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Tuning electrical conduction along endothelial tubes of resistance arteries through Ca^{2+}-activated K^+ channels

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Abstract

Rationale—Electrical conduction through gap junction channels between endothelial cells of resistance vessels is integral to blood flow control. Small and intermediate-conductance Ca^{2+}-activated K^+ channels (SK_{Ca}/IK_{Ca}) initiate electrical signals in endothelial cells but it is unknown whether SK_{Ca}/IK_{Ca} activation alters signal transmission along the endothelium.

Objective—We tested the hypothesis that SK_{Ca}/IK_{Ca} activity regulates electrical conduction along the endothelium of resistance vessels.

Methods and Results—Freshly isolated endothelial cell tubes (60 μm wide; 1–3mm long; cell length, ~35 μm) from mouse skeletal muscle feed (superior epigastric) arteries were studied 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). SK_{Ca}/IK_{Ca} activation (NS309, 1 μmol/L) reduced the change in V_m along endothelial cell tubes by ≥50% and shortened the electrical length constant (λ) from 1380 to 850 μm ($P<0.05$) while intercellular dye transfer (propidium iodide) was maintained. Activating SK_{Ca}/IK_{Ca} with acetylcholine or SKA-31 also reduced electrical conduction. These effects of SK_{Ca}/IK_{Ca} activation persisted when hyperpolarization (>30 mV) was prevented with 60 mM $[K^+]_o$. Conversely, blocking SK_{Ca}/IK_{Ca} (apamin + charybdotoxin) depolarized cells by ~10 mV and enhanced electrical conduction (i.e., changes in V_m) by ~30% ($P<0.05$).

Conclusions—These findings illustrate a novel role for SK_{Ca}/IK_{Ca} activity in tuning electrical conduction along the endothelium of resistance vessels by governing signal dissipation through changes in membrane resistance. Voltage-insensitive ion channels can thereby tune intercellular electrical signaling independent from gap junction channels.

Keywords

conducted vasodilation; hyperpolarization; gap junctions; potassium channels

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Introduction

Electrical conduction through gap junction channels (GJCs) coordinates vasomotor responses in vascular resistance networks.[1–3] Through axial orientation and robust expression of GJCs, endothelial cells (ECs) serve as the primary pathway for cell-to-cell conduction along the vessel wall[4–6] with electrical signals transmitted to smooth muscle cells (SMCs) via myoendothelial GJCs.[3, 7–9] Once initiated, hyperpolarization (and vasodilation) can travel along the resistance vasculature for millimeters.[10–13] As shown in exercising skeletal muscle, vasodilation originating downstream within the microcirculation can ascend the resistance network via conduction along the endothelium to encompass feed arteries upstream and thereby increase total blood flowing into the active muscle.[14]

In accord with the cable properties of electrical conduction, the distance that signals travel along the endothelium is determined by the length constant ($\lambda = r_m/r_a)^{1/2}$, where r_m = membrane resistance and r_a = axial resistance to current flow.[15, 16] With negligible resistance of cytoplasm, intercellular r_a is determined primarily by GJCs.[8, 16] Previous studies have focused on alterations of conducted vasodilation through manipulating the functional integrity of GJCs[5, 14, 17] or the expression profile of their connexin subunits.[18–21] Alternatively, changes in r_m may also provide a mechanism for governing electrical conduction independent of manipulating GJCs. In turn, changes in r_m should reflect the activation of ion channels in the plasma membrane,[15, 16] which otherwise insulates the cell interior from the extracellular milieu. Remarkably, the effect of governing r_m through ion channel activation has not been studied in light of electrical conduction along the endothelium of resistance vessels.

The small (K_{Ca} 2.3; KCNN3)-and intermediate (K_{Ca} 3.1; KCNN4)-conductance Ca^{2+}-activated K^+ channels (SK_{Ca}/IK_{Ca}) are abundantly expressed in EC membranes.[22–24] Upon activation of SK_{Ca}/IK_{Ca} the efflux of K^+ promotes hyperpolarization.[22, 25] While this response may promote vasodilation and tissue perfusion,[24] a reduction in r_m along the endothelium could dissipate electrical signals and thereby compromise electrical conduction and impair ascending vasodilation. Given the prominent role of SK_{Ca}/IK_{Ca} in the initiation of EC hyperpolarization,[9, 24] we questioned whether activation of these ion channels impacts electrical conduction along the endothelium.

Understanding how the activation of SK_{Ca} /IK_{Ca} may govern electrical conduction along the endothelium of intact vessels is limited by myoendothelial coupling to SMCs, perivascular nerve activity, and circulating vasoactive agents along with the shear stress exerted by blood flow. Further, the initiation of an electrical signal to trigger conduction has typically consisted of an agonist (e.g., ACh[4, 10, 26]) or electrical field stimulation[27, 28] via micropipettes. However, such “local” stimuli can activate multiple cells simultaneously, with uncertainty in the precise origin and intensity of the actual stimulus. To avoid such confounding influences, we have freshly isolated EC tubes from feed arteries of mouse abdominal skeletal muscle.[29, 30] Intact EC tubes from these resistance vessels can exceed 3 mm in length, enabling the efficacy of electrical conduction along the endothelium to be evaluated. Using sharp intracellular microelectrodes, current microinjection into a single EC alters membrane potential (V_m) independent of agonists or ion channel activation. By simultaneously recording V_m at defined distances from the site of current injection, we tested the hypothesis that SK_{Ca}/IK_{Ca} activity can `tune' electrical conduction along the endothelium.

Findings reported here are the first to show that activating SK_{Ca}/IK_{Ca} with either pharmacological agents (NS309, SKA-31) or a physiological agonist (ACh) impairs electrical conduction along the endothelium of a resistance artery. Concomitantly,

intercellular coupling through GJCs remains intact as confirmed by robust dye transfer between ECs. We further demonstrate that blocking constitutively open SK_{Ca}/IK_{Ca} enhances electrical conduction. Thus altering r_m via opening and closing plasma membrane ion channels effectively tunes the conduction of electrical signals along the endothelium.

Methods

Tissue preparation and intracellular recording

Procedures were approved by the Institutional Animal Care and Use Committee and performed in accord with the National Research Council's Guide for the Care and Use of Laboratory Animals (8th Ed. 2011). Male C57BL/6 mice (bred at the University of Missouri; age: 3–6 months; n=45) were anesthetized (pentobarbital; 60 mg/kg intraperitoneal injection. Abdominal muscles were removed bilaterally; feed arteries (superior epigastric) were dissected free from surrounding tissue. Using gentle trituration following mild enzymatic digestion, SMCs were dissociated to produce intact EC tubes (width: 60 μm, length: 1–3 mm).[29, 30] Experiments were performed at 32 °C and completed within 3 h.[29, 30]

A freshly-isolated EC tube was secured at each end in a tissue bath on the stage of an inverted microscope and superfused continuously with physiological salt solution [PSS; composition in mM: 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 N-2-Hydroxyethylpiperazine-m′-2-ethanesulfonic acid (HEPES), 10 Glucose]. A pair of intracellular microelectrodes (borosilicate glass; tip resistance, 150–200 MΩ) coupled to independent amplifiers were inserted into ECs along the midline of the tube with a separation distance of 50–2000 μm. With individual ECs ~35 μm long,[29] 50 μm separation enabled `local' responses to be recorded from Site 2 as close to the signal origin (Site 1) as possible while ensuring that microelectrodes were in separate (adjacent) cells.[30] Successful dual impalements were indicated by correspondence of electrical events between microelectrodes.

Figure 1 illustrates the experimental approach and summarizes the analyses employed. To study electrical conduction over distance (Figs. 2 and 3), impalement at Site 1 was maintained while the microelectrode for recording V_m at Site 2 was repositioned at the next distance (order randomized across experiments). As standard procedure for evaluating interventions, separation distance was maintained at 500 μm (Figs. 4–8)[7, 30] corresponding to ~15 ECs placed end-to-end. Current was microinjected (±0.1–3 nA; 2s pulse) at Site 1 while recording V_m at Site 2 under control conditions, during activation of SK_{Ca}/IK_{Ca} with NS309, SKA-31, or ACh and during inhibition of SK_{Ca}/IK_{Ca} with apamin + charybdotoxin (Ap + ChTX). Entire EC tubes were exposed by adding agents to the superfusion solution and V_m was confirmed to be the same between microelectrodes to ensure that the entire EC tube was isopotential before current injections.

Data analyses

One EC tube was studied per mouse. Analyses included: Resting V_m (mV) under Control conditions; Change in V_m (Δ mV) = peak response V_m – preceding baseline V_m; Conduction Amplitude (CA, mV/nA) = V_m at Site 2 / current injected at Site 1; Fraction of Control CA = CA during treatment/preceding control CA; Conduction Efficiency = CA at each distance/CA at 50 μm separation; Length constant (λ) = distance over which the electrical signal decayed to 37% (1/e) of the `local' value. Data were analyzed using Analysis of Variance with Bonferroni post-hoc comparisons, regression analyses, and paired t-tests. Differences were accepted as statistically significant with $P < 0.05$. Summary data are presented as means ± S.E. Values for n refer to the number of EC tubes studied under respective conditions.

Results

Effects of SK_{Ca}/IK_{Ca} activation on electrical conduction over distance

Resting V_m was −26±2 mV at Sites 1 and 2 independent of separation distance (n=11). As shown in Figure 2, ΔV_m at Site 2 increased in direct proportion to current injected irrespective of polarity. The amplitude of $\Delta V_m 2$ for each current decreased when separation distance between microelectrodes was increased from 500 to 1500 μm (Figs. 2A–2C) yet the I–V relationship remained linear (Fig. 2C). At 500 μm separation, the slope of the I–V relationship through ± 0.1–3 nA was 7.5±0.5 mV/nA. When calculated for −1 nA, CA (500 μm) was not different (7.7±0.5 mV/nA; n=11), substantiating −1 nA as a standard reference.

The ΔV_m (−37 mV) in response to 1 μmol/L NS309 corresponds to the peak hyperpolarization obtained with ACh (3 μmol/L).[30] At each distance, opening SK_{Ca}/IK_{Ca} with 1 μmol/L NS309 reduced CA (Figs. 2A,2B) yet the I–V relationship remained linear (Fig. 2D). Thus NS309 decreased CA at 500 μm to nearly the same extent as did increasing distance to 1500 μm under Control conditions (Fig. 2C). Following continuous paired recordings (Control, NS309) at each distance, washout of NS309 (10–15 min) restored Control V_m and CA (e.g., CA at 500 μm: Control, 7.7 ± 0.4 mV/nA; NS309 washout, 7.9 ± 0.4 mV/nA, n=16), confirming the reversibility of SK_{Ca}/IK_{Ca} activation. The next separation distance was evaluated in the same manner.

During current injections, NS309 (1 μmol/L) reduced $\Delta V_m 2$ at the `local' site by half and decreased λ by ~40% (Control: 1380±80 μm; NS309: 850±60 μm; Fig. 3A, $P<0.05$). By normalizing CA at each separation distance to respective `local' values, Conduction Efficiency was also reduced at each distance ($P < 0.05$; Fig. 3B). The injection current required for the same local change in V_m obtained with −1 nA under Control conditions increased to −2 nA with 1 μmol/L NS309, consistent with a corresponding decrease in r_m. When local hyperpolarization was thereby matched between conditions, the decay in $\Delta V_m 2$ over distance was again greater ($P<0.05$) with NS309 (Fig. 3C; Supplemental Table I). Thus, activating SK_{Ca}/IK_{Ca} impaired electrical conduction irrespective of ΔV_m at the site of current injection.

For the following experiments, current was injected at Site 1 while V_m was recorded at Site 2 with 500 μm separation maintained between microelectrodes.[7, 30] The stability of impalements enabled multiple treatments to be evaluated during continuous (paired) recordings. Responses were evaluated through the full range of current microinjection with data presented for −1 nA.

Progressive inhibition of electrical conduction during graded activation of SK_{Ca}/IK_{Ca}

A question central to these experiments was: Can electrical conduction be `tuned' according to the level of SK_{Ca}/IK_{Ca} activation? Current was injected at Site 1 and V_m recorded at Site 2 (500 μm) before and during increments in SK_{Ca}/IK_{Ca} activation with recovery between each treatment. Hyperpolarization began at 100 nmol/L NS309 and increased with [NS309] (Fig. 4A; $P < 0.05$); maximal response at 10 μmol/L corresponded to $V_m = −81 ± 1$ mV. Impairment of CA; $P<0.05$) began at 300 nmol/L NS309 and CA decreased as [NS309] increased with conduction abolished at 10 μmol/L (Figs. 4B, 4C). At 1 μmol/L, NS309 reduced CA by half (Figs. 4B, 4C). In separate experiments, using SKA-31 to activate SK_{Ca}/IK_{Ca}[24] produced effects similar to NS309 though with attenuated potency and efficacy (compare Figs. 4D–4F with Figs. 4A–4C). Nevertheless, for both pharmacological agents, a `threshold' V_m associated with significant reduction in CA occurred at ~−40 mV (Figs. 4A, 4D). These findings indicate that the efficacy of electrical conduction along the endothelium can be tuned according to the level of SK_{Ca}/IK_{Ca} activation.

Maintenance of dye transfer during inhibition of electrical conduction

Impairment of electrical conduction during SK_{Ca}/IK_{Ca} activation is interpreted to reflect greater current dissipation along the endothelium. Thus an essential question is: Does intercellular coupling persist when electrical conduction is inhibited? We used dye transfer as a functional qualitative index of GJC patency between neighboring ECs.[4, 7, 10, 27, 30] An EC tube was exposed to the NS309 concentration that inhibited conduction (10 μmol/L) beginning 5 min before and throughout recordings. With 0.1% propidium iodide dye (MW = 668.4 Da) in the microelectrode, fluorescent images were acquired following 30 min of continuous recording. Under Control conditions (Fig. 5A) and during hyperpolarization (to −81 ± 4 mV) with inhibition of electrical conduction [CA (500 μm): 0.3 ± 0.1 mV/nA)], dye microinjected into one EC readily spread to neighboring ECs (Fig. 5B; n=3). Thus dye transfer during maximal activation of SK_{Ca}/IK_{Ca} with NS309 was qualitatively similar to Control, confirming that intercellular coupling through GJCs remained patent during absence of electrical conduction. For reference, in EC tubes exposed to carbenoxolone (100 μmol/L) or β-glycyrrhetinic acid (40 μmol/L) to block GJCs, dye transfer was inhibited reversibly along with electrical conduction.[30]

Inhibition of electrical conduction independent of hyperpolarization

The activation of SK_{Ca}/IK_{Ca} consistently hyperpolarized ECs, raising the question: Does loss of electrical conduction during SK_{Ca}/IK_{Ca} activation depend upon hyperpolarization? According to the Nernst relationship, raising $[K^+]_o$ from 5 (Control) to 60 mmol/L (equimolar replacement of NaCl with KCl) reduces E_K from −89 mV to −23 mV and should thereby "clamp" V_m near Control (V_m= −23±1 mV, n=7) and prevent hyperpolarization during K^+ channel activation. To test this effect, current was injected and V_m recorded before and during superfusion with either NS309 (1 μmol/L), 60 mmol/L $[K^+]_o$, or both together. Despite lack of hyperpolarization, the decrease in CA (500 μm) during NS309 + 60 mmol/L $[K^+]_o$ was not different from that during NS309 alone (Figs. 6A, 6B). However with 60 mmol/L $[K^+]_o$ alone the I–V relationship deviated ($P<0.05$) from linearity when negative current exceeded −1.5 nA (Fig. 6C), attributable to the corresponding reduction in E_K. By evaluating V_m2 responses to −1 nA, comparisons between conditions were maintained within the linear range of the I–V relationship. Treatment with NS309 consistently reduced CA (500 μm) by half (Fig. 6D), confirming that inhibition of electrical conduction during SK_{Ca}/IK_{Ca} activation was independent of a change in V_m.

Inhibition of conduction by ACh

To determine whether a physiological agonist that activates SK_{Ca}/IK_{Ca} exerts similar effects, EC tubes were superfused with ACh (3 μmol/L).[30] During peak hyperpolarization, responses at Site 2 decreased by ~70% versus Control (Fig. 7). Exposure to ACh + 60 mmol/L $[K^+]_o$ confirmed that ACh inhibited CA (500 μm) irrespective of hyperpolarization (Supplemental Fig. I).Thus activating SK_{Ca}/IK_{Ca} with a classic vasodilator also impaired electrical conduction irrespective of hyperpolarization.

Enhanced electrical conduction with SK_{Ca}/IK_{Ca} blockade

Progressive activation of SK_{Ca}/IK_{Ca} impaired electrical conduction in a graded manner (Fig. 4). Thus if a subpopulation of SK_{Ca}/IK_{Ca} were open at rest than inhibition of these channels should enhance electrical conduction. This prediction was tested by injecting current and recording V_m before and during exposure to Ap + ChTX. Exposure to these SK_{Ca}/IK_{Ca} blockers depolarized ECs (Control: −28±2 mV; Ap + ChTX: −18±2 mV; n=6; $P<0.05$) and increased CA (500 μm) by ~30% ($P<0.05$; Fig. 8). Further, the relationship between ΔV_m at Site 2 (500 μm) and current injected at Site 1 remained linear (with greater slope) through the entire range of current injection ($R^2 \geq 0.99$; n = 6).

A summary of the treatment effects of the preceding interventions (Figures 4, 6, 7, 8) on electrical conduction at a standard reference distance (500 μm) is given in Supplemental Table II.

Negligible role for K_{ATP} or BK_{Ca} channels

If manipulating SK_{Ca}/IK_{Ca} activity alters electrical conduction than complementary effects would be expected for other K^+ channels. We tested for such an effect of ATP-regulated K^+ channels (K_{ATP}) using levcromakalim (10 μmol/L; n=5). However this K_{ATP} opener[22, 25] had no significant effect on resting V_m (−27±1 mV) or CA (500 μm; Control, 8.4 ± 1.1, levcromakalim: 8.6 ± 1.1 mV/nA). Complementary experiments investigating a role for the large conductance Ca^{2+} (and voltage) - sensitive K^+ channel (BK_{Ca}) using the activator NS1619[22, 25] (10 or 30 μmol/L; n=4) were also without effect on resting V_m (−27±2 mV) or CA (500 μm; Control, 8.7±1.3, NS1619: 9.3±1.1 mV/nA). These findings indicate that neither K_{ATP} nor BK_{Ca} are functionally expressed in EC tubes.

Negligible role for nitric oxide

Endothelial cell hyperpolarization may also promote nitric oxide (NO) synthesis.[31, 32] We therefore tested whether inhibition of NO synthase altered the effect of SK_{Ca}/IK_{Ca} activation on electrical conduction. Irrespective of treatment with the inhibitor N^{ω}-nitro-L-arginine (100 μmol/L), exposure to NS309 (1 μmol/L) or to ACh (3 μmol/L) suppressed CA (500 μm) to the same extent (Supplemental Fig. II).

Discussion

This study has revealed the ability of membrane ion channel activation to tune the efficacy of electrical conduction along the endothelium of resistance vessels. Using intact EC tubes freshly isolated from feed arteries of mouse skeletal muscle, findings illustrate that activation of SK_{Ca}/IK_{Ca} impairs axial signal transmission. This effect is explained by a fall in membrane resistance (r_m) that dissipates charge as current flows from cell to cell along the endothelium. During intracellular microinjection of propidium iodide, robust dye transfer between neighboring ECs confirmed that cell-cell coupling through GJCs was maintained in the absence of electrical conduction. Thus, irrespective of SMCs or of changing cell-cell coupling through GJCs, dynamic changes in r_m can profoundly alter the efficacy of electrical signal transmission along the endothelium of resistance vessels. Remarkably, such tuning of electrical conduction was governed by ion channels that are insensitive to voltage.

The nature of electrical conduction along EC tubes

Conduction amplitude was defined as the slope of the I–V relationship with responses routinely evaluated at −1 nA; i.e., within the linear range throughout our experiments. At a standard reference distance of 500 μm, our values of CA (~8 mV/nA; Figures 2C–D, 3A) compare favorably with values (~7 mV per 0.8 nA) calculated for the conduction of hyperpolarization along resistance arteries of hamster skeletal muscle.[7] Moreover, hyperpolarizing and depolarizing signals conducted with similar efficacy along EC tubes as shown by the linear (i.e., ohmic) changes in V_m throughout the range of currents injected (±0.1–3 nA) at each distance (Fig. 2). Such behavior indicates low functional expression of voltage-sensitive ion channels and is consistent with the lack of effect of NS1619 on V_m or CA. In turn, the deviation from linearity observed during hyperpolarization with >1.5 nA in the presence of 60 mmol/L $[K^+]_o$ (Fig. 6C) can be explained by the reduction in E_K. In contrast to the present findings, depolarizing currents injected into ECs (or SMCs) of feed arteries from hamsters evoked less than half of the absolute change in V_m than did hyperpolarizing current of equal intensity.[7] Such deviation observed for intact vessels

(versus the linearity of responses for EC tubes) may be attributed to the influence of SMCs through myoendothelial coupling;[7, 9] e.g., through voltage gated K^+ channels (K_v) that may not otherwise be present in ECs.[25]

Whereas activating SK_{Ca}/IK_{Ca} with NS309 (1 μmol/L) reduced CA by 50% or more at each distance (Fig. 3A), comparing absolute values does not resolve changes in the nature of electrical conduction because the local response to current injection (i.e., change in V_m) is reduced. Expressing responses at each distance relative to the local response provides a normalized index of "Conduction Efficiency" and illustrates significantly greater decay in electrical conduction during SK_{Ca}/IK_{Ca} activation when compared to Control (Fig. 3B). However, it is important to evaluate the effect of SK_{Ca}/IK_{Ca} activation when conduction is initiated from a similar local change in V_m. We therefore compared responses to −2 nA current during NS309 with responses to −1 nA under Control conditions. With local responses (ΔV_m) not different between conditions, the decay in V_m over distance remained significantly greater during SK_{Ca}/IK_{Ca} activation (Fig. 3C; Supplemental Table I).

Along the entire distance (2000 μm) studied, responses decayed by 4.4% per 100 μm. This value is consistent with ~5% per 100 μm reported for the decay of electrical conduction along the endothelium of retinal arterioles.[33] Further, the consistency of such behavior suggests that the biophysical properties of EC tubes determined here can be applied to the endothelium of vessels in other resistance networks. Indeed, λ determined here for electrical conduction (~1.4 mm; Fig. 3) is consistent with control values reported for intact guinea pig arterioles (~1.1 to 1.6 mm)[15] and hamster retractor feed arteries (1.2 mm).[16] A limitation of the present study is the lack of SMCs, which precludes resolution of how SK_{Ca}/IK_{Ca} activation may influence electrical signaling through myoendothelial GJCs. Nevertheless, even with SMCs present in earlier studies,[15, 16, 33] the similarity in values for λ across preparations and laboratories supports the role of the endothelium as the primary cellular pathway for axial current flow. In turn, minimal charge loss through myoendothelial GJCs is explained by the high input resistance of SMCs.[8]

Tuning electrical conduction independent of GJC regulation

Electrical conduction along the endothelium is promoted by high r_m preventing signal dissipation and low r_a through GJCs between neighboring cells. Whereas GJCs have been a focus for regulating vascular conduction,[5, 17–21] the present study demonstrates that electrical conduction along the endothelium can be progressively inhibited by graded activation of SK_{Ca}/IK_{Ca} (Fig. 4). This interpretation is consistent with the robust expression and ionic conductance of both $K_{Ca}2.3$ (SK_{Ca}) and $K_{Ca}3.1$ (IK_{Ca}) channels estimated from patch clamp studies of native ECs freshly isolated from mouse aortae[23] and the established role of SK_{Ca}/IK_{Ca} in governing EC hyperpolarization.[9, 22, 25]. From a typical resting V_m of ~−25 mV, incremental activation of SK_{Ca}/IK_{Ca} with NS309 approached E_K and coincided with loss of conduction (Fig. 4A). In a reciprocal manner, blocking SK_{Ca}/IK_{Ca} with Ap + ChTX depolarized EC tubes by ~10 mV with a ~30% increase in CA (500 μm; Fig. 8). This modest effect of SK_{Ca}/IK_{Ca} inhibition compared to SK_{Ca}/IK_{Ca} activation on electrical conduction can be explained by a low percentage of the total SK_{Ca}/IK_{Ca} in EC membranes being open under Control conditions.

The SK_{Ca}/IK_{Ca} opener SKA-31 had less potency and reduced efficacy compared to NS309 (Figs. 4A, 4D). Nevertheless, the apparent threshold of channel activation required to significantly reduce CA corresponded to a V_m of ~−40 mV for both agents (0.3 μmol/L NS309, 3 μmol/L SKA-31; Fig. 4). Hyperpolarization may also promote the production of NO[31, 32] which may in turn alter r_m[34] or cell-to-cell coupling through acting on GJCs.[35] Nevertheless, the inhibition of electrical conduction by NS309 was maintained when hyperpolarization was prevented (Fig. 6) and when NO synthesis was inhibited

(Supplemental Fig. II). Importantly, and similar to the direct activation of SK_{Ca}/IK_{Ca} by NS309 and SKA-31, CA (500 μm) was reduced ~70% during indirect activation of these channels by ACh (Fig. 7). Further, as seen with NS309, preventing hyperpolarization to ACh (Supplemental Fig. I) or inhibiting NO synthesis (Supplemental Fig. II) did not alter the ability of ACh to suppress electrical conduction. Thus the modulation of r_m (and tuning of electrical conduction) along the endothelium may reflect a more generalized response to physiological agonists that signal through G protein-coupled receptors[36] irrespective of NO.

The present findings support the hypothesis that opening ion channels in the plasma membrane impairs electrical conduction along the endothelium. As dye transfer remained intact during exposure to NS309 when conduction was abolished (Fig. 4), loss of conduction cannot be attributed to closure of GJCs. In turn, despite the lack of effect for levcromakalim or NS1619, we infer that the activation or inhibition of other ion channels that are functionally expressed in membranes of electrically coupled cells can have effects consistent with those shown here for SK_{Ca}/IK_{Ca}. Consistent with this inference are recent findings from rat mesenteric arteries, where spreading vasodilation in response to ACh (or isoproterenol) was enhanced when K^+ channels (BK_{Ca} and K_v) were inhibited in SMCs that were electrically coupled to ECs.[37] Hyperpolarization can activate other K^+ channels (e.g., inward rectifying, K_{IR}) to increase V_m and enhance electrical conduction.[11, 38] Nevertheless, it is unlikely that such a role would be manifest in EC tubes given the linearity of the I–V relationship (Fig. 2) and a resting V_m (~−25mV) above that associated with the negative slope conductance of K_{IR}.[25] Further, given the same decrease in CA to NS309 or ACh when hyperpolarization was prevented (Figs. 6D and Supplemental Fig. I, respectively), it appears unlikely that that activation of K_{IR} contributes to electrical conduction along the endothelium. However, this conclusion does not preclude a role for K_{IR} expressed in SMCs to contribute to conducted vasodilation along intact vessels.[11, 38]

Summary and conclusions

Electrical signaling underlies the correspondence between changes in V_m and diameter along the resistance vasculature.[6, 7, 10] Previous studies have focused on the role of intercellular coupling through GJCs in determining the efficacy of electrical conduction,[1–3, 8] particularly as it applies to conducted vasodilation along arterioles and ascending vasodilation during exercise.[2,14] Paradigms for studying conducted responses have typically involved stimulating a vessel segment (or the tissue in which vessels are embedded) while monitoring vasomotor responses at sites remote from the region of stimulation.[14, 18, 19, 21, 37, 39, 40] However there is ambiguity in defining the number of cells activated, the precise stimulus intensity at the signal origin, and the specific role(s) of respective cell layers. In the present study, the endothelium of resistance arteries was isolated as an intact tube to eliminate the influence of blood flow or surrounding cells and tissue. Intracellular microelectrodes enabled electrical conduction to be initiated from a point source (single cell) within the electrical syncytium using prescribed current pulses while monitoring V_m at defined distances. In turn, selective activation (or inhibition) of SK_{Ca}/IK_{Ca} along the endothelium resolved how electrical conduction can be tuned through changes in membrane resistance while individual cells remain coupled to each other through GJCs.

This study presents two novel findings. First, that electrical conduction can be finely tuned through the activity of voltage-insensitive membrane ion channels. Thus our experiments are the first to demonstrate an integral role for SK_{Ca}/IK_{Ca} activation in governing EC signaling that is distinct from their established role of initiating EC hyperpolarization[9, 24] (Fig. 9). Second, that SK_{Ca}/IK_{Ca} can define the spatial domain of electrical signaling along the endothelium (Fig. 9). The SK_{Ca}/IK_{Ca} have gained recognition as therapeutic targets in light of their ability to initiate hyperpolarization and promote vasodilation to increase tissue blood

flow.[24, 41, 42] The present findings imply that selectively targeting ion channels for therapeutic intervention should account for integrated effects throughout resistance networks as well as local effects on vasomotor tone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations and Acronyms

Ap	apamin
ACh	acetylcholine
BK_{Ca}	large conductance Ca^{2+} activated K^+ channels
CA	conduction amplitude
ChTX	charybdotoxin
EC	endothelial cell
SK_{Ca}/IK_{Ca}	small- and intermediate-conductance Ca^{2+} activated K^+ channels
K_{ATP}	ATP-regulated K^+ channels
K_{IR}	inward-rectifying K^+ channels
K_v	voltage-gated K^+ channels
NO	nitric oxide
NS1619	1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one
NS309	6,7-dichloro-1H-indole-2,3-dione 3-oxime
SMC	smooth muscle cell
SKA-31	naptho[1,2-*d*]thiazol-2-ylamine
V_m	membrane potential

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Novelty and Significance

What is known?

- The endothelium conducts electrical signals along resistance vessels to coordinate relaxation of smooth muscle cells.
- Gap junctions provide a low-resistance pathway for current to flow between endothelial cells.
- Small- and intermediate- Ca^{2+}-activated K^+ channels (SK_{Ca}/IK_{Ca}) are highly expressed in endothelial cells and initiate hyperpolarization.

What new information does this article contribute?

- Electrical conduction of hyperpolarization and depolarization along the endothelium produce equivalent (but opposite) changes in membrane potential.
- Irrespective of gap junctions or charge polarity, opening voltage-insensitive ion channels dissipates electrical signals along the endothelium.
- Activation of SK_{Ca}/IK_{Ca} channels effectively “tunes” the ability of the endothelium to conduct electrical signals.

The endothelium is recognized as the principal pathway for initiating vasodilation through SK_{Ca}/IK_{Ca} activation and for the conduction of hyperpolarization in resistance networks. In regulating conducted vasodilation, attention has focused on the role and manipulation of intercellular gap junctions to alter the resistance to current flow between cells. In contrast, we studied whether altering the electrical resistance of endothelial plasma membranes via activation or blockade of ion channels can regulate electrical conduction.. Our new findings demonstrate that, irrespective of charge polarity, electrical conduction along the endothelium is impaired during SK_{Ca}/IK_{Ca} activation and is enhanced by SK_{Ca}/IK_{Ca} blockade. At the same time, neighboring cells remain well coupled to each other through gap junctions. We suggest that impaired tissue blood flow during endothelial dysfunction may reflect enhanced ion channel activation (i.e. “leaky” plasma membranes) that impairs coordinated control of vascular resistance. Therapeutic interventions designed to improve tissue blood flow by altering ion channel activation should take into account the effects on electrical conduction in resistance networks.

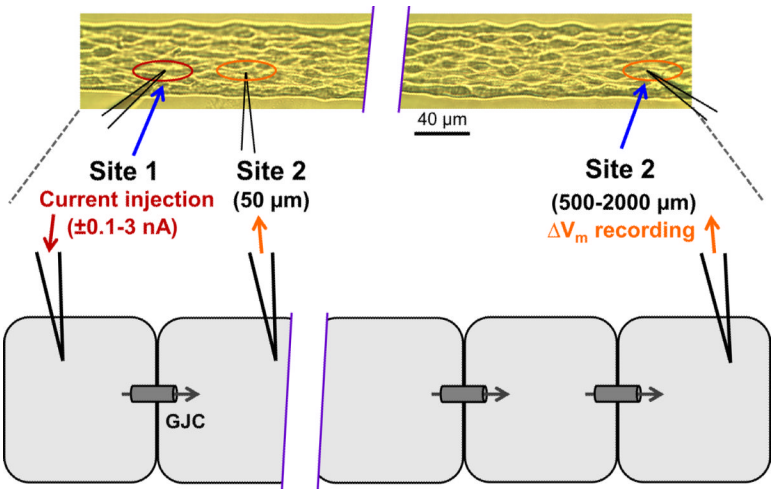

Figure 1. Experimental design

Inset at top: dual intracellular electrodes positioned in an EC tube (Differential Interference Contrast image). Site 1 and Site 2 shown within ovals correspond to cartoon below. Current (±0.1 – 3.0 nA, 2s pulses) was injected at Site 1 while V_m was recorded with separation distances between microelectrodes of 50–2000 µm. To determine the role of SK_{Ca}/IK_{Ca} in tuning electrical conduction, SK_{Ca}/IK_{Ca} on all cells were activated (NS309, SKA-31, ACh) or inhibited (apamin + charybdotoxin) by addition of respective agents to the superfusion solution. With no change in axial resistance ($\mathbf{r}_a$) to current flow through gap junction channels (GJC), changes in conduction amplitude (CA; ΔV_m at Site 2/current injected at Site 1; mV/nA), reflect changes in membrane resistance ($\mathbf{r}_m$) associated with activation or inhibition of SK_{Ca}/IK_{Ca}. Data in Figs. 3, 4, 6–8 reflect CA responses to −1 nA current injection (note that $-\Delta V_m/-1$ nA = positive CA value). The Fraction of Control CA (Figs. 4C, F and 8C) was defined as CA during treatment/CA under control conditions at the same 500 µm site. Conduction Efficiency (Fig. 3B) was defined as (CA at X µm) / (CA at 50 µm), where X= 50–2000 µm.

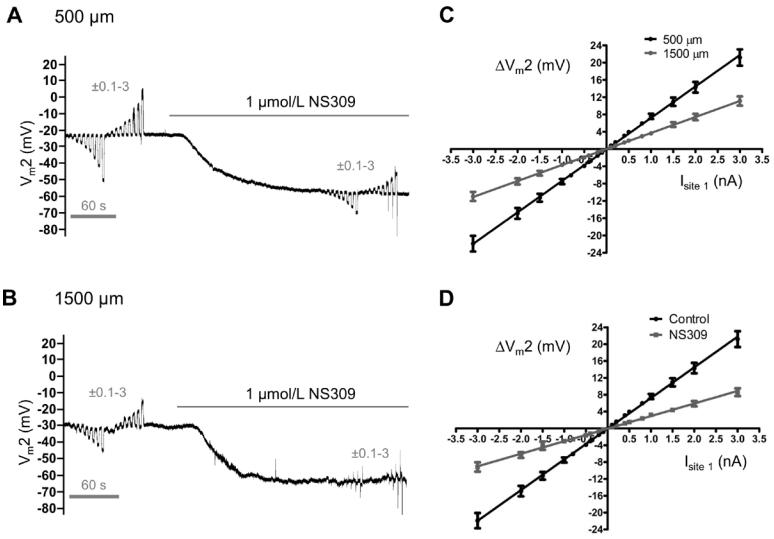

Figure 2. Effect of distance and SK_{Ca}/IK_{Ca} activation on electrical conduction

At defined separation distances between intracellular microelectrodes, continuous (paired) recordings at Site 2 during current microinjection at Site 1 were obtained under Control conditions and during NS309 treatment to activate SK_{Ca}/IK_{Ca}. After each set of paired recordings, washout of NS309 (10–15 min) restored Control V_m before recording at the next distance. **A,** Representative V_m recording at Site 2 during ±0.1–3 nA microinjected at Site 1 (distance = 500 μm) before and during NS309. From Control V_m of ~−25 mV, NS309 hyperpolarized ECs to ~−60 mV and decreased V_m2 responses. **B,** As in A with separation distance = 1500 μm. Note reduction in Control V_m2 responses compared to **A** followed by loss of V_m2 responses during NS309. **C,** Summary data illustrating the effect of microelectrode separation distance on the change in V_m at Site 2 (ΔV_m2) under Control conditions with 500 μm (black line) or 1500 μm (grey line) separation. With reduced slope at greater distance, ΔV_m2 responses remained linear through the full range of current injected ($R^2 = 1$). With −1 nA current, absolute ΔV_m2 decreased from 7.7 ± 0.6 mV at 500 μm to 3.8 ± 0.3 mV at 1500 μm. **D,** With separation maintained at 500 μm for the same EC tubes in **C**, activation of SK_{Ca}/IK_{Ca} with NS309 (1 μmol/L) reduced CA to 3.1 ± 0.3 mV. Summary data in C and D are means ± S.E.; n = 11.

Note: Panels C and D include (with permission: British Journal of Pharmacology © 2011) control data from 8 experiments presented in Figure 2B,C of Behringer et al.[30]

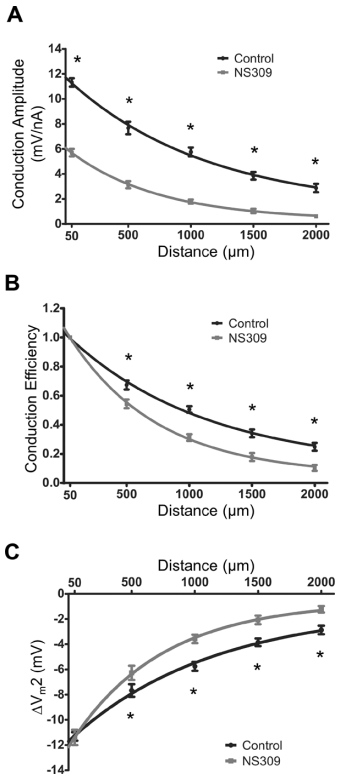

Figure 3. Effect of SK_{Ca}/IK_{Ca} activation on spatial decay of electrical conduction

Summary data (means ± S.E.) illustrating electrical conduction versus distance between intracellular microelectrodes before and during treatment with NS309 (1 μmol/L). At each distance, continuous (paired) recordings were obtained under Control conditions and during NS309. **A,** For Conduction Amplitude (−1 nA microinjected at Site 1), NS309 reduced the local response by half and λ by ~40% (Control: 1380±80 μm; NS309: 850±60 μm). **B,** Conduction Efficiency = data from **A** normalized to respective values at 50 μm before and during NS309; note greater decay with NS309. **C,** With current microinjection adjusted to produce the same local ΔV_m (Control: −1 nA; NS309: −2 nA), the $\Delta V_m 2$ (= resting V_m - peak response V_m) with distance indicates greater decay of hyperpolarization with NS309. For these experiments, n = 11 at 50 – 1,500 μm; n=7 at 2,000 μm). *Control significantly different from NS309, $P < 0.05$

Note: Panel A includes (with permission: British Journal of Pharmacology © 2011) control data from 8 experiments presented in Figure 2B,C of Behringer et al.[30]

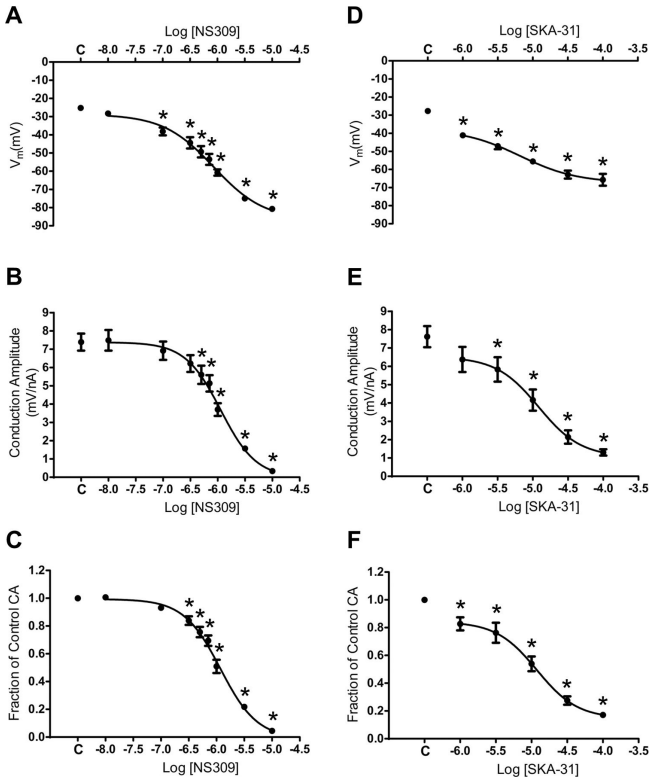

Figure 4. Effect of progressive activation of SK_{Ca}/IK_{Ca} on electrical conduction

Summary data (means ± S.E.) for continuous recordings at Site 2 located 500 μm from current microinjection (−1 nA) at Site 1. **A,** resting V_m with increasing [NS309]; C indicates Control. **B,** Conduction Amplitude as a function of [NS309]; C indicates Control. **C,** As in **B** indicating effect of SK_{Ca}/IK_{Ca} activation with NS309 relative to Control (Fraction of Control CA = CA during respective [NS309] / Control CA). **D,** as in A for SKA-31. **E,** as in **B** for SKA-31. **F,** as in **C** for SKA-31. *Significantly different from Control, $P < 0.05$, n = 6–8 in each panel. Note reduced potency and efficacy for SKA-31 versus NS309.

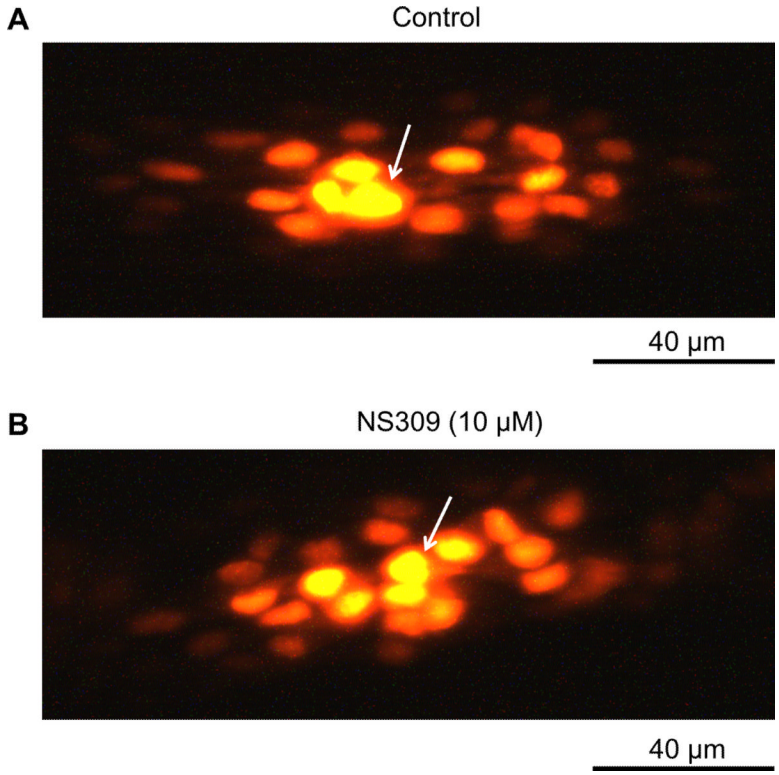

Figure 5. Intercellular dye transfer through GJCs is maintained during SK_{Ca}/IK_{Ca} activation with NS309

Propidium iodide dye (0.1% in 2M KCl) was included in microelectrode during intracellular recording to evaluate intercellular coupling through gap junction channels. Arrows indicate impaled cell in each panel and recordings lasted 30 min. **A,** Dye transfer during Control conditions. **B,** As in **A** with NS309 (10 μmol/L) introduced 5 min prior to cell penetration. With 500 μm separation between microelectrodes, cells hyperpolarized to −81 ± 1 mV and electrical conduction was abolished (Figs. 4A–4C) yet robust dye transfer to surrounding cells was maintained. Images are representative of n=3 experiments.

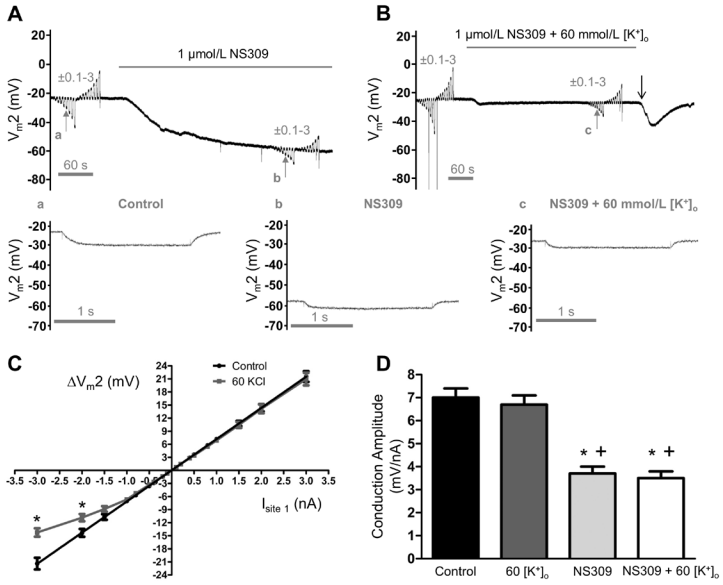

Figure 6. Impaired electrical conduction during SK_{Ca}/IK_{Ca} activation is independent of hyperpolarization

Continuous (paired) recordings of V_m at Site 2 (V_m2) located 500 μm from current microinjections at Site 1. **A,** V_m2 during ±0.1–3 nA before and during SK_{Ca}/IK_{Ca} activation with NS309 (1 μmol/L). During NS309, note hyperpolarization (to ~−60 mV) and decrease in V_m2 responses. **B,** As in **A** before and during treatment with NS309 + 60 mmol/L $[K^+]_o$ to prevent hyperpolarization to NS309. Note decrease in V_m2 responses during NS309 similar to findings in **A**. Transient hyperpolarization near end of recording ("↓") attributable to slower washout of NS309 versus KCl. **a**, **b** and **c** correspond to small vertical arrows in **A** and **B** showing individual recordings of V_m2 during −1 nA injected at Site 1 for (**a**) control (ΔV_m = −6 mV), (**b**) NS309 (ΔV_m = −3 mV), and (**c**) NS309 + 60 mmol/L $[K^+]_o$ (ΔV_m = −3 mV; note lack of hyperpolarization to NS309 with reduced hyperpolarization to −1 nA current). **C,** Summary data for ΔV_m2 responses to full range of current injection (± 0.1–3 nA) during Control and during 60 mmol/L $[K^+]_o$. Note deviation from linearity for ΔV 2 responses to >−1.5 nA during 60 mmol/L $[K^+]_o$. *Significantly different from Control, $P < 0.05$. **D,** Summary data (means ± S.E.) for CA at Site 2 to −1 nA current injection at Site 1 during Control, during 60 mmol/L $[K^+]_o$, during NS309 (1 μmol/L), and during NS309 (1 μmol/L) + 60 mmol/L $[K^+]_o$. *Significantly different from Control ($P < 0.05$). +Significantly different from 60 mmol/L $[K^+]_o$, $P < 0.05$. Summary data (means ± S.E.) in **C** and **D** are for n = 7.

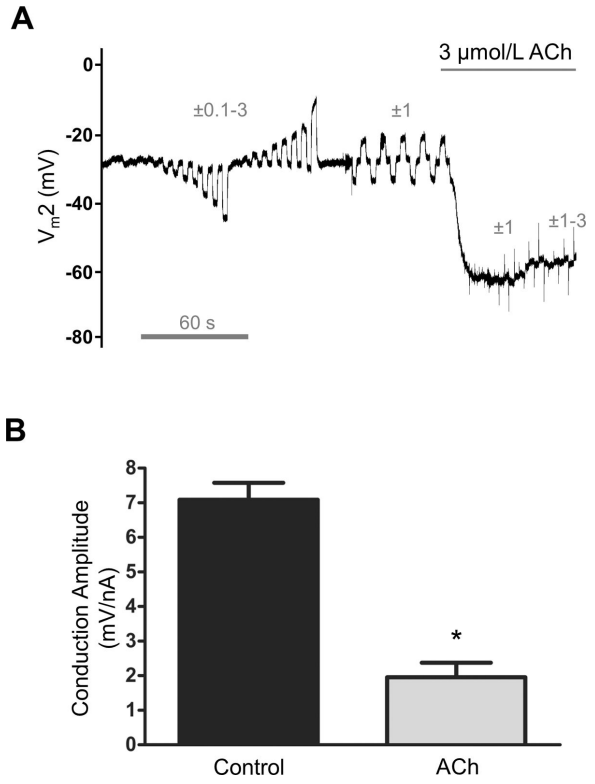

Figure 7. Acetylcholine impairs electrical conduction

Data are from continuous (paired) recordings of V_m at Site 2 located 500 µm from current microinjection (−1 nA). **A,** Representative recording illustrating responses to ±0.1–3 nA before and to ±1, 2, and 3 nA during ACh (3 µmol/L). The V_m during ACh was −67 ± 5 mV (n=6). **B,** Summary data (means ± S.E.; n=6) for Conduction Amplitude (CA). Note decrease in CA during ACh. Hyperpolarization to −2 nA during ACh (−3.9 ± 0.8 mV, n=6) was significantly less ($P < 0.05$) than hyperpolarization to −1 nA under Control conditions (−7.1 ± 0.5 mV, n=6). *Significantly different from Control, $P < 0.05$. Following washout of ACh, CA returned to 7.2 ± 0.5 mV/nA (n=6).

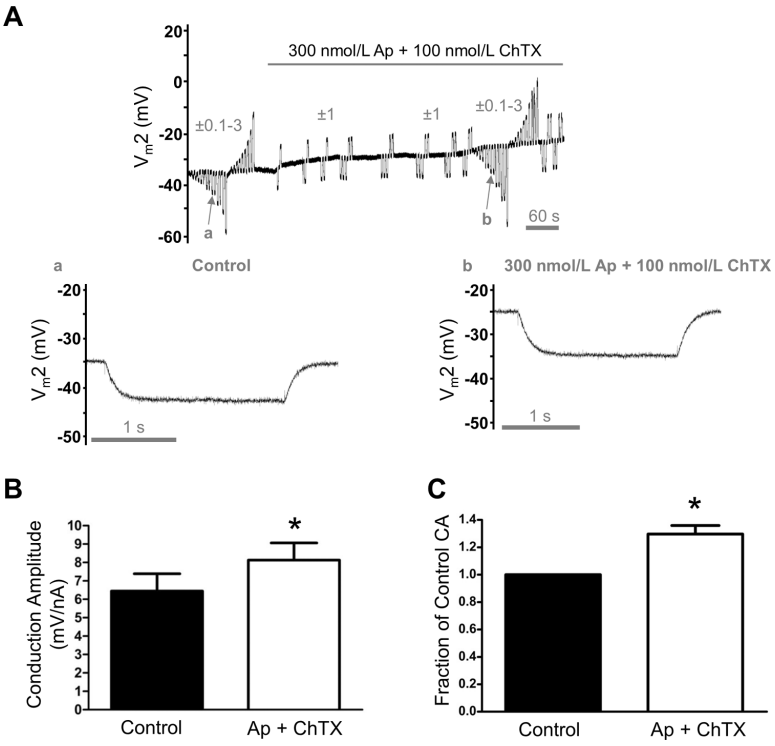

Figure 8. Enhanced electrical conduction during SK_{Ca}/IK_{Ca} blockade

All data are from continuous (paired) recordings 500 μm from the site of current microinjection. **A,** Representative recording indicating V_m2 responses to ±0.1–3 nA microinjections before and during SK_{Ca}/IK_{Ca} block with Ap (300 nmol/L) + ChTX (100 nmol/L). Note depolarization (~10 mV) and increases in V_m2 responses during Ap + ChTX. **a** and **b** correspond to arrows in **A** and illustrate V_m2 responses to −1 nA injected at Site 1 during Control and subsequent exposure to Ap + ChTX: ΔV_m2 was −8 mV in **a** and −10 mV in **b**. Records are from EC tube with resting V_m = −34 mV to illustrate depolarization and increased CA associated with blocking SK_{Ca}/IK_{Ca}. **B,** Summary data (means ± S.E.) for Control and with Ap + ChTX. **C,** Data from **B** expressed as Fraction of Control Conduction Amplitude (= CA during Ap + ChTX /Control CA) to illustrate relative effect of SK_{Ca}/IK_{Ca} inhibition. *Significantly different from Control, $P < 0.05$ (n = 6).

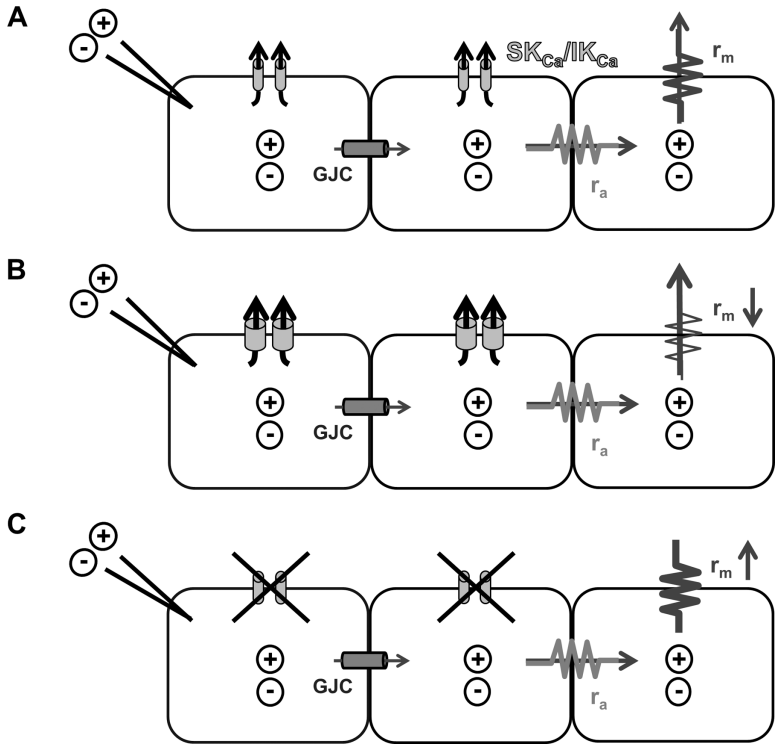

Figure 9. Role for SK_{Ca}/IK_{Ca} in tuning electrical conduction along EC tubes

Cartoon illustrating biophysical determinants of electrical signaling along neighboring cells in an EC tube. **A,** Electrical current (+/-) microinjected into one cell spreads to neighboring cells through gap junction channels (GJC). **B,** Activation of SK_{Ca}/IK_{Ca} (e.g., with NS309, SKA-31 or ACh; Figs. 3, 4, 7) increases current leak through the plasma membrane (i.e., $\mathbf{r}_m$ decreases), thus less current is available to spread to neighboring cells through GJC. **C,** Blocking SK_{Ca}/IK_{Ca} (e.g., with apamin + charybdotoxin; Fig. 8) reduces current leak across the plasma membrane (i.e., $\mathbf{r}_m$ increases) thus more current is available to spread to neighboring cells through GJC. Axial resistance to current flow between cells through GJC ($\mathbf{r}_a$) is assumed to remain constant. With respective agents superfused over the entire EC tube, SK_{Ca}/IK_{Ca} channels were either opened (e.g. via NS309; **B**) or closed (Ap + ChTX; **C**) along the entire EC tube.