Cyclooxygenase, p38 Mitogen-Activated Protein Kinase (MAPK), Extracellular Signal-Regulated Kinase MAPK, Rho Kinase, and Src Mediate Hydrogen Peroxide-Induced Contraction of Rat Thoracic Aorta and Vena Cava

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ABSTRACT

In hypertension, blood vessels exhibit increased reactive oxygen species production that may alter vascular tone. We previously observed that H2O2 contracted rat thoracic vena cava under resting tone and aorta contracted with KCl. In arteries but not veins, H2O2-induced contraction required extracellular Ca2+ influx. Because of this difference in Ca2+ utilization, we hypothesized that signaling pathways mediating H2O2-induced contraction in vena cava under resting tone differed from those mediating H2O2-induced contraction in aorta contracted with KCl. Inhibitors of cyclooxygenase (COX) 1 and 2 (indomethacin, 10 μM), thromboxane A2 (TXA2) receptors [ICI185282 (2RS,4RS,5S)-4-4-hydroxyphenyl-2-trifluoromethyl-1,3-dioxan-5-yl heptenoic acid, 10 μM], p38 mitogen-activated protein kinase (MAPK) [SB203580 (2’-amino-3’-methoxyflavone), 10 μM], src [PP1 (4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine, 10 μM], and rho kinase [Y27632 (trans-4-[[1(R)-1-aminoethyl]-N-4-pyridinylcyclohexanecarboxamide dihydrochloride), 10 μM] significantly reduced H2O2-induced contraction in vena cava under resting tone and aorta after KCl (30 mM) contraction. In contrast, the phosphatidylinositol 3-kinase (PI3-K) inhibitor LY294002 [2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one, 20 μM] did not reduce aortic or venous H2O2-induced contraction. p38 MAPK, Erk MAPK, and src inhibition did not reduce aortic or venous contraction to the TXA2 receptor agonist U46619 (9,11-dideoxy-9α,11α-methaneoxypGF2α, 1 μM), whereas rho kinase inhibition significantly reduced aortic and venous contraction to U46619, and PI3-K inhibition reduced venous contraction to U46619. Our data suggest that, in rat thoracic aorta and vena cava, a COX-derived metabolite is one important mediator of H2O2 contraction, possibly via rho kinase activation, and that H2O2-induced contraction via p38 and Erk MAPK probably occurs independently of TXA2 receptor activation.

Our laboratory is interested in understanding the role of veins in blood pressure regulation, specifically under pathological conditions, such as hypertension, where oxidative stress is elevated (Lassague and Griending, 2004; Cai, 2005). Because arterial tone determines total peripheral resistance, the venous circulation is involved in blood pressure regulation has historically been overlooked. The venous circulation contains approximately 60 to 70% total blood volume. Increased constriction of splanchnic veins can cause a shift of blood from the abdominal cavity to the thoracic cavity, increasing venous return, cardiac output, and ultimately blood pressure (Guyton, 1955; Martin et al., 1998; Johnson et al., 2001). Mean circulatory filling pressure, an in vivo measurement of venous tone, is elevated in animal models of hypertension, supporting the idea that veins are important in blood pressure regulation (Martin et al., 1998; Johnson et al., 2001).

H2O2 has been proposed to be the most likely reactive oxygen species (ROS) involved in signal transduction because it is not a

ABBREVIATIONS: ROS, reactive oxygen species; COX, cyclooxygenase; TXA2, thromboxane A2; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PKC, protein kinase C; U46619, 9,11-dideoxy-9α,11α-methaneoxypGF2α; ICI185282, 2RS,4RS,5S-4-4-hydroxyphenyl-2-trifluoromethyl-1,3-dioxan-5-yl heptenoic acid; PD98059, 2’-amino-3’-methoxyflavone; SB203580, 4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine; PP1, 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine; Y27632, trans-4-[[1(R)-1-aminoethyl]-N-4-pyridinylcyclohexanecarboxamide dihydrochloride; LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; DMSO, dimethylsulfoxide; TXB2, thromboxane B2; EIA, enzyme immunoassay; PI3-K, phosphatidylinositol 3-kinase; PGH2, prostaglandin H2.
free radical and is inherently more stable than other ROS (Wolin et al., 2002; Ardanaz and Pagano, 2006). H$_2$O$_2$ can also freely pass through membranes and has a longer diffusion distance than superoxide (Wolin et al., 2002; Ardanaz and Pagano, 2006). H$_2$O$_2$ modifies vascular smooth muscle tone, causing contraction and relaxation, depending on experimental conditions, vascular bed, and species (Lucchesi et al., 2005; Thakali et al., 2006). Multiple signal transduction pathways have been reported to mediate H$_2$O$_2$-induced contraction, including (but not limited to) COX-stimulated thromboxane A$_2$ (TXA$_2$) production (Rodriguez-Martinez et al., 1998; Gao and Lee, 2001), tyrosine kinases (Jin and Rhoades, 1997), mitogen-activated protein kinases (MAPKs), phospholipase C (Sheehan et al., 1993), and rho kinase (Thakali et al., 2005), although the progression of signaling has not been established. TXA$_2$ receptors signaling also occurs via the activation of many of the same pathways including Erk MAPK, p38 MAPK, PKC (Bos et al., 2004), tyrosine kinases, and rho kinase (Tazzeo et al., 2003). Thus, one can envision H$_2$O$_2$ stimulating COX to produce vasoactive eicosanoids, specifically the vasoconstrictor TXA$_2$. However, many of these signaling elements, including Erk MAPK and p38 MAPK, are also redox-sensitive such that oxidation of active site thiol residues can activate kinases and inactivate phosphatases (Rhee et al., 2000, 2005; Ockler et al., 2005).

Presently, we are comparing H$_2$O$_2$-induced contraction in a model artery and vein, and the rat thoracic aorta and the thoracic vena cava, respectively. We previously reported that, under basal conditions, veins but not arteries robustly contract to exogenous H$_2$O$_2$. However, if arteries were either depolarized with KCl (30 mM) or K$^+$ channels were inactivated (tetraethylammonium, 10 mM), H$_2$O$_2$-induced contraction was significantly potentiated (Thakali et al., 2006). We observed that, although extracellular Ca$^{2+}$ influx was necessary for aortic (KCl-potentiated) H$_2$O$_2$-induced contraction, venous H$_2$O$_2$-induced contraction (under basal conditions) occurred in the absence of extracellular Ca$^{2+}$ influx (Thakali et al., 2006), suggesting mechanistic differences in arterial and venous H$_2$O$_2$-induced contraction. The goal of this work was to determine whether the signal transduction pathways mediating venous H$_2$O$_2$-induced contraction under basal conditions were the same pathways mediating aortic H$_2$O$_2$-induced contraction after KCl (30 mM) contraction.

Materials and Methods

Isolated Tissue Bath Protocol. All animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals at Michigan State University (East Lansing, MI). Thoracic aorta and vena cava were removed from anesthetized male Sprague-Dawley rats (200–250 g) (50 mg/kg i.p. pentobarbital) and placed in physiological salt solution containing 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH$_2$PO$_4$, 1.17 mM MgSO$_4$, 7H$_2$O, 1.6 mM CaCl$_2$, 2H$_2$O, 14.9 mM NaHCO$_3$, 5.5 mM dextrose, and 0.03 mM Ca$_{Na}$EDTA, pH 7.2. Vessels were cleaned of fat, and rings (3–4 mm) of aorta and vena cava were prepared for measurement of isometric tension as described previously (Thakali et al., 2006). In brief, rings were placed between two wire hooks, one that was attached to a stationary glass rod and the other that was connected to a force transducer to measure isometric contraction. Passive tension was pulled (aorta, 4 g; vena cava, 1 g), and vessels were equilibrated for 1 h in warmed (37°C), aerated (95% O$_2$, 5% CO$_2$) physiological salt solution, with frequent buffer changes. Tissue viability was assessed by contraction to an adrenergic agonist (aorta, phenylephrine, 10 µM; vena cava, norepinephrine, 10 µM). Norepinephrine was used to contract vena cava because phenylephrine did not reproducibly contract vena cava, and phenylephrine was used to contract aorta to compare results with past experiments.

Cumulative concentration response curves to H$_2$O$_2$ (1 µM–1 mM) were performed in aorta after KCl (30 mM) contraction [69 ± 8% phenylephrine (10 µM) contraction] and vena cava under quiescent conditions (under passive tension). Cumulative concentration response curves to U46619 (1 nM–1 µM) were performed in aorta and vena cava under passive tension. When inhibitors were used (10 µM indomethacin, 10 µM IC1185282, 10 µM PD98059, 10 µM SB203580, 10 µM PPI, 10 µM Y27632, and 20 µM LY294002), an inhibitor or vehicle (0.1% ethanol for indomethacin, 0.1% DMSO for IC1184282, PD98059, SB203580, and PPI; 0.2% DMSO for LY294002; and deionized water for Y27632) was added for 1 h before performing cumulative concentration response curves to agonists. Cumulative concentration response curves to KCl (6–100 mM) were also performed in aorta in the presence of the signaling inhibitors. We found that Y27632 (10 µM) significantly reduced aortic KCl-induced contraction; thus, in the presence of Y27632 (10 µM), aorta were contracted with 100 mM KCl to more closely match the contraction induced by 30 mM KCl in vehicle-incubated aorta. The signaling inhibitors and concentrations of inhibitors were chosen, because we have demonstrated previously specificity and effectiveness of these inhibitors in arterial smooth muscle cell cultures (Watts, 1996; Banes et al., 1999; Florian and Watts, 1999; Gao and Lee, 2001; Northcott et al., 2002).

Tissue Thromboxane B$_2$ Measurement. Thromboxane B$_2$ (TXB$_2$) levels were measured in rat thoracic aorta and vena cava using an enzyme immunoassay (EIA) kit purchased from Cayman Chemicals (Ann Arbor, MI). Briefly, clean rat thoracic vena cava were cut in half and incubated in 150 µl of EIA buffer, with one-half incubated in H$_2$O$_2$ (1 mM) and another with vehicle (water) for 10 min at 37°C. Cleaned rings (3–4 mm) of rat thoracic aorta were incubated in 150 µl of EIA buffer plus KCl (30 mM), and one ring was incubated with H$_2$O$_2$ (1 mM) and another with vehicle (water) for 10 min at 37°C. Microcentrifuge tubes containing vessels and EIA buffer/H$_2$O$_2$ or vehicle were frozen at ~80°C for 4 h. The tubes were thawed, sonicated for 3 s, and centrifuged (1 min, 14,000 rpm). Fifty microliters of the supernatant was added to a secondary antibody-coated 96-well plate to determine the amount of TXB$_2$ present in each sample. A standard TXB$_2$ curve was also performed and is described below in the data analysis section. Total protein was determined after dissolving vessel pellets in NaOH (1 N) and performing a Lowry’s protein assay.

Data Analysis. Data are presented as mean ± S.E. of the percentage of the initial contraction to phenylephrine (aorta, 10 µM) or norepinephrine (vena cava, 10 µM) for n experiments, where n indicates the number of rats used. In aorta contracted with KCl (30 mM), contraction to H$_2$O$_2$ was calculated as the contraction above the maximal KCl response. When comparing multiple concentration response curves with H$_2$O$_2$ or U46619, two-way analysis of variance with Bonferroni’s post hoc test was performed. For quantification of TXB$_2$ EIA, a standard curve using TXB$_2$ was run along with arterial and venous samples. For the standard curve, the ratio of absorbance of bound ligand (TXB$_2$) to the absorbance of total binding was plotted against the log of the concentration of TXB$_2$, and linear regression was performed to determine TXB$_2$ levels in aortic and venous samples. TXB$_2$ levels were normalized to total protein in each sample and are reported as picograms of TXB$_2$ per milligram of total protein. In all cases, p ≤ 0.05 was considered statistically significant.

Chemicals. Acetylcholine, H$_2$O$_2$ (30%), norepinephrine, and phenylephrine were solubilized in water, and indomethacin was solubilized in ethanol and were purchased from Sigma Chemical Co. (St. Louis, MO). PD98059, SB203580, LD294002, PPI, IC1185282, and U46619 were solubilized in DMSO, and Y27632 was solubilized in water and purchased from Biomol (Plymouth Meeting, PA).
Results

COX-Derived TXA₂ Mediates Aortic H₂O₂-Induced Contraction after KCl (30 mM) and Basal Venous H₂O₂-Induced Contraction. The nonspecific COX inhibitor, indomethacin (10 μM; Kalgutkar et al., 2000), significantly reduced aortic H₂O₂-induced contraction after KCl (30 mM) and basal venous H₂O₂-induced contraction (Fig. 1A). TXA₂ has been reported to mediate H₂O₂-induced contraction in rat aorta (Rodriguez-Martinez et al., 1998; Gao and Lee, 2001); thus, we investigated the role of the TXA₂ receptor in aortic and venous H₂O₂-induced contraction using the TXA₂ receptor antagonist ICI185282 (Fig. 1B). ICI185282 (10 μM) significantly reduced aortic H₂O₂-induced contraction after KCl (30 mM) and basal venous H₂O₂-induced contraction (Fig. 1B). As expected, the same concentration of ICI185282 (10 μM) significantly inhibited U46619-induced contraction (1 nM–1 μM) in rat thoracic aorta (not KCl-contracted) and vena cava (Fig. 1C).

MAPKs (p38 and Erk), Src, and Rho Kinase but Not PI3-K Mediate Aortic and Venous H₂O₂-Induced Contraction. The p38 MAPK inhibitor SB203580 (10 μM), Erk MAPK inhibitor PD98059 (10 μM), and src inhibitor PP1 (10 μM) significantly reduced KCl (30 mM)-potentiuated aortic H₂O₂-induced contraction and basal venous H₂O₂-induced contraction to the TXA₂ receptor agonist U46619. Data are represented as means ± S.E.M. for the number (N) of animals in parentheses. EtOH, ethanol, NE, norepinephrine, PE, phenylephrine. *, statistically significant difference from vehicle (p < 0.05).
contraction (Fig. 2A). KCl (6–100 mM)-induced contraction of rat thoracic aorta was significantly reduced by Y27632 (10 μM) (Fig. 3C). Thus, Y27632 (10 μM)-incubated aorta were contracted with a higher concentration of KCl (100 mM) before performing H2O2 concentration response curves to match the contraction to KCl (30 mM) in vehicle-incubated aorta. Y27632 (10 μM), a rho kinase inhibitor, significantly reduced KCl (100 mM)-potentiated aortic H2O2-induced contraction (Fig. 3A), and we previously observed a similar inhibition in vena cava (Fig. 3A; reproduced with permission from Thakali et al., 2005). The PI3-K inhibitor LY294002 (20 μM) did not significantly alter aortic H2O2-induced contraction after KCl (30 mM) contraction or basal venous H2O2-induced contraction (Fig. 4A). Inhibition of p38 MAPK, Erk MAPK, and src had no effect on maximal U46619 (1 μM) contraction in aorta and vena cava (Fig. 2B). Similarly to H2O2-induced contraction, rho kinase inhibition reduced maximal aortic and venous U46619-induced contraction (Fig. 3B). Unlike H2O2-induced contraction, PI3-K inhibition modestly reduced maximal venous but not aortic U46619-induced contraction (Fig. 4B).

**TXB2 Levels in Rat Thoracic Aorta and Vena Cava.** To determine whether H2O2 stimulates COX to synthesize TXA2, aorta and vena cava were incubated with H2O2 (1 mM, 10 min, 37°C), and TXB2 (a stable metabolite of TXA2) levels were determined using a quantitative enzyme immunoassay kit. This length of time of H2O2 incubation was chosen because it was sufficient to observe H2O2-induced contraction in both aorta and vena cava. Basal levels of TXB2 were higher in vena cava compared with aorta (vena cava, 146 ± 11 pg of TXB2/mg protein; aorta, 29 ± 1 pg of TXB2/mg protein) (Fig. 5). H2O2 incubation significantly decreased TXB2 levels in vena cava (control, 146 ± 11 pg of TXB2/mg protein; H2O2, 108 ± 10 pg of TXB2/mg protein) but did not alter TXB2 levels in aorta (control, 29 ± 1 pg of TXB2/mg protein; H2O2, 25 ± 2 pg of TXB2/mg protein) (Fig. 5).

**Discussion**

**Common Signal Transduction Pathways Mediate H2O2-Induced Contraction in Arteries and Veins.** Multiple signaling pathways have been reported to mediate H2O2-induced contraction, and this current study affirms the idea that H2O2 is a physiologically relevant signaling molecule capable of activating multiple signal transduction pathways. We observed that Erk MAPK, p38 MAPK, src, rho kinase, and a COX-derived metabolite mediate H2O2-induced contraction in rat thoracic vena cava under basal conditions and in rat thoracic aorta after KCl contraction. The absolute magnitude of inhibition of H2O2-induced contraction seems to be different between arteries and veins. It possible that better penetration or a higher concentration of indomethacin, PD98059, PP1, or SB203580 is achieved in the smooth muscle of rat thoracic vena cava compared with aorta because of differences in wall structure between the two vessel types, although we have not tested this possibility experimentally. Nonetheless, the same concentrations of indomethacin, ICI185282, PD98059, PP1, SB203580, and Y27632 inhibited...
H$_2$O$_2$-induced contraction in both arteries and veins, suggesting that within the context of these signaling pathways, H$_2$O$_2$-induced contraction in arteries and veins occurs via similar signal transduction pathways.

Interestingly, the PI3-K pathway was not involved in mediating H$_2$O$_2$-induced contraction in vena cava under basal conditions or in KCl-contracted aorta. We have previously verified inhibition of PI3-K activity with LY294002 (20 μM) in cultured rat aortic smooth muscle cells (Northcott et al., 2002). H$_2$O$_2$ stimulation reportedly increases phosphorylation of Akt, a commonly evaluated downstream target of PI3-K in cultured rat aortic smooth muscle cells and perfused mouse mesenteric resistance arteries (Griendling et al., 2000; Lucchesi et al., 2005). From our experiments, we concluded that PI3-K is not involved in H$_2$O$_2$-induced contraction, but we cannot discount the possibility that acute H$_2$O$_2$ exposure in aorta and vena cava may activate Akt independently of PI3-K or may activate PI3-K to induce changes in other endpoints, such as cell proliferation, growth and apoptosis, or vascular remodeling.

In the current study, we compared H$_2$O$_2$ signaling in vena cava under quiescent conditions to aorta contracted with KCl. We previously observed that aorta under quiescent conditions contracted minimally to exogenous H$_2$O$_2$ and that depolarization of aorta (by increasing the concentration of extracellular K$^+$ or nonspecific potassium channel blockade)
potentiated the aortic contraction to H₂O₂ (Thakali et al., 2006). The magnitude of H₂O₂-induced contraction in vena cava under quiescent conditions (when normalized to contraction to an adrenergic agonist) is much larger than H₂O₂-induced contraction in aorta contracted with KCl. This could be due to differences in the efficacy of H₂O₂ to elicit contraction in aorta and vena cava or even differences in H₂O₂ penetration through the blood vessel wall. It is also possible that aortic KCl preconditioning may preferentially activate some signaling pathways or alter sensitivity to signaling inhibitors compared with U46619-induced contraction in aorta under quiescent conditions. However, we previously observed that aortic KCl-induced contraction was not reduced by PD98059, SB203580, or PP1 (Watts, 1996; Banes et al., 1999; Florian and Watts, 1999; Northcott et al., 2002), suggesting that for p38 MAPK, Erk MAPK, and src activity, KCl preconditioning does not result in the activation of different contractile pathways compared with contraction induced by U46619 alone. We observed that maximal aortic contraction to KCl was significantly reduced by Y27632 (10 μM), a rho kinase inhibitor. Thus, Y27632 (10 μM)-incubated aorta were contracted with 100 mM KCl to reach a magnitude of contraction comparable with vehicle (30 mM KCl)-incubated aorta.

Currently, there is no consensus on the signal transduction cascade mediating vascular H₂O₂-induced contraction. Our studies were performed with the intention of clarifying H₂O₂-mediated signaling but instead highlight the complexity of ROS signaling. Yang et al. (1998) reported that aortic H₂O₂-induced contraction was mediated by COX, PKC, and protein tyrosine kinases and was Ca²⁺-dependent. Wolin et al. (2002) observed that pulmonary arterial contraction to H₂O₂ was reduced by Erk MAPK inhibition, whereas Lucchesi et al. (2005) observed that mouse mesenteric artery contraction to H₂O₂ after KCl depolarization was mediated by p38 MAPK, but not Erk MAPK. In context of the above-mentioned sig-
naling pathways, Pelaez et al. (2000a) reported that in the pulmonary vasculature of the rat, $\text{H}_2\text{O}_2$-induced contraction under basal conditions was independent of myosin light chain phosphorylation and extracellular Ca$^{2+}$ influx, and in porcine pulmonary arteries, $\text{H}_2\text{O}_2$-induced contraction was independent of Erk MAPK and PKC activity (Pelaez et al., 2000b). We conclude that species, vascular bed, and experimental conditions probably dictate the contractile signaling pathways activated by $\text{H}_2\text{O}_2$.

**Role of COX and TXA$_2$ in Mediating Aortic and Venous $\text{H}_2\text{O}_2$-Induced Contraction.** One possible scheme of $\text{H}_2\text{O}_2$ signal transduction is that $\text{H}_2\text{O}_2$ activates COX to produce prostanoid metabolites including TXA$_2$. Cyclooxygenase, also known as prostaglandin endoperoxide H synthase, catalyzes two reactions, a peroxidase reaction and a cyclooxygenase reaction in the conversion of arachidonic acid into prostaglandin H$_2$ (PGH$_2$), the precursor to prostanoids. The cyclooxygenase reaction mediated by COX is peroxide-dependent; hydroperoxides such as $t$-butyl peroxide, peroxynitrite, and $\text{H}_2\text{O}_2$ increase cyclooxygenase activity (although not as efficiently as aliphatic hydroperoxides) (Smith et al., 2000; Kulmacz, 2005), whereas scavengers of peroxides like glutathione peroxidase but not scavengers of superoxide reduce cyclooxygenase activity (Kulmacz, 2005). Rodriguez-Martinez et al. (1998) observed that $\text{H}_2\text{O}_2$-induced contraction in aorta from normotensive Wistar Kyoto and spontaneously hypertensive rats was reduced by a PGH$_2$/TXA$_2$ receptor antagonist and by nonspecific COX inhibition. The hypothesis that $\text{H}_2\text{O}_2$ could stimulate TXA$_2$ synthesis and thus vascular contraction was confirmed by Gao and Lee (2001), where they demonstrated that nonspecific COX inhibitors, TXA$_2$ synthase inhibitors, and a phospholipase A$_2$ inhibitor reduced $\text{H}_2\text{O}_2$-stimulated TXB$_2$ production (a stable metabolite of TXA$_2$) and that these inhibitors and TXA$_2$ receptor antagonists reduced $\text{H}_2\text{O}_2$-induced contraction of rat mesenteric arteries. Through the use of a nonspecific COX inhibitor and a TXA$_2$ receptor antagonist, our contractility data suggest that in both rat thoracic aorta and vena cava, $\text{H}_2\text{O}_2$ stimulates the production of the vasoconstrictor TXA$_2$. However, when TXB$_2$ (a stable metabolite of TXA$_2$) levels were measured in aorta and vena cava, $\text{H}_2\text{O}_2$ did not increase TXB$_2$ levels and in fact decreased TXB$_2$ levels in vena cava, contrary to studies by Rodriguez-Martinez et al. (1998) and Gao and Lee (2001). One limitation of our TXB$_2$ EIA was that because the assay was incompatible with the physiological salt solution used in contractility experiments, $\text{H}_2\text{O}_2$ incubations were performed in EIA buffer from the kit, the composition of which is proprietary information. Thus, our experimental conditions may not have been optimal for detection of $\text{H}_2\text{O}_2$-stimulated TXB$_2$.

It is also possible that TXB$_2$ was the incorrect endpoint to measure because PGH$_2$, the actual product of COX activity, causes contraction via TXA$_2$ receptor binding (Davidive, 2001). We observed that U46619, a TXA$_2$ receptor-specific agonist, induced concentration-dependent contraction of both rat thoracic aorta and vena cava, verifying that in rat thoracic aorta and vena cava, TXA$_2$ receptors couple to contraction. We observed that U46619-induced contraction, although inhibited by a TXA$_2$ receptor antagonist, is not dependent on p38 MAPK, Erk MAPK, and src signaling pathways in a similar fashion to $\text{H}_2\text{O}_2$-induced contraction. U46619-mediated contraction occurred via PI3-K and rho kinase activation in vena cava and rho kinase activation in aorta. It is possible that the dichotomy between the signaling pathways mediating $\text{H}_2\text{O}_2$-induced contraction and U46619-induced contraction in aorta and vena cava occurs because although U46619 causes contraction via TXA$_2$ receptor activation, U46619 may induce TXA$_2$ receptor-dependent signaling in a manner different from TXA$_2$ or U46619 may have some unknown effects independent of TXA$_2$ receptor activation. It is also possible that the signaling inhibitors and concentrations used are nonspecific. Although we have previously demonstrated the specificity of the concentrations of inhibitors used in aortic smooth muscle cell cultures (Watts, 1996; Banes et al., 1999; Florian and Watts, 1999; Gao and Lee, 2001; Northcott et al., 2002), a study by Davies et al. (2000) suggests that many commonly used signaling inhibitors inhibit two or more kinases, depending on the assay used to determine inhibitor specificity. Our data suggest that COX activity is partially responsible for mediating $\text{H}_2\text{O}_2$-induced contraction in arteries and veins. From these current studies, we cannot determine whether $\text{H}_2\text{O}_2$-induced contraction via p38 MAPK, Erk MAPK, and src occurs independently of TXA$_2$ receptor activation.

**Redox Signaling: A Possible Mechanism Mediating $\text{H}_2\text{O}_2$-Induced Contraction?** It is likely that $\text{H}_2\text{O}_2$-stimulated TXA$_2$ receptor activation does not wholly account for $\text{H}_2\text{O}_2$-induced contraction, and other mechanisms may mediate aortic and venous $\text{H}_2\text{O}_2$-induced contraction. Increased intracellular Ca$^{2+}$ is another possible mediator of $\text{H}_2\text{O}_2$-induced contraction, although the focus of the current study was not to investigate differences in Ca$^{2+}$ handling between arteries and veins. Although $\text{H}_2\text{O}_2$ is not a free radical like many other reactive oxygen species, it is an oxidant and can participate in redox signaling (Wolin et al., 2002; Cai, 2005; Ardanaz and Pagano, 2006). Three potential mechanisms by which $\text{H}_2\text{O}_2$ could modify vascular tone via redox signaling are thiol oxidation and activation of kinases mediating vascular contraction, thiol oxidation and inhibition of phosphatases that normally inhibit contraction, and direct oxidation and activation of contractile proteins. Receptor tyrosine kinases (e.g., epidermal growth factor receptor), nonreceptor tyrosine kinases (e.g., Src, Lck, and Abl), and other kinases, including MAPKs, Akt, Janus kinase, and upstream regulators of these kinases (e.g., Ras) can be activated by oxidation of cysteine residues (Rhee et al., 2000, 2005; Oeckler et al., 2005). Besides activating kinases, $\text{H}_2\text{O}_2$ can also inhibit the activity of both protein tyrosine phosphatases and serine-threonine phosphatases via thiol oxidation of active site cysteine residues (Rhee et al., 2000; Oeckler et al., 2005). The oxidative inactivation of phosphatases is kinetically favored over the activation of kinases (Oeckler et al., 2005); thus, redox inhibition of phosphatases regulating kinase activity may be another important mechanism of $\text{H}_2\text{O}_2$ signaling. The ATPase activity of myosin is redox-sensitive, such that thiol oxidation induces myosin ATPase activity independent of Ca$^{2+}$ binding and phosphorylation of the regulatory myosin light chain$_{\alpha}$ (Sparrow et al., 1970; Chandra et al., 1985; Ngai and Walsh, 1987). $\text{H}_2\text{O}_2$ can also alter gene transcription, although the acute contractile effects of $\text{H}_2\text{O}_2$ are not likely mediated by changes in gene transcription. To date, most of the studies regarding redox signaling have been performed in cell lines in the context of studying apoptosis, growth, and proliferation. Rogers et al. (2006) recently reported that in
dog and rat coronary arterioles, H$_2$O$_2$-mediated vasodilation was reversed by dithiothreitol, a reducing agent, suggesting that H$_2$O$_2$ modification of vascular tone was redox-sensitive. Another possible explanation of our confusing and contradictorv results could be due to concurrent TXA$_2$ receptor activation and redox signaling by H$_2$O$_2$.

Our studies underscore the complexity of H$_2$O$_2$ signaling and the difficulty in predicting the physiological actions of ROS. Of the pathways investigated, their involvement in H$_2$O$_2$-induced contraction does not differ between arteries and veins. Our data suggest that ROS can alter both venous and arterial tone. We speculate that in hypertension, a disease characterized by increased oxidative stress, both the arterial and venous circulation are likely targets of elevated ROS levels.

References


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