidant diamide (1 μM) elicited what appears to be a peroxide-independent increase in force and ERK phosphorylation that were both attenuated by PD-98059. Thus pulmonary arteries possess a peroxide-elicited contractile mechanism involving ERK MAP kinase, which is stimulated by stretch and regulated through the control of Nox oxidase activity by the availability of cytosolic NADH.

Materials and Methods

Force measurement. Isolated endothelium-denuded arterial rings of 3–4 mm in length and diameter were prepared from secondary branches of the bovine pulmonary arteries of calf lungs obtained from a slaughterhouse immediately after slaughter, by adaptation of previously discussed methods (3, 21, 22). In all experiments, the endothelium was denuded by gentle rubbing of the lumen of the vessel. In brief, arterial rings were mounted on wire hooks attached to Coulbourn force displacement transducers for measurement of changes in isometric force via an AD Instrument’s Powerlab system running Chart 5 software. Arteries were incubated for 1 h at an optimal passive tension of 5 g in individually thermostated 10-ml tissue baths containing Krebs-bicarbonate buffer, pH 7.4, containing (in mM): 118 NaCl, 4.7 KCl, 1.5 CaCl2, 25 NaHCO3, 1.1 MgSO4, 1.2 KH2PO4, and 5.6 glucose, at 37°C gassed with 21% O2, 5% CO2, balance N2. After 1 h of equilibration, the vessels were depolarized with Krebs-bicarbonate buffer containing KCl in place of NaCl (final concentration 130 mM KCl). The vessels were then re-equilibrated with Krebs-bicarbonate for 1 h. The rings were then contracted with 30 mM KCl (prepared by substituting NaCl with KCl, “Pre” in Figs. 3A and 4A) under 21% O2, to generate a control contraction. In some arteries, stretch was used to increase the passive force to 20 g by lengthening the arterial rings with the rack and pinon manipulator used to adjust the position of the force transducer for 20 min. Force was re-adjusted with 10 mM lactate, 10 mM apocynin, and 10 μM PD-98059, then with 10 mM pyruvate and 10 mM lactate, to lactate, a 30-min treatment, and 30 mM KCl. When Cu,Zn-superoxide-dismutase activity is inhibited, relaxation of bovine pulmonary arteries to nitric oxide is prevented by apocynin and diphenyliodonium (DPI). Thus the LDH reaction can control superoxide generation by a Nox-type oxidase, which was inhibited by apocynin and diphenyliodonium (DPI). The LDH reaction appears to be able to control the generation of vascular smooth muscle through its influence on cytosolic NAD(H) redox, and H2O2 has the ability to regulate processes that promote both relaxation and contraction.

In this study, we examine whether bovine pulmonary arteries show a peroxide-mediated contractile response that functions through the ERK MAP kinase pathway in addition to the previously studied cGMP-associated relaxation response (3) and whether a passive stretch treatment enhances contractile force through activation of this pathway. Then the actions of pyruvate and lactate are examined to determine whether the hypothesized effects of these metabolites on cytosolic NAD(H) redox regulate basal and stretch-enhanced force generation through changes in the ERK MAP kinase pathway. While studying the vasoactive actions of the thiol oxidant diamide, we observed this agent also caused a direct contractile response correlated with activation of the ERK MAP kinase system, and we examined this response to determine whether it functioned through modulating the effects of endogenous H2O2.

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to compensate for stretch-induced decays in force, as needed, and then passive force was returned to the basal level (5 g) before the second exposure to 30 mM KCl (“Post” in Fig. 3A). After force generation elicited by a second exposure to 30 mM KCl in the absence or presence of drugs indicated in the results reached a steady state, the vessels were rapidly frozen in liquid nitrogen and analyzed further via Western blotting. In some studies, pretreatment with 10 mM lactate or 10 mM pyruvate was used to examine redox modulation, and an atmosphere of 95% N2-5% CO2 was used for probing the effects of hypoxia. As described previously (21), 100 μM apocynin (Fluka, St. Louis, MO), 100 μM ebselen, and 10 μM PD-98059 (Sigma, St. Louis, MO) were used to examine the influence of Nox-type oxidases, intracellular peroxide, and ERK MAP kinase, respectively, on the enhancement of contraction to 30 mM KCl caused by stretch or lactate. The change in force developed by the Post (in Figs. 3A and 4A) contraction to 30 mM KCl was compared with the initial Pre contraction and expressed as a percentage. In other studies, arterial rings precontracted with 30 mM KCl were exposed to peroxide (100 μM or 1 mM H2O2) and the response compared in the presence or absence of the mitogen-activated protein kinase kinase (MEK) inhibitor 10 μM PD-98059.

Detection of changes in superoxide. Changes in superoxide levels in endothelium-denuded pulmonary arteries resulting from exposure to the stretch protocol were quantified by the methods previously described (18, 21) for measurement of force in a single photon-counting chemiluminescence detection apparatus constructed in a light-tight box. In these experiments, pulmonary artery rings were incubated in Krebs bicarbonate buffer containing 5 μM lucigenin in a cuvette continuously gassed with 21% O2. The chemiluminescence observed at 20 and 60 min after exposure to stretch was reported as a percentage of the chemiluminescence observed before stretch in a protocol that included measurements of contractions to 30 mM KCl before and 60 min after exposure to the stretch protocol. Pulmonary arteries exposed to the same protocol omitting exposure to stretch were employed as controls for examining the effects of stretch.

Western blotting. Studies comparing force generation to ERK phosphorylation utilized arteries that were rapidly removed, blotted, and frozen for Western blot analysis usually during the second 30 mM KCl contractile response after force had reached a steady state under the conditions reported in RESULTS. Frozen arterial segments were pulverized under liquid nitrogen and placed in a homogenization buffer [60 mM Tris, 10 mM EGTA, 2 mM EDTA, protease and phosphatase inhibitors (Sigma), pH 7.5]. The tubes were centrifuged, the supernatant was isolated, and protein levels were assayed by a modified Bradford method (2) for each sample. Ten micrograms of each sample were loaded and run on 12% SDS-PAGE gels, transferred to supported nitrocellulose membranes, subsequently exposed to primary and secondary antibodies in 5% milk/TBS-Tween buffers, and detected by Amersham Biosciences ECL detection kits on autoradiography film. Densitometric analysis via the Kodak 1D system was used to quantitate protein levels. Phosphorylated ERK measurements reported in RESULTS were normalized to the total ERK form measured after stripping and reblotting the gels and then expressed as percentages of the control condition used in each experimental group. The detection of total ERK did not appear to be altered by any of the conditions examined. Total and phosphorylated forms of ERK antibodies were from Sigma/RBI (St. Louis, MO).

Statistics. Student’s two-tailed t-tests were used to assess significance of changes in force generation to 30 mM KCl of the treatments examined compared with the control contraction to KCl. ANOVA with a post hoc Student’s t-test employing a Bonferroni correction was used to determine significance between experimental groups. Values were represented as means ± SE, and P < 0.05 was used to determine statistical significance.

RESULTS

H2O2 elicits pulmonary artery contraction through activating ERK MAP kinase. Endothelium-denuded bovine pulmonary arteries precontracted with KCl were reported in a previous study to show minimal relaxation to H2O2 and an increase in force when they were exposed to 1 mM peroxide (3). Figure 1A contains a typical Western analysis showing that ERK MAP kinase phosphorylation appears increased in endothelium-denuded bovine pulmonary arteries precontracted with 30 mM KCl when they are exposed to 0.1 and 1 mM H2O2. As shown in Fig. 1B, attenuating the activation of ERK with 10 μM PD-98059, an inhibitor of its phosphorylation by MEK, promotes relaxation to 0.1 mM H2O2 and converts contraction to the 1 mM dose of H2O2 to a relaxation. Summary data in Fig. 1C indicate that 1 mM H2O2 increased the phosphorylation of ERK compared with vessels not exposed to H2O2, whereas vessels pretreated with PD-98059 and then exposed to 1 mM peroxide demonstrated levels of ERK phosphorylation that were significantly below that of control vessels. Although the 0.1 mM dose of H2O2 appears to cause a modest relaxation and an increase in ERK, these responses were somewhat variable and did not reach statistical significance.

Alteration of cytosolic NAD(H) redox through the LDH reaction modulates ERK activity. Since we previously provided evidence that bovine pulmonary arteries possess an oxidase generating superoxide and relaxant levels of H2O2, which is controlled by NADH availability, based on studying the influence of modulating cytosolic NAD(H) redox with lactate and pyruvate through the LDH reaction (9, 22), we hypothesized that modulating this redox system could function to regulate the control of ERK by endogenous H2O2. As shown in Fig. 2, exposure of bovine pulmonary arteries to 10 mM lactate or 10 mM pyruvate was observed to cause changes in the levels of ERK phosphorylation in vessels that were freeze-clamped after a 30-min incubation period and subsequently analyzed by Western blot analyses. The data in Fig. 2 show a concentration-dependent increase in the levels of phosphorylated ERK by lactate and a decrease or inhibition of phosphorylation by pyruvate.

Stretch causes enhanced force generation and ERK activation in pulmonary arteries. Our initial studies on the effects of stretch were performed in the coronary artery (21), and we were interested to determine whether a similar phenomenon is present in the pulmonary circulation as well. A 20-min period of stretch was chosen for the study using the protocol shown in Fig. 3A, because this stretch treatment caused prolonged increases in superoxide for over a 1-h period as determined by lucigenin chemiluminescence, similar to the response previously described in bovine coronary arteries (21). The level of superoxide detected by measuring chemiluminescence derived from 5 μM lucigenin was significantly increased to 148 ± 9% (n = 4) of the prestretch chemiluminescence at 20 min after the release of stretch and remained elevated (171 ± 42%) 60 min
after the release of stretch, whereas superoxide levels were not significantly elevated in control arteries (108 ± 16%, n = 4) that were not exposed to the stretch treatment at the time point corresponding to 60 min. Time-controlled contractions in unstretched pulmonary arterial segments developed 104 ± 6% (Fig. 3B) and 107 ± 4% (n = 4) of the prestretch 30 mM KCl contraction at the 20- and 60-min time points when re-exposed to 30 mM KCl at these time points. In arteries exposed to stretch, contractile force to 30 mM KCl averaged 70% greater than that seen in prestretched controls at the 20-min time point (Fig. 3B) and was 71 ± 11% greater (P < 0.05, n = 4) than the control contraction at 60 min after exposure to stretch. Vessels stretched while being continuously gassed under nitrogen did not demonstrate an enhancement in contractile function to 30 mM KCl (95 ± 11% of control), yet interestingly upon reoxygenation and repeat of the stretch under normal aeration, the stretch response returned (166 ± 17% of control), suggesting a role for oxygen in the changes that are observed. Furthermore, Western analysis of stretched pulmonary arteries demonstrated a twofold increase in the levels of phosphorylated ERK (see Fig. 3C).

Stretch-enhanced contractile function is dependent on NADH oxidase and ERK MAP kinase signaling. To develop evidence for a role of NADH oxidase and the ERK pathway in the response to stretch, we incubated vessels with the Nox oxidase inhibitor 100 μM apocynin, an enhancer of intracellular peroxide metabolism, 100 μM ebselen, 10 mM lactate, or 10 mM pyruvate. Additionally, to confirm the dependence of Nox oxidase-derived reactive oxygen species (ROS) signaling specifically through the ERK MAP kinase pathway, we exposed another subset of vessels to the MEK inhibitor 10 mM PD-98059. All vessels were then subjected to the passive stretch protocol. Figure 3, B and C, contains summary data for the enhancement of force development and the increase in ERK MAP kinase phosphorylation caused by stretch. The increase in force generation was attenuated 88% by PD-98059 and was not observed in the presence of apocynin, ebselen, and pyruvate. With the exception of ebselen, these agents did not have a statistically significant effect on force generation to 30 mM KCl in unstretched, incubated time controls. Ebselen may...
be increasing force in these arteries by removing a basal peroxide-mediated relaxation, which we have characterized in previous studies (19, 22). Vessels incubated with lactate retained the stretch-induced enhancement in contractile function and had a statistically significant increase in the contractile response to 30 mM KCl in incubated time-control arteries not exposed to stretch.

The origins of increases in ERK phosphorylation associated with enhanced force generation elicited by exposure to the stretch protocol were studied in Fig. 3C. In a pattern similar to that of the force changes (compare with Fig. 3B), the approximate doubling of ERK phosphorylation caused by stretch is prevented by PD-98059, apocynin, ebselen, and pyruvate. Additionally, PD-98059 and pyruvate decreased ERK phosphorylation, whereas lactate increased phosphorylation by ~75% above baseline levels seen in control incubated tissues not exposed to the stretch protocol. Although ebselen appeared to be increasing ERK phosphorylation, the changes observed were not statistically significant.

**Role of lactate and pyruvate modulation of ERK in pulmonary arterial contractile function.** As demonstrated in Figs. 2 and 3, it appears that lactate increases ERK phosphorylation and force development by KCl in a manner consistent with its modulating the availability of NADH. To investigate whether NADH oxidase activity is important in responses elicited by the experimental addition of lactate, we utilized preincubation with 0.1 mM apocynin to investigate the role of a Nox-type oxidase. Although apocynin had no significant effect on force phosphorylation...
generation or ERK phosphorylation in control vessels, as seen in Fig. 4, B and C, it abolished the lactate-induced increases in both ERK phosphorylation and contractile function.

We have previously demonstrated that ROS activation of the ERK family of MAP kinases is involved in the changes in contractility to stretch. To determine whether this pathway is involved in the redox-modulated changes as well, the responses to lactate were examined in the presence of hypoxia, 100 μM ebselen, and under conditions where the activation of ERK by MEK was inhibited with 10 μM PD-98059. Hypoxia increased force without affecting ERK phosphorylation, and lactate did not alter contraction to 30 mM KCl or ERK phosphorylation under hypoxia (Fig. 4, B and C). The contraction to 30 mM KCl (122 ± 13% of control, n = 12) and level of ERK phosphorylation (ERK-Pi = 132 ± 29% of control, n = 9) observed in the presence of 100 μM ebselen were not significantly altered by 10 mM lactate in the presence of ebselen [KCl = 124 ± 15% (n = 12), ERK-Pi = 176 ± 32% (n = 9)]. The data in Fig. 4 also demonstrate that PD-98059 attenuates the lactate-induced increase in force generation and ERK phosphorylation. Neither the MEK inhibitor nor the vehicle 0.1% DMSO significantly altered the contractile response to 30 mM KCl in arteries that were not exposed to redox modulation (data not shown).

Fig. 4. Influence of Nox-derived ROS, hypoxia, and ERK activation on a lactate-mediated enhancement of pulmonary arterial force generation. A: representative tracing from the Powerlab Chart system demonstrating force development to KCl in bovine pulmonary arterial rings. Force development is measured in grams increasing along the y-axis as a function of time (in min). Note the indicated additions of drugs at point X along the time frame of the protocol, followed by the 30-min period of incubations in the absence or presence of 10 mM lactate between Pre, the preincubation control contraction to 30 mM KCl, and Post, the postincubation contraction to 30 mM KCl in the absence or presence of lactate, drugs, and/or hypoxia. The difference is then calculated and expressed as a percentage of the preincubation contraction to assess alterations in contractile function. B and C: summary data for changes in force generation and ERK phosphorylation, respectively (n = 8). Bovine pulmonary arterial ring segments were incubated for 30 min in the absence or presence of 10 mM lactate between Pre, the preincubation control contraction to 30 mM KCl, and Post, the postincubation contraction to 30 mM KCl in the absence or presence of lactate, drugs, and/or hypoxia. The difference is then calculated and expressed as a percentage of the preincubation contraction to assess alterations in contractile function.
Thiol oxidation activates an ERK-mediated contraction. The role of thiol modification as the potential protein-oxidant interaction or “entry point” into the kinase signaling cascade was further investigated. Vessels were incubated with the thiol oxidant 1 μM diamide for 5, 30, and/or 60 min, freeze clamped, and analyzed for changes in ERK phosphorylation. There is a rapid activation of the ERK MAP kinase pathway at 5 min (175 ± 21%) that appears to remain maximally elevated at the 60-min time point (206 ± 24% of time control, n = 6). Because preliminary experiments suggested the thiol modification leading to ERK activation appeared to be oxidase independent, because it was not prevented by inhibition of pulmonary artery flavoprotein containing oxidases with 1 μM diphenyliodonium (19) or by a hypoxic atmosphere of 95% N2-5% CO2 (data not shown), we attempted to localize the thiol modification site pharmacologically. As seen in Fig. 5, A and C, the increase in ERK phosphorylation by diamide at the 60-min time point was attenuated by the MEK inhibitor PD-98059, but not by the src inhibitor PP2 (1 μM), suggesting the existence of a thiol redox-sensitive site between these two kinases, which are thought (21) to be on the pathway regulating the activation of ERK by stretch and peroxide. Coinciding with this increase in ERK phosphorylation is a small, but significant diamide-elicited contraction (3.6 ± 0.3 g, n = 11 compared with force changes 0.8 ± 0.4 g, n = 11 in baseline time controls) that develops over the 60-min period after the addition of diamide. This contraction is attenuated by the ERK pathway inhibitor PD-98059 but not by inhibition of src signaling with PP2. Summary data for these changes in force are shown in Fig. 5B.

DISCUSSION

The results of this study provide evidence for the presence in bovine pulmonary arteries of an ERK MAP kinase-mediated contractile mechanism that is regulated by exogenous and endogenously formed H2O2. This mechanism appears to function in a manner that opposes a previously studied relaxing mechanism to peroxide that appears to function through cGMP. The effects of lactate and pyruvate on modulating the mechanism to peroxide that appears to function through a manner that opposes a previously studied relaxing process involving thiol oxidation.

Peroxide regulates pulmonary artery force through simultaneously activating both relaxing and ERK-mediated contracting mechanisms. In this study we developed evidence for a role for stimulation of ERK-mediated contraction as an explanation for the previously observed (3) biphasic concentration-dependent relaxation-contraction response of precontracted endothelium-denuded bovine pulmonary arteries elicited by exposure to H2O2. KCl was selected in the present study as the contractile agent because it minimizes expression...
H2O2 leads to an increased activation of the ERK MAP kinase through a arteries caused by exposure to increasing doses of H2O2. like the recently reported (21) ERK-mediated contractile com- mitted. The role of ERK in this response of pulmonary arteries is higher dose of peroxide when the activation of ERK is inhib- lower dose and reversal from contraction to relaxation in the peroxide there appears to be an activation of an alternative phosphorylation.

of relaxation to H2O2. Under increasing concentrations of peroxide there appears to be an activation of an alternative contractile pathway mediated through ERK MAP kinase, as demonstrated in Fig. 1 by the observation of a relaxation at the lower dose and reversal from contraction to relaxation in the higher dose of peroxide when the activation of ERK is inhib- ited. The role of ERK in this response of pulmonary arteries is like the recently reported (21) ERK-mediated contractile com- ponent of a similar biphasic response of bovine coronary arteries caused by exposure to increasing doses of H2O2. Relaxing mechanisms including stimulation of soluble gua- nylate cyclase and cGMP (3), opening potassium channels (27), and additional redox mechanisms controlling calcium caused by oxidizing cytosolic NADPH (8) could be contribut- ing factors to the relaxing component of the response of pulmonary arteries to peroxide. Stimulation of tyrosine kinases has been reported to contribute contractile responses elicited by peroxide (11). Because multiple tyrosine kinases participate in the src-EGF receptor pathway previously identified in vascular tissue linking peroxide to the stimulation of ERK, mechanisms examined in this study could be an important contributing factor to processes through which redox regulation of tyrosine phosphorylation contributes to the regulation of force in pul- monary arteries. Force regulation by MAP kinase systems has been a topic of much research and debate, and there is evidence linking ERK to control of the contractile apparatus in smooth muscle through regulation of caldesmon and calponin (17, 20).

Cytosolic NADH regulates force generation through a Nox oxidase-mediated activation of ERK. The data in this study provide evidence that lactate and pyruvate have opposing effects on ERK phosphorylation that can be associated with their effects on the regulation of force generation. The data in Figs. 3 and 4 provide evidence that prolonged incubation with lactate increases force through stimulating the ERK MAP kinase pathway. On the basis of our previous work demon- strating that lactate causes a rapidly developing relaxation of pulmonary arteries through a mechanism that appears to in- volve endogenous H2O2 generation, it is likely that lactate is also simultaneously activating a relaxing mechanism that pre- vents the full expression of its ERK-mediated contracting mechanism in a manner similar to the effects of exposing pulmonary arteries to exogenous H2O2. Because lowering basal levels of ERK activation with pyruvate did not depress force, it is likely that the basal level of phosphorylation of ERK is below the threshold for influencing the contraction to 30 mM KCl. Our studies in bovine coronary arteries (21) were also consistent with force generation to 30 mM KCl not being influenced by the basal levels of ERK phosphorylation, whereas increased ERK activation by peroxide or stretch pro- moted an enhancement of force in a manner similar to peroxide and lactate observed in the present study. However, when stretch promoted an increase in activation of ERK, the inhibi- tion of this activation of ERK by pyruvate was associated with an attenuation of the stretch-induced increase in force. Because the increase in force and ERK activation caused by lactate were prevented by an inhibitor of Nox-type oxidases, the increase in cytosolic NADH that is hypothesized to be caused by lactate is likely to be a controlling factor in the generation of peroxide, which is thought to initiate the activation of ERK.

Stretch activates an ERK-mediated contraction-enhancing mechanism in pulmonary arteries that appears to be regulated by the availability of cytosolic NADH. Stretch was observed to cause an enhancement of contraction in bovine pulmonary arteries through a mechanism that appears to involve activation of a Nox oxidase-mediated stimulation of ERK MAP kinase in a manner similar to the response to stretch that was observed in bovine coronary arteries (21). This is based on the studies in bovine coronary arteries showing that the response to stretch was associated with an apocynin-inhibited membrane binding of the p47phox Nox oxidase subunit (21) and by observing in pulmonary arteries that the stretch-induced enhancement of force and ERK phosphorylation were attenuated by apocynin, presumably through a similar inhibition of Nox-activation by its p47phox subunit. Stretch increased the detection of super- oxide by lucigenin chemiluminescence, and increasing the intracellular metabolism of peroxide with ebselen also ap- peared to prevent observation of the ERK-associated force enhancing effects of stretch. The role of ERK in the response is supported by stretch causing an increase in its phosphoryla- tion and by inhibition of the stretch induced increases in force
Thiol redox changes may stimulate peroxide-independent mechanisms of ERK activation. The process through which peroxide interacts with the pathway linked to ERK activation is not known, and it could be hypothesized to originate from a peroxide-induced change in thiol redox. Because we observed that, in the bovine pulmonary arteries examined in this study, very low doses of diamide activated a slowly developing contraction, which was attenuated by MEK inhibition, we began to investigate if this drug provides evidence on how peroxide is activating ERK. Our previous studies (21) suggested that the src-EGF receptor region of the pathway activating ERK shown in Fig. 6 is the earliest step detected on the pathway to ERK activation by stretch after the formation of peroxide by oxidase activation. Thus after confirming that diamide was not functioning through a peroxide-dependent pathway by determining that its contractile response was not attenuated by preventing peroxide generation with hypoxia and inhibition of essentially all flavoprotein oxidases with DPI, we examined whether inhibition of src with PP2 altered the contraction to diamide. Since it did not, a process between the activation of src and the control of the phosphorylation of ERK by MEK is being activated by the thiol oxidant diamide. Because diamide has many potential actions that could function to influence the ERK pathway, including inhibiting thiol-containing protein phosphatases (6, 14, 16, 23), additional studies are needed to elucidate how diamide is activating ERK. Although the actions of diamide observed in this study do not appear to provide new information on how peroxide is activating ERK, they raise the possibility that thiol redox–regulated processes may have additional sites through which they regulate ERK activation.

Role of peroxide regulation of ERK in the control of pulmonary vascular function. The ERK MAP kinase system is a regulator of both pulmonary endothelial cell function (1, 13, 15, 26) and vascular smooth muscle growth (5, 10, 12, 24, 28), which could be of importance in remodeling that occurs in pulmonary hypertension. Observations made in this study demonstrate that peroxide causes pulmonary arterial smooth muscle contraction through activation of ERK and that cytosolic NADH redox is a key regulator of the basal activity of this system. The contraction to hypoxia in bovine pulmonary arteries is not associated with increases in ERK phosphorylation, and hypoxia was observed to inhibit the lactate–elicited activation of this system. Exposure of pulmonary arteries to a brief period of stretch, which might occur under pulmonary hypertensive conditions, stimulated the ERK pathway through the effects of stretch on oxidase activation, resulting in a subsequent enhancement of force generation. Although the role of ERK stimulation by the redox and stretch-activated processes examined in this study are not understood, it is likely that these are fundamental regulatory systems that contribute to pulmonary vascular control processes associated with pathophysiological situations that alter oxidant production and redox, as well as conditions such as increased pressure, which would activate the ERK-mediated response to stretch.

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