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Aging Impairs Electrical Conduction Along Endothelium of Resistance Arteries Through Enhanced Ca^{2+}-Activated K^+ Channel Activation

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Abstract

Objective—Intercellular conduction of electrical signals underlies spreading vasodilation of resistance arteries. Small and intermediate-conductance Ca^{2+} activated K^+ channels (SK_{Ca}/IK_{Ca}) of endothelial cells serve a dual function by initiating hyperpolarization and modulating electrical conduction. We tested the hypothesis that the regulation of electrical signaling by SK_{Ca}/IK_{Ca} is altered with advancing age.

Approach and Results—Intact endothelial tubes (60 μm wide; 1-3 mm long) were freshly isolated from male C57BL/6 mouse (Young: 4-6 months; Intermediate: 12-14 months; Old: 24-26 months) superior epigastric arteries. Using dual intracellular microelectrodes, current was injected (±0.1-3 nA) at site 1 while recording membrane potential (V_m) at site 2 (separation distance: 50-2000 μm). Across age groups, greatest differences were observed between Young and Old. Resting V_m in Old (−38±1 mV) was more negative ($P<0.05$) than Young (−30±1 mV). Maximal hyperpolarization to both direct (NS309) and indirect (acetylcholine) activation of SK_{Ca}/IK_{Ca} was sustained ($\Delta V_m \sim -40$ mV) with age. The length constant (λ) for electrical conduction was reduced ($P<0.05$) from 1630±80 μm (Young) to 1320±80 μm (Old). Inhibiting SK_{Ca}/IK_{Ca} with apamin + charybdotoxin or scavenging H_2O_2 with catalase improved electrical conduction ($P<0.05$) in Old. Exogenous H_2O_2 (200 μM) in Young evoked hyperpolarization and impaired electrical conduction; these effects were blocked by apamin + charybdotoxin.

Conclusions—Enhanced current loss through K_{Ca} activation impairs electrical conduction along the endothelium of resistance arteries with aging. Attenuating the spatial domain of electrical signaling will restrict the spread of vasodilation and thereby contribute to blood flow limitations associated with advanced age.

Keywords

endothelial dysfunction; ion channels; oxidative stress

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Disclosures
None.

Introduction

Aging is associated with endothelial dysfunction [12, 3], a disorder characterized by impaired vasodilation in response to acetylcholine (ACh) [4-6], to muscular exercise [7], or to heating the skin [8]. As a stimulus that is well-defined in its actions, ACh application triggers endothelium-dependent vasodilation by increasing the production of nitric oxide (NO) and/or activating small (KCa2.3, KCNN3)- and intermediate (KCa3.1, KCNN4)-conductance calcium-activated K^+ channels (SK_{Ca}/IK_{Ca}). The bioavailability of NO decreases with advancing age [5, 11] and the function of endothelial SK_{Ca}/IK_{Ca} may be altered in pathological states [12, 13]. However the effect of aging on endothelial SK_{Ca}/IK_{Ca} function has not been determined, particularly in light of impairments in blood flow that accompany advancing age [7, 14, 15]. Cell-to-cell signaling through gap junctions is integral to endothelial function. Once initiated, hyperpolarization spreads rapidly along the endothelium and through myoendothelial junctions to relax smooth muscle cells (SMCs) [16, 17]. By synchronizing vasomotor responses in resistance networks, the conduction of electrical signals along the endothelium serves to coordinate blood flow control along and among vessel branches [18, 19]. Nevertheless, the spatial domain of endothelial signaling has received little attention in the context of aging.

In previous studies, conducted vasodilation in response to ACh [20] and ascending vasodilation in response to skeletal muscle contraction [14] were decreased in Old (20 month) vs. Young (3 month) male C57BL/6 mice. While the mechanism underlying this functional deficit has remained undefined, altered cell-to-cell coupling through gap junctions [21] could underlie impaired conduction. An alternative mechanism entails greater leakage of current through ion channels in plasma membranes, thereby precluding transmission of electrical signals along the endothelium [22]. The activation of SK_{Ca}/IK_{Ca} initiates endothelial cell (EC) hyperpolarization and vasodilation [9, 10, 16, 23]. Recent findings have revealed a role for SK_{Ca}/IK_{Ca} activation in modulating the spread of electrical signals along the endothelium of resistance arteries [22]. Thus, changes in SK_{Ca}/IK_{Ca} function with advancing age may alter the ability of electrical signals to travel along the endothelium and thereby affect vasomotor control.

The present experiments were designed to define the ability of the endothelium to initiate and conduct electrical signals with advancing age. Using endothelial tubes freshly isolated from resistance arteries of skeletal muscle from Young (4-6 months), Intermediate (12-14 months) and Old (24-26 months) mice we tested the hypothesis that the dual function of SK_{Ca}/IK_{Ca} to initiate and modulate electrical signaling along the endothelium is altered with aging. Findings reported here are the first to show that, by enhancing ion channel activation (particularly IK_{Ca}) aging promotes hyperpolarization of the endothelium while impairing its ability to conduct electrical signals. This increase in ion channel activation is attributable to oxidative stress manifested through the actions of H_2O_2.

Methods and Materials

Animal care and use were approved by the University of Missouri Animal Care and Use Committee and comply with the *Guide for the Care and Use of Laboratory Animals* (National Research Council; 8th Ed., revised 2011). Male C57BL/6 mice were studied at 3-6 (n=60), 12-14 (n=9), and 24-26 (n=39) months of age. Mice received standard chow and tap water ad libitum. For experiments, endothelial tubes were freshly isolated from superior epigastric arteries of abdominal skeletal muscle. Details are provided in the online Supplemental Materials and Methods.

Results

Figures 1-7 illustrate Old vs. Young mice. Key values for the Intermediate age group are stated in the text with their summary data shown in Supplemental Figures II, III and V. Across experiments, resting V_m was more negative ($P < 0.05$) in Old (−38 ± 1; n=28) vs. Young (−30 ± 1; n=29) or Intermediate (−31 ± 1 mV; n=9).

Expression of connexins and SK_{Ca}/IK_{Ca} in endothelial tubes

The mRNA transcript expression for SK_{Ca} and IK_{Ca} (Figure 1A) and connexins (Cx37, Cx40, and Cx43) (Supplemental Figure IA) was similar between Young and Old. Fluorescence immunolabeling confirmed the presence of respective K_{Ca} (Figures 1B-1E) and connexin proteins (Supplemental Figures IB-IG).

Endothelial hyperpolarization to acetylcholine is sustained with aging while intercellular transmission of electrical signals is reduced

It is unknown whether the ability of ACh to initiate hyperpolarization in resistance artery endothelium is altered with advancing age. Thus, we determined whether hyperpolarization to ACh was affected by age. Figure 2A illustrates progressive hyperpolarization in response to cumulative [ACh] from 10^{-8} to 10^{-5} M with washout and recovery between exposures. Neither the pEC_{50} for hyperpolarization (Young: 7.29 ± 0.10, Intermediate: 7.48 ± 0.11, Old: 7.16 ± 0.11) nor the maximum response to ACh (ΔV_m; Young: −38 ± 3 mV, Intermediate: −41 ± 3 mV, Old: −39 ± 1 mV; n=6 per group) differed between groups (Figures 2B, 2C; Supplemental Figures IIA, IIB).

To test the efficacy of cell-to-cell electrical coupling along the endothelium, current (±1-3 nA) was injected into one EC (site 1) while V_m was recorded from site 2 at constant separation distance (500 μm). Under control conditions conduction amplitude (CA) for Old (5.9 ± 0.5 mV/nA) was ~66% of Young (8.9±0.7 mV/nA) and ~57% of Intermediate (10.4 ± 0.7 mV/nA) (Figure 2D; Supplemental Figure IIC). Acetylcholine reduced CA in all groups in a concentration-dependent manner (Figure 2D; Supplemental Figure IIC). Attributable to their lower initial values for CA (Figure 2D), endothelial tubes from Old tended to maintain a higher Fraction of Control CA compared to Young during exposure to submaximal concentrations (< 1 μmol/L) of ACh (Figure 2E). This difference between age groups was significant ($P < 0.05$) for Old vs. Intermediate (Supplemental Figure IID).

Aging decreases the length constant for electrical conduction

In light of electrical conduction along the endothelium being integral to conducted vasodilation [23, 24], and finding that CA at a constant separation distance was impaired in Old during control conditions, we investigated whether aging would alter the effective distance of electrical conduction. As shown in Figures 3B and 3D, ΔV_m at site 2 ($\Delta V_m 2$) was related linearly ($R^2 \geq 0.99$) to the amplitude and polarity of current injected at site 1 for Young and Old. The slope of the current-voltage (I-V) relationship decreased with age and with distance (Figure 3B vs. 3D; Supplemental Figure IIIA vs. IIIB). Nevertheless, CA at each distance was reduced ($P < 0.05$) for Old compared to Young (Figure 3E; Table 1) or Intermediate (Supplemental Figure IIIC). The calculated length constant for electrical conduction (λ) was greater ($P < 0.05$) in Young (1630 ± 80 μm, n=12) and Intermediate (1900 ± 90, n=8) compared to Old (1320 ± 80 μm, n=9). When CA at each distance was normalized to respective local values, Conduction Efficiency was reduced ($P<0.05$) at 1500-2000 μm for Old vs. Young (Figure 3F) or Intermediate (Supplemental Figure IIID). Increasing the amount of current injected for Old (by 40%) to achieve the same absolute CA at the local site as Young confirmed significantly greater spatial decay in Old (Table 1).

Aging decreases the effect of SK_{Ca}/IK_{Ca} activation on electrical conduction

Given that aging reduced CA and increased spatial decay, we tested whether the effect of direct SK_{Ca}/IK_{Ca} activation on electrical conduction would vary with age. NS309 (1 μmol/L) reduced CA at all distances ($P < 0.05$) for endothelial tubes from Young and Old (Figures 4C, 4E. See Supplemental Figures IVC, IVD for concentration-dependent effects of NS309 on CA at 500 μm) as well as from the Intermediate group (Supplemental Figure V). For all age groups, the reduction in electrical conduction during NS309 treatment was also significant ($P<0.05$) with data expressed as Conduction Efficiency (Figures 4D, 4F; Supplemental Figure VB). The difference between Control and NS309 treatment was greater in Young than in Old (Figure 4). Thus the reduction in λ by NS309 was also greater in Young vs. Old (diminished by 690 ± 110 μm vs. 320 ± 90 μm, respectively; $P < 0.05$). Nevertheless, and consistent with ΔV_m responses to ACh, the magnitude of hyperpolarization to NS309 did not differ between age groups ($\Delta V_m = -33 \pm 2$ mV each; See Supplemental Figures IVA, IVB for concentration-dependent effects of NS309 on V_m).

SK_{Ca}/IK_{Ca} blockade restores electrical conduction in Old

In light of evidence indicating greater SK_{Ca}/IK_{Ca} activity in Old, we hypothesized that blocking SK_{Ca}/IK_{Ca} would enhance electrical conduction to a greater extent in endothelial tubes of Old vs. Young. This was tested by injecting current and recording V_m2 continuously (at distance = 500 μm) before and during exposure to apamin (Ap; 300 nmol/L) and/or charybdotoxin (ChTx; 100 nmol/L) (Figures 5A and 5B). Blockade of SK_{Ca}/IK_{Ca} depolarized Old by 16 ± 2 mV (to −24 ± 2 mV; n=11) which was a greater ($P < 0.05$) effect than for Young (10±1 mV depolarization to −22 ± 1 mV; n=11). During Ap + ChTx, CA increased more ($P < 0.05$) in Old (57 ± 6%) vs. Young (24 ± 4%) thus CA in Old was restored to that of Young (Figure 5C). Expressing CA values as Fraction of Control (Figure 5D) illustrated the relatively greater effect of SK_{Ca}/IK_{Ca} blockade on electrical conduction in Old vs. Young. Experiments in which apamin or charybdotoxin were applied individually indicated a prominent role for IK_{Ca} in dissipating injected current (Figure 5C and 5D).

Scavenging H_2O_2 with catalase improves electrical conduction in Old

Aging and endothelial dysfunction have been attributed to heightened oxidative stress [11, 25, 26] including excess production of hydrogen peroxide (H_2O_2) [11, 27, 28]. As H_2O_2 may alter the activity of K_{ca} [29, 30], we tested whether scavenging H_2O_2 with membrane-permeant catalase would improve electrical conduction. Catalase (500 U/ml) depolarized V_m by 3 ± 1 mV in Young (n=6; e.g., Figure 6A) and by 9 ± 1 mV in Old (n=7; e.g., Figure 6B) such that resting V_m (~ −28 mV) was no longer different between age groups. With negligible effect on CA of Young, catalase increased both absolute (Figure 6C) and relative CA (Figure 6D) of Old by ~30% ($P<0.05$). In complementary experiments (Supplemental Figure VI), the presence of catalase had little effect on hyperpolarization to ACh (3 μmol/L) for either age group (n=4 each). In response to NS309 (1 μmol/L), the presence of catalase reduced hyperpolarization of Young by 8 ± 1 mV and increased hyperpolarization of Old by 4 ± 1 mV (Supplemental Figure VI).

Inhibiting endothelial nitric oxide synthase does not improve electrical conduction in Old

As the bioavailability of endothelium-derived NO decreases with advancing age [5, 11], we tested whether uncoupled endothelial nitric oxide synthase was a source of H_2O_2 [31]. Endothelial tubes from Old treated were treated with the inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 100 μmol/L, 20 minutes). We found no significant effect on either resting V_m (Control: −41 ± 2 mV; L-NAME: −40 ± 4 mV, n = 3) or CA (at 500 μm: Control = 7.0 ± 1.0 mV/nA; L-NAME = 7.6 ± 1.2 mV/nA, n = 3).

SK_{Ca}/IK_{Ca} activation by H_2O_2 impairs electrical conduction

In light of catalase restoring V_m and conduction of Old to approximate values of Young, we tested whether exogenous H_2O_2 would hyperpolarize endothelial tubes and impair electrical conduction of Young. Addition of H_2O_2 (200 μmol/L) hyperpolarized V_m progressively over time, approximating the equilibrium potential for K^+ (E_K; ~−90 mV) after ~ 20 minutes (Figures 7A and 7B). Conduction Amplitude decreased as hyperpolarization increased (Figures 7A and 7C). In separate experiments, inclusion of Ap + ChTx prevented changes in V_m and CA during H_2O_2 exposure (Figures 7D and 7E; Supplemental Figures VII and VIII). Upon washout of Ap + ChTx, H_2O_2 evoked hyperpolarization and inhibited CA (Figures 7D and 7E; Supplemental Figures VII and VIII).

Discussion

Blood flow to skeletal muscle is attenuated with aging but the underlying mechanisms have remained poorly defined. Evidence has pointed to a role for enhanced sympathetic neuroeffector signaling [14, 15] however little is known of changes that may occur within the vascular wall. Intrinsic to blood flow control in resistance networks is the conduction of electrical signals along the endothelium to coordinate SMC relaxation [17, 32, 33]. The present study has determined that the ability of the endothelium of skeletal muscle resistance arteries to conduct electrical signals is impaired with aging. The use of intact endothelial tubes freshly isolated from mouse superior epigastric arteries [22, 34, 35] enabled these changes to be resolved independent of blood flow or surrounding cells. Remarkably, conduction along the endothelium of Old was restored to that of Young upon selective blockade of SK_{Ca}/IK_{Ca}, particularly IK_{Ca}. Complementary experiments demonstrate that either direct (with NS309) or indirect (with ACh) activation of SK_{Ca}/IK_{Ca} in endothelial tubes of Young produced effects that mimicked the behavior of Old. In light of the association between aging and oxidative stress [11, 25-27] and reports that H_2O_2 may alter the activity of K_{Ca} [29, 30], treating endothelial tubes of Old with catalase restored electrical conduction in a manner consistent with the effects of blocking IK_{Ca} with charybdotoxin. In a reciprocal manner, treating endothelial tubes of Young with H_2O_2 impaired electrical conduction and this effect was also inhibited with SK_{Ca}/IK_{Ca} blockade. These data collectively support the hypothesis that, via the actions of H_2O_2, more K_{Ca} channels are open under resting conditions in Old vs. Young. In turn, the diminished resistance of cell membranes enables electrical signals to "leak" as they travel along the endothelium, reducing the spatial domain of electrical signaling [22].

Impact of age on electrical conduction: Effects of SK_{Ca}/IK_{Ca} activation

Endothelial dysfunction is characterized by impaired endothelium-dependent vasodilation [1]. While such changes have been attributed to impaired NO bioavailability and signaling [2, 5], endothelium dependent hyperpolarization (EDH) initiated through SK_{Ca}/IK_{Ca} activation predominates as a signal (via myoendothelial coupling) for SMC relaxation in resistance vessels [10, 17, 32, 33]. Alterations in SK_{Ca}/IK_{Ca} function have been associated with vascular disease [12, 13]; nevertheless, it has not been determined how these ion channels may be affected by aging. Nor has it been determined what consequences such changes may have on endothelial function, particularly in regard to the initiation and conduction of electrical signals. We show here that the ability of either direct (NS309; Supplemental Figure IVB) or indirect (ACh; Figure 2C) SK_{Ca}/IK_{Ca} activation to produce hyperpolarization was preserved in endothelial tubes of Old. Despite a more negative resting V_m in Old vs. Young, the consistency of hyperpolarization to ACh indicates that the G-protein coupled signaling events underlying SK_{Ca}/IK_{Ca} activation [9, 10, 12] were maintained in endothelial tubes of Old. This finding is in contrast to reports that ACh-induced hyperpolarization of mesenteric arteries preconstricted with norepinephrine was greater in Young (1-8 month) vs. Old (20-26

month) rats [36, 37]. Others have reported diminished sensitivity for relaxation to SK_{Ca}/IK_{Ca} activation (NS309) in saphenous arteries of 64-week vs. 12-week male mice [38]. However a differential effect of NS309 was not apparent for hyperpolarization of endothelial tubes of Old vs. Young (Supplemental Figure IV). Such differences between preparations illustrates the use of the endothelial tube as a model to evaluate properties intrinsic to the endothelium to avoid the influence of smooth muscle activation, which can alter endothelial function via signaling through myoendothelial gap junctions [39].

In accordance with the biophysical determinants of the electrical length constant [$\lambda = (r_m/r_a)^{1/2}$], the ability of electrical signals to spread along the endothelium reflects: (1) the axial resistance to current flow between cells (i.e., r_a), which is determined primarily by the patency of gap junctions; and (2) the "leakiness" of plasma membranes (i.e., r_m; the membrane resistance to current flow), which can be determined by the activation of ion channels (e.g., SK_{Ca}/IK_{Ca} [22]). Respective signaling proteins are well-expressed in endothelial tubes of both Old and Young (Figure 1, Supplemental Figure I). Throughout our experiments, resting V_m was consistently 5-10 mV more negative in endothelial tubes of Old vs. Young. These findings are consistent with the ~6 mV more negative resting V_m of hippocampal pyramidal neurons from Old (>36 months) vs. Young (2-3 months) rabbits, also attributed to enhanced Ca^{2+}-activated K^+ current in neurons of Old [40].

The linearity and stability of electrical responses (Figures 3B and 3D; Supplemental Figures IIIA and IIIB) enabled electrical recordings throughout a full range of current injections (±0.1-3 nA) at multiple distances (50 to 2000 μm). Under resting conditions, the λ we determined for endothelial tubes of Old was depressed by ~20% compared to Young (Figures 3E and 3F; Supplemental Figure III). Our functional experiments illustrate that activating SK_{Ca}/IK_{Ca} reduced λ (and CA) to a greater extent in Young (~40%) when compared to Old (~25%) (Figure 4). Further, depolarization and augmentation of electrical conduction during selective blockade of SK_{Ca}/IK_{Ca} with Ap + ChTx was significantly greater in Old vs. Young and attributable primarily to actions on IK_{Ca} (Figure 5). We therefore suggest that, compared to Young (or Intermediate, which exhibited properties close to those of Young; Supplemental Figures II and III), the endothelium of Old has more IK_{Ca} open at rest, thereby allowing greater current leak and signal dissipation [22]. In addition, the restoration of electrical conduction along endothelial tubes of Old to levels not different from Young upon selective channel blockade (Figure 5C) indicates that gap junction patency was sufficient across age groups to maintain intercellular electrical coupling along the endothelium.

The endothelium can conduct depolarization as effectively as hyperpolarization (Figures 3A-3D; Supplemental Figures IIIA and IIIB) [22, 34]. Such linearity of the I-V relationship indicates a negligible functional expression of voltage-sensitive ion channels [e.g. large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca})] within EC plasma membranes. However, this behavior is not the case in intact feed arteries of hamster skeletal muscle, where depolarization conducted with less efficacy compared to hyperpolarization [32]. This deviation from linearity can also be explained by myoendothelial coupling to SMCs [32], as the latter express voltage-gated K^+ channels which increase conductance (i.e., current leakage) upon depolarization [41]. Further, given the ability of α_1-adrenoreceptor activation on SMCs to activate SK_{Ca}/IK_{Ca} of ECs via myoendothelial coupling [39], the impairment of conducted vasodilation along intact vessels [20] can be depressed even further than the effects of aging on SK_{Ca}/IK_{Ca} by an associated increase in sympathetic drive [42]. In turn, the present findings suggest how impaired ability of the endothelium to conduct electrical signals may underlie earlier *in vivo* observations in mouse skeletal muscle of decreased conducted vasodilation along arterioles [20] and impaired ascending dilation of feed arteries [14] with aging.

Collectively, such effects may contribute to the restriction of blood flow and compromised oxygen delivery during physical activity in older (e.g., > 60 years) humans [2, 7].

Impact of oxidative stress on an isolated endothelial syncytium

Aging and endothelial dysfunction are associated with increased oxidative stress [11, 25, 26, 43-45]. Lower production of reactive oxygen species (ROS; e.g. superoxide) was reported in aortae from long-living mice (*Peromyscus leucopus*) vs. conventional house mice (Mus musculus) [25]. The ROS signaling pathway begins with superoxide produced by mitochondria, NADPH/xanthine oxidases, and uncoupled eNOS [11, 27, 31, 46]. Excessive and highly reactive superoxide levels are converted to the stable intermediate H_2O_2 either spontaneously or through actions of superoxide dismutases [11, 27, 28]. Catalase and glutathione peroxidase convert H_2O_2 into water and these enyzmes are expressed at higher levels in *P. leucopus* vs. *M. musculus* [25], underscoring the ability to metabolize H_2O_2 as a determinant of maximum lifespan potential.

Intracellular H_2O_2 can alter protein thiol groups to form disulfide bridges in and between proteins [28]. Thus, the actions of H_2O_2 may be promiscuous in modifying ion channel function. The physiological actions of H_2O_2 may thereby be manifested through its actions on the ion channels that are expressed in a given cell type (Figure 1). This reasoning is consistent with the actions of H_2O_2 on endothelial tubes from Young (Figure 7), where the consequences of activating SK_{Ca}/IK_{Ca} (i.e., hyperpolarization and impaired electrical conduction) were similar to the actions of either indirect (with ACh; Figure 2) or direct (with NS309; Figure 4) channel activation. In turn, these findings are consisted with the ability of catalase to restore resting V_m and electrical conduction of Old to values not different from Young (Figure 6). The concentration of H_2O_2 used in the present study (200 μmol/L) is consistent with that used by others (~100 to 300 μmol/L) to evoke dilation of coronary arterioles [30, 47]. Further, the effects we observed for H_2O_2 (as well as aging) were sensitive to SK_{Ca}/IK_{Ca} blockade with Ap + ChTx (Figures 7D and 7E; Supplemental Figures VII and VIII). Altogether, our study indicates that H_2O_2 activate SK_{Ca}/IK_{Ca} of resistance artery endothelium. However, it should also be recognized that the effects of oxidative stress on the endothelium shown here may be “masked” in intact vessels by the presence of SMCs and their activation of BK_{Ca} channels in response to H_2O_2 [30]. As the inhibition of eNOS had no effect (see Results), key questions raised by the present findings point to resolving mitochondrial vs. non-mitochondrial sources of H_2O_2 and direct vs. indirect (i.e., via increases in $[Ca^{2+}]_i$ [48] or altered phosphorylation by protein kinase G I-α [49]) activation of K_{Ca} by oxidative stress.

Summary

Our goal in this study was to determine the ability of the endothelium of resistance arteries from mouse skeletal muscle to initiate and conduct electrical signals with advancing age independent from the prevailing influence of blood flow, smooth muscle cells or other vasoactive stimuli. We focused on the roles of SK_{Ca}/IK_{Ca} to initiate hyperpolarization and to modulate the transmission of electrical signaling. Our findings demonstrate that the function of SK_{Ca}/IK_{Ca} to generate hyperpolarization was sustained with advancing age. However, more SK_{Ca}/IK_{Ca} (particularly IK_{Ca}) were open at rest in the endothelium of old animals which resulted in a more negative resting membrane potential and diminished electrical conduction, attributable to greater signal dissipation via charge loss through the plasma membrane. Scavenging H_2O_2 or blocking SK_{Ca}/IK_{Ca} channels (particularly IK_{Ca}) depolarized the endothelium of Old and restored electrical conduction to values not different from the endothelium of Young. Conversely, exposing endothelial tubes of Young to H_2O_2 produced hyperpolarization and reduced electrical conduction and these effects were also prevented by blocking IK_{Ca} alone or together with SK_{Ca}. The present findings are the first to

highlight the effect of aging on the role of K_{Ca} channels in governing the initiation [9, 10] and transmission of electrical signals [22] within vascular endothelium. Endothelial SK_{Ca}/IK_{Ca} function thereby serves both to generate hyperpolarization underlying smooth muscle relaxation and to modulate the spread of vasodilatation along resistance networks. With aging, attenuating the spatial domain of electrical signaling will restrict spreading vasodilation and thereby contribute to blood flow limitations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACh	acetylcholine
Ap	apamin
CA	conduction amplitude
ChTx	charybdotoxin
EC	endothelial cell
eNOS	endothelial nitric oxide synthase
H_2O_2	hydrogen peroxide
SK_{Ca}/IK_{Ca}	small- and intermediate Ca^{2+}-activated K^+ channels
NO	nitric oxide
NS309	6,7-dichloro-1H-indole-2,3-dione 3-oxime
r_i	internal resistance to current flow between cells
r_m	membrane resistance to current flow
V_m	membrane potential

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Significance

Aging is associated with endothelial dysfunction, a disorder contributing to restricted muscle blood flow and compromised oxygen delivery during physical activity. The endothelium is instrumental in coordinating dilation within resistance networks by conducting electrical signals [e.g. hyperpolarization via activation of small- and intermediate- Ca^{2+} activated K^+ channels (SK_{Ca}/IK_{Ca})] that dilate resistance arteries to increase peak tissue blood flow. How advancing age impacts electrical signals underlying vasodilation is unknown. Using endothelial tubes freshly isolated from mouse superior epigastric arteries, we show that the initiation of hyperpolarization through SK_{Ca}/IK_{Ca} activation is sustained in old age while the spread of electrical signals is impaired. This functional decrement in electrical conduction along the endothelium is explained by loss of current through activated SK_{Ca}/IK_{Ca} (particularly IK_{Ca}) in response to oxidative stress. Attenuating the spatial domain of electrical signaling will impair spreading dilation of resistance arteries and can thereby restrict tissue blood flow.

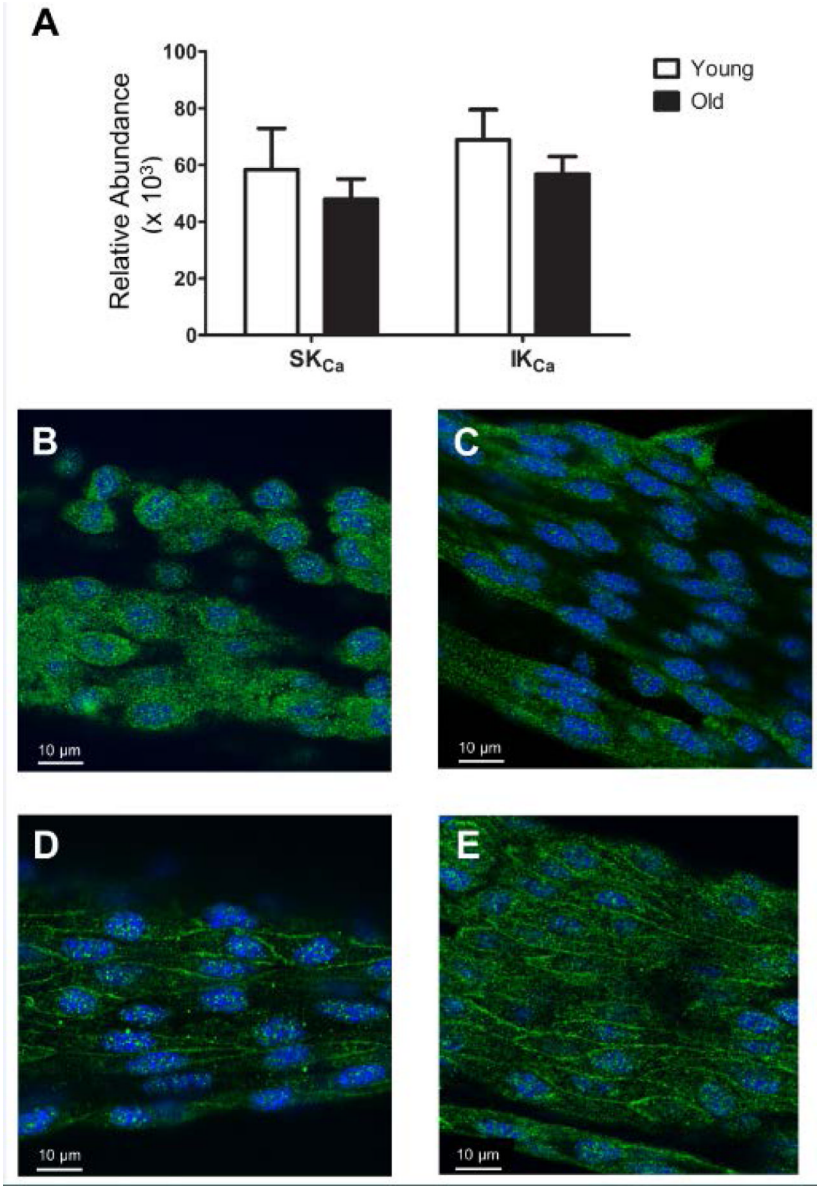

Figure 1. Expression of SK_{Ca}/IK_{Ca} in endothelial tubes of resistance arteries from Young and Old mice

A, Abundance of mRNA for SK_{Ca} and IK_{Ca} relative to the expression of glucuronidase β (Gusb) in endothelium of Young and Old (n=5 per group). Summary data are means ± S.E. **B**, Single confocal slice image of an isolated endothelial tube from Young indicating SK_{Ca} in green and nuclei in blue. **C,** As in **B**, for Old. **D,** As in **B** for IK_{Ca} in Young. **E**, As in **D** for Old; each image represents at least three independent experiments.

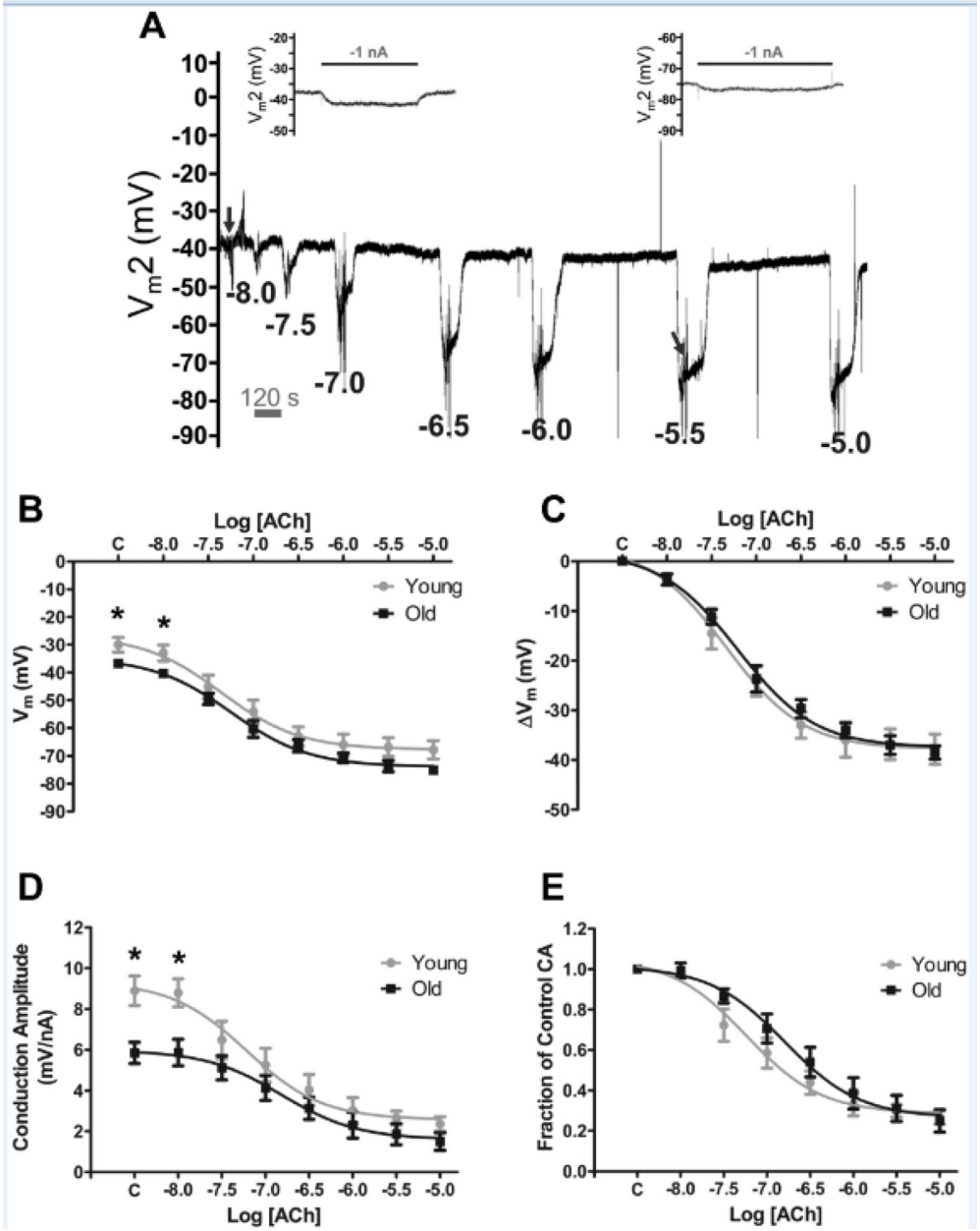

Figure 2. Effects of ACh on membrane potential and electrical conduction in endothelial tubes of resistance arteries from Young and Old mice

A, Representative membrane potential recording within an endothelial tube from an Old mouse illustrating hyperpolarization to increasing [ACh] with washout and recovery between each [ACh]. Electrical responses to current injected (±1-3 nA pulses, 2s each) at site 1 were recorded at site 2 (V_m2) with separation distance = 500 μm during control (left inset corresponds to trace under left arrow; see Figure 3A for expanded trace) and during peak hyperpolarization to each [ACh] (right inset corresponds to trace under right arrow). During hyperpolarization, note loss of V_m2 responses with residual capacitance spikes. For B-E, "C" on X-axes refers to Control values at rest; **B**, Effect of [ACh] on resting V_m in Young and Old. **C**, Effect of [ACh] on the change in V_m (ΔV_m) from Control for Young and Old. **D**, Effect of [ACh] on Conduction Amplitude (CA) in Young and Old (for −1nA). **E**,

Effect of [ACh] on Fraction of CA (= CA at each [ACh] / respective Control value in **D**. ***P** < 0.05, Young vs. Old. (n = 6 per group). Summary data are means ± S.E.

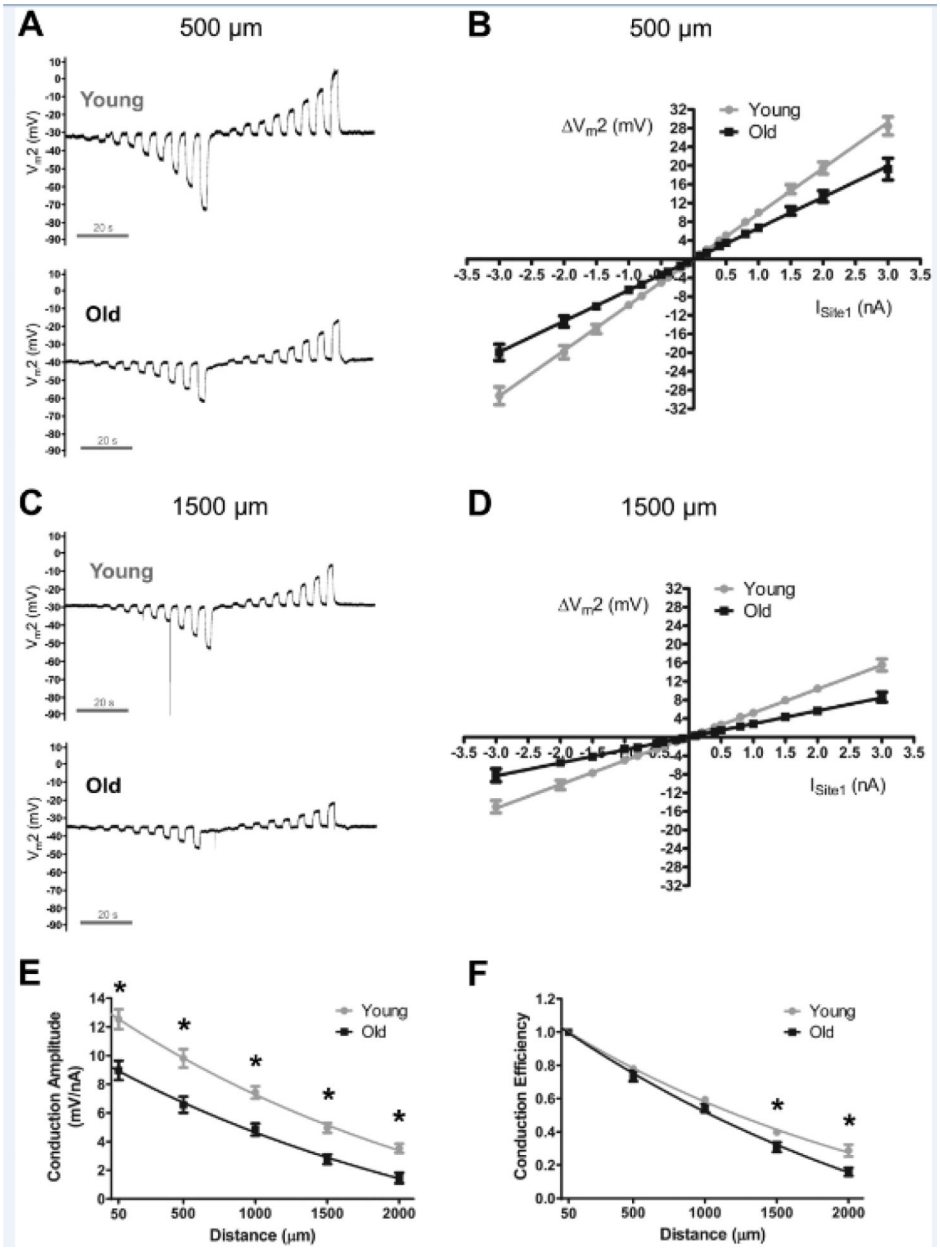

Figure 3. Electrical conduction along the endothelium of resistance arteries is impaired in Old vs. Young mice

A, Membrane potential was recorded at site 2 (V_m2) located 500 µm from current injected at site 1 (±0.1 to 3 nA). Changes in V_m2 were related linearly to the amplitude and polarity of current injected at site 1. Note more negative V_m and diminished (~30%) ΔV_m responses in Old vs. Young at each level of current injection. **B**, Summary data for experiments illustrated in **A** at 500 µm distance. Note lower ($P < 0.05$) slope of Old vs. Young (Young: 9.8 ± 0.7 mV/nA, n=12; Old: 6.6 ± 0.6 mV/nA, n=9). **C**, As in **A** with site 2 located 1500 µm from site 1. Note lower responses compared to **A**. **D**, As in **B** for site 2 at distance = 1500 µm [(Young: 5.0 ± 0.3 mV/nA (n=12), Old: 2.8 ± 0.3 mV/nA (n=9)]; the slope of respective I-V relationships decreased as distance increased. **E**, Conduction Amplitude vs. distance. At each distance, values for Old were depressed relative to values for Young; data are in response to −1 nA current injection. **F**, Conduction Efficiency = data from **E**

normalized to respective CA at local (50 μm) site; note relatively greater decay with distance in Old. Calculated length constant for electrical conduction ($\hat{\lambda}$) was greater ($P < 0.05$) in Young (1630 ± 80 μm, n=12) versus Old (1320 ± 80 μm, n=9). *****$P < 0.05$, Young vs. Old. Resting V_m was greater ($P < 0.05$) in Old (−36 ± 2 mV) vs. Young (−28 ± 2 mV). Summary data are means ± S.E.

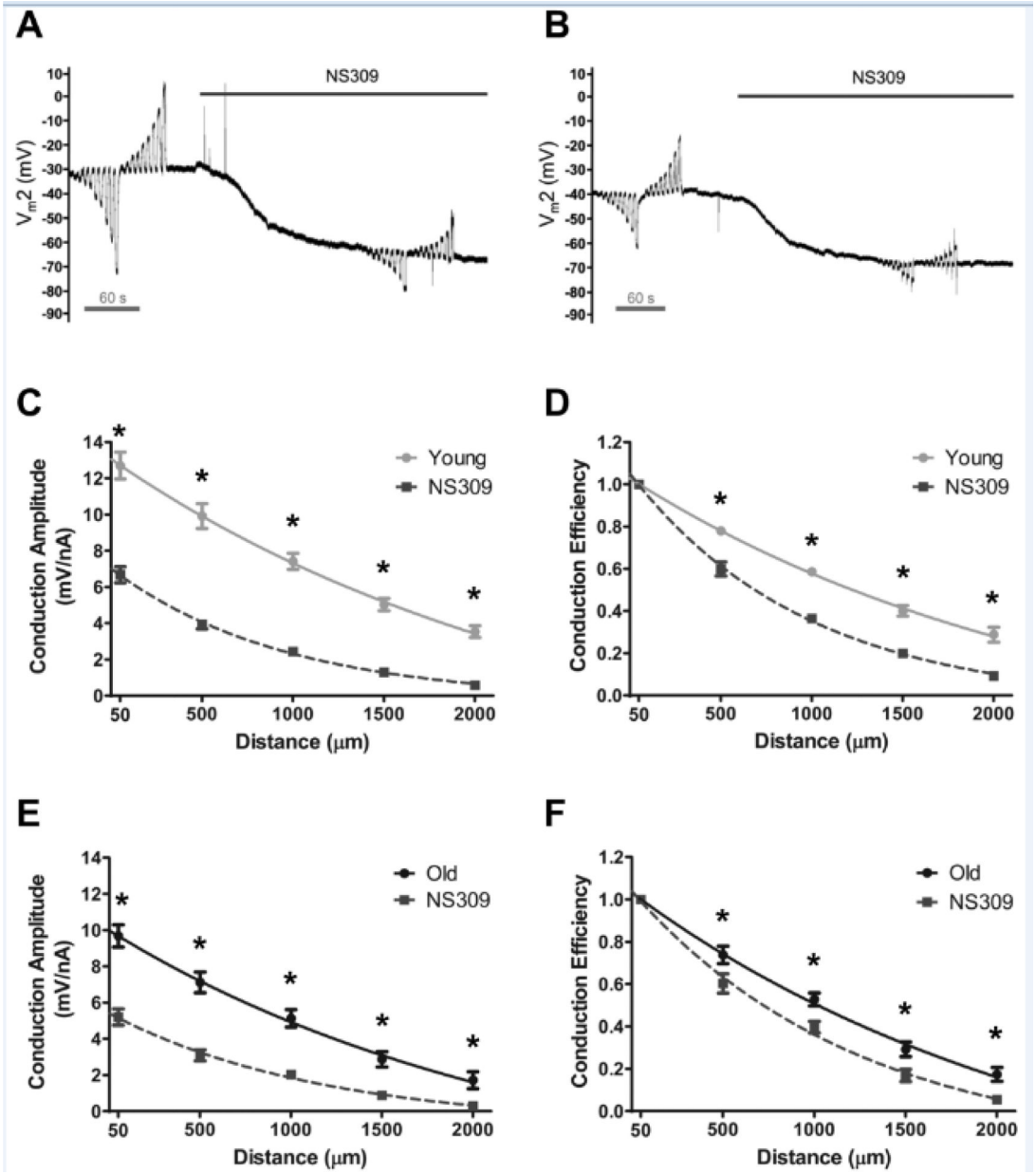

Figure 4. Impairment of electrical conduction during SK_{Ca}/IK_{Ca} activation is greater along endothelium of Young vs. Old mice

A, Representative recording of membrane potential responses at 500 μm (V_m2) from current injected at site 1 (±0.1 to 3 nA) before and during SK_{Ca}/IK_{Ca} activation with NS309 (1 μmol/L) in endothelial tube of Young. **B**, As in **A** for Old. The effect of SK_{Ca}/IK_{Ca} activation with NS309 on Conduction Amplitude (Panels **C** and **E**) and Conduction Efficiency (Panels **D** and **F**) vs. distance. Panels **C** and **D**, Young; Panels **E** and **F**, Old. NS309 reduced the amplitude and efficiency of electrical conduction across age groups with relatively greater effects in Young vs. Old (n=7 for Old, n=10 for Young). *****$P < 0.05$ vs. NS309. Summary data are means ± S.E.

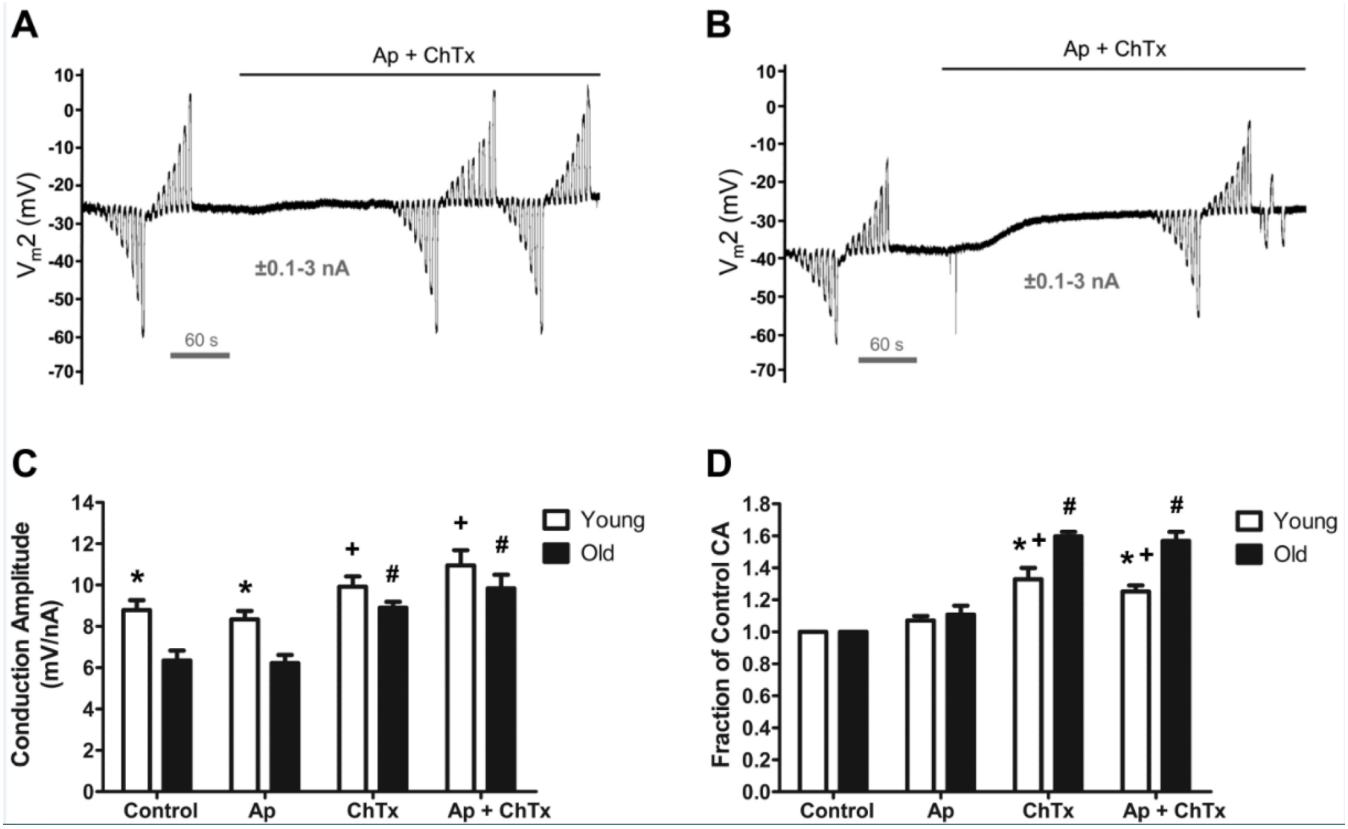

Figure 5. Enhanced electrical conduction during SK_{Ca}/IK_{Ca} blockade is greater along endothelium of Old vs. Young mice

A, Representative recording of membrane potential responses at 500 μm (V_m2) from current injected at site 1 (±0.1 to 3 nA) in endothelial tube of Young. Note slight depolarization and enhanced V_m2 responses during apamin (Ap, 300 nmol/L) + charybdotoxin (ChTx, 100 nmol/L). **B**, As in **A** for Old. Note more negative resting V_m with reduced V_m2 responses vs. Young. During Ap + ChTx, note greater depolarization and enhancement of V_m2 responses vs. Young. **C**, Summary data (means ± S.E.) for conduction amplitude (CA) at rest (Control) and during Ap alone (n=5), ChTx alone (n=5) and in combination (n=11) within respective age groups. **D**, Data from **C** expressed as Fraction of Control CA within respective age groups. In **C** and **D**, note greater effect of ChTx vs, Ap on restoring CA in Old. *****P<0.05 vs. Old for respective condition; +P<0.05 vs. respective Young Control; #P<0.05 vs. respective Old Control.

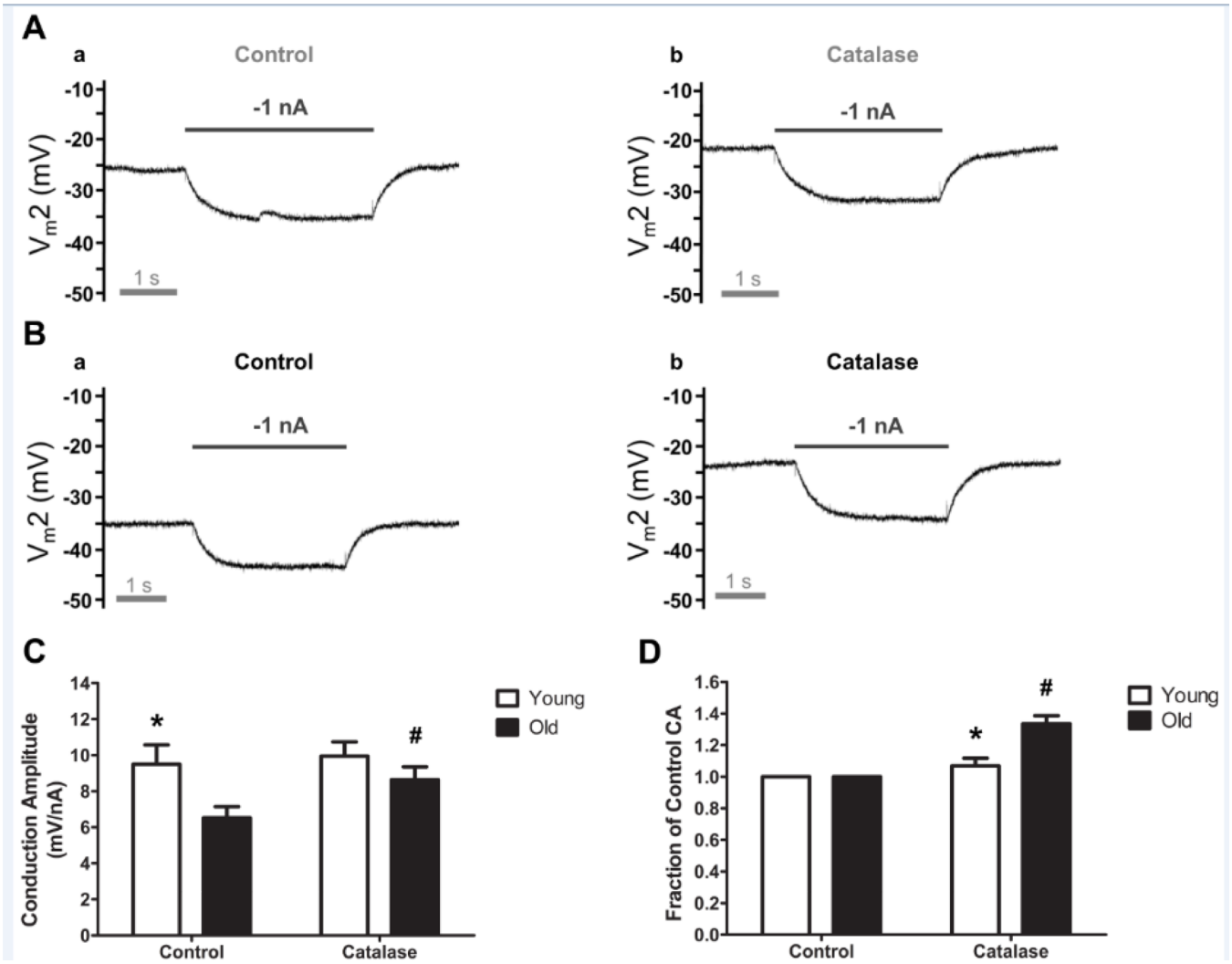

Figure 6. Catalase improved electrical conduction along endothelium of Old vs. Young mice

A, Representative recording of membrane potential responses at 500 μm (V_m2) from current injected at site 1 in Young illustrating response to −1 nA before (**a**) and during catalase (500 U/ml, 20 minutes) to scavenge H_2O_2. (**b**). Note slight depolarization during catalase while V_m2 response (−10 mV) to −1 nA current was maintained. **B**, As in **A** for Old. Note greater depolarization from Control vs. Young and enhanced V_m2 responses during catalase (Control: −7 mV, catalase: −10 mV). **C**, Summary data (means ± S.E.) for Conduction Amplitude at 500 μm distance under respective conditions for Young (n=6) and Old (n=7). **D**, Data from **C** normalized as Fraction of Control. * P<0.05 vs. Old for respective condition; #P<0.05 vs. Old Control.

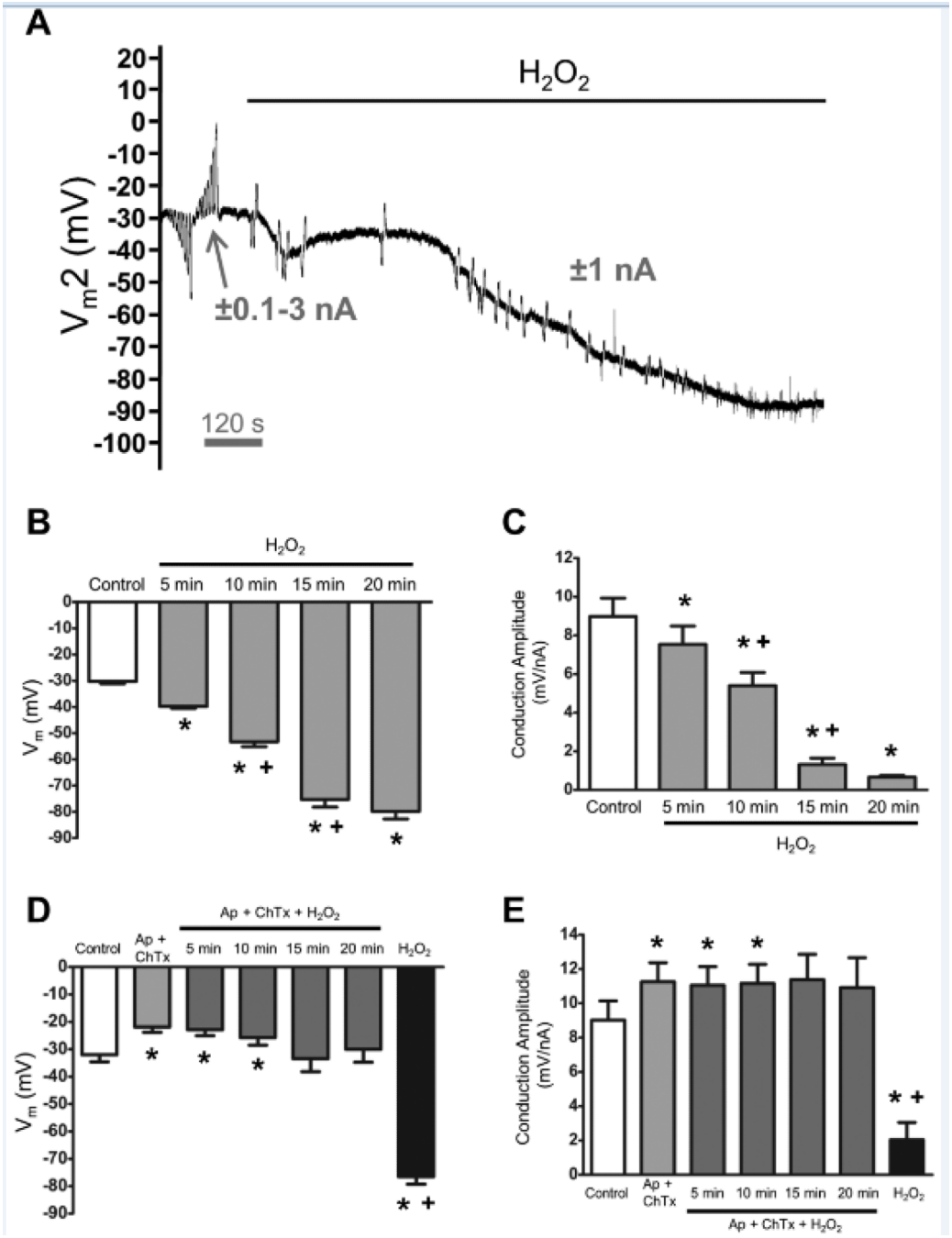

Figure 7. Endothelial hyperpolarization and loss of electrical conduction via SK_{Ca}/IK_{Ca} activation with H_2O_2

A, Representative recording of membrane potential responses at 500 μm (V_m2) from current injected at site 1 before and during H_2O_2 (200 μmol/L) exposure. Note progressive hyperpolarization and loss V_m2 responses (with residual capacitance spikes). **B**, Summary data before (Control) and during effect of H_2O_2 on resting V_m over 20 minutes. **C**, Summary data before (Control) and during effect of H_2O_2 on Conduction Amplitude (distance = 500 μm) at times corresponding to those in **B**. **D**, Summary data for V_m before (Control) and during Ap (300 nmol/L) + ChTx (100 nmol/L), during H_2O_2 with Ap + ChTx for 20 minutes (note lack of hyperpolarization), and after washout of Ap + ChTx with H_2O_2

still present (note hyperpolarization to ~−80 mV). **E**, Conduction Amplitude (distance = 500 μm) at times corresponding to those in **D**. During H_2O_2 exposure, note maintenance of CA with Ap + ChTx present and loss of CA following their washout. *P < 0.05 vs. Control; +P < 0.05 vs. preceding time point. Summary data are means ± S.E.; n=6-8 per group. Data in **B** and **C** were obtained together in one set of experiments; Data in **D** and **E** were obtained together in a separate set of experiments. All data in this Figure are based upon continuous recordings from endothelial tubes of Young mice. See complementary data in Supplemental Figure VIII.

Table 1

Spatial decay of electrical conduction is greater in Old vs. Young.

Distance (μm)	Young (-1nA) ΔV_m2 (mV)	Old (-1nA) ΔV_m2 (mV)	Old (-1.4nA) ΔV_m2 (mV)
50	-12.5 ± 0.7	-9.0 ± 0.7*	-12.5 ± 0.9
500	-9.8 ± 0.6	-6.6 ± 0.6*	-9.1 ± 0.8
1000	-7.4 ± 0.4	-4.8 ± 0.4*	-6.7 ± 0.6
1500	-5.0 ± 0.3	-2.7 ± 0.3*	-3.8 ± 0.5*
2000	-3.5 ± 0.3	-1.5 ± 0.4*	-2.0 ± 0.5*

The standard current pulse microinjected at site 1 to evaluate a change in membrane potential at site 2 (ΔV_m2) at distances of 50-2000 μm was -1 nA. The ΔV_m2 response to -1 nA was reduced at all distances in Old (Column 3) vs. Young (Column 2). To achieve the same ΔV_m2 at the nearest distance (50 μm) required ~ 40% more current (-1.4 nA; Column 4) in Old. Despite the same ΔV_m2 at 50 μm; note progressively greater signal loss with distance in Old vs. Young (compare Column 2 with Column 4). These data are complementary to Figure 3E, F.

*$P < 0.05$ vs. Young ΔV_m2 responses to -1 nA at the same distances (n = 12 for Young, n = 9 for Old).