Nitration and Functional Loss of Voltage-Gated K^+ Channels in Rat Coronary Microvessels Exposed to High Glucose

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Coronary microvessels generate reactive oxygen species in response to high glucose (HG), resulting in vasodilator defects involving an impaired function of vascular K^+ channels. Inhibition of voltage-gated K^+ (K_v) channels by peroxynitrite ($ONOO^-$), formed by the interaction of superoxide and nitric oxide, may contribute to impaired dilation. The present study investigated whether HG induces $ONOO^-$ formation to mediate nitration and impairment of K_v channels in rat small coronary arteries (RSCAs). Exposure to $ONOO^-$ reduced the dilator influence of K_v channels in RSCAs. Patch-clamp studies revealed that $ONOO^-$ diminished whole-cell and unitary K_v currents attributable to the K_v1 gene family in smooth muscle cells. Subsequently, immunohistochemically detected enhancement of nitrotyrosine residues in RSCAs that were cultured in HG (23 mmol/l) compared with normal glucose (5.5 mmol/l) for 24 h correlated with the nitration of $K_v1.2$ channel α-subunits. HG-induced nitrotyrosine formation was partially reversed by scavenging $ONOO^-$. Finally, RSCAs that were exposed to HG for 24 h showed a loss of K_v channel dilator influence that also was partially restored by the $ONOO^-$ scavengers urate and ebselen. We conclude that $ONOO^-$ generated by HG impairs K_v channel function in coronary microvessels, possibly by nitrating tyrosine residues in the pore-forming region of the K_v channel protein. *Diabetes* 53:2436–2442, 2004

In type 2 diabetes, endothelium-dependent vasodilator responses are impaired in both the macro- and the microvasculature, possibly related to the formation of reactive oxygen species (ROS). The presence of hyperglycemia per se fosters the local formation of superoxide ($O_2^{\cdot -}$) (1,2), which may interact with nitric oxide (NO) to form the highly reactive oxidant peroxynitrite ($ONOO^-$). Indeed, recent evidence has emphasized that NO derived from inducible NO synthase under conditions of high glucose (HG) may contribute to vasodilator dysfunction by driving the generation of $ONOO^-$ (3). In turn, $ONOO^-$ may promote oxidative and nitrosative tissue damage (4), in part by nitration of tyrosine residues to impair the function of multiple proteins, including K^+ channels that mediate vasodilation (5,6).

In this regard, most studies have focused on $ONOO^-$-induced inhibition of high-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels as a mechanism of vasodilator impairment (5,6). However, a targeted inhibition of K_{Ca} channels by $ONOO^-$ cannot account for some of the profound vasodilator defects that are induced by HG and seem to relate more closely to an impaired function of voltage-gated K^+ (K_v) channels. For example, vasodilator responses to forskolin and isoproterenol rely on cAMP-induced activation of K_v channels to mediate relaxation, but these responses are markedly blunted in rat small coronary arteries (RSCAs) that are exposed to HG for 24 h (7). In addition, the activity and expression levels of K_v channels are sensitive to oxidant stress (8), and although K_v channels emanate from at least 11 gene families (K_v1–K_v11), the K_v1 "Shaker-type" family channels that are densely expressed in vascular smooth muscle cells (VSMCs) may be particularly susceptible to open-channel block by redox agents (9,10). Collectively, these findings provide a compelling reason to investigate whether ROS associated with HG alter K_v channel function. The present study tested the hypothesis that HG induces endogenous $ONOO^-$ formation in RSCAs, resulting in nitration and impairment of K_v channels and vasodilator function.

RESEARCH DESIGN AND METHODS

Preparation of RSCAs. Seven-week-old male Sprague-Dawley rats (Harlan, Madison, WI) were anesthetized with sodium pentobarbital (60 mg/kg i.p.). RSCAs (internal diameter 150–200 μm) were dissected from the left ventricle. Some RSCAs were incubated in culture media supplemented with either 5.5 mmol/l D-glucose (normal glucose [NG]) or 23 mmol/l D-glucose (high glucose [HG]), or 5.5 mmol/l D-glucose plus 17.5 mmol/l L-glucose (LG) for osmotic control as described previously (11). All rats were housed in the Association for Assessment and Accreditation of Laboratory Animal Care–approved Biomedical Resource Center at the Medical College of Wisconsin, and all protocols were approved by the Animal Care Committee.

Formation of $ONOO^-$. Authentic $ONOO^-$ was synthesized according to published methods (5,6). The amount of $ONOO^-$ in the stock solution was determined spectrophotometrically using the reported extinction coefficient for $ONOO^-$ (1,670 $mol \cdot l^{-1} \cdot cm^{-1}$). Before each application, an aliquot of the stock solution was diluted in 1 mmol/l NaOH and rapidly added to the vessel chamber to achieve a final concentration of 5 μmol/l. Decomposed $ONOO^-$

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4-AP, 4-aminopyridine; COR, correolide; HG, high glucose; K_{Ca}, Ca^{2+}-activated K^+; K_v, voltage-gated K^+; LG, L-glucose; L-NAME, N^{ω}-nitro-L-arginine methyl ester; MnTBAP, manganese [III] tetrakis 4-benzoic acid porphyrin; NG, normal glucose; ROS, reactive oxygen species; RSCA, rat small coronary artery; VSMC, vascular smooth muscle cell.

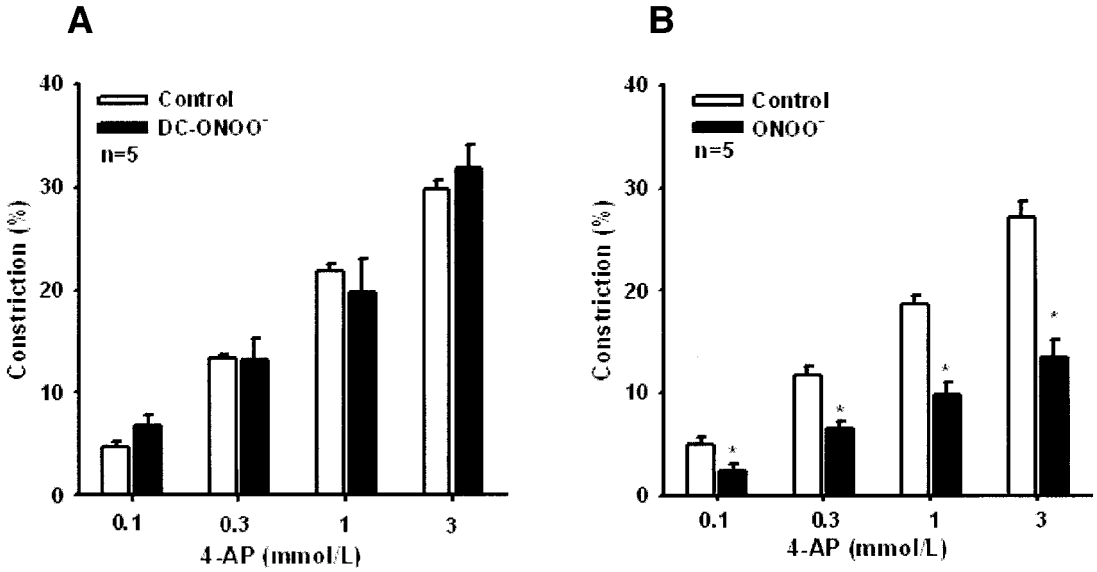

FIG. 1. The effect of authentic $ONOO^-$ on the contractile response to 4-AP in RSCAs. *A*: DC-$ONOO^-$ had no effect on 4-AP–induced contractions. *B*: $ONOO^-$ (5 μmol/l) reduced constriction to 4-AP. *$P < 0.05$ vs. control.

($DC\text{-}ONOO^-$) was made by leaving $ONOO^-$ at room temperature for at least 2 h. The decay of $ONOO^-$ was confirmed spectrophotometrically.

Videomicroscopy. RSCAs were cannulated on glass micropipettes in an organ chamber filled with physiological salt solution as described previously (11). The physiological salt solution was warmed to 37°C, continuously circulated, and bubbled with 21% O_2, 5% CO_2, and 74% N_2. Most vessels that were equilibrated for 1 h at an intraluminal pressure of 60 mmHg developed spontaneous tone averaging 70% of the passive diameter, which was defined as the maximal diameter assessed in Ca^{2+}-free solution at the end of the experiment. RSCAs that did not develop this degree of spontaneous tone were constricted by U46619 (10 nmol/l) to 70% of the passive diameter.

Patch-clamp recording of K_v current. Enzymatic isolation of single VSMCs was performed according to published methods (12). Whole-cell patch-clamp recordings were obtained using standard pulse protocols and instrumentation (5). Briefly, families of K^+ currents were generated by stepwise 10-mV depolarizing pulses (400-ms duration, 5-s intervals) from a holding potential of −60 mV to 50 mV in cells that were dialyzed with 10 nmol/l calcium. Trials were performed in triplicate, and peak current amplitudes were divided by membrane capacitance to obtain K^+ current density (12).

Unitary K_v currents were obtained in inside-out membrane patches of VSMCs from RSCAs, which were bathed in symmetrical 145 mmol/l K^+ solutions. For eliminating interfering currents from K_{Ca} and ATP-sensitive K^+ channels, 100 nmol/l iberiotoxin was added to the pipette solution and 1 μmol/l apamin and glibenclamide was added to the bath solution. The effect of authentic $ONOO^-$ on unitary K_v currents was evaluated by 2-min recording intervals before and after drug application. Averaged current amplitudes were obtained at stepwise patch potentials for calculation of single-channel conductance.

Immunohistochemical detection of nitrotyrosine. For determining the effect of HG on nitrotyrosine formation, RSCAs were exposed to NG or HG for 24 h. In some arteries, urate (100 μmol/l) was applied every 8 h to scavenge $ONOO^-$. The joint effect of the $O_2^{\cdot-}$ scavenger manganese [III] tetrakis 4-benzoic acid porphyrin (MnTBAP; 300 μmol/l) and the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 μmol/l) on nitrotyrosine formation was examined in other arteries. Freshly isolated RSCAs were used as controls. All RSCAs were prepared as frozen sections (5 μm thick). The immunodetection of nitrotyrosine residues has been described (5,6).

Immunoprecipitation and Western blot. For obtaining adequate vascular tissue for immunoanalysis, VSMCs were enzymatically dissociated from RSCAs and cultured in RPMI-1640 medium that contained 20% FBS, 100 units/ml penicillin G, and 100 μg/ml streptomycin. The first passage of VSMCs was divided into six plates and permitted to reach 80% confluence. In other plates, the purity of VSMCs was verified by immunostaining with α-smooth muscle actin. Subsequently, cells were treated for 24 h with DMEM that contained NG, HG, or LG. Urate (100 μmol/l) was added to some HG plates to scavenge $ONOO^-$. After incubation, total or membrane proteins were collected for immunoprecipitation and Western blot analysis, respectively (13).

Proteins (100–200 μg/reaction) were immunoprecipitated by incubations at 4°C with antinitrotyrosine (4 μl/reaction) for 2 h, and then incubated in protein A for 1 h. The immunoprecipitates were centrifuged and washed three times with ice-cold lysis buffer, and Western blots were performed using antibodies directed against $K_v1.2$ and $K_v1.5$ channel α-subunits (Upstate Biotechnology, Lake Placid, NY) (13). Anti-$K_v1.2$ and anti-$K_v1.5$ were also used to compare $K_v1.2$ and $K_v1.5$ expression at different glucose levels. β-Actin was used as an internal standard to ensure equal lane loading.

Chemicals. All chemicals were purchased from Sigma. Correolide was provided by Drs. Maria Garcia and Gregory Kaczorowski (Merck Research Laboratories, Rahway, NJ). Correolide was prepared as a 20-mmol/l stock in DMSO and stored at 4°C.

Statistical analysis. All data are expressed as mean ± SE. Percentage of constriction was defined as the percentage of reduction from control internal diameter. Data from videomicroscopy, patch-clamp, and Western blot studies were compared using one-way ANOVA, with repeated measures for dose and condition. Differences between individual means were determined by Newman-Keuls test. All differences were judged to be significant at $P < 0.05$.

RESULTS

$ONOO^-$ blunts the vasodilator influence of K_v channels. Initial experiments were designed to determine whether $ONOO^-$ inhibited K_v channel function in RSCAs. Graded concentrations of 4-aminopyridine (4-AP), a broad-spectrum K_v channel blocker, constricted RSCAs in control conditions (Fig. 1; $n = 5$). These findings suggested that K^+ efflux through K_v channels contributed significantly to resting diameter. Notably, decomposed $ONOO^-$ ($DC\text{-}ONOO^-$) had no effect on the constriction of RSCAs to 4-AP (Fig. 1*A*). However, 4-AP–induced contractions were attenuated by incubation of RSCAs in authentic $ONOO^-$ (5 μmol/l), indicating a loss of K_v channel dilator function (Fig. 1*B*). Figure 2*A* shows similar U46619-induced constriction (10^{-9} to 10^{-7} mol/l) in RSCAs that were exposed to $DC\text{-}ONOO^-$ or $ONOO^-$. Arteries that were incubated in $DC\text{-}ONOO^-$ or $ONOO^-$ showed similar dilation to the L-type Ca^{2+} channel antagonist nifedipine (10^{-8} to 10^{-6} mol/l; Fig. 2*B*). These results indicate that $ONOO^-$ selectively impairs vasoactive responses that are dependent on functional K_v channels.

$ONOO^-$ inhibits whole-cell and unitary K_v1 currents. K_v1 "Shaker-type" gene family members are redox targets in some VSMCs (8). To determine whether $ONOO^-$ specifically impairs K_v1 channels in RSCAs, we used the

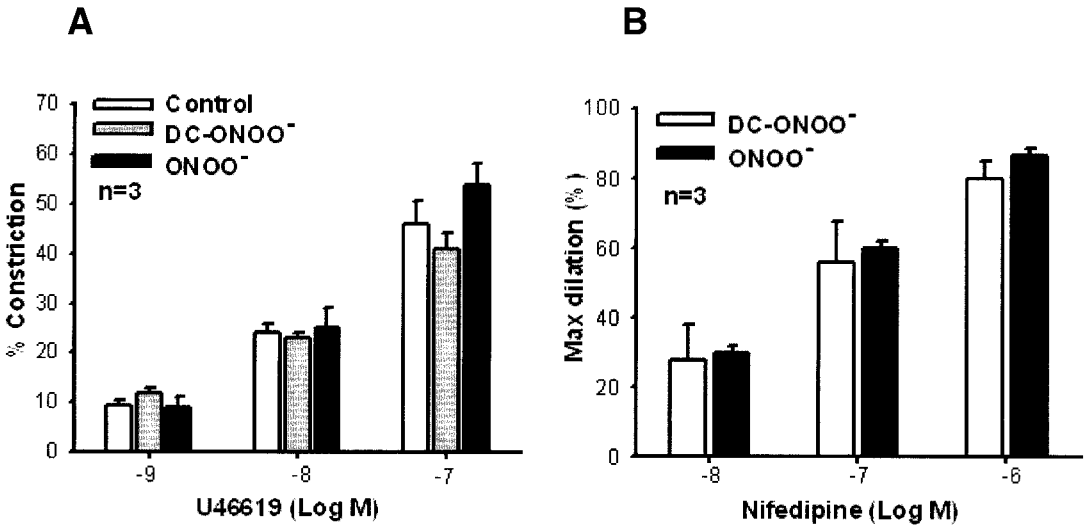

FIG. 2. *A*: **Similar contractile responses to graded doses of U46619 were observed in arteries that were incubated with either DC-ONOO⁻ or ONOO⁻ compared with control (n = 3; NS).** *C*: **Nifedipine induced similar dilator responses in arteries that were incubated with DC-ONOO⁻ and ONOO⁻ (n = 3; NS).**

specific K_v1 channel blocker correolide (COR) (13). DC-ONOO⁻ did not significantly affect K_v current in freshly isolated VSMCs, but COR (1 μmol/l) blocked a large component, indicating the presence of K_v1 family channels (Fig. 3*A*). In contrast, authentic ONOO⁻ (5 μmol/l) reduced total K_v current amplitude, and COR-sensitive K_v1 current was not detected after exposure to ONOO⁻ (Fig. 3*B*). Current-voltage relations averaged from five cells verified that DC-ONOO⁻ did not significantly alter K_v current density in coronary VSMCs (Fig. 3*C*), whereas authentic ONOO⁻ fully eliminated the COR-sensitive K_v1 current component (Fig. 3*D*).

Subsequent studies determined whether ONOO⁻ directly inhibited K_v channels in cell-free, inside-out patches of coronary VSMCs. A prominent K_v channel that progressively activated in response to stepwise patch depolarization was detected (Fig. 4*A*) and showed a unitary conductance of ~75 pS (Fig. 4*B*). Exposing the cytosolic patch surface to ONOO⁻ (5 μmol/l) profoundly inhibited K_v channel activity, whereas the unitary amplitude was

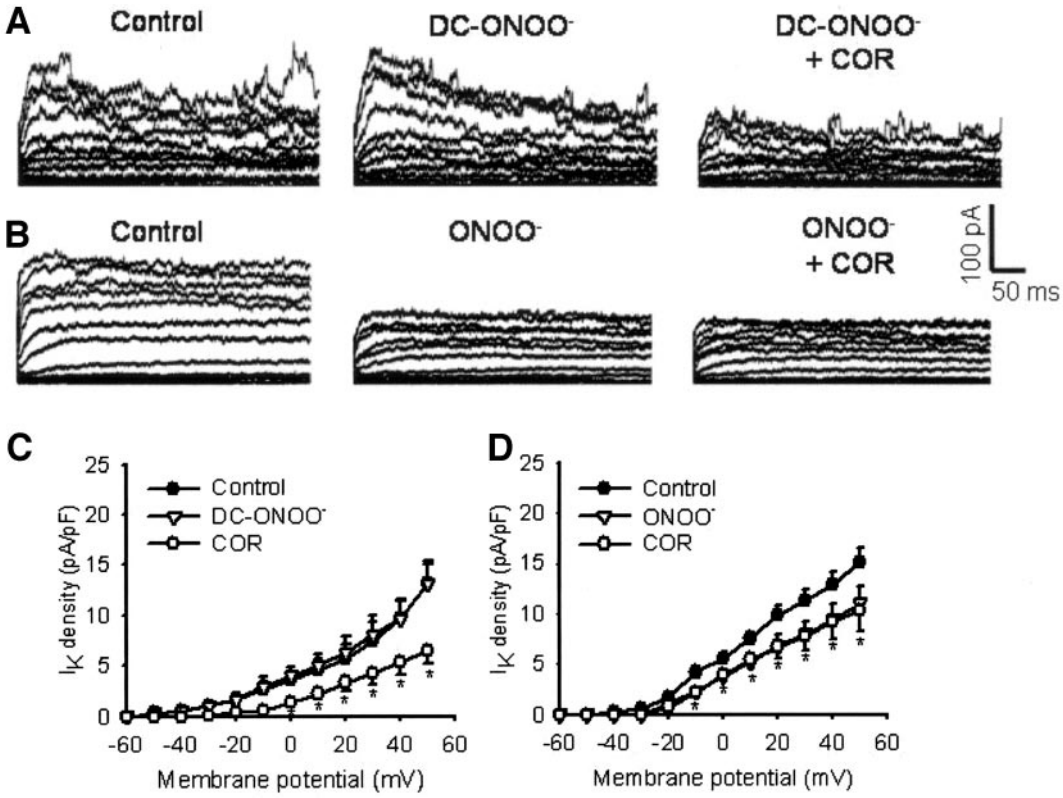

FIG. 3. *A* **and** *B*: **Sample traces of whole-cell K^+ current in VSMCs. Currents were elicited by 10-mV depolarizing steps from −60 mV to 50 mV. Cell capacitance was 7 pF (DC-ONOO⁻) and 8 pF (ONOO⁻).** *C* **and** *D*: **I-V relationships of K^+ current (I_K) density in cells that were treated with either DC-ONOO⁻ or ONOO⁻, respectively. *P < 0.05 vs. control.**

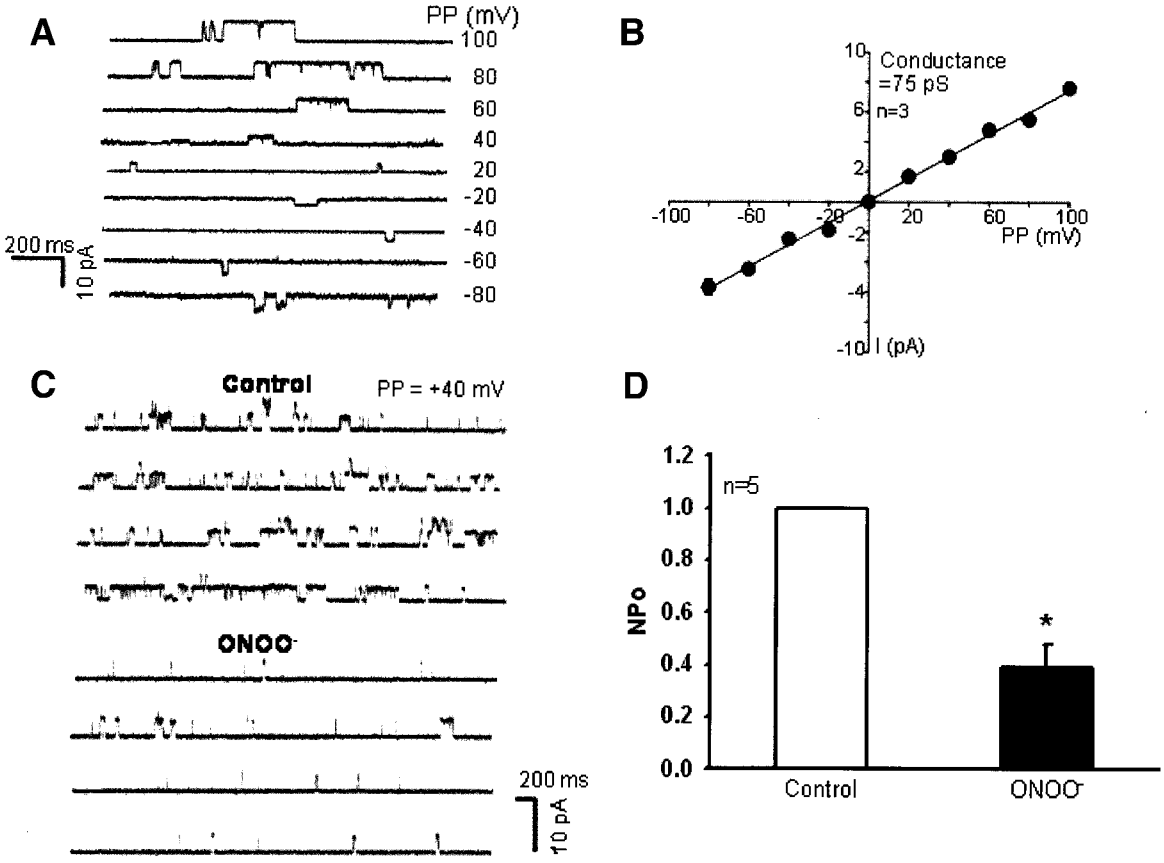

FIG. 4. ***A*****: Unitary K_v current elicited at different patch potentials (PP).** ***B*****: K_v current amplitudes (I) plotted as a function of PP revealed a single-channel conductance of 75 pS ($n = 3$).** ***C*****: Recording of unitary K_v currents before and after authentic 5 μmol/l $ONOO^-$.** ***D*****: Average open-state probability (NPo) before and after $ONOO^-$ ($n = 5$). *$P < 0.05$ vs. control.**

unaffected (Fig. 4*C*). In five different cells, $ONOO^-$ reduced the open-state probability of single K_v channels by 40 ± 8% (Fig. 4*D*).

HG enhances $ONOO^-$ production. On the basis of initial findings indicating that authentic $ONOO^-$ impaired K_v1 channel function, new studies investigated whether HG promotes $ONOO^-$ formation in RSCAs. Nitrotyrosine, a footprint of $ONOO^-$, was used to assess $ONOO^-$ production in RSCAs that were exposed to NG or HG (5). Sample images from five representative experiments indicate that staining was more prominent in RSCAs that were incubated in HG media (Fig. 5*A* and *B*). Nitrotyrosine levels were reduced after treatment with 100 μmol/l urate, a scavenger of $ONOO^-$ (Fig. 5*C* and *D*). The nitrotyrosine signal also was decreased after dual incubation with MnTBAP (300 μmol/l) and L-NAME (100 μmol/l), drugs that scavenge $O_2^{\cdot -}$ and inhibit NO synthesis, respectively (Fig. 5*E* and *F*). A low level of nitrotyrosine reactivity was observed in freshly isolated RSCAs (Fig. 5*G*), and elimination of the primary antinitrotyrosine antibody resulted in signal loss (Fig. 5*H*). These studies suggest that HG increases tyrosine nitration via endogenous $ONOO^-$ that is formed from the reaction of $O_2^{\cdot -}$ with NO.

HG enhances nitration of K_v channels. The K_v1 channels in VSMCs represent tetrameric structures composed of $K_v1.2$ and $K_v1.5$ α-subunits (13,14). Western blots indicated that the expression of $K_v1.2$ and $K_v1.5$ α-subunits was similar between RSCAs that were incubated for 24 h in NG, LG, or HG media (Fig. 6*A* and *B*). Averaged densitometric values from five studies confirmed these findings in which the expression of β-actin, a 42-kDa structural protein, provided an internal standard (Fig. 6*C*). However, nitrotyrosine immunoprecipitates revealed an elevated nitration of $K_v1.2$ α-subunits in RSCAs that were incubated in HG for 24 h, a response that was partially normalized by urate (Fig. 6*D*). In contrast, HG did not promote nitration of $K_v1.5$ α-subunits, and urate had little effect on basal nitration of this protein (Fig. 6*E*). Averaged data from five samples of immunoprecipitates indicated that HG enhanced nitration of $K_v1.2$ but not $K_v1.5$ α-subunits (Fig. 6*F*). Increased nitration of $K_v1.2$ α-subunits was also observed in $K_v1.2$ immunoprecipitates that were probed with nitrotyrosine antibody (data not shown).

Impaired K_v channel function is partially restored by scavengers of $ONOO^-$. A final set of experiments investigated whether incubation in HG for 24 h impairs K_v channel dilator function via generation of $ONOO^-$. Compared with RSCAs that were exposed to NG levels (Fig. 7*A*), those that were exposed to HG constricted less to 4-AP (Fig. 7*B*), indicating a reduced dilator influence of K_v channels. Scavenging of $ONOO^-$ by urate (100 μmol/l) restored 30% constriction to 4-AP in HG but not NG arteries. Ebselen (100 μmol/l), a chemically distinct $ONOO^-$ scavenger, also partially (25%) and selectively restored the impaired constrictor response to 4-AP observed in RSCAs that were incubated in HG (Fig. 7*C* and *D*).

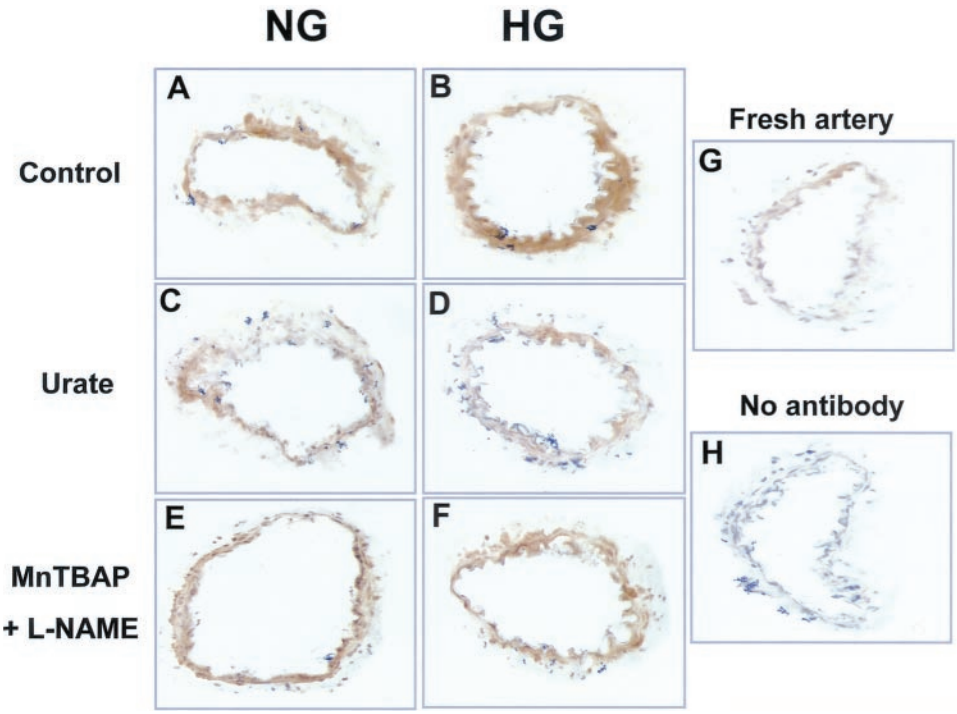

FIG. 5. Nitrotyrosine (NT) levels in RSCAs. The findings are representative of five different preparations. *A* and *B*: Arteries were incubated for 24 h in NG or HG. Brown staining indicates NT residues. Staining was more prominent in HG. *C* and *D*: Immunoreactivity was reduced by 100 μmol/l urate, a scavenger of $ONOO^-$. *E* and *F*: Immunoreactivity also was reduced by 300 μmol/l MnTBAP and 100 μmol/l L-NAME. *G*: A freshly isolated artery was used as a control. *H*: Immunoreactivity was lost in the absence of anti-NT.

DISCUSSION

The present study provides several new findings. First, our data demonstrate that authentic $ONOO^-$ attenuates the dilator function of K_v channels in RSCAs and reduces macroscopic and single-channel K_v1 current. Second, short-term exposure of RSCAs to HG promotes $ONOO^-$ formation, an event that is associated with the nitration of $K_v1.2$ but not $K_v1.5$ pore-forming α-subunits. Third, RSCAs that are incubated in HG demonstrate impaired dilator function of K_v channels, and this defect is partially restored by $ONOO^-$ scavengers. Overall, these findings suggest potentially important roles for $ONOO^-$ production directly or through elevations in ambient glucose in the control of coronary vasomotor tone.

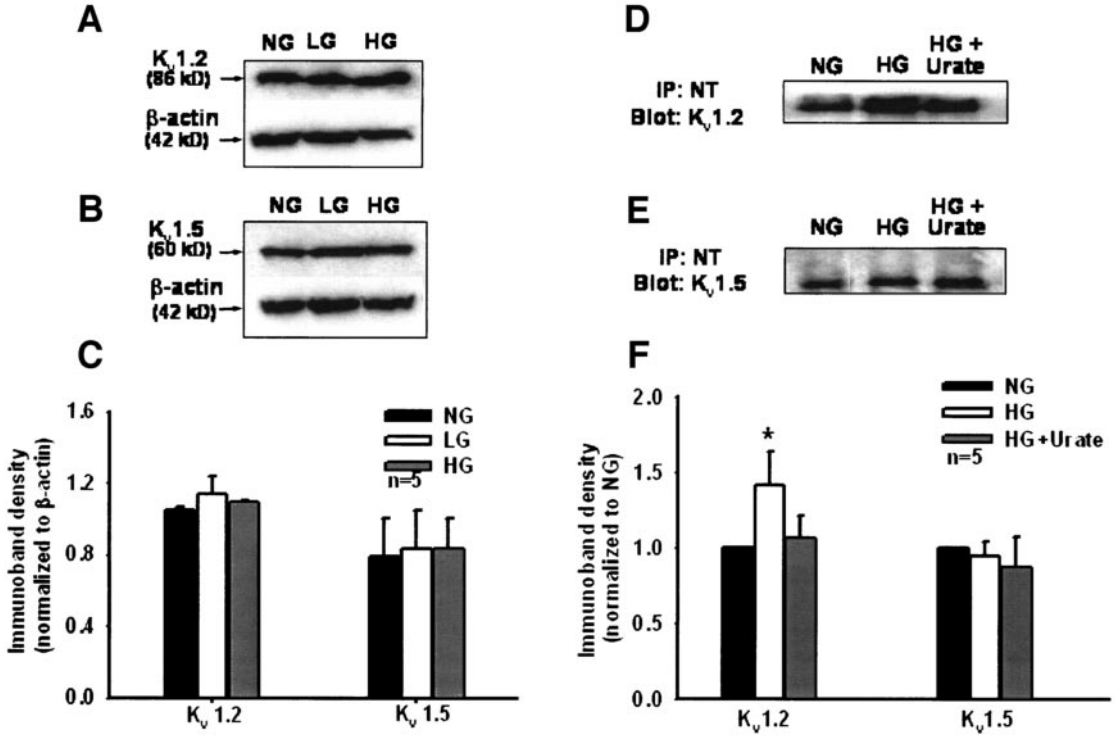

FIG. 6. *A* and *B*: Western blots demonstrate similar expression of $K_v1.2$ and $K_v1.5$ α-subunits, respectively, in RSCAs that were incubated for 24 h in NG, LG, or HG. β-Actin was used as an internal standard. *C*: Averaged values for five Western blots. *D*: Immunoprecipitates of $K_v1.2$ showed elevated NT residues in HG compared with NG arteries. Scavenging of $ONOO^-$ by 100 μmol/l urate reduced nitration levels in HG vessels. *E*: Similar levels of NT residues were detected in $K_v1.5$ immunoprecipitates from LG and HG arteries. Urate did not alter the immunoreactive signal. *F*: Average band densities normalized to the NG signal ($n = 5$). *$P < 0.05$ vs. NG.

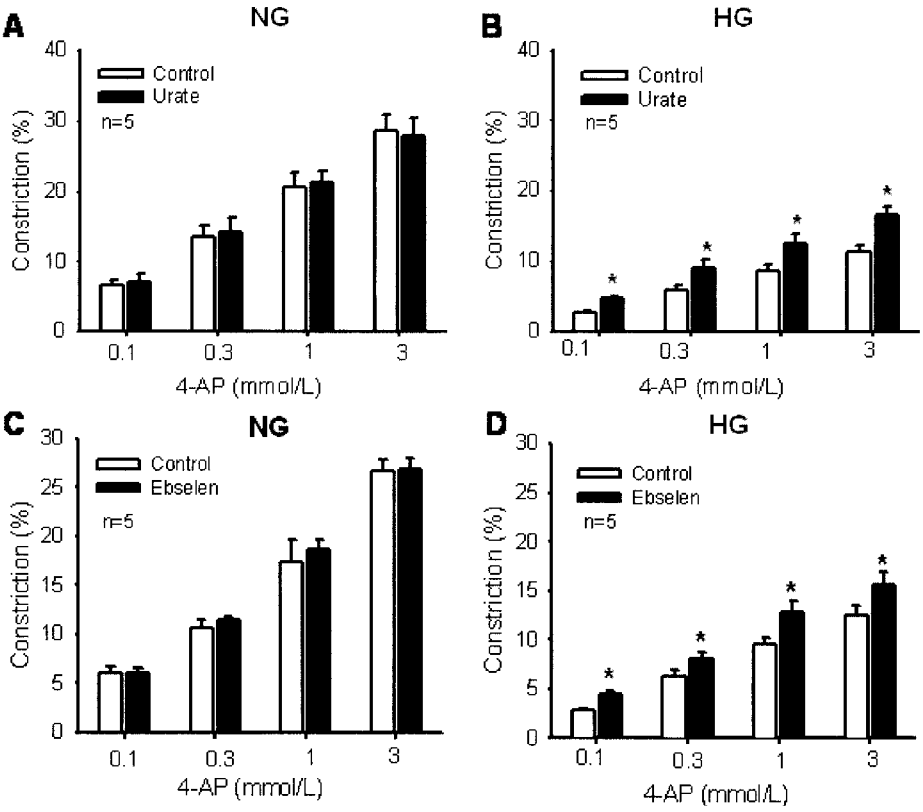

FIG. 7. *A* and *B*: RSCAs that were incubated for 24 h in NG constricted more to 4-AP than RSCAs that were exposed to HG, respectively. Scavenging of $ONOO^-$ by 100 μmol/l urate partially restored 4-AP constrictions in HG preparations. *C* and *D*: Scavenging of $ONOO^-$ by 100 μmol/l ebselen also partially restored responses in HG arteries. *$P < 0.05$ vs. NG.

$ONOO^-$ attenuates K_v channel function. The formation of $ONOO^-$, driven by the interaction of $O_2^{\cdot -}$ and NO, has been proposed as a contributing factor in the pathogenesis of vasodilator dysfunction (5). Recently, our laboratory reported that vasodilator responses that are dependent on cAMP activation of K_v channels were attenuated in RSCAs that were exposed to HG for 24 h (7). The findings of the present study directly demonstrate that authentic $ONOO^-$ reduces K_v channel dilator function in cannulated and pressurized RSCAs. In addition, patch-clamp studies revealed that $ONOO^-$ blocks a COR-sensitive component of K_v1 current in single VSMCs from RSCAs, suggesting that K_v1 family channels are susceptible to $ONOO^-$ inhibition. Finally, our data demonstrate that the open-state probability of K_v channels in cytosol-free membrane patches of RSCAs is reduced in response to authentic $ONOO^-$, suggesting that $ONOO^-$ may directly inhibit K_v1 channels in VSMC membranes independent of intracellular signaling mechanisms. Thus, it seems that $ONOO^-$ may powerfully inhibit coronary K_v1 channel activity.

In this study, urate effectively prevented formation of $ONOO^-$ in RSCAs that were exposed to HG (Fig. 5) but partially restored the impaired 4-AP constriction in HG vessels. This may be due to a higher threshold for detection of $ONOO^-$ by immunohistochemistry than for its inhibition of K_v channels, which may occur at concentrations not detected by immunohistochemistry. Alternatively, it may be that factors other than $ONOO^-$ contribute to impaired K_v channel function during hyperglycemia. We previously demonstrated that superoxide dismutase, a $O_2^{\cdot -}$ scavenger, partially restored impaired K_v channel activation in RSCAs that were exposed to HG, indicating an inhibitory role of $O_2^{\cdot -}$ on K_v channels (11). The relative contribution of $O_2^{\cdot -}$ and $ONOO^-$ in the reduction of K_v current in HG is not known. On the basis of our previous and present studies, we believe that both $O_2^{\cdot -}$ and $ONOO^-$ contribute to the impaired K_v channel function during hyperglycemia.

HG-induced $ONOO^-$ formation is associated with nitration and inhibition of K_v1 channels. The present study also provides initial evidence that HG promotes $ONOO^-$ formation in RSCAs, an event associated with nitration of tyrosine residues in VSMCs. In support of this hypothesis, scavenging of $ONOO^-$ by urate reduced the immunoreactivity correlating to nitrotyrosine residues in RSCAs that were exposed to HG for 24 h. Similarly, the use of MnTBAP and L-NAME jointly to reduce the availability of $O_2^{\cdot -}$ and NO to form $ONOO^-$-attenuated nitrotyrosine formation. Subsequent findings revealed that elevation of $ONOO^-$ by HG selectively increased the number of nitrotyrosine residues in $K_v1.2$ but not $K_v1.5$ α-subunits, an event that was partially reversed by the $ONOO^-$ scavenger urate. These findings suggest that $ONOO^-$ may specifically nitrate particular proteins that compose the K_v1 channel pore, providing initial insight into the molecular basis of $ONOO^-$-induced inhibition of coronary K_v channels. Notably, short-term exposure to HG has been linked to an increased $ONOO^-$ production in the endothelial cells of human aorta and rat retinal arteries (15–17), and $ONOO^-$ may inhibit prostacyclin synthase in these endothelial cells (15). Thus, several lines of evidence suggest that the elevation of $ONOO^-$ in response to HG may impair the function of at least several vasodilator mechanisms in the arterial wall, including K_v channels in VSMCs.

Study limitations. Several limitations of the present study should be acknowledged. First, our findings have not established a causal relationship between $ONOO^-$-in-

duced nitration of $K_v1.2$ α-subunits and impaired K_v1 channel function. Mutagenesis will be required to confirm the molecular basis of inhibition, which may involve single or multiple nitrations of the 17 tyrosine residues that reside in the $K_v1.2$ α-subunit. Second, although nitrotyrosine is widely used as a marker for $ONOO^-$ formation, other mechanisms of tyrosine nitration exist, including H_2O_2-NO_2-hemeperoxidase and NO_2-mediated nitrosylation (18). However, because H_2O_2-NO_2-hemeperoxidase occurs primarily in inflammatory cells (19) and nitrotyrosine formation by NO_2 is a limited reaction (20), $ONOO^-$ most likely was responsible for K_v channel nitration in our study. Finally, short-term exposure to HG may not produce the same complexity or degree of vascular dysfunction as in diabetes, where alternative mechanisms could mask or aggravate vasodilator dysfunction. However, the advantage of this study is the ability to directly examine K_v channel function in response to HG without confounding influences, such as circulating plasma constituents and neurohumoral factors. Future studies will examine the overall effect of $ONOO^-$ in diabetes-induced K-channel–mediated vascular dysfunction.

Physiological relevance. K_v channels are a major contributor to the resting tone of small coronary arteries and represent a powerful dilator influence in the coronary circulation in vivo (for review, see 21). These channels participate in coronary vasodilator responses to acidosis, cAMP-dependent agonists such as β-adrenergic activation, and endothelial factors. Under these conditions, in which resting vascular tone relies on K_v channel function, the generation of $ONOO^-$ during hyperglycemia impairs channel availability and may compromise coronary blood flow and myocardial perfusion.

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