Pleiotropic Effects of Hydrogen Peroxide in Arteries and Veins From Normotensive and Hypertensive Rats

Keshari Thakali, Lauren Davenport, Gregory D. Fink, Stephanie W. Watts

Abstract—Hydrogen peroxide causes vascular contraction and relaxation and contributes to the pathogenesis of hypertension. We hypothesized that the contractile state of blood vessels governs whether \( \text{H}_2\text{O}_2 \) causes contraction or relaxation. Hydrogen peroxide (1 \( \mu\text{mol/L} \) to 1 mmol/L) concentration-dependently contracted thoracic aorta and vena cava from sham normotensive and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The maximal contraction to \( \text{H}_2\text{O}_2 \) was 3 times greater in DOCA aorta compared with sham aorta but unchanged in DOCA vena cava compared with sham vena cava. In prostaglandin \( \text{F}_{2\alpha} \) (20 \( \mu\text{mol/L} \)-contracted aorta and vena cava from sham and DOCA rats, \( \text{H}_2\text{O}_2 \) (1 \( \mu\text{mol/L} \) to 1 mmol/L) induced a concentration-dependent relaxation that was impaired in DOCA aorta but not DOCA vena cava. In contrast, in KCl (30 mmol/L)-contracted vessels, maximal \( \text{H}_2\text{O}_2 \)-induced contraction was enhanced 15-fold in sham aorta and 5-fold in DOCA aorta but only 2-fold in sham vena cava. Tetraethylammonium (10 mmol/L), BAY K 8644 (100 nmol/L), and ouabain (1 mmol/L) all enhanced maximal aortic \( \text{H}_2\text{O}_2 \)-induced contraction, whereas only ouabain enhanced venous \( \text{H}_2\text{O}_2 \)-induced contraction. The removal of extracellular \( \text{Ca}^{2+} \) reduced \( \text{H}_2\text{O}_2 \)-induced contraction in KCl-contracted aorta, whereas maximal venous \( \text{H}_2\text{O}_2 \)-induced contraction (under basal conditions) was unchanged. Our data suggest that differences in arterial and venous \( K^+ \) channel activity and extracellular \( \text{Ca}^{2+} \) influx are responsible for differences in arterial and venous contraction to \( \text{H}_2\text{O}_2 \). In DOCA-salt hypertension, arterial but not venous contraction to \( \text{H}_2\text{O}_2 \) is enhanced, and relaxation to \( \text{H}_2\text{O}_2 \) is reduced. (Hypertension. 2006;47:482-487.)

Key Words: contraction ■ relaxation ■ arteries ■ veins

\( \text{H}_2\text{O}_2 \) is capable of modifying vascular tone in complex manners. Whereas vessels in some vascular regions contract to \( \text{H}_2\text{O}_2 \), others relax in the presence of \( \text{H}_2\text{O}_2 \). Lucchesi et al.6-10 observed in mouse mesenteric resistance arteries that the same concentration of \( \text{H}_2\text{O}_2 \) could induce either contraction or relaxation, depending on \( K^+ \) channel activity. Because of differences in species, vascular bed studied, and experimental design, no consensus has been reached on the mechanisms that govern the vasoactive properties of \( \text{H}_2\text{O}_2 \). We reported previously that \( \text{H}_2\text{O}_2 \) contracts both rat thoracic aorta and vena cava but that aortic contraction to \( \text{H}_2\text{O}_2 \) was minimal (<10% of maximal contraction to an adrenergic agonist) compared with venous \( \text{H}_2\text{O}_2 \)-induced contraction (>80% of maximal contraction to an adrenergic agonist).11 The focus of the present study was to determine why there are differences in arterial and venous contraction to \( \text{H}_2\text{O}_2 \). We hypothesized that in rat thoracic aorta, we previously observed minimal \( \text{H}_2\text{O}_2 \)-induced contraction, because the predominant arterial response to \( \text{H}_2\text{O}_2 \) is relaxation.

Hypertension is associated with changes in vascular reactivity. Specifically, vasoconstriction to agonists, such as serotonin,12 phenylephrine,13 and \( \text{H}_2\text{O}_2 \),14 is enhanced, whereas endothelial-dependent relaxation is reduced. Changes in vascular smooth muscle membrane potential15 and \( \text{Ca}^{2+} \) handling13 have been proposed to account for enhanced vasoconstriction in hypertension and endothelial dysfunction responsible for reduced endothelial-dependent relaxation.13,16 However, most of these experiments were performed in arteries, and it is unclear whether similar changes in venous reactivity occur in hypertension. Increasing evidence suggests that increased venous tone, in addition to increased arterial resistance, is important in hypertension,17 because mean circulatory filling pressure, an index of venomotor tone, is increased in hypertension.18,19 Increased vasoconstriction in splanchnic veins can lead to significant increases in cardiac output and, consequently, increases in blood pressure. We questioned how arterial and venous reactivity to \( \text{H}_2\text{O}_2 \) changed in hypertension and hypothesized that the vascular response to \( \text{H}_2\text{O}_2 \), be it contraction or relaxation, would change in the established phase of mineralocorticoid hypertension.

Methods

Animals

All of the animal studies were performed in accordance with institutional guidelines of Michigan State University. Male Sprague-Dawley rats (250 to 300 g; Charles River Laboratories, Inc) underwent uninephrectomy and implantation of a deoxycorticosterone acetate (DOCA) pellet (200 mg/kg) under isoflurane anesthesia. DOCA-treated rats drank water supplemented with 1.0% NaCl and 0.2% KCl, whereas sham rats drank normal tap water. Systolic blood pressures were measured using the standard tail-cuff method. In experiments com-
paring sham and DOCA responses, sham normotensive and DOCA-salt hypertensive rats were paired.

Isolated Tissue Bath Protocol

Thoracic aorta and vena cava were removed from anesthetized male Sprague-Dawley rats (pentobarbital, 60 mg/kg, IP); placed in physiological salt solution (PSS) containing (in mmol/L): NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄, 7.6; Na₂HPO₄, 1.17; CaCl₂, 2H₂O, 1.6; NaHCO₃, 14.9; dextrose, 5.5; and CaNa₂EDTA, 0.03 (pH 7.2); and prepared for measurement of isometric tension as described previously. Cumulative concentration response curves to H₂O₂ (1 μmol/L to 1 mmol/L) were performed in aorta and vena cava as follows: (1) under quiescent conditions; (2) after KCl (30 mmol/L) contraction; or (3) after prostaglandin (PGF₂α) (20 μmol/L) contraction. When inhibitors were used, they or vehicle were added for 1 hour before performing cumulative H₂O₂ (1 μmol/L, to 1 mmol/L) concentration response curves. In Ca²⁺-free experiments, tissues were washed 3 times at 10-minute intervals with Ca²⁺-free PSS plus EGTA (0.15 mmol/L) and then were washed 3 times at 10-minute intervals with Ca²⁺-free PSS before performing cumulative H₂O₂ concentration response curves.

Data Analysis

Data are presented as mean±SE of the percentage of the initial contraction to phenylephrine (PE; aorta, 10 μmol/L) or norepinephrine (NE; vena cava, 10 μmol/L). In tissues contracted with KCl (30 mmol/L), contraction to H₂O₂ was calculated as the contraction above the maximal KCl response. In tissues contracted with PGF₂α (20 μmol/L), relaxation was calculated as a percentage of contraction to PGF₂α (20 μmol/L). Agonist EC₅₀ values were calculated using a nonlinear regression analysis using the algorithm (effect=maximum response×(1+EC₅₀/agonist concentration); GraphPad Prism). When a clear maximum response was not obtained, the EC₅₀ values calculated were considered estimates, with the true EC₅₀ value greater than or equal to the calculated value. When comparing 2 groups, the appropriate Student t test was used, and when comparing ≥3 groups, 1-way ANOVA with Bonferroni’s post-hoc test was performed. In all of the cases, a P value ≤0.05 was considered statistically significant.

Chemicals

Acetylcholine, H₂O₂ (30%), norepinephrine, and phenylephrine were solubilized in water; tetrathyammonium and ouabain were solubilized in dimethylsulfoxide; 4-aminopyridine (4-AP) was solubilized in 1N HCl (pH 7.4); and BAY K 8644 was solubilized in ethanol and were purchased from Sigma Chemical Co. Endothelin 1 was purchased from Bachem. Gilbenclamide was solubilized in DMSO and purchased from Calbiochem.

### Results

H₂O₂-Induced Contraction Was Enhanced In Aorta From Hypertensive Rats

Systolic blood pressure was significantly higher in DOCA salt rats (176±6 mm Hg) compared with sham rats (112±3 mm Hg). Aorta and vena cava from sham normotensive and DOCA-salt hypertensive rats were contracted to norepinephrine (NE; vena cava; 10 μmol/L) or PE (aorta; 10 μmol/L) contraction. Relaxation is represented as a percentage of PGF₂α (20 μmol/L) contraction. *Statistically significant difference between sham and DOCA.

**Table 1. Maximal H₂O₂-Induced Contraction of Aorta and Vena Cava From Sham Normotensive and DOCA-Salt Hypertensive Rats Under Basal Conditions and After KCl (30 mmol/L) Contraction and Maximal H₂O₂-Induced Relaxation of Aorta and Vena Cava From Sham Normotensive and DOCA-Salt Hypertensive Rats After PGF₂α (20 μmol/L) Contraction**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Contraction Under Basal Conditions</th>
<th>Contraction After KCl (30 mmol/L)</th>
<th>Relaxation After PGF₂α (20 μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham aorta</td>
<td>3.7±0.7</td>
<td>53.8±6.5</td>
<td>16.8±8.0</td>
</tr>
<tr>
<td>DOCA aorta</td>
<td>11.3±2.5*</td>
<td>51.0±8.2</td>
<td>28.6±3.4</td>
</tr>
<tr>
<td>Sham vena cava</td>
<td>84.9±13.4</td>
<td>175.4±67.7</td>
<td>26.3±4.6</td>
</tr>
<tr>
<td>DOCA vena cava</td>
<td>72.7±16.9</td>
<td>23.2±15.0</td>
<td></td>
</tr>
</tbody>
</table>

Contraction is represented as a percentage of either NE (vena cava; 10 μmol/L) or PE (aorta; 10 μmol/L) contraction. Relaxation is represented as a percentage of PGF₂α (20 μmol/L) contraction.

**Table 2. Estimated EC₅₀ Values (−log mol/L) for H₂O₂-Induced Contraction of Aorta and Vena Cava From Sham Normotensive and DOCA-Salt Hypertensive Rats Under Basal Conditions and After KCl (30 mmol/L) Contraction and Estimated EC₅₀ Values (−log mol/L) for H₂O₂-Induced Relaxation of Aorta and Vena Cava From Sham Normotensive and DOCA-Salt Hypertensive Rats After PGF₂α (20 μmol/L) Contraction**

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<th>Contraction After KCl (30 mmol/L)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sham aorta</td>
<td>3.8±0.3</td>
<td>3.9±0.1</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>DOCA aorta</td>
<td>3.8±0.1</td>
<td>3.8±0.1</td>
<td>3.4±0.1*</td>
</tr>
<tr>
<td>Sham vena cava</td>
<td>4.4±0.1</td>
<td>4.1±0.3</td>
<td>4.1±0.2</td>
</tr>
<tr>
<td>DOCA vena cava</td>
<td>4.5±0.2</td>
<td>3.7±0.1</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant difference between sham and DOCA.

![Figure 1. H₂O₂ (1 μmol/L to 1 mmol/L) contracted both rat thoracic aorta (A) and vena cava (B) in a concentration-dependent manner, and this contraction was enhanced in aorta but not vena cava from DOCA-salt hypertensive rats (aorta: n=9 to 11; vena cava: n=4). Points represent mean±SEM. *Statistically significant difference between Sham and DOCA (P<0.05).](image-url)
findings, we observed that aortic H$_2$O$_2$-induced contraction was significantly smaller than venous H$_2$O$_2$-induced contraction when H$_2$O$_2$-induced contraction was calculated as a percentage of initial adrenergic challenge.

**Vascular Response to H$_2$O$_2$ Varied Depending on the Contractile State of the Blood Vessels**

H$_2$O$_2$ (1 μmol to 1 mmol/L) induced relaxation of aorta (Figure 2A) and vena cava (Figure 2B) when vessels were contracted with PGF$_{2\alpha}$ (20 μmol/L; data summarized in Tables 1 and 2). H$_2$O$_2$-induced relaxation was significantly rightward shifted in aorta from DOCA-salt hypertensive rats [sham aorta estimated EC$_{50}$ (−log mol/L): 4.0±0.1; DOCA aorta estimated EC$_{50}$ (−log mol/L): 3.4±0.1] but was unchanged in vena cava from DOCA-salt hypertensive rats [sham vena cava estimated EC$_{50}$ (−log mol/L): 4.0 (0.1; DOCA vena cava estimated EC$_{50}$ (−log mol/L): 4.1±0.2; DOCA vena cava estimated EC$_{50}$ (−log mol/L): 3.7±0.1]. When PGF$_{2\alpha}$ cumulative concentration response curves were performed in sham and DOCA aorta, there were no differences in EC$_{50}$ values or maximal PGF$_{2\alpha}$-induced contraction (data not shown), suggesting that differences in PGF$_{2\alpha}$ contraction were not responsible for differences in sham and DOCA aortic H$_2$O$_2$-induced relaxation after PGF$_{2\alpha}$ contraction.

The same concentrations of H$_2$O$_2$ (1 μmol/L to 1 mmol/L) induced contraction of aorta (Figure 2C) and vena cava (Figure 2D) when vessels were contracted with KCl (30 mmol/L; data summarized in Tables 1 and 2). Maximal H$_2$O$_2$-induced contraction was enhanced 15-fold in KCl (30 mmol/L)-contracted sham aorta (sham control: 4.9±0.4% of initial PE contraction; sham KCl: 53.8±6.5) and enhanced 5-fold in DOCA aorta (DOCA control: 14.3±3.3% of initial PE contraction; DOCA KCl: 51.0±8.2) compared with maximal aortic H$_2$O$_2$-induced contraction under basal conditions. Maximal H$_2$O$_2$-induced contraction was enhanced 2-fold in KCl (30 mmol/L)-contracted sham vena cava (sham control: 80.0±15.7% of initial NE contraction; sham KCl: 175.4±67.7), although venous H$_2$O$_2$-induced contraction above KCl-induced contraction was highly variable. Enhanced aortic contraction after KCl (30 mmol/L) contraction occurred specifically for H$_2$O$_2$, because KCl (30 mmol/L) contraction did not potentiate maximal aortic contraction to other agonists, such as norepinephrine (Figure 2E) or endothelin 1 (Figure 2F).

**Nonspecific K$^+$ Channel and Na$^+/K^+$ ATPase Blockade Potentiated Aortic H$_2$O$_2$-Induced Contraction**

When aorta were incubated with tetraethylammonium (TEA; 10 mmol/L), a nonspecific K$^+$ channel blocker, maximal H$_2$O$_2$-induced contraction was significantly enhanced in sham and DOCA aorta (Figure 3A; sham control: 3.0±1.4% of initial PE contraction; sham TEA: 61.4±21.0; DOCA control: 13.2±3.1; DOCA TEA: 46.5±9.6) but was unchanged in vena cava (Figure 3B; sham control: 80.7±13.4% of initial NE contraction; sham TEA: 98.0±4.1). Specific K$^+$ channel inhibitors, TEA (1 mmol/L) to inhibit Ca$^{2+}$-dependent K$^+$ (BK$_{Ca}$) channels, glibenclamide (2 μmol/L) to inhibit ATP-dependent K$^+$ (K$_{ATP}$) channels, and 4-AP (3 mmol/L) to inhibit voltage-gated K$^+$ channels, were used individually to determine whether a specific family of K$^+$ channels was involved in the potentiation of aortic H$_2$O$_2$-induced contraction, but none of these inhibitors potentiated aortic H$_2$O$_2$-induced contraction (data not shown). A combination of TEA (1 mmol/L), glibenclamide (2 μmol/L), and 4-AP (3 mmol/L) to inhibit voltage-gated K$^+$ channels, were used individually to determine whether a specific family of K$^+$ channels was involved in the potentiation of aortic H$_2$O$_2$-induced contraction, but none of these inhibitors potentiated aortic H$_2$O$_2$-induced contraction (data not shown). TEA (10 μmol/L to 50 mmol/L) caused a concentration-dependent contraction of thoracic aorta from DOCA-salt hypertensive rats and had no effect on thoracic aorta from normotensive rats or vena cava from normotensive and DOCA-salt hypertensive rats (data not shown).

Na$^+/K^+$ ATPase blockade using ouabain (1 mmol/L) significantly enhanced maximal sham aortic H$_2$O$_2$-induced contraction (Figure 3C; sham control: 2.3±0.9% of initial PE contraction; sham ouabain: 29.3±5.6) and maximal sham venous H$_2$O$_2$-induced contraction (Figure 3D; sham control: 19.1±9.3% of initial NE contraction; sham ouabain: 249.2±41.6). Ouabain
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Extracellular Ca\(^{2+}\) Influx Was Required for Aortic but Not Venous H\(_2\)O\(_2\)-Induced Contraction

Removal of extracellular Ca\(^{2+}\), using Ca\(^{2+}\)-free PSS in the presence of EGTA, significantly reduced aortic H\(_2\)O\(_2\)-induced contraction in the presence of elevated extracellular KCl (30 mmol/L) from sham normotensive rats (sham KCl/control: 36.8±6.1% of initial PE contraction; sham KCl/Ca\(^{2+}\)-free: 3.8±1.5; Figure 4A). This maneuver also significantly reduced KCl-induced contraction (data not shown). Removal of extracellular Ca\(^{2+}\) did not significantly reduce maximal venous H\(_2\)O\(_2\)-induced contraction (Figure 4B) from sham normotensive rats (sham control: 71.1±14.5% of initial PE contraction; sham Ca\(^{2+}\)-free: 72.0±7.9). L-type Ca\(^{2+}\) channel activation using BAY K8644 (100 mmol/L), which did not contract sham normotensive aorta or vena cava, significantly potentiated maximal sham aortic H\(_2\)O\(_2\)-induced contraction (sham control: 4.5±0.7% of initial PE contraction; sham BAY K8644: 19.9±5.5; Figure 4C) but not maximal sham venous H\(_2\)O\(_2\)-induced contraction (sham control: 84.6±17.3% of initial NE contraction; sham BAY K8644: 76.6±11.9; Figure 4D).

Discussion

Pleiotropic Effects of H\(_2\)O\(_2\) on Vascular Tone

H\(_2\)O\(_2\) is a complex signaling molecule capable of having multiple seemingly contradictory effects on vascular smooth muscle tone. Why H\(_2\)O\(_2\) causes relaxation in some vessels and contraction in others has been the topic of many investigations, but as to date no definitive conclusion has been reached as to how H\(_2\)O\(_2\) causes such pleiotropic effects. Moreover, there has not been a comparison of the vasoactive properties of H\(_2\)O\(_2\) in arteries and veins. Lucchesi et al\(^9\) observed that when K\(^+\) channel function was compromised, (ie, in the presence of elevated extracellular [K\(^+\)], H\(_2\)O\(_2\) caused contraction. However, if K\(^+\) channel function was not compromised (in phenylephrine-contracted vessels), H\(_2\)O\(_2\) induced relaxation. Our results suggest that a common phenomenon—we propose K\(^+\) channel blockade–induced by elevated extracellular [K\(^+\)], K\(^+\) channel blockade, or Na\(^+\)/K\(^+\) ATPase blockade allows H\(_2\)O\(_2\) to cause contraction. Stimulation of K\(^+\) channel opening, specifically, K\(_{\mathrm{Ca}}\), K\(_{\mathrm{ATP}}\), and K\(_{\mathrm{AT}}\), has been implicated as a mechanism of H\(_2\)O\(_2\)-induced relaxation.\(^20\)

Thus, we hypothesize that H\(_2\)O\(_2\) normally opens some K\(^+\) channels, but when these K\(^+\) channels are unable to open, the contractile pathways activated by H\(_2\)O\(_2\) are revealed. Another possible explanation for our data are that elevated extracellular [K\(^+\)], K\(^+\) channel blockade, or Na\(^+\)/K\(^+\) ATPase blockade depolarize smooth muscle, and this depolarization mediates H\(_2\)O\(_2\)-induced contraction. Like KCl-induced contraction, PGF\(_{2\alpha}\)-induced contraction is accompanied by smooth muscle depolarization, but PGF\(_{2\alpha}\)-induced depolarization involves Cl\(^-\) efflux and Na\(^+\) influx, with no reported changes in K\(^+\) channel activity.\(^21\) In PGF\(_{2\alpha}\)-contracted aorta and vena cava, K\(^+\) channel function may be uncompromised, permitting
H$_2$O$_2$-induced relaxation to be observed. Thus, our data suggest that K$^+$ channel function determines the end vascular response, contraction or relaxation, to H$_2$O$_2$, and we observed that this dependence on K$^+$ channel function does not differ between arteries and veins.

To our surprise, specific pharmacological inhibition of BK$_{Ca}$, K$_{ATP}$, or voltage-dependent K$^+$ channels (K$_v$) or a combination of these K$^+$ channel inhibitors did not potentiate aortie H$_2$O$_2$-induced contraction as did TEA (10 mmol/L) or KCl (30 mmol/L). We speculate that TEA (10 mmol/L) and KCl (30 mmol/L) caused a greater degree of K$^+$ blockade than individual K$^+$ channel blockers and that greater K$^+$ channel blockade was necessary for enhanced aortic H$_2$O$_2$-induced contraction. However, without electrophysiological data, we cannot compare the degree of K$^+$ channel blockade between the different treatments, and this idea must remain a speculation.

**Differences in Arterial and Venous H$_2$O$_2$-Induced Contraction**

Under basal conditions, veins contract more robustly to H$_2$O$_2$ than do arteries, and why this occurs is unknown. Differences in the activity of catalase and glutathione peroxidase (antioxidant enzymes that degrade H$_2$O$_2$) in arteries and veins may explain why basal contractile responses to H$_2$O$_2$ varied between arteries and veins. However, because pharmacological manipulations elicited both contraction and relaxation to H$_2$O$_2$ in arteries and veins, we chose to take a more mechanistic rather than a molecular approach to understanding differences in arterial and venous H$_2$O$_2$-induced contraction.

Increasing extracellular K$^+$ and Na$^+$/K$^+$ ATPase blockade both potentiated aortic and venous H$_2$O$_2$-induced contraction, possibly through indirectly increasing intracellular Ca$^{2+}$. Interestingly, K$^+$ channel blockade did not potentiate venous H$_2$O$_2$-induced contraction to the same degree that it potentiated aortic H$_2$O$_2$-induced contraction. This suggests that if compromising K$^+$ channel activity reveals H$_2$O$_2$-induced contraction, then veins have less tonic K$^+$ channel activity than arteries under basal conditions. Differences in K$^+$ channel expression in arteries and veins may explain why K$^+$ channel blockade failed to significantly potentiate venous H$_2$O$_2$-induced contraction. Li et al. observed differences in mRNA expression of K$_{ATP}$ channels in arteries and veins; specifically, rat aorta expressed low levels of K$_{ATP}$ channel mRNA, whereas vena cava had no detectable K$_{ATP}$ channel mRNA. To our knowledge, it is unknown whether there are other arterial and venous differences in K$^+$ channel expression and whether different ion channels determine membrane potential, although membrane potential is the same in arterial and venous smooth muscle cells.

Differences in arterial and venous Ca$^{2+}$ handling may contribute to differences in arterial and venous contraction to H$_2$O$_2$. The literature regarding the role of Ca$^{2+}$ in H$_2$O$_2$-induced contraction is controversial. In pulmonary arteries, H$_2$O$_2$-induced contraction was independent of extracellular Ca$^{2+}$ influx, whereas in canine basilar arteries and rat thoracic aorta, H$_2$O$_2$-induced contraction was dependent on extracellular Ca$^{2+}$ influx and release of intracellular Ca$^{2+}$.

In perspective with the other studies examining the role of Ca$^{2+}$ in H$_2$O$_2$-induced contraction, there appears to be species, experimental condition–specific, and vessel–specific differences in the role of extracellular influx of [Ca$^{2+}$] in H$_2$O$_2$-induced contraction. We observed that H$_2$O$_2$-induced contraction in rat thoracic vena cava did not require extracellular Ca$^{2+}$ influx, whereas aortic H$_2$O$_2$-induced contraction was dependent on extracellular Ca$^{2+}$ influx. We also observed that L-type Ca$^{2+}$ channel activation potentiated aortic H$_2$O$_2$-induced contraction (but to a lesser extent than with TEA or increased extracellular K$^+$ treatment) and had no effect on venous H$_2$O$_2$-induced contraction. Thus, we concluded that differences in K$^+$ channel activity and extracellular Ca$^{2+}$ influx account for differences in arterial and venous contraction to H$_2$O$_2$.

**DOCA-Salt Hypertension Is Associated With Changes in Arterial Reactivity to H$_2$O$_2$**

We observed that H$_2$O$_2$-induced contraction was significantly enhanced in aorta but not vena cava from DOCA-salt hypertensive rats compared with sham normotensive rats, consistent with observations that arterial contraction to other agonists, such as norepinephrine and serotonin, are enhanced in DOCA-salt hypertension. Enhanced H$_2$O$_2$-induced contraction has been observed in arteries from spontaneously hypertensive rats. Enhanced arterial vasconstriction in hypertension has largely been attributed to increased Rho kinase and L-type Ca$^{2+}$ channel activity. Changes in membrane potential and K$^+$ channel activity also occur in hypertension and may be involved in arterial hyperresponsiveness. Arterial smooth muscle cells from spontaneously hypertensive rats are depolarized compared with normotensive Wistar-Kyoto rats. The depolarization of hypertensive arterial smooth muscle cells may mediate increased Rho kinase activity and L-type Ca$^{2+}$ channel activity in hypertension, because arterial smooth muscle depolarization with high concentrations of KCl increases RhoA activity and expression of the pore-forming α$_{1C}$ subunit of the L-type Ca$^{2+}$ channel. We observed that aortic H$_2$O$_2$-induced contraction requires extracellular Ca$^{2+}$ influx and conclude that both enhanced Rho kinase and L-type Ca$^{2+}$ channel activity may account for enhanced aortic but not venous H$_2$O$_2$-induced contraction in DOCA-salt hypertension.

In addition to enhanced sensitivity to a number of vasoconstrictors, reduced vasodilation because of endothelial dysfunction is another vascular contractile change that accompanies hypertension. We observed reduced H$_2$O$_2$-induced relaxation in PGF$_2$α-contracted aorta from hypertensive rats compared with normotensive rats, and our results are consistent with the observation that H$_2$O$_2$-induced relaxation is reduced in mesenteric arteries from spontaneously hypertensive rats. It is interesting to note that the contractile changes that occurred in arteries from DOCA-salt hypertensive rats (enhanced contraction to H$_2$O$_2$ and reduced relaxation to H$_2$O$_2$) were not observed in vena cava from hypertensive rats.

Changes in K$^+$ channel activity may drive changes in arterial contractility observed in hypertension. Total K$^+$ currents are reduced in arterial smooth muscle cells from spontaneously hypertensive rats, but the expression and functional activity of Ca$^{2+}$-activated K$^+$ channels, specifically BK$_{Ca}$ channels, is
increased in hypertension, whereas $K_v$ channel activity is reduced.\textsuperscript{16,26,30} We observed that TEA (10 $\mu$mol/L to 50 $\mu$mol/L) caused a concentration-dependent contraction of thoracic aorta from DOCA-salt hypertensive rats, suggesting that arteries from DOCA-salt hypertensive rats display increased BK$_{Ca}$ channel activity as demonstrated by Xu et al.\textsuperscript{31} However, we have not performed electrophysiological experiments to confirm our tissue bath results. Despite increased BK$_{Ca}$ channel activity in arteries from DOCA-salt hypertensive rats (which would tend to hyperpolarize smooth muscle cells and prevent contraction), basal H$_2$O$_2$-induced contraction was enhanced in arteries from hypertensive rats, and we hypothesize that this was because of either increased Rho kinase or L-type Ca\textsuperscript{2+} channel activity. Our data also suggest that H$_2$O$_2$-induced relaxation in aorta likely does not occur via BK$_{Ca}$ channel opening, because in DOCA aorta where there was an apparent increase in BK$_{Ca}$ channel activity, reduced H$_2$O$_2$-induced relaxation was observed. Interestingly, we did not observe similar changes in K$^+$ channel activity in veins from DOCA-salt hypertensive rats.

**Perspectives**

The vascular effects of H$_2$O$_2$ are complex, and the mechanisms of H$_2$O$_2$-induced contraction differ between arteries and veins, specifically the dependence on extracellular Ca\textsuperscript{2+} influx and K$^+$ channel activity differ in arteries and veins. DOCA-salt hypertension is accompanied by increased H$_2$O$_2$-induced contraction and reduced H$_2$O$_2$-induced relaxation in arteries but not in veins. It is difficult to predict the in vivo vascular response to H$_2$O$_2$ from in vitro experiments; however, our data suggests that whereas veins contract more robustly to H$_2$O$_2$, in hypertension, arterial but not venous reactivity to H$_2$O$_2$ is altered such that elevated plasma H$_2$O$_2$ will lead to increases in arterial but not venous tone. This work highlights the complex functions of reactive oxygen species in vascular tissue and continues to support a profound difference in arterial versus venous function both in normal and high blood pressure.

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**References**


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