Endothelin-1-induced contraction in veins is independent of hydrogen peroxide

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Thakali, Keshari, Stacie L. Demel, Gregory D. Fink, and Stephanie W. Watts. Endothelin-1-induced contraction in veins is independent of hydrogen peroxide. Am J Physiol Heart Circ Physiol 289: H1115–H1122, 2005. — Reactive oxygen species (ROS), such as superoxide and H2O2, are capable of modifying vascular tone, although the response to ROS can vary qualitatively among vascular beds, experimental procedures, and species. Endothelin-1 (ET-1) induces superoxide production, which can be dismutated to H2O2. The RhoA/Rho kinase pathway partially mediates ET-1-induced contraction and recently was implicated in superoxide-induced contraction. We hypothesized that H2O2, not superoxide, mediates venous ET-1-induced contraction. Rat thoracic aorta and vena cava contracted to exogenously added H2O2 (1 µM–1 mM), whereas the aorta and vena cava contracted to ET-1 (30 nM) or BQ-788 (100 nM), respectively, reduced ET-1 (100 nM)-induced increases in venous H2O2. In summary, ET-1 increased venous contractility; reactive oxygen species

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Endothelin-1 (ET-1) stimulates the production of ROS. Specifically, ET-1 induces NADPH oxidase to stimulate production of superoxide from endothelial and smooth muscle cells. The ETA receptor blockade, but not ETB receptor blockade, reduces elevated superoxide levels in the aorta and vena cava from DOCA-salt hypertensive rats and reduces ET-1-stimulated superoxide production, suggesting that the ETA receptor couples to superoxide production. Elevated ROS levels are important in hypertension because chronic administration of apocynin, which prevents NADPH oxidase assembly, attenuates the increase in blood pressure in the DOCA-salt rat (1). This has been largely attributed to decreases in arterial tone. The precise relationship between venous contractility and blood pressure is not fully understood. Understanding signaling mechanisms underlying venous contractility, one goal of this present work, will provide more information about venous mechanisms in determining blood pressure.

The Rho kinase pathway is involved in ET-1-induced contraction of rabbit basilar artery (16) and is involved in ET-1-induced Ca2+ sensitization in rabbit cavernosal smooth muscle (29). Superoxide-induced contraction in the rat thoracic aorta occurs via activation of the Rho kinase pathway (9). To our knowledge, it is unknown whether H2O2-induced contraction can occur via activation of the Rho kinase pathway. Superoxide is extremely reactive and has a small diffusion distance compared with H2O2, which is readily diffusible and easily crosses membranes. Thus, we hypothesize that the more stable ROS H2O2 is a likely mediator of smooth muscle contraction and other ROS signaling. We envision ET-1 stimulating NADPH oxidase to produce superoxide, which dismutates into H2O2 that can activate Rho kinase, causing myosin phosphatase phosphorylation and inactivation, resulting in venous contractility.

MATERIALS AND METHODS

Isoolated tissue bath protocol. The thoracic aorta and thoracic vena cava were dissected from anesthetized male Sprague-Dawley rats (pentobarbital sodium, 60 mg/kg ip) and placed in a physiological salt solution (PSS) containing (in mM) 130 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4·7H2O, 1.6 CaCl2·2H2O, 14.9 NaHCO3, 5.5 dextrose, and 0.03 CaNa2EDTA (pH 7.2). Tissues were cleaned of extraneous tissue and fat, cut into 3 to 4-mm rings, and hung between two wire hooks with one end attached to a stationary glass rod and the other end attached to a force transducer. The tissues were placed into warmed (37°C) aerated (95% O2-5% CO2), 30-ml isolated tissue baths for measurement of isometric contractile force.

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Each ring was placed under optimum resting tension (previously determined, 4,000 mg for rat aorta, 1,000 mg for rat vena cava) and allowed to equilibrate for 1 h with frequent buffer changes. Arteries were challenged with a maximal concentration of the α-adrenergic agonist phenylephrine (PE; 10 μM), and veins were challenged with a maximal concentration of the α-adrenergic agonist norepinephrine (NE; 10 μM) to initiate a maximal contraction, and tissues were washed repeatedly until tone returned to baseline. Veins were challenged with NE because PE did not elicit a reproducible contraction in these tissues. Arteries were challenged with PE to relate current findings to previous studies. In some studies, the endothelium of the aorta was removed by gently rubbing the intima of the aortic rings with a metal wire. Endothelial removal was verified as a <20% relaxation to acetylcholine (ACH, 1 μM) in PE (10 nM)-contracted aorta, and endothelial integrity was verified by a >80% relaxation to ACH (1 μM) in PE (10 μM)- or NE (10 μM)-contracted aorta and vena cava, respectively. Afterward, tissues were again washed until baseline was reached, and then a cumulative concentration response curve to an agonist was performed or the tissues were incubated with antagonists or vehicle for 1 h before performing cumulative concentration response curves to ET-1 or exogenous H₂O₂.

We observed that high concentrations of catalase (2,000 U/ml) caused excess bubble formation that interfered with measuring contraction. Thus Antifoam B (0.001%), a silicon-based emulsifier, was added to control and catalase (2,000 U/ml)-incubated baths to reduce bubbles. After cumulative H₂O₂ concentration response curves were performed, the vena cava was washed several times with PSS and a subsequent NE (10 μM) challenge was performed to assess tissue viability after exposure to millimolar concentrations of H₂O₂.

Measurement of arterial and venous H₂O₂ production. H₂O₂ production from the rat aorta and vena cava was assessed by using an Amplex Red H₂O₂ assay kit (Molecular Probes, Eugene, OR). Three-to-four-millimeter segments of the thoracic aorta and thoracic vena cava were dissected from rats; cleaned of fat, connective tissue, and blood; and then incubated in 500 μl of Krebs-HEPES buffer containing (in mM) 20 HEPES, 119 NaCl, 4.6 KCl, 1.0 MgSO₄, 7.5 H₂O₂, 0.15 Na₂HPO₄, 0.4 KH₂PO₄, 5 NaHCO₃, 1.2 CaCl₂, and 5.5 dextrose at 37°C for 1 h. Antagonists or vehicle was incubated during the 1-h Krebs-HEPES buffer equilibration. Krebs-HEPES buffer was carefully removed, and 200 μl of Amplex Red reaction solution was added to the tissues. Tissues were incubated with ET-1 (1, 10, and 100 nM) or ET-1 (100 nM) plus antagonists [atrasentan (10 nM); ETA receptor antagonist BQ-788 (100 nM); ET₄ receptor antagonist or diethylthiocarbamate (DCC, 10 mM) a SOD inhibitor] for 4 h at 37°C. Li et al. (12, 13) previously determined that maximal ET-1-stimulated superoxide production occurred after a 4-h incubation; thus we chose to measure ET-1-stimulated H₂O₂ production after a 4-h incubation. The Amplex Red solution was then transferred to a 96-well plate, and fluorescence emission was measured (excitation = 544 nm; emission = 584 nm) on a SpectraMax Gemini plate reader (Molecular Devices, Sunnyvale, CA) by using SoftMaxPro software. A standard curve with the use of known H₂O₂ concentrations was performed with each experiment and was used to determine H₂O₂ concentration. After each experiment, aortic and venous segments were homogenized, and protein concentrations were determined by using the bicinchoninic assay for protein determination. H₂O₂ production from the rat aorta and vena cava is represented as nanomole per milligram protein or as a percentage of control response.

Measurement of arterial and venous superoxide production. Superoxide production in the rat aorta and vena cava was assessed by using lucigenin-enhanced chemiluminescence as described previously (12, 13). Briefly, 4-mm segments of thoracic aorta and vena cava were dissected from rats; cleaned of fat, connective tissue, and blood; and then equilibrated in 950 μl of Krebs-HEPES buffer for 30 min at 37°C. DDC (10 mM) was then added to the buffer with segments of tissue and incubated for 4 h at 37°C. Tissues were then transferred to a new tube containing 1 ml of Krebs-HEPES buffer with lucigenin (5 μM) and incubated at 37°C for 10 min, and then 10 luminescence readings were measured by using a luminometer (TD-20/20 Luminometer, Turner Designs, Sunnyvale, CA). 4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt (Tiron, 10 mM) was added to the tubes, and after a 10-min incubation, 10 luminescence readings were taken. Tissues were blotted dry and weighed. The superoxide signal from the tissues was determined as the average difference in luminescence before and after the addition of Tiron, and superoxide levels [in nmol superoxide/(min·mg)] were determined by using a standard curve generated with xanthine-xanthine oxidase.

Data analysis. Data are presented as means ± SE and as a percentage of the initial response to PE (10 μM for arteries) or NE (10 μM for veins) for the number of animals indicated in parentheses. When two groups were compared, the appropriate Student’s t-test was used. When more than two groups were compared, ANOVA was performed by using Bonferroni’s post hoc test. When H₂O₂ levels were compared, repeated measured ANOVA was performed with Bonferroni’s post hoc test. In all cases, a P value ≤0.05 was considered statistically significant.

Chemicals. ACH, 3-amino triazole (3-AT), Antifoam B emulsion, catalase, polyethylene glycol (PEG)-catalase, DDC, H₂O₂ (30%), lucigenin, NE, PE, and Tiron were solubilized in water and purchased from Sigma Chemical (St. Louis, MO). ET-1 was obtained from Peninsula Laboratories (Belmont, CA) and solubilized in deionized water. Y-27632 was purchased from BioMol Research Laboratories (Plymouth Meeting, PA) and solubilized in dimethyl sulfoxide (DMSO). Atrasentan (solubilized in DMSO) was a gift from Abbott Laboratories.

RESULTS

Rat thoracic aorta and vena cava contract to exogenous H₂O₂. Arteries and veins both contracted to exogenous H₂O₂ (1 μM–1 mM) (Fig. 1). Whereas H₂O₂ contracted both the aorta and vena cava, H₂O₂ had a larger maximal contraction in the vena cava than in the aorta when contraction was measured as a percentage of the initial contraction to NE (10 μM, vena cava) or PE (10 μM, aorta) (maximal venous contraction to
H$_2$O$_2$ = 85 ± 13% of maximal NE contraction; maximal arterial contraction to H$_2$O$_2$ = 10 ± 3% of maximal PE contraction). Endothelial removal did not significantly alter aortic H$_2$O$_2$-induced contraction (Fig. 1), suggesting that the aortic endothelium plays little or no role in modifying H$_2$O$_2$-induced contraction. Removal of vena cava endothelium was difficult because current methods of endothelial removal significantly damage underlying smooth muscle cells; thus these experiments were not performed in endothelium-denuded vena cava.

**The RhoA/Rho kinase pathway is involved in venous H$_2$O$_2$- and ET-1-induced contraction.** The data in Fig. 2A suggest that the RhoA/Rho kinase pathway may be an important signaling mechanism involved in H$_2$O$_2$-induced venous contraction because Y-27632 (5 and 10 μM) caused a 73% and 88% reduction, respectively, in maximal venous H$_2$O$_2$-induced contraction. Similarly, the RhoA/Rho kinase pathway may play a role in ET-1-induced contraction in veins because venous ET-1-induced contraction was significantly reduced by Y-27632 (10 μM, Fig. 2B), a concentration that maximally inhibited H$_2$O$_2$-induced contraction.

**H$_2$O$_2$ is not involved in venous ET-1-induced contraction.** To examine the role of H$_2$O$_2$ in venous ET-1-induced contraction, we measured the effects of catalase, a H$_2$O$_2$ scavenger, on ET-1-induced contraction in veins. Neither catalase (100 U/ml, Fig. 3A and 2,000 U/ml, Fig. 3B) nor the cell-permeable PEG-catalase (163 and 326 U/ml, Fig. 3C) significantly reduced venous ET-1-induced contraction, suggesting that H$_2$O$_2$ is not a downstream effector mediating ET-1-induced venous contraction. PEG-catalase (326 U/ml) potentiated maximal ET-1-induced contraction, suggesting that ET-1-stimulated H$_2$O$_2$ may actually function as a venous vasodilator. However, inhibition of catalase activity with 3-AT (50 mM) had no effect on venous ET-1-induced contraction (Fig. 3D), suggesting that endogenous H$_2$O$_2$ does not cause venous relaxation under these conditions.

We were concerned that millimolar concentrations of H$_2$O$_2$ would cause vessel injury because several groups reported observing reductions in contractility after exposure to H$_2$O$_2$ (1 mM) (4, 15, 21). We compared initial NE (10 μM) contraction in the vena cava at the beginning of an experiment to a subsequent NE (10 μM) contraction after cumulative exogenous H$_2$O$_2$ concentration response curves had been performed, and we considered H$_2$O$_2$-induced vessel injury to be a reduction in NE (10 μM) contraction compared with the initial NE contraction. Catalase (100 U/ml) or PEG-catalase (163 U/ml) exposure before cumulative H$_2$O$_2$ concentration response curves prevented H$_2$O$_2$-mediated reductions in NE contraction (Fig. 4), suggesting that catalase protected the vena cava from H$_2$O$_2$-induced vessel injury. Incubation with 3-AT (50 mM), a catalase inhibitor, did not prevent H$_2$O$_2$-mediated reductions in NE contraction as did catalase but further depressed subsequent NE contraction after H$_2$O$_2$ exposure (Fig. 4).

**ET-1 increases H$_2$O$_2$ in rat thoracic vena cava but not aorta.** The Amplex Red H$_2$O$_2$ assay was used to assess the ability of ET-1 to increase endogenous H$_2$O$_2$ production in rat thoracic aorta and vena cava. Basal endogenous H$_2$O$_2$ production was three times higher in the vena cava than in the aorta (vena cava, 0.74 ± 0.09 nmol H$_2$O$_2$/mg protein; aorta, 0.24 ± 0.05 nmol H$_2$O$_2$/mg protein) (Fig. 5A), whereas basal superoxide production was five times lower in the vena cava than in the aorta [vena cava, 0.06 ± 0.05 nmol superoxide/(min·mg)]; aorta, 0.32 ± 0.03 nmol superoxide/(min·mg)] (Fig. 5B). Both venous and arterial H$_2$O$_2$ were superoxide derived because incubation with DDC, a superoxide dismutase inhibitor, significantly reduced basal H$_2$O$_2$ (Fig. 5A). ET-1 (100 nM) stimulated a significant increase in H$_2$O$_2$ production in the vena cava but not in the aorta (Fig. 5C), although the ET-1-induced increase...
in \( \text{H}_2\text{O}_2 \) in the vena cava did not appear to be concentration dependent within the concentrations tested (Fig. 6A). ET-1-induced increases in \( \text{H}_2\text{O}_2 \) production were reduced by either \( \text{ETA} \) or \( \text{ETB} \) receptor antagonism with the use of atrasentan (30 nM) or BQ-788 (100 nM), respectively, whereas neither of these drugs had any effect on basal venous \( \text{H}_2\text{O}_2 \) levels (Fig. 6B).

**DISCUSSION**

ROS are involved in the pathophysiology of ET-1-dependent hypertension. \( \text{H}_2\text{O}_2 \) and other ROS are vasoactive and can modulate blood vessel contractility. Several groups have reported elevations in arterial tissue levels of ET-1 and arterial production of superoxide in endothelin-dependent models of hypertension, such as DOCA-salt hypertension (8, 12, 25). The increase in arterial superoxide production in DOCA-salt hypertension is mediated by increased NADPH oxidase activity (1, 12) and is attenuated by \( \text{ETA} \) receptor blockade (12). ET-1 infusion increased blood pressure and increased vascular superoxide (5, 23), suggesting that ET-1 is directly or at least closely involved with increasing superoxide in hypertension. However, there are conflicting results regarding the role of ROS (superoxide and/or \( \text{H}_2\text{O}_2 \)) in hypertension induced by...
ET-1 infusion. In a study performed by Sedeek et al. (23), 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (Tempol, a SOD mimetic) reduced ET-1-induced increases in blood pressure and ET-1-induced increases in vascular superoxide, suggesting that ET-1-stimulated ROS directly have a pressor response. In a conflicting study performed by Elmarakby et al. (5), neither Tempol nor apocynin (an inhibitor of NADPH oxidase assembly) reduced ET-1-induced increases in blood pressure, but both Tempol and apocynin reduced vascular superoxide production and other markers of oxidative stress, suggesting that ET-1-stimulated ROS do not directly increase blood pressure but may be a result of elevated blood pressure. Our data suggest that endogenous H2O2 production does not mediate ET-1-induced contraction or relaxation and thus is likely not involved in the pressor response to ET-1.

H2O2 can modulate vascular tone. Most of the studies pertaining to the ability of H2O2 to modify vascular tone have been performed in arteries, although one study has been performed in rat pulmonary veins (19). H2O2 elicits vascular contraction and relaxation with the response depending on the vascular bed studied and experimental conditions. Rat aorta (6, 21), rat pulmonary arteries and veins, abdominal aorta, and inferior vena cava contract to H2O2 (1 μM–1 mM) (19). We observed a concentration-dependent contraction to H2O2 (1 μM–1 mM) in thoracic aorta and vena cava. We did not perform experiments to examine the ability of H2O2 to cause relaxation. The H2O2-induced contraction observed in thoracic vena cava was larger in magnitude than that in the aorta when this contraction was represented as a percentage of initial adrenergic agonist challenge. Our experiments were performed with the endothelium intact so as to more closely mimic how H2O2 might affect contractility in vivo, and we did not observe any significant difference in aortic H2O2-induced contraction with endothelial removal, suggesting that aortic contraction to H2O2 is not affected by release of endothelium-derived factors but is due to H2O2 acting directly on vascular smooth muscle cells. However, Rodriguez-Martinez et al. (21) observed that endothelial removal enhanced aortic contraction to H2O2. Discrepancies in the magnitude of aortic H2O2-induced contraction may be due to differences in the strain of rat used.

Rho kinase pathway: the link between H2O2- and ET-1-induced contraction? As an initial step to determine whether H2O2 was involved in ET-1-induced contraction, we examined the role of the Rho kinase pathway as a potential point of convergence of signaling in H2O2 and ET-1-induced contraction. Contraction via the RhoA/Rho kinase pathway involves Ca2+/sensitization such that small increases in intracellular Ca2+ will elicit smooth muscle contraction (26). Recently, Jin et al. (9) reported that superoxide-induced contraction of the rat thoracic aorta occurred via the Rho kinase pathway as xanthine-xanthine oxidase-induced contraction was reduced by the Rho kinase inhibitor Y-27632. Catalase only partially reduced xanthine-xanthine oxidase-induced contraction; thus Jin et al. (9) concluded that superoxide, as opposed to H2O2, mediated contraction in arteries via activation of the Rho kinase pathway. An alternative means by which superoxide and H2O2 may cause contraction is via superoxide and H2O2 reducing bioavailable nitric oxide (NO) to form peroxynitrite and hydroxyl radical (17), respectively. NO inhibits Ca2+/sensitization and actin reorganization induced by RhoA (22). Functionally, NO-mediated vasodilation of rat aorta partially occurs via NO inhibition of Rho kinase as Y-27632, a Rho kinase inhibitor, is...
less effective in the absence of NO, suggesting that there is more Rho kinase activity in the absence of NO (2). The Rho kinase-specific inhibitor Y-27632 (1–10 μM) significantly inhibited venous H₂O₂-induced contraction, suggesting that the RhoA/Rho kinase pathway is likely an important mechanism for H₂O₂-induced contraction.

The Rho kinase pathway has been implicated in ET-1-induced contraction (16), the high blood pressure of the DOCA-salt rat (28), and in the pathogenesis of human essential hypertension (14). The Rho kinase inhibitor Y-27632 (10 μM) significantly reduced venous contraction to ET-1, suggesting that the Rho kinase pathway is involved in venous ET-1-induced contraction. The concentration of Y-27632 used (10 μM) has been shown to completely reduce xanthine-xanthine oxidase-induced contraction in rat thoracic aorta, and Y-27632 (1 μM) did not affect PKC-mediated contraction (9). From the above experiments the Rho kinase pathway seemed to be a logical point of convergence for H₂O₂ and ET-1-induced contraction, and we hypothesized that ET-1 could stimulate superoxide and H₂O₂ production, which could in turn activate the Rho kinase pathway to mediate ET-1-induced contraction.

H₂O₂ does not mediate ET-1-induced contraction in rat thoracic vena cava. To determine whether endogenous H₂O₂ production mediated venous ET-1-induced contraction, we examined the effect of catalase, a H₂O₂ scavenger, on ET-1-induced contraction. We did not observe a reduction in ET-1-induced contraction in the presence of catalase (100 and 2,000 U/ml) or PEG-catalase (163 and 326 U/ml), suggesting that in normal vena cava, endogenous H₂O₂ does not mediate ET-1-induced contraction. PEG-catalase (326 U/ml) significantly potentiated maximal ET-1-induced contraction, suggesting that endogenous venous H₂O₂ production may play a vasodilatory role. However, 3-AT, a catalase inhibitor, had no effect on maximal ET-1-induced contraction. These data suggest that ET-1-induced superoxide and H₂O₂ production do not have immediate contractile effects and are consistent with the observation that Tempol does not markedly reduce ET-1-induced contraction in vena cava from normotensive rats (13).

To further dissociate ET-1-induced ROS production from ET-1-induced contraction, we compared basal endogenous H₂O₂ production to ET-1 (100 nM)-stimulated H₂O₂ production in the rat thoracic aorta and vena cava. We observed that the vena cava had significantly more basal endogenous H₂O₂ than the aorta and that ET-1 increased H₂O₂ production in the vena cava but not in the aorta. One possible explanation as to why we did not observe an ET-1-mediated increase in aortic H₂O₂ production could be because the Amplex Red H₂O₂ assay kit was not sensitive enough to measure comparatively small increases in H₂O₂, and this is a noted limitation in the interpretation of these experiments. Another explanation is that the low contractile reactivity to H₂O₂ and the low basal H₂O₂ production in the aorta may be indicative of generally lower H₂O₂-producing capabilities and is reflected in our inability to

Fig. 5. A: basal H₂O₂ production was significantly higher in veins compared with arteries, and this H₂O₂ production was superoxide derived as incubation with diethyldithiocarbamate (DDC, 10 mM) significantly reduced H₂O₂ production. Data are represented as nmol of H₂O₂/mg protein. *P < 0.05 represents a statistically significant difference in the presence of DDC; #P < 0.05 represents a statistically significant difference between aorta and vena cava.

B: basal superoxide production (measured with lucigenin-enhanced chemiluminescence) was significantly higher in arteries compared with veins. Data are represented as nmol of superoxide/min * mg. *P < 0.05 represents a statistically significant difference between aorta and vena cava. C: ET-1 increased endogenous H₂O₂ production in veins but not arteries. Data are represented as a percentage of the control response (n = 9–14). *P < 0.05 represents a statistically significant difference in the presence of ET-1.
measure ET-1-induced increases in H$_2$O$_2$. Future studies using the intracellular fluorescent ROS probe dichlorodihydrofluorescein (DCFH) may be useful for comparing the spatial relationship of arterial and venous H$_2$O$_2$ production, but such experiments are not feasible to perform in aortic and venous tissue sections because of high elastin autofluorescence. Also, DCFH is not a specific probe for H$_2$O$_2$ but will also fluorescence in the presence of hypochlorous acid and peroxynitrite (27).

Not surprisingly, the H$_2$O$_2$ in rat thoracic aorta and vena cava was primarily derived from superoxide as DDC, a Cu/ZnSOD inhibitor, significantly reduced basal H$_2$O$_2$ levels in the aorta and vena cava, although other mechanisms of H$_2$O$_2$ production are likely present. Whereas rat thoracic aorta and vena cava appear to have similar sources of H$_2$O$_2$, one report (7) suggests that the source of superoxide differs in human arteries and veins. Guzik et al. (7) report that NADPH oxidase is the major source of superoxide in human saphenous veins, whereas xanthine oxidase is the primary source of superoxide in human internal mammary artery. When aortic and venous levels of superoxide were measured by using lucigenin-enhanced chemiluminescence, we observed that rat thoracic aorta has approximately five times as much superoxide as rat thoracic vena cava. It is possible that arteries compared with veins have lower SOD enzymatic activity or that catalase activity is high in arteries and low in veins such that H$_2$O$_2$ levels remain low in arteries but high in veins. Perhaps the complement of antioxidant enzymes in arteries and veins differs such that ET-1 induces the production of different ROS in arteries and veins; specifically, in rat arteries superoxide is predominantly formed, whereas in rat veins, H$_2$O$_2$ is predominantly formed. Whether the antioxidant systems and their activities differ in

![Fig. 6.](image_url)

A: ET-1 (100 nM) increased venous H$_2$O$_2$ production, though the increase in H$_2$O$_2$ was not dependent on ET-1 concentration (n = 5–7). B: ET-1 (100 nM)-induced increases in venous H$_2$O$_2$ were reduced by ET$_A$ receptor blockade (30 nM atrasentan) or ET$_B$ receptor blockade (100 nM BQ-788), whereas atrasentan and BQ-788 alone had no effects on control H$_2$O$_2$ production. Data are represented as a percentage of control response (n = 5–6). *P < 0.05 represents a statistically significant difference from control.
arteries and veins is a question that warrants future investigation.

The majority of studies to date on the role of ROS in modulating vascular smooth muscle tone and structure have been performed in arteries because they are primarily responsible for controlling total peripheral resistance. Reduced vascular capacitance or increased venomotor tone (i.e., decreased venous compliance) is observed in animal models of hypertension as well as human hypertension (20). In mineralocorticoid hypertension, mean circulatory filling pressure, a measure of venous tone, is elevated, suggesting that there is an increase in venous tone in hypertension, but what causes vascular capacitance to decrease is still under investigation (11, 18). The increase in mean circulatory filling pressure in this ROS-dependent model of hypertension is also dependent on activation of endothelin receptors, making it important to understand the potential of ET-1 in modifying venous contractility through mechanisms that depend on ROS. Thus we contend that ET-1 in veins plays an important role in raising blood pressure, but its actions via H$_2$O$_2$ may be unrelated to contractility.

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