Peroxynitrite (ONOO\textsuperscript{−}) is a strong and relatively stable oxidant species capable of causing lipid peroxidation and nitration of tyrosine residues on key cellular proteins.\textsuperscript{1,2} Although it is a weak vasodilator in some vascular beds,\textsuperscript{3,4} ONOO\textsuperscript{−} impairs relaxation to endothelial dependent and independent vasodilators.\textsuperscript{5} Because ONOO\textsuperscript{−} is formed in tissue by the nonenzymatic interaction between superoxide (O\textsubscript{2}·\textsuperscript{−}) anion and nitric oxide (NO),\textsuperscript{6,7} elevated levels may be expected when O\textsubscript{2}·\textsuperscript{−} formation is increased such as in diabetesthe, atherosclerosis, hypertension, and ischemia.\textsuperscript{8} The impairment in conduit artery endothelium-dependent dilation observed in these conditions may be due in part to reduction in levels of the dilator NO, as a result of generation of either O\textsubscript{2}·\textsuperscript{−} or ONOO\textsuperscript{−}, both of which can reduce bioavailability of NO.

Both animal, such as dog\textsuperscript{9} and pig,\textsuperscript{10,11} and human\textsuperscript{12} studies indicate that in the microvasculature endothelial-derived hyperpolarization factor (EDHF) contributes more than NO to vasodilation. However, the effects of ONOO\textsuperscript{−} on EDHF-mediated vasodilation is not known. Previous studies suggest that ONOO\textsuperscript{−} impairs K\textsubscript{Ca} channel activity in rat cerebral vascular smooth muscle cells (VSMCs).\textsuperscript{13} There is a paucity of information about the effects of ONOO\textsuperscript{−} on the activity of K\textsubscript{Ca} channels in human coronary arterioles. This is important because K\textsubscript{Ca} mediates the EDHF-induced dilation in human coronary microcirculation.\textsuperscript{14} Therefore, we hypothesized that ONOO\textsuperscript{−} inhibits K\textsubscript{Ca} channel activity in human coronary arteriolar VMSC membranes, which in turn reduces hyperpolarization-mediated vasodilation. We tested this hypothesis by examining vasodilator responses to K\textsubscript{Ca} channel openers, whole-cell K\textsuperscript{+} current density, and K\textsubscript{Ca} single-channel properties. The results indicate that ONOO\textsuperscript{−} inhibits K\textsubscript{Ca} channel-mediated vasodilation in human coronary arterioles. The impaired dilation is associated with a reduction in activity of K\textsubscript{Ca} channels in the vascular smooth muscle cells and may explain impaired vasomotor responses in disease states characterized by elevated levels of ONOO\textsuperscript{−}.

**Materials and Methods**

**Preparation of Coronary Arteries**

**Human Coronary Arterioles**

Human coronary arterioles (HCAs, 124±7 μm) were derived from the right atrial appendage of human subject undergoing cardiopulmonary bypass surgery. Tissue was obtained from 31 patients (aged 62±3 years, male=27, female=4). After surgical removal, atrial appendage tissue was placed in oxygenated Krebs buffer with the following composition (in mmol/L): NaCl 118, KCl 4.7, CaCl\textsubscript{2} 2.5,
KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 20, Na$_2$EDTA 0.026, and dextrose 11, pH 7.4. Coronary arteries were carefully dissected from the endocardial surface of the atrial appendage and prepared for videomicroscopic, immunohistochemistry (IHC), or patch-clamp studies.

**Rat Small Coronary Arteries (RCAs)**

Seven-week-old male Sprague Dawley rats (Harlan, Wis) were anesthetized with sodium pentobarbital (60 mg/kg, IP). Small coronary arteries (ID, 150 to 200 μm), supported with a thin layer of myocardial tissue to prevent collapse during sectioning, were dissected from the ventricle and prepared for IHC. We chose RCAs for the majority of IHC experiments because (1) five treatments were applied for each IHC experiment, which requires 5 vessels derived from the same tissue in order to maintain the same basal conditions and to optimize comparisons. Insufficient numbers of HCs were obtained from a single piece of tissue due to the small size of atrial appendage provided; (2) use of RCAs would provide more consistent and comparable results. These coronary vessels have similar physiological responses as HCs.

For all studies of cannulated microvessels, the vessel chamber was bubbled with 21% O$_2$, 5% CO$_2$, and 74% N$_2$ at 37°C.

**Formation of ONOO$^-$**

**Authentic ONOO$^-$ and Decomposed ONOO$^-$**

Authentic ONOO$^-$ was synthesized according to published methods. The amount of ONOO$^-$ in the stock solution was determined spectrophotometrically using the reported extinction coefficient for ONOO$^-$ (1670 mol/L$^{-1}$.cm$^{-1}$). Before each application, an aliquot of the stock solution was diluted in 1 mmol/L NaOH and rapidly added to either vessel or patch clamp chamber to achieve a final concentration of 1 or 5 μmol/L. Decomposed ONOO$^-$ (DC-ONOO$^-$) was made by leaving ONOO$^-$ at room temperature for about 2 hours. The decay of ONOO$^-$ was confirmed spectrophotometrically.

**Exogenous Generation of ONOO$^-$**

ONOO$^-$ was also formed in the vessel chamber by adding 10 mmol/L of sodium nitroprusside (SNP) or spermine nonoate (SN) plus xanthine (XA, 0.1 mmol/L) and xanthine oxidase (XO, 10 mm/mL).

**Immunohistochemistry for Detecting Nitrotyrosine, a Marker of ONOO$^-$ Interaction With Tissue**

To determine whether ONOO$^-$ was indeed generated and interacted with microvascular tissue. RCAs were used in IHC studies. RCAs were dissected from fresh hearts and treated for 2 minutes with authentic ONOO$^-$, DC-ONOO$^-$, SNP+XA+XO, or SNP+XA+XO+SOD, respectively. Frozen sections 5-μm thick were cut and mounted onto glass slides. After 5 minutes of washing with phosphate buffered saline (PBS), slides were treated with 10% goat serum/0.3% Triton X-100 in PBS for 1 hour at room temperature. Slides were then incubated for 2 hours with monoclonal mouse anti-nitrotyrosine antibodies (Upstate Biotechnology, NY), followed by two 10-minute washes with 0.3% triton X-100 in PBS. Slides were then incubated for 1 hour in biotin-conjugated secondary antibody and 30 minutes with streptavidin-HRP conjugated solution. The bound antibody was visualized by addition of 0.5 mg/mL diaminobenzidine and 0.01% hydrogen peroxide in PBS, which formed the insoluble brown precipitate (positive staining). Control slides (no primary antibody in incubation solution) were prepared to test the specificity of staining. IHC was also performed on RCAs treated with DC-ONOO$^-$, authentic ONOO$^-$, or no treatment (control) to confirm the interaction of ONOO$^-$ in human as well as rat coronary arterioles.

**Generation of Superoxide**

O$_2^-$ was produced by the enzymatic reaction of XA with XO. Either vessels or cells were incubated in buffer containing XA (0.1 mmol/L) and catalase (CAT, 500 U/mL). After incubation, O$_2^-$ was generated by the addition of XO (10 μU/mL). All experiments involving either vessel diameter measurements or patch clamp recordings were performed within 25 minutes, which corresponds to the minimum duration of steady state O$_2^-$ concentrations generated by the XA/XO system as determined by ferricytochrome c measurements (data not shown).

**Videomicroscopy**

HCs were placed into an organ chamber filled with physiological salt solution (PSS), cannulated with glass micropipettes, and secured with 10-0 nylon suture as described previously. Each pipette was attached to a pressure reservoir. The PSS in the organ chambers was warmed to 37°C, bubbled with 21% O$_2$, 5% CO$_2$, and 74% N$_2$, and continually circulated with a rotary pump. Vessels were allowed to equilibrate for 60 minutes at an intraluminal pressure of 60 mm Hg. Internal diameters were measured with a calibrated manual videomicroscope with a resolution of 2 μm attached to an inverted microscope. Most arterioles developed spontaneous tone averaging 30% of the passive diameter. If not, vessels were treated with endothelin-1 (10$^{-10}$ to 5$\times$10$^{-10}$ mol/L) to contract the vessel by 30% from the passive diameter. At the end of each experiment, vessels were maximally dilated with papaverine (10$^{-4}$ mol/L), and the percent vessel dilation to agonists was normalized to the maximal diameter assessed in the presence of papaverine. In some vessels, endothelium was denuded with air. The efficacy of endothelial denudation of HCs was verified by showing (1) failure of vessels to dilate to 10$^{-4}$ mol/L ADP and (2) preserved dilation to a maximal dose of papaverine. Viability of the vascular smooth muscle constrictor capacity was confirmed by evidence of constriction to endothelin-1 or presence of prominent myogenic tone.

**Patch Clamp Recording of K$_c$ Currents**

Enzymatic isolation of single VSMCs was performed according to published methods. Whole-cell patch-clamp recordings were obtained using standard pulse protocols and instrumentation as previously described. Briefly, families of K$^+$ currents were generated by stepwise 10-mV depolarizing pulses (400-ms duration, 5-second intervals) from a holding potential of −60 mV in cells dialyzed with 100 mmol/L ionized Ca$^{2+}$. Seal resistance was 2 to 10 GΩ. Peak current elicited at a single membrane potential was defined as the average of 500 sample points encompassing the maximal current point. In a single cell, K$_c$ currents were defined as the difference between outward current recorded in drug-free bath solution and after superfusion with 100 mmol/L ibetoxin (IBTX), a K$_c$ channel blocker. Trials were performed in triplicate and averaged to estimate peak current amplitudes (picoampere per picofarad) to normalize for cellular membrane area. For each cell, 10-mV hyperpolarizing steps were averaged to account for capacitance and leak compensation values.

Unitary K$_c$ currents were obtained in inside-out patches of human coronary arterial smooth muscle membranes, which were bathed in symmetrical 145 mmol/L KCl and subjected to membrane potential of +40 mV. Unitary currents were recorded for 2 minutes before and after application of authentic ONOO$^-$ or DC-ONOO$^-$ . Averaged current amplitudes were obtained for calculation of single-channel conductance.

**Statistics**

All data are expressed as mean±SEM. Percent dilation was calculated as the percent change from control internal diameter (in the presence of either myogenenic tone or endothelin-1) to maximal diameter measured after papaverine. All statistics were performed in paired fashion so that each vessel served as its own control. Data from vessels exposed to DC-ONOO$^-$, authentic, or exogenously generated ONOO$^-$ and studied by either patch-clamp or videomicroscopy were compared using a one-way ANOVA with repeated measures for dose and condition (ONOO$^-$ exposure). Differences between individual means were determined by Newman-Keuls test. All differences were judged to be significant at the level of $P<0.05$. 

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Results

Immunohistological Detection of Nitrotyrosine in Coronary Arteries

Because ONOO$^{-}$ decomposes very quickly at physiological pH, we first verified that our methods actually produced ONOO$^{-}$ in sufficient concentration and proximity to interact with exposed arterioles. Figure 1A and 1B show the effect of incubating RCAs with either decomposed ONOO$^{-}$ (Figure 1A), or 5 μmol/L authentic ONOO$^{-}$ (Figure 1B). Nitrotyrosine residues (brown staining) are markedly increased after exposure for 2 minutes to authentic ONOO$^{-}$ compared with decomposed ONOO$^{-}$. Figure 1C illustrates the effect of simultaneous treatment with an NO donor, SNP, and O$_2^-$, generated by XA and XO. Nitrotyrosine residues are prominently seen in the vasculature indicating the endogenous formation of ONOO$^{-}$. Treatment with SOD eliminated the formation of nitrotyrosine (Figure 1D). Specificity of antibody staining is shown in Figure 1E. No staining is seen in the absence of primary antibody. Vessels not exposed to either authentic ONOO$^{-}$ or ONOO$^{-}$-generating systems (control) showed similar amounts of staining as the vessels treated with DC-ONOO$^{-}$ (data not shown). Similar to RCAs, strong staining of nitrotyrosine residues was observed in HCAs treated with ONOO$^{-}$ compared with HCAs treated with DC-ONOO$^{-}$ or no treatment (n=2, data not shown).

Effect of ONOO$^{-}$ on Bradykinin-Induced Human Coronary Arteriolar Dilation

Figure 2A shows that incubation of HCAs for 2 minutes in DC-ONOO$^{-}$ did not significantly alter dilation to bradykinin (BK) compared with control (control versus DC-ONOO$^{-}$: $10^{-10}$ mol/L, 19±2% versus 26±6%; $10^{-8}$ mol/L, 53±10% versus 65±8%). ONOO$^{-}$ (5 μmol/L) significantly reduced dilation at both doses of BK (3±5% and −2.5±8%; n=6, *P<0.05 versus DC-ONOO$^{-}$). BK dilation was also tested with 1 μmol/L ONOO$^{-}$. Reduced dilation (37±6%) was observed at the higher (10$^{-8}$ mol/L) but not lower dose (20±4%; n=3). Because 5 μmol/L ONOO$^{-}$ elicited greater inhibition of dilation, and because this dose is in the range used by others,3,25 this concentration was applied for the rest of experiments. Both DC-ONOO$^{-}$ and authentic ONOO$^{-}$ had little effect on basal tone (before versus after treatment: DC-ONOO$^{-}$, 99±13 versus 99±13 μm; ONOO$^{-}$, 91±15 versus 100±13 μm). A similar reduction in dilation to both doses of BK was observed when ONOO$^{-}$ was generated in the vessel chamber by mixing either SNP (Figure 2B; 5±5% and 7±8% versus control of 20±11% and 42±14%; *P<0.05,
n=6) or SN (Figure 2C; 1±1% and 9±2% versus control of 10±2% and 37±3%; P<0.05, n=6) with XA and XO.

Reversibility of ONOO−-induced impairment of dilation to BK was assessed 2 and 4 hours after exposure to ONOO−. Similar degrees of impaired dilation to BK were observed at both recovery periods, indicating that the effect of ONOO− (which is rapidly decomposed at physiological pH) persists for at least 4 hours.

Effect of ONOO− on KCa Channel Opener-Induced Dilation

HCA dose-dependently dilated to NS1619, a Kc-channel opener, (10−6 mol/L, 13±3%; 10−5 mol/L, 39±5%). This dilator response was significantly blocked by iberiotoxin (IBTX), a specific Kc-channel blocker (10−6 mol/L, 4±1.9%; 10−5 mol/L, 15±5%; n=4, P<0.05 versus before IBTX). Because BK-induced dilation is mediated by activation of Kc channels in vascular VSMCs, the effect of ONOO− on NS1619-induced dilation was examined after endothelial denudation. In the presence of DC-ONOO−, HCAS dilated to NS1619 (10−6 mol/L, 24±7%; 10−5 mol/L, 61±10%). After administration of ONOO−, dilation to NS1619 was abolished (10−6 mol/L, −6.5±17%; 10−5 mol/L, −18±19%; n=10, P<0.05 versus DC-ONOO−) suggesting that ONOO− directly inhibits Kc channels in human coronary arteriolar VSMCs (Figure 3A).

Specificity of the ONOO−-induced impairment in dilation to BK and NS1619 was assessed using papaverine (10−3 and 10−4 mol/L). Papaverine maximally dilated HCA (control) from 63±6 to 102±9 μm. The percent maximal dilation to papaverine was similar in control, ONOO−, and DC-ONOO−-treated groups (Figure 3B; n=4, P=NS).

Effect of ONOO− on Whole-Cell K+ Current Densities

Inhibition of dilation to both BK and NS1619 by ONOO− implicates a direct effect on Kc channels. To directly determine the effect of ONOO− on Kc-channel activity in VSMC membranes, patch-clamp techniques were used to measure Kc currents in freshly isolated HCA VSMCs. Figure 4A shows families of voltage-gated K+ currents generated by incremental 10-mV depolarizing steps from −60 mV to +60 mV in VSMCs isolated from HCAs. Peak K+ current amplitude was similar between arteries exposed to regular bath solution (control) or DC-ONOO−. The currents were reduced by 100 nmol/L IBTX, identifying Kc channels as the primary conducting pathway in HCAs. Whole-cell Kc current was suppressed in VSMCs from HCAs exposed to 5 μmol/L ONOO−. Figure 4B compares K+ current densities between VSMCs exposed to DC-ONOO− and ONOO− in the presence and absence of IBTX (n=10 each). The IBTX-sensitive current density corresponding to the Kc current component was greatly reduced in cells exposed to ONOO−.

Effect of ONOO− on Single-Channel Properties of Kc Channels

Subsequent experiments examined the direct effect of ONOO− on Kc-channel activity in VSMCs. Intracellular components were eliminated by forming inside-out patches detached from the cell. Figure 5A shows sample recordings in the presence of either DC-ONOO− or ONOO−. DC-ONOO− had little effect on Kc-channel open state probability (NPo). However, ONOO− greatly reduced NPo of Kc-channel in VSMCs of HCAs (Figure 5B). Open state probability of Kc-channel was significantly reduced by ONOO− (0.02±0.003; n=6, P<0.05 versus control) compared with DC-ONOO− (0.12±0.009; n=6, P=NS versus control).

Effect of Superoxide on Human Coronary Arteriolar Dilation to Bradykinin

Because O2− is a precursor to the formation of ONOO− in vivo, it is important to know whether O2− might also alter Kc-channel function in HCAs. Dilation to BK, which operates through a mechanism involving activation of Kc, was compared in the absence and presence of O2−, generated by XA+XO. N4-nitro-l-arginine methyl ester (L-NAME, 10−4 mol/L) suppressed in VSMCs exposed to DC-ONOO− or ONOO− by 80±3% (n=6, P<0.05 versus control). BK-induced dilation was abolished by 100 nmol/L L-NAME (n=6, P<0.05 versus control).

Figure 3. A, Effect of authentic ONOO− on NS1619-induced dilation in human coronary arterioles. Compared with DC-ONOO−, ONOO− greatly reduced dilation to NS1619 (n=6, *P<0.05 vs DC-ONOO−). B, Comparison of dilations to papaverine in human coronary arterioles incubated with no ONOO− (control), DC-ONOO−, or authentic ONOO−. Dilations to papaverine were similar among all 3 groups (n=4, P=NS).

Figure 4. A, Sample traces of whole-cell K+ current in human coronary arteriolar VSMCs. Currents were elicited by incremental 10-mV depolarizing steps from −60 mV to +60 mV. Cell capacitance was similar between cells: DC-ONOO− was 10 pF and ONOO− was 8 pF. B, Current voltage relationships of K+ current density in cells treated with either DC-ONOO− (left) or ONOO− (right). DC-ONOO− had no effect on whole-cell K+ current. IBTX blocked a large component of the outward current in cells exposed to DC-ONOO−. ONOO− reduced whole-cell K+ current by 30%. IBTX had no further effect in the presence of ONOO−.
clamp configuration. NP_o of K_Ca channel was compared in
ined in human arteriolar VSMCs using an inside-out patch
/XO. O_2 between XA and XA
ence of O_2
authentic ONOO
Figure 5. A, Sample recordings of single-channel K_Ca current
peroxide. Similar dilations to BK (10^{-9} mol/L, 7±4% versus 7±5%; 10^{-8} mol/L, 22±10% versus 19±7%; 10^{-7} mol/L, 89±4% versus 79±7%; n=8, P=NS) were observed in
of NO on cytochrome P450 epoxygenase,30 the enzyme
previously, products of NOS and COX do not substantially
VASMCs. Fourth, the direct effect of ONOO^· inhibits dilation to NS1619, a direct opener of K_Ca channels. Because these channels are responsible for dilation to BK, this finding implicates K_Ca channels in the mechanism of impaired BK-induced dilation after exposure to ONOO^·. Third, the direct effect of ONOO^· on K_Ca channels is inhibitory. This finding is supported both by whole-cell and isolated inside-out patches from HCA VSMCs. Fourth, the effect of ONOO^· on K_Ca is in direct contrast to another free-radical, O_2^·, which did not affect dilation to BK or activity of K_Ca channels. These findings may help to explain the ability of EDHF to compensate for NO in disease states characterized by enhanced production of superoxide,26,27 whereas in conditions characterized by excess production of ONOO^·, the compensation could be prevented, thereby reducing myocardial perfusion.1

BK-Mediated Vasodilation and Superoxide
The inhibitory effect of O_2^· on NO-mediated vasodilation is well established. In subjects with risk factors for coronary artery disease (CAD), elevated levels of O_2^· reduce NO-mediated vasodilation in the coronary circulation even before lesions are evident.28 However in vessels from some species loss of NO-mediated dilation is not associated with reduced vasodilator capacity, possibly due to a compensatory dilation by other factors. Such is the case with BK-induced response in rabbit aorta where NO mediates dilation under normal conditions, but in hypercholesterolemia, where elevated production of O_2^· inactivates NO-mediated vasodilation.27 In response, non-NO mechanisms compensate to maintain dilation to BK.29 This compensation likely involves enhanced release of other vasodilator compounds such as EDHF. The mechanism may involve release from the inhibitory influence of NO on cytochrome P450 epoxygenase,30 the enzyme

Discussion
There are 4 major new findings of the present study. First, ONOO^· inhibits dilation to BK in HCAs. As we observed

Figure 6. Effect of superoxide on BK-induced dilation in human coronary arterioles. Superoxide was generated by the reaction of XA+XO in the vessel chamber. Dilation to BK was preserved in the presence of superoxide.
responsible for generating epoxyeicosatrienoic acid, a likely candidate for EDHF. In contrast to NO-mediated dilation, the hyperpolarization-mediated vasodilatation is not inhibited by exaggerated production of superoxide as occurs with hypercholesterolemia. The present study confirms the maintenance of BK-induced vasodilation in the presence of exogenously generated superoxide. Similar concentrations of $O_2^-$ did suppress dilation to NO-mediated responses in rabbit aorta.31,32

BK dilates both conduit and resistance vessels in the heart.33,34 In both cases the endothelium plays a necessary role, liberating primarily NO in conduit arteries and EDHF in resistance arterioles, although this may vary depending on the species.35-37 In hearts from human subjects with CAD or its risk factors, BK-induced dilation almost exclusively involves release of EDHF, which opens $K_{Ca}$ channels in adjacent VSMCs, inducing hyperpolarization and relaxation.12 This contrasts with subjects who do not have CAD, where NO synthase participates in the dilation.38 The present findings are consistent with a shift toward hyperpolarization-mediated dilation in subjects with CAD, even though a prominent component of the dilation in subjects without CAD also involves hyperpolarization.12

The failure of exogenously administered $O_2^-$ to inhibit dilation to BK suggests that the formation, release, and vascular action of EDHF is not affected by $O_2^-$. It is possible that $O_2^-$ does inhibit EDHF and that alternate dilator mechanisms offset the loss of EDHF. However this is unlikely for two reasons. First, other primary mechanisms of endothelium-dependent dilation (prostacyclin, NO) were inhibited in this study. Thus the source of an alternate mechanism of compensation is not clear. Second, direct activation of $K_{Ca}$ channels in VSMCs was not affected by $O_2^-$. Thus, the present data are consistent with EDHF as a viable alternative mechanism of vasodilation when elevated levels of $O_2^-$ are present.

**Effect of ONOO$^-$ on $K_{Ca}$ Channel Activity**

In contrast to $O_2^-$, ONOO$^-$ did inhibit BK-mediated dilation in subjects with CAD or its risk factors. ONOO$^-$ is a more reactive ROS than $O_2^-$ and in physiological systems has a longer half-life and greater volume of distribution than $O_2^-$. ONOO$^-$ is both nitrosylating and oxidizing, and may affect a wide range of cell proteins. The present study showed that ONOO$^-$ not only impaired coronary dilation to BK, but also inhibited dilation to NS1619, an activator of $K_{Ca}$ channels. This would suggest that it is not necessary to invoke inhibition of endothelial release of EDHF as a mechanism by which ONOO$^-$ inhibits dilation to BK.

To examine this mechanism from a more direct approach, we tested the effect of ONOO$^-$ on $K_{Ca}$ currents in whole-cell patches. ONOO$^-$, but not decomposed ONOO$^-$, reduced $K_{Ca}$ whole-cell currents in isolated HCA VSMCs. This finding of reduced channel activity could result from alterations in the channel itself or from effects on cytosolic components that regulate channel function. To minimize possible effects of cytosolic components, we further demonstrated that ONOO$^-$ reduces channel opening probability in isolated inside-out patches from VSMCs, suggesting that ONOO$^-$ has a direct effect on the $K_{Ca}$ channel itself, or possibly a membrane channel-associated protein.

**Study Limitations**

Peroxynitrite is rapidly decomposed at physiological pH and may not reach the tissue in high enough concentrations to interfere with $K^+$ channel function. It is possible that some byproduct of ONOO$^-$ decomposition is responsible for altered channel activity. This is not likely for at least two reasons. First, decomposed ONOO$^-$ did not alter dilation to BK or $K_{Ca}$ currents in VSMCs. Second, immunohistochemistry demonstrated prominent nitrotyrosine accumulation in both rat and human coronary vessels exposed to ONOO$^-$. Nevertheless we cannot exclude an effect of ONOO$^-$ on cell membranes that inhibits opening of $K_{Ca}$ channels.

Nitrosylation of proteins is very slowly reversible. Our studies were not of sufficient duration (longer than 4 hours) to determine the duration of the impairment in $K_{Ca}$ function after exposure to ONOO$^-$. Although there is substantial evidence for ONOO$^-$ accumulation in the tissue of subjects with cardiovascular disease,40 it is difficult to estimate the local concentrations of this radical species. In conditions such as ischemia and reperfusion or inflammation, both $O_2^-$ and NO may accumulate in significant amounts within cells.8 Depending on the stoichiometry, this may substantially increase production of ONOO$^-$. The concentrations of ONOO$^-$ used in this study were based on similar concentrations used by others to effect changes in cell function.2,3 The use of SNP and XA/XO was an attempt to more closely mimic the in vivo situation where ONOO$^-$ is formed by a similar reaction of $O_2^-$ with NO, and to avoid possible damage from very high local concentrations of ONOO$^-$ that could arise from nonhomogeneous mixing of the concentrated solution applied to the bath. Similar nitrotyrosine staining and functional inhibition of $K_{Ca}$ was observed with this technique. However, data must be interpreted with caution because we cannot be sure to have mimicked pathophysiological conditions where ONOO$^-$ is generated.

We observed that endothelial denudation enhanced the dilator response of HCAs to NS1619. This enhancement was not due to the presence of DC-ONOO$^-$, because a similar dilator response to bradykinin or papaverine was seen in controls or in vessels incubated with DC-ONOO$^-$. However, the precise mechanism by which endothelial denudation enhances dilation of HCAs to NS1619 is not clear. Future studies will be needed to explore the mechanisms of differential effect of NS1619 on vascular endothelial and smooth muscle cells. In this study, vessels were denuded to minimize effects of NS1619 on the endothelium.

**Summary**

Although it is well described that $O_2^-$ impairs NO-mediated vasodilation, the effect on other mechanisms of vasodilation is not well understood. We determined that BK-induced dilation is not reduced by $O_2^-$. However, ONOO$^-$, another ROS formed by the interaction between NO and $O_2^-$, does inhibit dilation to BK. The mechanism appears to involve a direct action of ONOO$^-$ to limit opening of the VSMC $K_{Ca}$ channel. These findings provide a potential mechanism for
compensatory dilation by EDHF when NO-mediated responses are reduced and could also explain an absolute reduction in vasodilation to many compounds when ONOO− generation is increased.

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Peroxynitrite Inhibits Ca\(^{2+}\)-Activated K\(^+\) Channel Activity in Smooth Muscle of Human Coronary Arterioles

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