HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2018 February 17.

Published in final edited form as:

Circ Res. 2017 February 17; 120(4): 658–669. doi:10.1161/CIRCRESAHA.116.309491.

Contribution of $K_V1.5$ Channel to H_2O_2-Induced Human Arteriolar Dilation and its Modulation by Coronary Artery Disease

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Abstract

Rationale—Hydrogen peroxide (H_2O_2) regulates vascular tone in the human microcirculation under physiological and pathophysiological conditions. It dilates arterioles by activating BK_{Ca} channels in subjects with coronary artery disease (CAD), but its mechanisms of action in subjects without CAD (non-CAD) as compared to those with CAD remain unknown.

Objective—We hypothesize that H_2O_2-elicited dilation involves different K^+ channels in non-CAD versus CAD, resulting in an altered capacity for vasodilation during disease.

Methods and Results—H_2O_2 induced endothelium-independent vasodilation in non-CAD adipose arterioles, which was reduced by paxilline, a BK_{Ca} channel blocker, and by 4-AP, a K_V channel blocker. Assays of mRNA transcripts, protein expression and subcellular localization revealed that $K_V1.5$ is the major K_V1 channel expressed in vascular smooth muscle cells (VSMCs) and is abundantly localized on the plasma membrane. The selective $K_V1.5$ blocker DPO-1 and the $K_V1.3/1.5$ blocker Psora-4 reduced H_2O_2-elicited dilation to a similar extent as 4-AP, but the selective $K_V1.3$ blocker PAP-1 was without effect. In arterioles from CAD subjects, H_2O_2-induced dilation was significantly reduced and this dilation was inhibited by paxilline but not by 4-AP, DPO-1 or Psora-4. $K_V1.5$ cell membrane localization and DPO-1-sensitive K^+ currents were markedly reduced in isolated VSMCs from CAD arterioles, although mRNA or total cellular protein expression were largely unchanged.

Conclusions—In human arterioles, H_2O_2-induced dilation is impaired in CAD, which is associated with a transition from a combined BK_{Ca}- and K_V ($K_V1.5$)-mediated vasodilation

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DISCLOSURES

None.

toward a BK_{Ca}-predominant mechanism of dilation. Loss of $K_V1.5$ vasomotor function may play an important role in microvascular dysfunction in CAD or other vascular diseases.

Keywords

Hydrogen peroxide; potassium channels; voltage-gated potassium channels; calcium-activated; vasodilation; endothelium-dependent hyperpolarization factor

Subject Terms

Vascular Biology; Ion Channels/Membrane Transport; Oxidant Stress; Coronary Artery Disease; Vascular Disease

INTRODUCTION

Hydrogen peroxide (H_2O_2), a diffusible reactive oxygen species (ROS), has been recognized as an important regulator of vascular tone and homeostasis under physiological and pathophysiological conditions.[1–3] As an endothelium-derived hyperpolarization (EDH) factor, H_2O_2 induces smooth muscle cell hyperpolarization and vasodilation in human coronary and adipose arterioles from subjects with coronary artery disease (CAD).[4–7] Other studies have also demonstrated H_2O_2-induced hyperpolarization and dilation in normal human and animal arteries.[8] The mechanisms of H_2O_2-induced vasodilation have not been fully elucidated; however, two main types of K^+ channels in vascular smooth muscle cells (VSMCs), large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels and voltage-gated K^+ (K_V) channels, have been variably implicated in different vascular beds.[9] For instance, BK_{Ca} channels contribute to H_2O_2-induced dilation in porcine coronary arteries.[10,11] In contrast, 4-aminopyridine (4-AP)-sensitive K_V channels mediate dilation to H_2O_2 in canine coronary arteries,[12] rat coronary and mesenteric arteries,[12, 13] and porcine coronary resistance arteries.[14] Using coronary arterioles from CAD subjects, we found that H_2O_2 opens smooth muscle BK_{Ca} channels to elicit smooth muscle hyperpolarization and relaxation.[6, 7] The mechanisms of dilation by H_2O_2 in subjects without CAD (non-CAD) versus those with CAD, as well as the functional consequences, remain unknown, but there is evidence that BK_{Ca} and K_V constitute two major K^+ currents in VSMCs isolated from non-CAD human arteries.[15]

In addition to H_2O_2-induced activation of K^+ channels, excessive and/or prolonged elevation of ROS can exert differential effects on vascular K^+ channel function in disease.[9, 16–18] Depending on the sensitivity of individual K^+ channels and the oxidative species involved, ROS can activate, inhibit, or leave unaltered K^+ channel function. [9] For example, impaired functions of K_V channels have been shown in various animal models of cardiovascular disease, while BK_{Ca} channels may exhibit either gain or loss of function under pathophysiological conditions.[9] The disease-associated alteration of vascular K^+ channel function in humans is less well understood.[19] In the present study, we tested the hypothesis that H_2O_2-elicited dilation involves different K^+ channels in non-CAD versus CAD arterioles, resulting in an altered vasodilatory response in CAD. Using an integrated approach comprising isolated vascular reactivity measurement, molecular and

immunohistochemical analyses, and electrophysiology, we assessed the role of two different types of K^+ channels (BK_{Ca} vs. K_V) in H_2O_2-induced dilation of human arterioles from non-CAD and CAD subjects. We further identified specific K_V1 channels, the major vascular K_V channel subfamily, in VSMCs and examined their functional contribution to H_2O_2-induced dilation. The impact of CAD on the function of K_V1 channels and potential underlying mechanisms were also determined.

METHODS

Tissue acquisition

Fresh human adipose tissues (pericardial, visceral and subcutaneous, *n*=24, 68 and 28, respectively.) were obtained as discarded surgical specimens from a total of 120 patients undergoing abdominal surgeries or cardiopulmonary by-pass procedures, and unused whole hearts (*n*=14) acquired from Donor Network. Patient demographic information is summarized in Online Table I.

Videomicroscopy

Arterioles (internal diameter, 100–250 μm) were carefully dissected from human adipose tissues and cannulated with two glass micropipettes for measurements of diameter with a video system as previously described.[20] Arterioles were preconstricted with endothelin-1 to approximately 30–50% of the baseline internal diameter. Relaxation responses to cumulative addition of H_2O_2 (1–100 μmol/L) to the vessel bath were determined in the absence and presence of 30 min preincubation with various modulators, including BK_{Ca} and K_V blockers. At the end of each experiment, papaverine (100 μmol/L) was added to determine the maximal internal diameter for normalization of dilator responses. Unless otherwise stated, experiments were performed on endothelium-intact arterioles and in the presence of L-NAME (100 μmol/L) and Indomethacin (10 μmol/L).

Enzymatic isolation of vascular cells

Vascular smooth muscle cells (VSMCs) were enzymatically dissociated from arteries as previously described.[7, 20] Cells were placed on ice and used the same day.

Patch-clamp recording of K^+ currents

Whole-cell K^+ currents were measured in freshly dissociated smooth muscle cells using the standard (ruptured-patch) or perforated patch-clamp method as previously described.[15, 21] The pipette solution contained (in mmol/L) 90 potassium aspartate, 30 KCl, 20 NaCl, 1 $MgCl_2$, 1 Mg-ATP, 1 EGTA, and 10 HEPES (pH 7.2 with KOH). The bath solution was composed of (in mmol/L) 140 NaCl, 5 KCl, 0.1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES (pH 7.4 with NaOH). Paxilline (100 nmol/L) was added in the bath solution to further minimize BK_{Ca} currents and thus allow relative isolation of K_V currents. Unless otherwise stated, all chemicals were applied to the bath through perfusion. Experiments were performed at room temperature.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from human adipose arteries and arterioles (200–500 μm) was extracted and cDNA was synthesized. The cDNA was amplified using a touch-down PCR protocol with gene-specific primers. Relative $K_V\alpha 1.5$ gene expression was quantified with real-time PCR using pooled sample cDNA from each non-CAD and CAD group. For primer sequences refer to Online Table III.

Immunoblotting

Human adipose arteries and arterioles (200–500 μm) and coronary arteries (200–2,000 μm) were dissected and membrane proteins were prepared with a differential centrifugation method as described previously.[22] Protein samples (20 μg) were separated by 10% SDS-PAGE. Membranes were blotted with a primary antibody against a specific K_V1 α-subunit, BK_{Ca}, and Na^+/K^+-ATPase (1:2,000 dilution), followed by a horseradish-peroxidase conjugated secondary antibody (1:20,000 dilution). Membranes were developed using the ECL Prime reagent (Amersham).

Immunohistochemistry

Freshly dissected arteries were embedded in OTC compound, frozen on dry-ice, and cut into 10-μm sections.[20] Sections were blocked with 5% normal goat serum and probed with a monoclonal or polyclonal antibody against a specific K_V1 α-subunit (1:200 dilution), followed by secondary probing with an Alexa Fluor 568-conjugated goat anti-rabbit or anti-mouse IgG antibody (1:400 dilution). Sections were counterstained with DAPI and mounted in SlowFade antifade medium (Invitrogen). Images were immediately captured using a confocal fluorescence microscope (model A1-R, Nikon).

Immunocytochemistry

Freshly isolated VSMCs were fixed with 4% paraformaldehyde and permeabilized with 0.1% saponin.[7] Cells were then incubated either with a monoclonal or polyclonal antibody specific to a K_V1 α-subunit (1:200 dilution), followed by an appropriate goat secondary antibody conjugated with Alexa Fluor 488 (1:400 dilution). Cells were then mounted and images were captured using a confocal fluorescence microscope.

Chemicals

DPO-1, paxilline, and Psora-4 were obtained from Tocris, stromatoxin from Alomone, and TCEP from Thermo Scientific. All other chemicals were purchased from Sigma. Stock solutions were prepared in distilled water, except for the following: CP339818, DPO-1, PAP-1, paxilline, and Psora-4 (ethanol); 4-AP (HCl, pH readjusted to 7.4); and indomethacin (0.1 mol/L Na_2CO_3).

Statistical analysis

All data are presented as mean±SEM. Comparisons of concentration-response curves of isolated vessels were performed using 2-way repeated measures analysis of variance (ANOVA), followed by the Student-Newman-Keuls multiple-comparison test. Other

comparisons were made using 1-way ANOVA or Student *t*-test. *P* values <0.05 were considered statistically significant.

RESULTS

K_V channels contribute to H_2O_2-induced vasodilation in non-CAD but not in CAD human arterioles

Previous studies have shown impaired function of smooth muscle K^+ channels in animal models of vascular disease, such as hypertension and metabolic syndrome. [9, 16–18] It remains largely unknown whether K^+ channel function is similarly altered in humans. We first examined the role of BK_{Ca} and K_V channels in H_2O_2-induced dilation using adipose arterioles from non-CAD and CAD subjects. Accumulating evidence indicates that peripheral arterioles such as those from adipose tissues, which are more readily available, can serve as a surrogate for assessing systemic and coronary arteriolar function.[19]

In non-CAD adipose arterioles (Figure 1A), H_2O_2 (1–100 μmol/L) induced vasodilation in a concentration-dependent manner (% maximal dilation, 96±2). Treatment of arterioles with paxilline (100 nmol/L), a potent BK_{Ca} channel blocker, induced a rightward shift of H_2O_2-induced dilation (% dilation at 10 μmol/L H_2O_2, 19±13 vs. 58±8 in control, and % dilation at 50 μmol/L H_2O_2, 66±12 vs. 90±3 in control, n=5, P<0.05). The general K_V channel blocker 4-AP (10 mmol/L) caused a similar rightward shift in the response to H_2O_2 (% dilation at 10 μmol/L H_2O_2, 19±5 vs. 57±10 in control, % dilation at 50 μmol/L H_2O_2, 49±8 vs. 85±4 in control, n=5, P<0.05). Combined blockade of BK_{Ca} and K_V channels abolished dilation up to 30 μmol/L H_2O_2 (Online Figure IA), indicating that these two K^+ channels mediate a major portion of H_2O_2-induced dilation in non-CAD human adipose arterioles. The potency of H_2O_2 (EC_{50}, 12±1 μmol/L; n=18, Online Figure IC) is similar to those reported in canine[12] and human[7] coronary arterioles.

In CAD arterioles (Figure 1B), H_2O_2-induced dilation was also blocked by paxilline (% dilation at 10 μmol/L, 1±6 vs. 41±8 in CAD control, % dilation at 50 μmol/L, 40±7 vs. 76±6 in CAD control, n=5, P<0.05). However, the dilation was not affected by 4-AP. These results indicate that K_V channel-dependent dilation in response to H_2O_2 is impaired in CAD, whereas consistent with our previous findings in coronary arterioles,[7] BK_{Ca} remains functional in disease.

Incubation of arterioles with 4-AP induced significant vasoconstriction in non-CAD but not in CAD subjects (Online Table II), indicating that K_V channels regulate basal vascular tone in non-CAD subjects.

H_2O_2-induced dilation involves smooth muscle hyperpolarization and is redox sensitive

H_2O_2-induced dilation was not affected by L-NAME (100 μmol/L), a NOS inhibitor, indomethacin (Indo, 10 μmol/L), a COX inhibitor, alone or in combination, suggesting that endothelial NO and prostacyclin do not contribute to H_2O_2-induced dilation. In addition, H_2O_2 induced similar dilation in endothelium-intact and denuded arterioles (Figure 2B), further inferring that the dilation is mediated via an endothelium-independent, likely smooth muscle mechanism. Next, the role of smooth muscle hyperpolarization in H_2O_2-induced

dilation was examined by using high K^+ (60 mmol/L)-Krebs-PSS in the presence of L-NAME and indomethacin (Figure 2B). Dilation to H_2O_2 was abolished by high K^+ in both endothelium-intact and -denuded arterioles (% dilation at 100 μmol/L, 8±4 and 4±6, respectively, n=3, P<0.05 vs. control), indicating H_2O_2-induced dilation is dependent on membrane hyperpolarization of VSMCs.

Treatment of adipose arterioles with exogenous catalase (1000 U/mL), a H_2O_2 metabolizing enzyme, also completely abolished H_2O_2-induced dilation (Online Figure II). An important mechanism by which H_2O_2 elicits biological effects is through oxidizing thiol groups of its target proteins.[23] Indeed, DTT (3 mmol/L), a membrane-permeant and thiol-specific reducing agent, quickly reversed H_2O_2-induced dilation within 3–5 min (% dilation at 100 μmol/L H_2O_2, 1±3 vs. 83±4 before DTT, n=3, P<0.05). However, TCEP (5 mmol/L), a membrane-impermeable thiol-reducing agent, had no effect. These data further confirm that H_2O_2 is a potent, specific and reversible smooth muscle relaxant, a characteristic consistent with its putative important signaling role in human arterioles.

Detection of K_V1 subunit mRNA and protein expression in non-CAD human arteries

The specific type(s) of K_V channels expressed in human arteries remains largely unexplored. We focused on K_V1 channels (*shaker*-related family), which are functionally significant K_V channels in most animal vascular beds studied[24] and are 4-AP-sensitive. Figure 3 shows mRNA expression of different K_V1 channel subunits in non-CAD adipose arterioles as assessed by RT-PCR. Several K_V1 α (pore-forming)-subunits, including 1.1, 1.2, 1.4, 1.5, as well as BK_{Ca} α-subunits, were consistently found in different samples including denuded vessels (n=5), with $K_V1.5$ being the most abundantly expressed K_V1 α-subunit. The two other K_V1 α-subunits (1.3 and 1.6) were variably expressed in some but not all samples. We also detected the α-subunits that form a second type of vascular K_V channels (2.1, 9.3), K_V1 β-(accessory)-subunits (1.1–1.3), although these subunits were not further pursued in the following immunoblotting assays.

Using subunit-specific antibodies, we examined the protein expression of $K_V1.1$–1.6 and BK_{Ca} α-subunits in the membrane fraction prepared from non-CAD adipose arteries/arterioles (n=2). Consistent with mRNA expression data, $K_V1.4$ and 1.5, and BK_{Ca} channels were readily detected at the protein level (Figure 4). $K_V1.3$ and 1.6 proteins were also found in non-CAD arteries/arterioles, whereas $K_V1.1$ and 1.2 proteins were not detected. The absence of $K_V1.2$ in human vascular samples was unexpected since it is a dominant K_V1 α-subunit that forms channel complex with $K_V1.5$ in rat cerebral arteries.[25] The failure to detect $K_V1.1$ or 1.2 in arterial samples does not seem to result from non-reactivity of the antibodies used because abundant expression of these two proteins was observed in human brain parallel controls.

K_V1 channels (except $K_V1.6$) are expressed as mature N-glycosylated proteins in native tissues such as brain. [26] As shown in Figure 4, the apparent molecular mass values of K_V1 proteins in human brain or adipose arterial tissues were approximately 75 kDa (1.1 and 1.2), doublet around 75 kDa (1.3 and 1.5), doublet around 110 kDa (1.4), and 60 kDa (1.6). Whereas $K_V1.6$ appears to be unmodified, $K_V1.1$–1.5 channels are of higher apparent

molecular weights corresponding to glycosylated or other post-translationally modified forms as previously reported in the brain or vascular tissues.[25,27]

Detection of K_V1 subunit protein localization in non-CAD human arteries

To determine the cell type-specific localization of K_V1 α-subunits in human arteries, we performed frozen-section immunofluorescence staining of human adipose arterioles. K_V1 proteins were labeled with red Alexa-568-conjugated secondary antibody and images were captured with a confocal fluorescence microscope. Thus, autofluorescence intrinsic to the internal elastic lamina can be detected in the green FITC channel and used to visually divide the endothelium and smooth muscle layers. As shown in Figure 5A, $K_V1.5$ and to a less amount $K_V1.4$ proteins were readily detected in the smooth muscle layer. Surprisingly, these two proteins were also abundantly expressed in endothelial cells (ECs). A small amount of $K_V1.3$ and 1.6 were also detected in ECs.

The limited resolution power of immunohistochemistry did not allow individual VSMCs to be distinguished even at 600X magnification. To further examine the subcellular localization of K_V1 proteins, we freshly dissociated VSMCs from adipose arterioles for immunocytochemistry. As shown in Figure 5B, $K_V1.5$ protein was predominantly localized on the plasma membrane of dissociated SMCs. Interestingly, $K_V1.5$ was also detected on the nuclei envelope, a cellular structure that is continuous with endoplasmic reticulum (ER) and serves as a site of initial protein synthesis for some membrane proteins.[28, 29] $K_V1.4$ protein was also detected in VSMCs, however the expression of $K_V1.4$ protein was lower than that of $K_V1.5$ and also seemed largely intracellular.

$K_V1.5$ as a major functional K_V1 channel in H_2O_2-induced dilation of non-CAD but not CAD human arterioles

Given that $K_V1.5$ is 4-AP-sensitive and abundantly expressed in VSMCs of human adipose arteries/arterioles, we subsequently examined whether this K_V1 channel is functionally involved in human arteriolar dilation. Adipose arterioles from non-CAD subjects were pretreated with subtype-specific K_V channel blockers and examined for H_2O_2-induced vasodilation. We found that selective $K_V1.5$ channel blocker DPO-1 (1 μmol/L) markedly reduced H_2O_2-induced dilation (Figure 6A; % dilation at 10 μmol/L, 13±6 vs. 55±7 in control, % dilation at 50 μmol/L, 53±9 vs. 86±1 in control, n=5, P<0.05), to a similar extent as after 4-AP treatment (Figure 1). Selective $K_V1.3/1.5$ blocker Psora-4 (30 nmol/L) caused a similar rightward shift in H_2O_2-induced dilation, but further reduced the maximal dilation to 100 μmol/L H_2O_2 (59±5% vs. 95±2% in control, n=5, P<0.05; Figure 6B). In contrast, selective $K_V1.3$ blocker PAP-1 (10 nmol/L) did not affect H_2O_2-induced dilation (Figure 6C; n=5). The $K_V1.3/1.4$ blocker CP-339818 (3 μmol/L) slightly attenuated the dilation only at 10 μmol/L H_2O_2 (Figure 6D), suggesting a minor contribution of $K_V1.4$ to H_2O_2-induced dilation. In addition, H_2O_2-induced and DPO-1-inhibitable dilation was significantly reduced in arterioles treated with $K_V1.5$ siRNA (Online Figure III). Together, these results suggest that $K_V1.5$ serves as a major 4-AP-sensitive K_V1 channel contributing to H_2O_2-induced dilation in non-CAD adipose arterioles.

Compared to non-CAD arterioles, there was a slight but statistically significant reduction of H_2O_2-induced dilation in CAD (% dilation at 10 μmol/L H_2O_2, 40±4 vs. 59±4 in non-CAD, % dilation at 50 μmol/L, 71±3 vs. 86±2 in non-CAD, n=18/group, P<0.05; Figure 7A). In contrast to non-CAD arterioles (Figure 6), K_V1.5 channel blockade by either DPO-1 or Psora-4 did not alter H_2O_2-induced dilation in CAD (Figure 7B and 7C), a finding consistent with ineffectiveness of 4-AP in CAD arterioles. These results indicate a loss of functional K_V channels, smooth muscle K_V1.5 in particular, in human arterioles during CAD.

Potential mechanism of impaired K_V1.5 function in CAD human arteries

We further examined the potential mechanisms responsible for impaired K_V1.5 function in CAD vessels. By immunostaining of freshly dissociated VSMCs, we found that the plasma membrane staining of K_V1.5 protein was markedly reduced in CAD subjects (Figure 8A). Analysis of plasma membrane/cytoplasmic ratio of K_V1.5 fluorescence further confirmed reduced plasma membrane distribution of this protein in VSMCs from CAD subjects as compared to non-CAD subjects (1.83±0.12 and 2.62±0.15, respectively; P<0.05). Intriguingly, K_V1.5 immunofluorescence from the nuclear envelope was comparable in CAD vs non-CAD samples.

In contrast to K_V1.5, BK_{Ca} protein localization on the plasma membrane was maintained in VSMCs from CAD subject as compared to non-CAD subjects (Online Figure IV-A; calculated plasma membrane/cytoplasmic ratio, 4.03±0.32 and 3.07±0.18, respectively). We also examined K_V1.5 and BK_{Ca} immunofluorescence in ECs isolated from adipose arterioles of non-CAD and CAD subjects (Online Figure IV-A and B). Whole cell immunofluorescence of K_V1.5 in ECs seemed comparable between CAD and non-CAD, although further analysis of plasma membrane/cytoplasmic ratio is difficult due to small cell size of isolated ECs. The immunoreactivity for BK_{Ca} channel α-subunits in ECs was minimal, which is in line with our previous findings that mRNA transcripts of BK_{Ca} were not detected in isolated ECs from human coronary arterioles.[7] The specificity of K_V1.5 antibodies was confirmed by using HEK293 cells with and without K_V1.5-DDK transfection and double-staining technique (Online Figure V).

Using end-point RT-PCR analysis, the mRNA level of K_V1.5 in CAD vessels was comparable to that of non-CAD tissues when normalized to the housekeeping gene β-actin (Figure 8B). These results were further confirmed by the quantitative analysis of mRNA expression using real-time PCR (Figure 8B, bar graph). Analysis of relative mRNA expression of K_V1.5 normalized to the mean of two housekeeping genes (β-actin and ATP5o) did not show marked change in non-CAD and CAD vessels (intact n=6/group, denuded n=4/group). A slight increase (7%) in intact CAD vessels but a moderate decrease (26%) in denuded CAD vessels was observed.

We also compared the expression of K_V1.5 protein in the membrane fraction of human coronary arteries (HCAs) from non-CAD and CAD patients (Figure 8C). Because vessels collected from CAD adipose tissues were usually limited and were not sufficient for membrane protein preparation, HCAs were used instead for these experiments. Our recent studies have shown that human conduit and resistance arteries express a similar profile of K_V1 channels at both mRNA and protein levels.[30] The average protein expression level of

$K_V1.5$ subunits normalized to Na^+/K^+-ATPase showed a trend toward an increase (but not statistically significant) in patients with CAD compared to those without (n=4 non-CAD and CAD subjects, respectively), although in 1 sample (out of 4) from CAD patients the level of $K_V1.5$ channel expression was much lower than average. The protein expression of BK_{Ca} did not differ in the two groups.

We next examined whole-cell K^+ currents in VSMCs freshly dissociated from non-CAD and CAD adipose arterioles (Figure 8D). In non-CAD VSMCs, progressive depolarizing steps from a holding potential of −70 mV elicited outward currents at membrane potentials positive to −40 mV that were subsequently reduced by DPO-1 (1 μmol/L). Compared to non-CAD, baseline K^+ currents in CAD were lower. Although K^+ currents were further reduced by DPO-1 in CAD, DPO-1-sensitive current density was markedly reduced as compared to non-CAD. Because K^+ currents were recorded with low-Ca^{2+} pipette and bath solutions and in the presence of the BK_{Ca} channel blocker paxilline (100 nmol/L), the contribution of BK_{Ca} to whole-cell K^+ currents was minimal. Recording of K_V currents were also confirmed by the findings that 4-AP concentration-dependently decreased the outward K^+ currents (Online Figure VI). Together, the above results suggest that mainly the reduced plasma membrane expression of smooth muscle $K_V1.5$ subunits, rather than a change of mRNA or total protein, contributes to impaired vasomotor function of $K_V1.5$ channels in human arteries with CAD.

DISCUSSION

The major new findings of this study are three-fold. First, H_2O_2 induces potent endothelium-independent vasodilation in adipose arterioles from non-CAD subjects that is largely mediated by BK_{Ca} and K_V channels. Second, $K_V1.5$ is the major type of smooth muscle K_V1 channel responsible for K_V-dependent H_2O_2 dilation in non-CAD arterioles. Third, the H_2O_2-elicited response is reduced in CAD arterioles and is accompanied by a loss of K_V (especially $K_V1.5$)- but not BK_{Ca}-dependent dilation. The impaired function of $K_V1.5$ may result from reduced cell surface localization of the channel protein without significant change of mRNA or total protein expression. Together, these results demonstrate a transition from BK_{Ca}- and K_V-mediated vasodilation toward a BK_{Ca}-predominant mechanism of dilation in the human microcirculation during CAD. While the pathophysiological significance of this K^+ channel transition remains to be explored, the loss of $K_V1.5$-mediated dilation to H_2O_2 or other vasodilators may represent an important mechanism contributing to microvascular dysfunction in humans with CAD or other vascular diseases.[31–33]

Smooth muscle K^+ channels in H_2O_2-induced dilation

Using HCAs from subjects with CAD, we previously reported that H_2O_2 induces vasodilation by activating smooth muscle BK_{Ca} channels, an effect secondary to H_2O_2-induced dimerization of protein kinase G.[7] BK_{Ca} channels have also been implicated in H_2O_2-induced dilation in different vascular beds such as porcine coronary arteries.[10,11,] However, other studies indicate that K_V channels but not BK_{Ca} channels contribute to the dilatory effect of H_2O_2 in canine and rat coronary arteries.[12] In the present study, we found that H_2O_2 induces high-K^+-sensitive dilation in non-CAD adipose arterioles that is inhibited

by blockers of both BK_{Ca} (paxilline and iberiotoxin) and K_V channels (4-AP). Furthermore, a combination of BK_{Ca} and K_V channel blockers almost completely abolished H_2O_2-induced dilation (up to 30 μmol/L H_2O_2), indicating that both BK_{Ca} and K_V mediates H_2O_2-induced dilation in non-CAD human arterioles. The mechanisms by which H_2O_2 dilation involves distinct K^+ channels in different species or vascular beds remain to be established.

There is evidence that endothelial pathways contribute to vasomotor effects of H_2O_2, including COX-derived prostacyclin.[34] However, in human adipose arterioles, H_2O_2-induced dilation was not affected by inhibition of either NO and COX or endothelial denudation, confirming that the dilation is mostly smooth muscle-dependent.

Membrane-permeant thiol reducing agent DTT completely blocked H_2O_2-induced dilation in human adipose arterioles, whereas membrane-impermeable thiol reagent failed to reverse the dilation. These data support a H_2O_2-mediated redox modification of K^+ channels proposed earlier[12] but further suggest an intracellular site of action. Indeed, H_2O_2 can alter the activities of ion channels such as L-type Ca^{2+} channel[35] and ATP-sensitive K^+ channel.[36] H_2O_2 also activates smooth muscle $K_V2.1$ channels in rat mesenteric arteries through a similar mechanism via S-glutathionylation.[13] It remains to be determined whether H_2O_2-induced dilation involves redox modification of K_V channels, or alternatively other intermediate signaling proteins as we reported previously for protein kinase G in H_2O_2-induced BK_{Ca} activation.[7]

K_V1 channel subtypes in human vasculature

The expression of specific K_V subunit channel gene products and proteins varies greatly among species and vascular beds,[24] and the molecular identity of K_V channels, especially 4-AP-sensitive K_V1 channels, in the human vasculature remains largely unknown. Using RT-PCR analysis of mRNA transcripts, we consistently detected $K_V\alpha1.1$, 1.2, 1.4, 1.5, 2.1, 9.3, and $K_V\beta1.1$–1.3 in both intact and denuded adipose arterioles from non-CAD subjects, while sample-to-sample variations were noted for $K_V\alpha1.3$ and 1.6 (Figure 3). Among six K_V1 α-subunits, $K_V1.3$–1.6 but not $K_V1.1$–1.2 were also detected at the protein level (Figure 4). Immunofluorescence assay of vessel sections and freshly dissociated VSMCs further revealed that $K_V1.5$ is the main subunit expressed on the plasma membrane of VSMCs. $K_V1.4$ seems mainly intracellular in adipose VSMCs. We also found $K_V1.5$, 1.4, and to a much less extent, $K_V1.3$ and 1.6 in the endothelium of adipose arterioles.

Pharmacological studies further demonstrated that $K_V1.5$ is a major functional K_V1 channel responsible for H_2O_2-induced dilation in non-CAD adipose arterioles (Figure 6). This is based on the findings that $K_V1.5$-selective blocker DPO-1 reduced H_2O_2-induce dilation by the same extent as the general K_V1 blocker 4-AP. $K_V1.5$ siRNA also significantly reduced H_2O_2-induced dilation in non-CAD arterioles (Online Figure III). Selective $K_V1.3/1.5$ blocker Psora-4 caused a similar rightward shift while $K_V1.3$ blocker failed to affect the dilation. Psora-4 further reduced the maximal dilation to 100 μmol/L H_2O_2, which may be because Psora-4 is >20-fold more potent for $K_V1.5$ (IC_{50}, 7.7 nmol/L[37]) than DPO-1 (IC_{50}, 0.2–0.3 μmol/L[38]). Finally, $K_V1.3/1.4$ blocker CP-339818 slightly reduced the vasodilator response to H_2O_2, suggesting a minor role of $K_V1.4$.

In non-CAD adipose arterioles, the dilation induced by the highest concentration of H_2O_2 (100 μmol/L) was blocked by high K^+ but only slightly blocked by the combination of BK_{Ca} and K_V channel blockers, indicating that potential involvement of other K^+ channels. Several studies reported that K_V2 α-subunits or $K_V2.1/9.3$ heterotetramers contribute to the regulation of vascular tone in rodent arteries.[39, 40] In the present study, we detected vascular $K_V2.1$ mRNA (Figure 3) and protein expression (data not shown); however, selective $K_V2.1$ and $K_V2.1/9.3$ blocker stromatoxin did not alter H_2O_2-induced dilation in non-CAD arterioles (Online Figure I). Redox-sensitive K_V7 channels represent another potential candidate, but a recent study indicate that they do not contribute to H_2O_2 dilation in porcine coronary arteries.[41] Further investigations are needed to elucidate the role of other K^+ channels in human arterioles.

Alteration of K^+ channel function in CAD

Compared to non-CAD arterioles, H_2O_2-induced dilation was significantly reduced in CAD arterioles (Figure 7A). Furthermore, H_2O_2-induced dilation of CAD adipose arterioles was inhibited by only BK_{Ca} but not K_V channel blockers. To the best of our knowledge, this is the first report on the recruitment of different smooth muscle K^+ channels in H_2O_2-induced vasodilation from health to disease. The expression of BK_{Ca} mRNA and protein, as well as protein localization, was not altered by CAD, a finding consistent with that of our previous study in HCAs.[7] We thus conclude that BK_{Ca} channels remain functional in CAD arterioles. In other vascular beds or species, the effect of disease on the expression and function of BK_{Ca} channels remain complex or controversial.[9, 16–18] For example, BK_{Ca} channel activity is increased in human VSMCs obtained from coronary atherosclerotic lesions.[42] Animal studies support that BK_{Ca} channel expression is increased in hypertension, possibly as a compensatory mechanism for the downregulation of K_V channel expression.[18] However, BK_{Ca} activity is reduced by exposure to high glucose or high concentrations of H_2O_2[43] and in the porcine model of metabolic syndrome.[44]

The reduction of H_2O_2-induced dilation in CAD arterioles can be mainly attributed to a loss of K_V, especially $K_V1.5$, channel function in VSMCs. In contrast to non-CAD adipose arterioles, blocking of $K_V1.5$ or other K_V1 channels did not affect H_2O_2-induced dilation in CAD vessels (Figure 1 and 7). Alternations in vascular K_V channel function and/or expression have also been reported in other pathological conditions such as pulmonary[45] and systemic[46] hypertension, metabolic syndrome[47] and diabetes,[48] but the identities of individual K_V channels involved haven't been well established. In human atrial myocytes, chronic atrial fibrillation, which is associated with oxidative stress or elevated ROS, reduces $K_V1.5$ (I_{Kur}) expression.[49]

The precise mechanisms of impaired K_V function under pathological conditions remain poorly understood. By examining $K_V1.5$ mRNA/protein expression and subcellular localization and $K_V1.5$ currents, we provide initial evidence that a reduced plasma membrane expression of $K_V1.5$ in VSMCs, rather than a change of mRNA and total protein synthesis, may be mainly responsible for the reduced $K_V1.5$ channel function in CAD. There is accumulating evidence that ion channels can recycle between the cytosol and cell membrane and this dynamic recycling determines the number of functional channels present

in the plasma membrane.[50–52] For example, a fraction of $K_V1.5$ channels on the cell membrane is rapidly internalized with a half time of ~10 min and some return to the surface with a half time of ~30 min.[52] In a HL-1 cell line, elevated ROS induces fairly rapid (within 60 min) reduction of surface expression of $K_V1.5$.[53] It remains to be tested in future studies whether prolonged elevation of ROS during CAD modulates cellular trafficking of smooth muscle $K_V1.5$ channels to reduce their membrane expression in human arterioles.

Potential study limitations

We found that $K_V1.5$ and $K_V1.4$ channels are expressed in ECs of human adipose arterioles. It is thus possible that H_2O_2 may activate endothelial K_V channels to induce endothelial hyperpolarization and subsequent vasodilation. Although the expression and function of endothelial K_V channels are poorly understood,[17] there is evidence on the role of K_V channels in endothelium-dependent hyperpolarization and dilation in arteries such as porcine coronary arteries[41,54] and guinea-pig coronary and carotid arteries.[55, 56] However, H_2O_2-induced dilation of adipose arterioles is largely smooth muscle-dependent, and therefore the contribution of endothelial K_V channels seems unlikely, at least in the present experimental settings. Nevertheless, we have not excluded a potential role for endothelial K_V channels in the dilation to H_2O_2 under other conditions (e.g., endogenous H_2O_2 generated in ECs).

The present study used arterial tissues from human subjects with a variety of conditions that can affect vasodilator responses. By necessity, the non-CAD tissue samples are often collected from subjects with diverse diseases and thus are not true normal controls. To minimize potential confounding effects of underlying disease, only subjects with no more than 1 risk factor for CAD and no evidence of CAD were classified as non-CAD for this study. We further addressed this limitation with a statistical approach, identifying and controlling the influence of individual risk factors. Analysis of risk factors for CAD did not show an impact due to hypertension, hyperlipidemia, sex, or age on H_2O_2-induced dilation. There is an interaction between CAD and BMI (body mass index) on EC_{50} of H_2O_2 response (Online Figure VIII); however, this interaction may require further investigation in another cohort with a larger sample size. We also used adipose arterioles from several regions of the body; however, pilot studies indicate that H_2O_2-induced dilation was similar among visceral, subcutaneous, or pericardial adipose arterioles within non-CAD and CAD subject groups (Online Figure VII).

A limitation of the functional studies with regard to H_2O_2-induced dilation is that we use a largely pharmacological approach, which may have off-target effects on other proteins such as other families of K_V channels. To mitigate this possibility, we used several chemically distinct blockers of K_V1 channels, and the results obtained with these blockers invariably pointed toward an important role of $K_V1.5$ in H_2O_2-induced dilation of non-CAD adipose arterioles. In addition, we found that $K_V1.5$-targeted blockers had no significant effects in CAD arterioles, suggesting that any non-specific effects should be minimal in the present study. A recent study also reported a preferential inhibition of $K_V1.5$ by DPO-1 using VSMCs dissociated from wild-type versus $K_V1.5$ knockout mice.[57] Nevertheless, a molecular approach using $K_V1.5$ siRNA was also included to determine the specific role of $K_V1.5$ in H_2O_2-induced dilation of non-CAD arterioles.

Clinical implications

Microvascular dysfunction has been implicated in a wide variety of pathologies including obesity-associated insulin resistance, inflammation in visceral fat, and ischemic heart disease.[31–33] In the absence of CAD or its risk factors, traditional vasodilator factors (i.e., NO and prostacyclin) are important for vasodilation in human coronary and adipose arterioles.[19] With the onset of CAD, the dilation is switched to a new mechanism requiring the release of H_2O_2 from endothelial cells and subsequent smooth muscle hyperpolarization.[4–6] In the present study, we demonstrate a reduced smooth muscle-mediated dilation resulting from a functional transition from BK_{Ca}- and K_V-mediated vasodilation to predominantly BK_{Ca}-mediated mechanism of dilation in human arterioles during CAD. These results thus reveal another potentially important aspect of microvascular dysfunction where cardiovascular disease not only changes the primary endothelial vasodilators (NO to H_2O_2) but also affects smooth muscle K^+ channels that respond to vasodilator factors. Our unpublished observations also show altered kinetics of dilation in healthy vessels in the presence of 4-AP, suggesting that in addition to reduced overall peak dilation in CAD versus health, the time-to-peak dilation may be altered due to changes in the expression/function of K_V channels.

Impaired K_V channel function will negatively impact local blood flow regulation in response to not only endothelial factors but also to tissue factors such as β-adrenergic transmitters and other metabolic factors.[9, 48, 58] This may induce deficit in regional blood supply and have detrimental effect on the function of tissues such as the heart where a tight coupling of blood perfusion and cell metabolism is essential.[58, 59] Regional organ perfusion, especially in the heart where near-maximal extraction of oxygen occurs at rest, requires tight, beat-to-beat regulation of blood flow in order to nearly instantaneously match oxygen supply with tissue's metabolic demand. In health, expression of different types and sub-types of K^+ channels and their axillary subunits with varying activation/inactivation kinetic properties contribute to this precise vasoregulatory control. With the onset/progression of disease, changes in function/expression of these channels would result in a dysregulation of blood flow and, in consequence, lead to a mismatch in oxygen supply and demand. Over time, these brief but repetitious states of tissue hypoxia could induce local inflammation, fibrosis and eventually adverse tissue remodeling.[19] The molecular mechanisms of impaired K_V function in CAD, as well as exact causal factors responsible for the K^+ channel remodeling, remain to be determined. A better understanding of these mechanisms may provide new strategies to improve or even restore normal K_V channels and cardiovascular function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the Division of Cardiothoracic Surgery at the Medical College of Wisconsin, the Cardiothoracic Surgery Group of Milwaukee, the Cardiovascular Surgery Associates of Milwaukee, the Midwest Heart Surgery Institute, Cardiothoracic Surgery division at the Zablocki VA Medical Center in Milwaukee, Froedtert Memorial Lutheran Hospital, Aurora St Luke's Medical Center, Wheaton Franciscan Healthcare's Elmbrook Memorial Hospital, St Joseph's Hospital, and the Wisconsin Heart Hospital, as well as the Wisconsin Donor Network, for providing human tissues. The authors thank Victoria Nasci for organizing tissue sample data, Dr. Paul Goldspink

for assistance with qPCR, Dr. Jian Zhang for assistance with immunohistochemistry, and Dr. Yanping Liu for critical review.

SOURCES OF FUNDING

Support for this research was provided by National Heart, Lung, and Blood Institute (HL096647 to D.X. Zhang; HL113612 to D.D. Gutterman), American Heart Association (0830042N to D.X. Zhang), and Advancing a Healthier Wisconsin Research and Educational program (9520311 to R.A. Sparapani).

Nonstandard Abbreviations and Acronyms

4-AP	4-aminopyridine
BK_{Ca} channel	large-conductance Ca^{2+}-activated K^+ channel
CAD	coronary artery disease
CP-339818	1-benzyl-4-pentylimino-1,4-dihydroquinoline
COX	cyclooxygenase
DPO-1	diphenyl phosphine oxide-1
DTT	DL-dithiothreitol
EC	endothelial cell
EDH	endothelium-derived hyperpolarization
HEK-293 cell	human embryonic kidney 293 cell
HCA	human coronary artery
H_2O_2	hydrogen peroxide
K_{Ca} channel	Ca^{2+}-activated K^+ channel
K_V channel	voltage-gated K^+ channel
L-NAME	N^G-nitro-L-arginine methyl ester
NOS	nitric oxide synthase
PAP-1	phenoxyalkoxypsoralen-1
Psora-4	5-, (4-phenylbutoxy)psoralen
PSS	physiological salt solution
ROS	reactive oxygen species
RT-PCR	reverse transcription-polymerase chain reaction
TCEP	tris(2-carboxyethyl)phosphine
VSMC	vascular smooth muscle cell

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Novelty and Significance

What Is Known?

- The primary mediator of shear stress-induced endothelium-dependent vasodilation is NO in patients without coronary artery disease (CAD) and hydrogen peroxide (H_2O_2) in those with CAD.
- The large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel and the voltage-gated K^+ (K_V) channel have been variably implicated in H_2O_2-induced dilation in both animals and humans.
- Kv1 family channels are known to be redox regulated.
- Kv1.5 channels in preclinical models mediate the actions of H_2O_2 and connect cardiac metabolism to myocardial blood flow

What New Information Does This Article Contribute?

- H_2O_2 induces potent smooth muscle-mediated vasodilation through BK_{Ca} and K_V (especially $K_V1.5$) channels in adipose arterioles from human subjects without CAD.
- H_2O_2-elicited dilation is reduced in arterioles from patients with CAD, and is accompanied by a loss of $K_V1.5$- but not BK_{Ca}-dependent dilation.
- $K_V1.5$ cell surface localization and channel currents are reduced in vascular myocytes from subjects with CAD, with no change of mRNA or total cellular protein expression.

Endothelium-derived vasodilator factors such as NO and PGI_2 play a key role in regulation of vascular tone and homeostasis under normal conditions. In subjects with CAD, the primary mediator changes to H_2O_2 in various vasodilator responses especially flow-mediated dilation. The present study shows a reduced smooth muscle-mediated dilation to H_2O_2 in arterioles from CAD compared with non-CAD subjects, resulting from a transition from a combined BK_{Ca}- and K_V ($K_V1.5$)-mediated vasodilation to a dilation mediated predominantly by BK_{Ca}. Therefore, the onset of cardiovascular disease not only changes the primary endothelial vasodilators (NO to H_2O_2) but also affects smooth muscle K^+ channels that respond to vasodilator factors. The loss of $K_V1.5$-mediated dilation to H_2O_2 or other vasodilators may represent an important mechanism contributing to microvascular dysfunction in humans with CAD or other cardiovascular diseases.

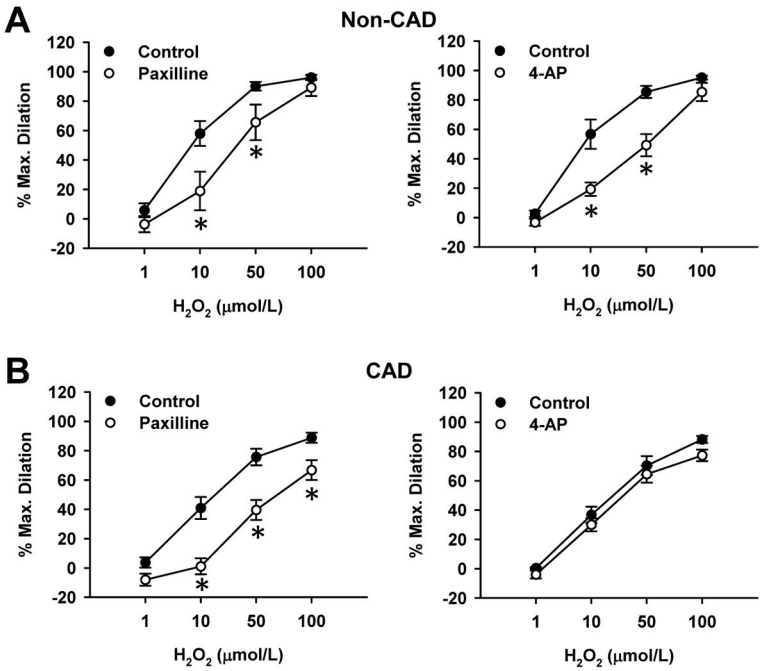

Figure 1. Role of BK_{Ca} and K_V channels in H_2O_2-induced dilation of human adipose arterioles from non-CAD and CAD subjects

H_2O_2 induced dose-dependent dilation in adipose arterioles. The dilation was reduced by paxilline (100 nmol/L), a BK_{Ca} channel blocker, in both non-CAD (A, left) and CAD (B, left) arterioles. In contrast, 4-AP (10 mmol/L), a general K_V channel blocker, reduced the dilation in non-CAD (A, right) but not CAD (B, right) arterioles, suggesting a loss of K_V channel function in disease. *P<0.05 versus control; n=5–6 vessels/group.

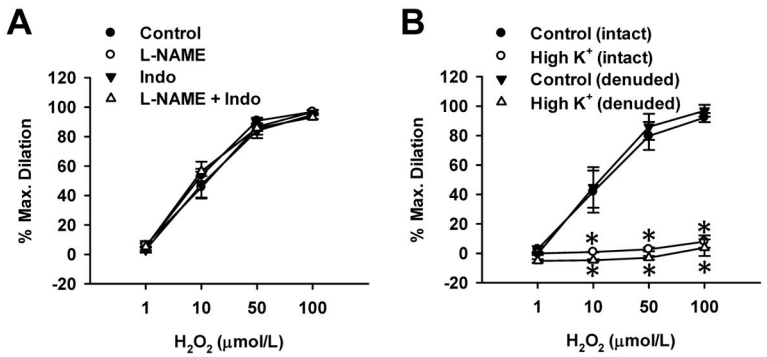

Figure 2. Effects of NOS and COX inhibition, endothelium denudation, and high K^+ on H_2O_2-induced dilation in non-CAD human adipose arterioles

A, The dilation was not affected by the nitric oxide synthase (NOS) inhibitor L-NAME (100 μmol/L), the cyclooxygenase inhibitor indomethacin (Indo, 10 μmol/L), alone or in combination. n=6 vessels/group. B, The dilation was not affected by removal of the endothelium, but was abolished by high K^+ (60 mmol/L). n=3 vessels/group; *P<0.05 versus control.

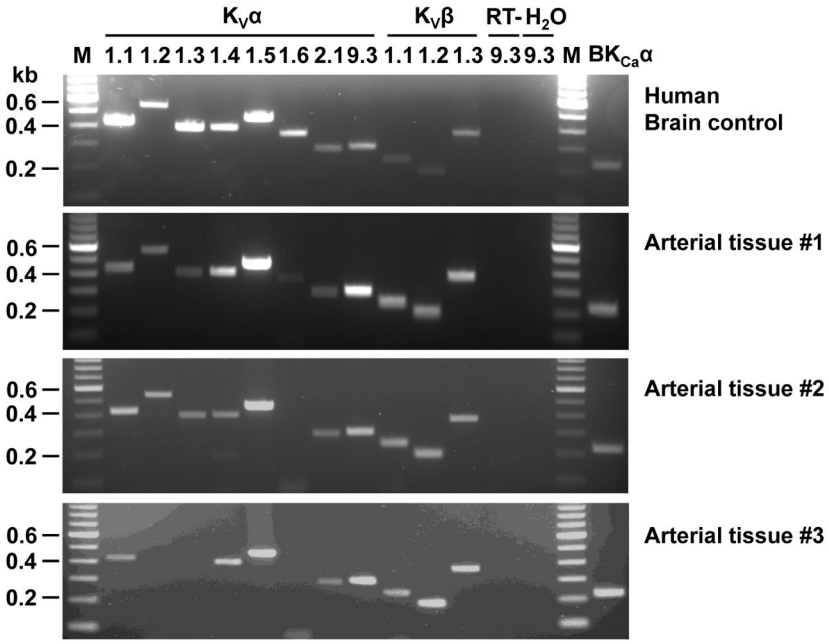

Figure 3. The mRNA expression of K_V1 and BK_{Ca} channel subunits in non-CAD human adipose arteries and arterioles

Three representative gel images of RT-PCR amplification products from adipose arteries and arterioles are shown (lower 3 panels). $K_V\alpha1.1$, $\alpha1.2$, $\alpha1.4$, $\alpha1.5$, $K_V\alpha2.1$, $K_V\alpha9.3$, $K_V\beta1.1$-$\beta1.3$, and BK_{Ca} were consistently found in different samples, whereas $K_V\alpha1.3$ and $\alpha1.6$ subunits were variably detected. As a positive control, human brain samples from a normal subject were found to express all K_V and BK_{Ca} channel subunits studied (top panel). RT-, without reverse transcription; H_2O, without template; M, marker.

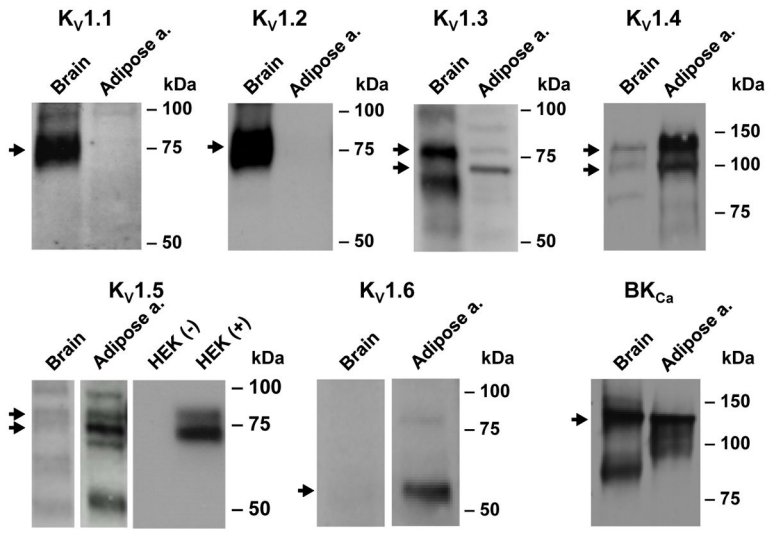

Figure 4. Western blot detection of K_V1 and BK_{Ca} channel α subunits

Representative images of protein expression of K_V1 and BK_{Ca} channel-forming α subunits in the membrane fraction of non-CAD human adipose arteries. Consistent with mRNA expression, $K_V1.5$ protein was detected in adipose arteries. $K_V1.3$, 1.4, and 1.6 were also detected in these samples. Human brain tissue (membrane fraction) was included as a positive control, which was found to abundantly express $K_V1.1$ and 1.2, as well as BK_{Ca}. Total lysates of HEK293 cells with (+) or without (-) exogenous $K_V1.5$ overexpression were also included as an additional control for $K_V1.5$. Arrow indicates mature forms of K_V1 or BK_{Ca} protein, with the approximate molecular mass values as follows: 75 kDa (1.1), 75 kDa (1.2), 75 kDa doublet (1.3), 110 kDa doublet (1.4), 75 kDa doublet (1.5), 60 kDa (1.6), and 130 kDa (BK_{Ca}).

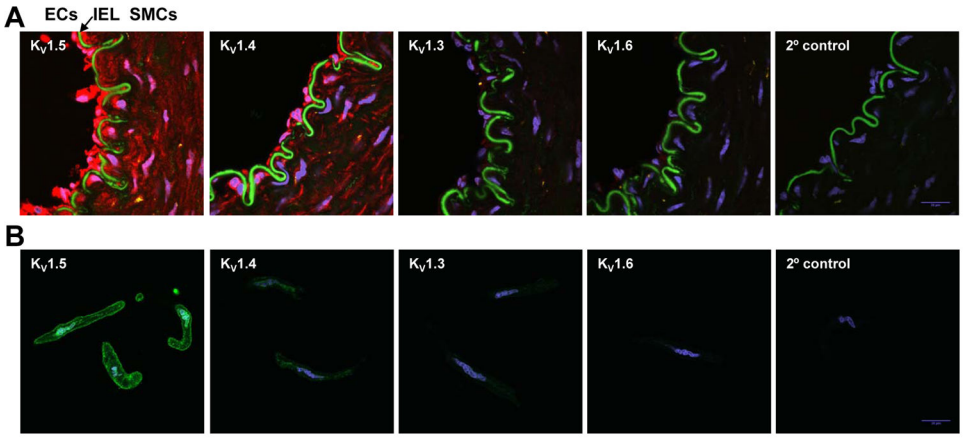

Figure 5. Immunofluorescence localization of K_V1 α-subunits in human adipose arterioles

$K_V1.5$ is the major K_V1 channel protein expressed in human adipose arteriolar smooth muscle cells. **A:** Confocal immunofluorescence images of K_V1 α-subunit proteins (red) in cross tissue sections (10 μm) of an intact human adipose arteriole. $K_V1.5$ and 1.4 of a lower level were detected in smooth muscle cells (SMCs) and endothelial cells (ECs). $K_V1.3$ and 1.6 subunits were also faintly visible in ECs. Cell nuclei were stained with DAPI (blue). IEL, internal elastic lamina (green auto-fluorescence). **B:** Confocal immunofluorescence images of corresponding K_V1 α-subunit proteins (green) in freshly dissociated SMCs from a human adipose arteriole. $K_V1.5$ protein was mainly localized on the cell membrane of SMCs. Cell nuclei was stained with DAPI (blue). Data are representative of >3 independent tissues.

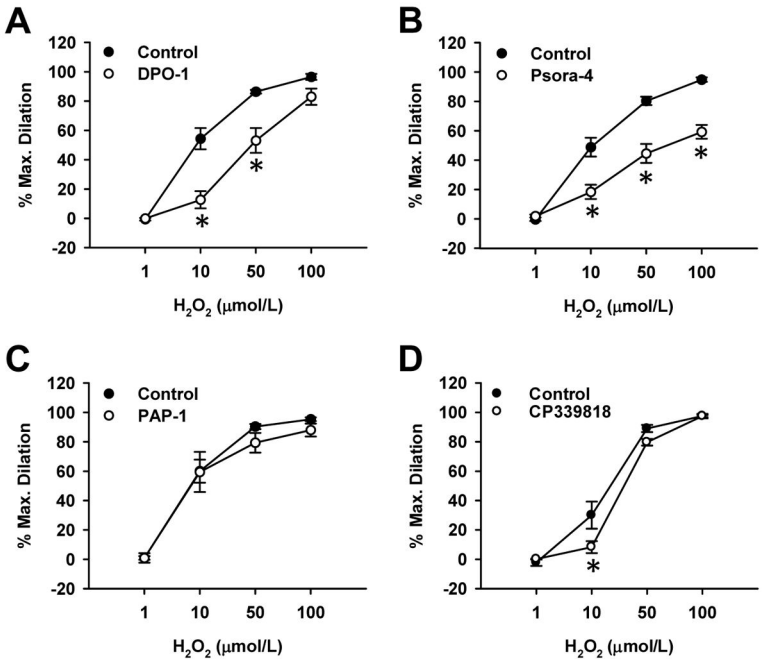

Figure 6. Role of $K_V1.5$ in H_2O_2-induced dilation of non-CAD human adipose arterioles

The dilation was blocked by DPO-1 (1 μmol/L, A), a selective $K_V1.5$ channel blocker, and Psora-4 (30 nmol/L, B), a $K_V1.3/1.5$ blocker. However, the dilation was not affected by PAP-1 (10 nmol/L, C), a $K_V1.3$ blocker. CP-339818 (3 μmol/L, D), a $K_V1.3/1.4$ blocker, slightly attenuated the dilation induced by 10 μmol/L H_2O_2 only. *P<0.05 versus control; n=5 vessels/group.

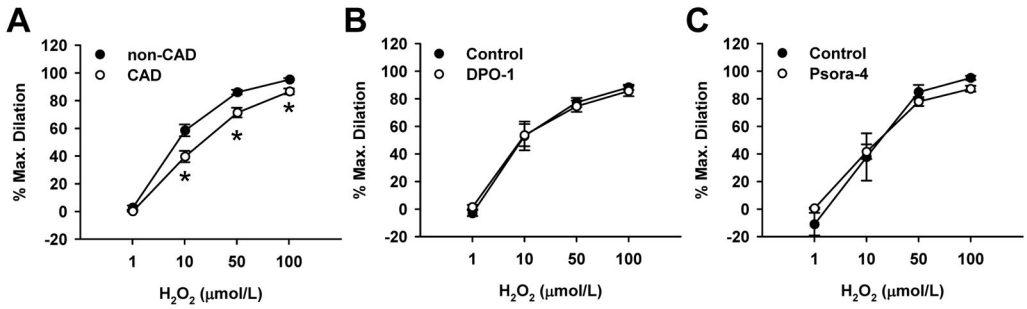

Figure 7. Role of $K_V1.5$ in H_2O_2-induced dilation of CAD adipose arterioles

Compared to non-CAD arterioles, H_2O_2-induced dilation was shifted rightward in CAD arterioles (A). DPO-1 (1 μmol/L, B) and Psora-4 (30 nmol/L, C) failed to alter the dilation in CAD arterioles. n=18 (A) or 3–6 (B, C) vessels/group; *P<0.05 versus control.

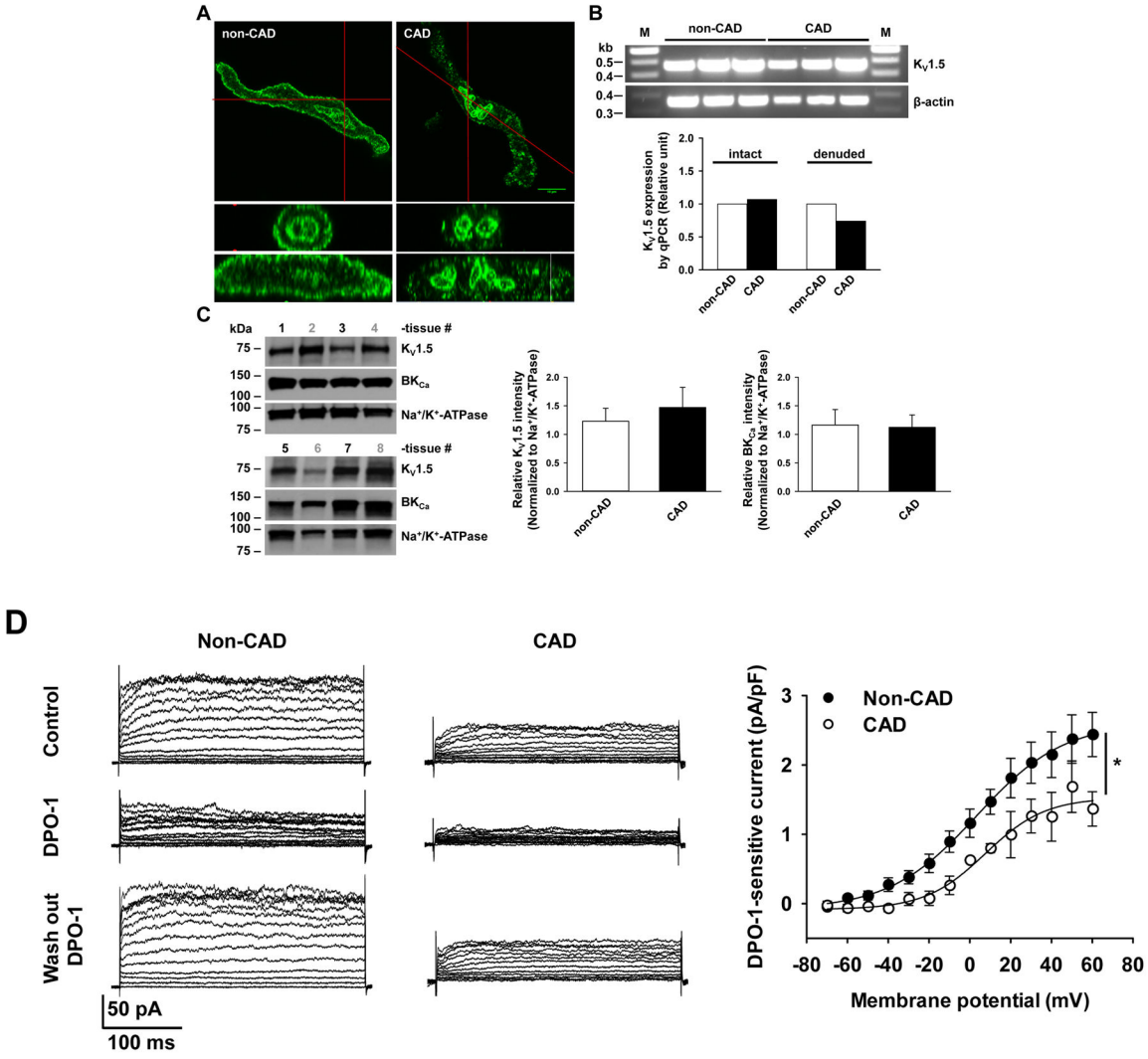

Figure 8. Comparison of $K_V1.5$ subcellular localization, total mRNA and protein expression, and K^+ current in non-CAD and CAD human arterioles

A: Immunofluorescence detection of $K_V1.5$ subunit proteins (green) in freshly dissociated VSMCs from non-CAD and CAD human adipose arterioles. The plasma membrane expression of $K_V1.5$ was markedly reduced in CAD as compared to non-CAD VSMCs. Lower two images represent cross-section views of top images vertically sectioned along red lines. Images are representative of results obtained from 3–4 each of non-CAD and CAD tissues. B: RT-PCR analysis of $K_V1.5$ mRNA. Top, end-point PCR gel images of $K_V1.5$, as well as β-actin control from non-CAD and CAD human adipose arterioles (n=3/group). Lower, relative abundance of $K_V1.5$ mRNA in non-CAD and CAD, normalized to the mean of β-action and ATP5o by quantitative PCR. Tissues were processed individually for mRNA extraction and cDNA synthesis before an equal amount of individual cDNA samples was pooled within each group for PCR analysis (n=6, 6, 4 and 4 for non-CAD intact, CAD intact, non-CAD denuded, and CAD denuded groups, respectively). C: Western blot detection of $K_V1.5$, BK_{Ca} and Na^+/K^+-ATPase proteins in non-CAD (tissue number in black) and CAD (tissue number in gray) human coronary arteries (n=4 tissues/group). Right,

summarized data. D: Effect of DPO-1 on voltage-elicited whole-cell K^+ currents in VSMCs freshly isolated from non-CAD and CAD human adipose arterioles. Currents were elicited by progressive 10 mV depolarizing steps from a holding potential of −70 mV to +60 mV. Left, representative traces recorded from two cells at baseline (control), 5–10 min after bath perfusion of 1 mmol/L DPO-1, and 5–10 min after DPO-1 washout, with a cell capacitance of 17 pF (non-CAD) and 18 pF (CAD), respectively. Right, averaged I-V relationships for DPO-1-sensitive K^+ currents (normalized to cell capacitance) in non-CAD and CAD myocytes; n=4–8 cells from each non-CAD (n=6) and CAD (n=4) subjects. *P<0.05 versus non-CAD.