Tuning Electrical Conduction Along Endothelial Tubes of Resistance Arteries Through Ca\(^{2+}\)-Activated K\(^{+}\) Channels

Erik J. Behringer, Steven S. Segal

**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 \(\mu\)m wide; 1–3 mm long; cell length, \(\approx\)35 \(\mu\)m) 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 \(\mu\)m). SK\(_{Ca}/IK_{Ca}\) activation (NS309, 1 \(\mu\)mol/L) reduced the change in \(V_m\) along endothelial cell tubes by \(\approx\)50\% and shortened the electrical length constant (\(\lambda\)) from 1380 to 850 \(\mu\)m (\(P<0.05\)) 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 mmol/L [K\(^{+}\)]\(_o\). Conversely, blocking SK\(_{Ca}/IK_{Ca}\) (apamin + charybdotoxin) depolarized cells by \(\approx\)10 mV and enhanced electrical conduction (ie, changes in \(V_m\)) by \(\approx\)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. (Circ Res. 2012;110:1311-1321.)

**Key Words:** conducted vasodilation ■ hyperpolarization ■ gap junctions ■ potassium channels ■ ion channels ■ membrane potential ■ conduction ■ vascular endothelium

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.\(^5\)-\(^7\)–\(^9\) 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.\(^8\)-\(^11\) 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 (KCa 2.3; KCNN3)- and intermediate (KCa 3.1; KCNN4)-conductance Ca2+-activated K+ channels (SKCa/IKCa) are abundantly expressed in EC membranes.22–24 On activation of SKCa/IKCa the efflux of K+ promotes hyperpolarization.22,23 Although this response may promote vasodilation,24 a reduction in rm along the endothelium could dissipate electrical signals and thereby compromise electrical conduction and impair ascending vasodilation. Given the prominent role of SKCa/IKCa in the initiation of EC hyperpolarization,22,24 we questioned whether activation of these ion channels affects electrical conduction along the endothelium.

Understanding how the activation of SKCa/IKCa 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., acetylcholine [ACh])4,10,26 or electric field stimulation27,28 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 (Vm) independent of agonists or ion channel activation. By simultaneously recording Vm at defined distances from the site of current injection, we tested the hypothesis that SKCa/IKCa activity can “tune” electrical conduction along the endothelium.

Findings reported in the present study are the first to show that activating SKCa/IKCa 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 SKCa/IKCa enhances electrical conduction. Thus, alter-

---

**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 illustration below. Current (±0.1–3.0 nA, 2-second pulses) was injected at site 1 while Vm was recorded with separation distances between microelectrodes of 50 to 2000 μm. To determine the role of SKCa/IKCa in tuning electrical conduction, SKCa/IKCa 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 (rm) to current flow through gap junction channels (GJC), changes in conduction amplitude (CA; ΔVm) at 2 current injected at site 1; mV/nA) reflect changes in membrane resistance (rm) associated with activation or inhibition of SKCa/IKCa. Data in Figures 3, 4, 6, 7, and 8 reflect CA responses to −1 nA current injection (note that −ΔVm/−1 nA = positive CA value). The fraction of control CA (Figure 4C, 4F, and 8C) was defined as CA during treatment/CA under control conditions at the same 500 μm site. Conduction efficiency (Figure 3B) was defined as (CA at X μm)/CA at 50 μm), where X = 50 to 2000 μm.
ing \( r_m \) via opening and closing plasma membrane ion channels effectively tunes the conduction of electrical signals along the endothelium.

**Methods**

An expanded Methods section is available in the Online Data Supplement.

**Tissue Preparation and Intracellular Recording**

Procedures were approved by the Institutional Animal Care and Use Committee with accord in the National Research Council’s Guide for the Care and Use of Laboratory Animals (8th edition, 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 re-separated (papaverine; 60 mg/kg) before current injections. Successful dual impalements were indicated by correspondence of electrical events between microelectrodes. With separation maintained at 50-2000 \( \mu \)m (Figures 4, 5, 6, 7, and 8) \( \Delta V_m \) remained linear through the full range of current injected (RF = 1). With \( \sim \) 1 nA, absolute \( \Delta V_m \) decreased from 7.7 \( \pm \) 0.6 mV at 500 \( \mu \)m to 3.8 \( \pm \) 0.3 mV at 1500 \( \mu \)m. With separation maintained at 500 \( \mu \)m for the same EC tubes in C, activation of \( \text{SK}_{Ca}/\text{IK}_{Ca} \) with NS309 (1 \( \mu \)mol/L) reduced CA to 3.1 \( \pm \) 0.3 mV. Summary data in C and D are mean \( \pm \) SE; \( n = 11 \). Note: C and D include (with permission: *British Journal of Pharmacology*, 2011) control data from 8 experiments presented in Figure 2B and 2C of Behringer et al. **

**Data Analyses**

One EC tube was studied per mouse. Analyses included resting \( V_m \) (mV) under control conditions; change in \( V_m \) (\( \Delta \) mV) = peak response \( V_m \) minus 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 mean±SE. Values for n refer to the number of EC tubes studied under respective conditions.

Results

Effects of SKCa/IKCa Activation on Electrical Conduction Over Distance

Resting Vm was −26±2 mV at sites 1 and 2 independent of separation distance (n=11). As shown in Figure 2, ΔVm at site 2 increased in direct proportion to current injected irrespective of polarity. The amplitude of ΔVm for each current decreased when separation distance between microelectrodes was increased from 500 to 1500 μm (Figure 2A through 2C), yet the I-V relationship remained linear (Figure 2C). At 500-μm separation, the slope of the I-V relationship through ±0.1 to 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 Vm (−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 SKCa/IKCa with 1 μmol/L NS309 reduced CA (Figure 2A and 2B), yet the I-V relationship remained linear (Figure 2D). Thus, NS309 decreased CA at 500 μm to nearly the same extent as did increasing distance to 1500 μm under control conditions (Figure 2C). After continuous paired recordings (control, NS309) at each distance, washout of NS309 (10–15 minutes) restored control Vm and CA (eg, 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 SKCa/IKCa activation. The next separation distance was evaluated in the same manner.

During current injections, NS309 (1 μmol/L) reduced ΔVm at the “local” site by half and decreased λ by ~40% (control: 1380±80 μm; NS309: 850±60 μm; Figure 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; Figure 3B). The injection current required for the same local change in Vm obtained with −1 nA under control conditions increased to −2 nA with 1 μmol/L NS309, consistent with a corresponding decrease in rm. When local hyperpolarization was thereby matched between microelectrodes,7,30 the I-V relationship remained linear (Figure 2D). Thus, activating SKCa/IKCa impaired electrical conduction irrespective of ΔVm at the site of current injection.

For the following experiments, current was injected at site 1 while Vm 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 SKCa/IKCa

A question central to these experiments was: Can electrical conduction be “tuned” according to the level of SKCa/IKCa activation? Current was injected at site 1 and Vm recorded at site 2 (500 μm) before and during increments in SKCa/IKCa activation with recovery between each treatment. Hyperpolarization began at 100 nmol/L NS309 and increased with [NS309] (Figure 4A; P<0.05); maximal response at

Figure 3. Effect of SKCa/IKCa activation on spatial decay of electrical conduction. Summary data (mean±SE) 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 injection adjusted to produce the same local ΔVm (control: −1 nA; NS309: −2 nA), the ΔVm2 (=resting Vm minus peak response Vm) with distance indicates greater decay of hyperpolarization with NS309. For these experiments, n=11 at 50 to 1500 μm; n=7 at 2000 μm. *Control significantly different from NS309, P<0.05. Note: A includes (with permission: British Journal of Pharmacology, 2011) control data from 8 experiments presented in Figure 2B and 2C of Behringer et al.30

1154 Circulation Research May 11, 2012

Downloaded from http://circres.ahajournals.org/ by guest on July 10, 2017
10 μmol/L corresponded to $V_m = -81 \pm 1$ mV. Impairment of CA ($P<0.05$) began at 300 mmol/L. NS309 and CA decreased as [NS309] increased with conduction abolished at 10 μmol/L (Figure 4B and 4C). At 1 μmol/L, NS309 reduced CA by half (Figure 4B and 4C). In separate experiments, using SKA-31 to activate $\text{SKCa}/\text{IKCa}^{b}$ produced effects similar to NS309 though with attenuated potency and efficacy (compare Figure 4D through 4F with Figure 4A through 4C). Nevertheless, for both pharmacological agents, a “threshold” $V_m$ associated with significant reduction in CA occurred at $-40$ mV (Figure 4A and 4D). These findings indicate that the efficacy of electrical conduction along the endothelium can be tuned according to the level of $\text{SKCa}/\text{IKCa}$ activation.

**Maintenance of Dye Transfer During Inhibition of Electrical Conduction**

Impairment of electrical conduction during $\text{SKCa}/\text{IKCa}$ 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 minutes before and throughout recordings. With 0.1% propidium iodide dye (MW = 668.4 Da) in the microelectrode, fluorescent images were acquired after 30 minutes of continuous recording. Under control conditions (Figure 5A) and during hyperpolarization (to $-81 \pm 4$ mV) with inhibition of electrical conduction (CA $500 \mu$m: 0.3 ± 0.1 mV/nA), dye microinjected into one EC readily spread to neighboring ECs (Figure 5B; n = 3). Thus, dye transfer during maximal activation of $\text{SKCa}/\text{IKCa}$ 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 $\gamma$-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 $\text{SKCa}/\text{IKCa}$ consistently hyperpolarized ECs, raising the question: Does loss of electrical conduction during $\text{SKCa}/\text{IKCa}$ activation depend on hyperpolarization? Accord-
Enhanced Electrical Conduction With SKCa/IKCa Blockade
Progressive activation of SKCa/IKCa impaired electrical conduction in a graded manner (Figure 4). Thus, if a subpopulation of SKCa/IKCa was open at rest, then 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 SKCa/IKCa 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; Figure 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 (R²=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 Online Table II.

Negligible Role for KATP or BKCa Channels
If manipulating SKCa/IKCa activity alters electrical conduction, then complementary effects would be expected for other K⁺ channels. We tested for such an effect of ATP-regulated K⁺ channels (KATP) using levcromakalim (10 μmol/L; n=5). However, this K⁺ opener 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 Ca²⁺- (and voltage)-sensitive K⁺ channel (BKCa) using the activator NS161922,25 (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 KATP nor BKCa are functionally expressed in EC tubes.

Negligible Role for Nitric Oxide
Endothelial cell hyperpolarization may also promote nitric oxide (NO) synthesis. We therefore tested whether inhibition of NO synthase altered the effect of SKCa/IKCa activation on electrical conduction. Irrespective of treatment with the inhibitor N⁴-nitro-o-arginine (100 μmol/L), exposure to NS309 (1 μmol/L) or to ACh (3 μmol/L) suppressed CA (500 μm) to the same extent (Online Figure 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 SKCa/IKCa 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, which falls within the linear range throughout our experiments. At a standard reference distance of 500 µm, our values of CA (≈7 mV/µA) are comparable with values (≈7 mV/0.8 nA) calculated for the conduction of hyperpolarization along resistance arteries of hamster skeletal muscle. Moreover, hyperpolarizing and depolarizing signals conducted with similar efficacy along EC tubes as shown by the linear (ie, ohmic) changes in $V_m$ throughout the range of currents injected (±0.1–3 nA) at each distance (Figure 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 $[K^+]_o$ (Figure 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. 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; for example, through voltage-gated K$^+$ channels (Kv) 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 (Figure 3A), comparing absolute values does not resolve changes in the nature of electrical conduction because the local response to current injection (ie, change in $V_m$) is reduced. Expressing responses at each distance relative to the local response provides a normalized index of “conduction efficiency” and

---

**Figure 6.** Impaired electrical conduction during SK$_{Ca}$/IK$_{Ca}$ activation is independent of hyperpolarization. Continuous (paired) recordings of $V_m$ at site 2 ($V_m^2$) located 500 µm from current injections at site 1. A, $V_m^2$ during 0.1 to 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 $V_m^2$ responses. B, As in A, before and during treatment with NS309+60 mmol/L $[K^+]_o$ to prevent hyperpolarization to NS309. Note decrease in $V_m^2$ 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 $V_m$ during −1 nA injected at site 1 for: a, control ($\Delta V_m$=-6 mV); b, NS309 ($\Delta V_m$=-3 mV); and c, NS309+60 mmol/L $[K^+]_o$ ($\Delta V_m$=-3 mV; note lack of hyperpolarization to NS309 with reduced hyperpolarization to −1 nA). C, Summary data for $\Delta V_m$ responses to full range of current injection (±0.1–3 nA) during control and during 60 mmol/L $[K^+]_o$. *Significantly different from control, $P<0.05$. D, Summary data for CA at site 2 to −1 nA injected 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 (mean±SE) in C and D are for n=7.
illustrates significantly greater decay in electrical conduction during SKCa/IKCa activation when compared with control (Figure 3B). However, it is important to evaluate the effect of SKCa/IKCa activation when conduction is initiated from a similar local change in Vm. We therefore compared responses to −2 nA current during NS309 with responses to −1 nA under control conditions. With local responses (ΔVm) not different between conditions, the decay in Vm over distance remained significantly greater during SKCa/IKCa activation (Figure 3C and Online 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. 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 in the present study for electrical conduction (∼1.4 mm; Figure 3) is consistent with control values reported for intact guinea pig arterioles (∼1.1–1.6 mm).15 A limitation of the present study is the lack of SMCs, which precludes resolution of how SKCa/IKCa 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 rm preventing signal dissipation and low rļ through GJCs between neighboring cells. Whereas GJCs have been a focus for regulating vascular conduction,3,17–21 the present study demonstrates that electrical conduction along the endothelium can be progressively inhibited by graded activation of SKCa/IKCa (Figure 4). This interpretation is consistent with the robust expression and ionic conductance of both KCa2.3 (SKCa) and KCa3.1 (IKCa) channels estimated from patch-clamp studies of native ECs freshly isolated from mouse aortae23 and the established role of SKCa/IKCa in governing EC hyperpolarization.22,25 From a typical resting Vm of ≈−25 mV, incremental activation of SKCa/IKCa with NS309 approached Ek and coincided with loss of conduction (Figure 4A). In a reciprocal manner, blocking SKCa/IKCa with Ap+ChTX depolarized EC tubes by ≈10 mV, with a ≈30% increase in CA (500 μm; Figure 8). This modest effect of SKCa/IKCa inhibition compared with SKCa/IKCa activation on electrical conduction can be explained by a low percentage of the total SKCa/IKCa in EC membranes being open under control conditions.

The SKCa/IKCa opener SKA-31 had less potency and reduced efficacy compared with NS309 (Figure 4A and 4D). Nevertheless, the apparent threshold of channel activation required to significantly reduce CA corresponded to a Vm of ≈−40 mV for both agents (0.3 μmol/L NS309, 3 μmol/L SKA-31; Figure 4). Hyperpolarization may also promote the production of NO,31,32 which may in turn alter rm14 or cell-to-cell coupling through acting on GJCs. Nevertheless, the inhibition of electrical conduction by NS309 was maintained when hyperpolarization was prevented (Figure 6) and when NO synthesis was inhibited (Online Figure II). Importantly, and similar to the direct activation of SKCa/IKCa by NS309 and SKA-31, CA (500 μm) was reduced ≈70% during indirect activation of these channels by ACh (Figure 7). Further, as seen with NS309, preventing hyperpolarization to ACh (Online Figure I) or inhibiting NO synthesis (Online Figure II) did not alter the ability of ACh to suppress electrical conduction. Thus, the modulation of rm (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 (Figure 4), loss of conduction cannot be attributed to closure of GJCs. In turn, despite the lack of effect for levromakalim 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 SKCa/IKCa. 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 (BKCa and Kv) were inhibited in SMCs that were electrically coupled to ECs. Hyperpolarization can activate other K⁺ channels (eg, inward rectifying, KIR) to increase Vₑm and enhance electrical conduction. Nevertheless, it is unlikely that such a role would be manifest in EC tubes, given the linearity of the I-V relationship (Figure 2) and a resting Vₑm (≈25 mV) above that associated with the negative slope conductance of KIR. Further, given the same decrease in CA to NS309 or ACh when hyperpolarization was prevented (Figure 6D and Online Figure I, respectively), it appears unlikely that that activation of KIR contributes to electrical conduction along the endothelium. However, this conclusion does not preclude a role for KIR expressed in SMCs to contribute to conducted vasodilation along intact vessels.

**Summary and Conclusions**

Electrical signaling underlies the correspondence between changes in Vₑm and diameter along the resistance vasculature. Previous studies have focused on the role of intercellular coupling through GJCs in determining the efficacy of electrical conduction, particularly as it applies to conducted vasodilation along arterioles and ascending vasodilation during exercise. 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. 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 Vₑm at defined distances. In turn, selective activation (or inhibition) of SKCa/IKCa 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 2 novel findings. First, 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 SKCa/IKCa activation in governing EC signaling that is distinct from their established role of initiating EC hyperpolarization9,24 (Figure 9). Second, SKCa/IKCa can define the spatial domain of electrical signaling along the endothelium (Figure 9). The SKCa/IKCa 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.

Acknowledgments
Dr William F. Jackson provided helpful comments during the initial stages of this study.

Sources of Funding
This work was supported by National Institutes of Health grants R37-HL041026, R01-HL086483, and F32-HL110701.

Disclosures
None.

References
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\(_{Ca}/IK_{Ca}\). 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.
Tuning Electrical Conduction Along Endothelial Tubes of Resistance Arteries Through Ca$^{2+}$-Activated K$^+$ Channels

Erik J. Behringer and Steven S. Segal

_Circ Res._ 2012;110:1311-1321; originally published online April 5, 2012; doi: 10.1161/CIRCRESAHA.111.262592

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/110/10/1311

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2012/04/05/CIRCRESAHA.111.262592.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIALS

for:

CIRCRESAHA/2011/262592/R2

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

Erik J. Behringer, Ph.D.\textsuperscript{1} and Steven S. Segal, Ph.D.\textsuperscript{1,2}

\textsuperscript{1}Medical Pharmacology and Physiology, University of Missouri, Columbia, MO 65212 USA
\textsuperscript{2}Dalton Cardiovascular Research Center, Columbia, MO 65211 USA

This Supplement contains:

Detailed Methods with References
Supplemental Tables I and II
Supplemental Figures I and II
Detailed Methods

Animal care and use.
All procedures were approved by the Animal Care and Use Committee of the University of Missouri and performed in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were housed on a 12:12-h light-dark cycle at ~23 °C with fresh water and food available ad libitum. Experiments were performed on C57BL/6 males bred at the University of Missouri (age, 3–6 months). Each mouse was anesthetized using pentobarbital sodium (60 mg/kg, intraperitoneal injection) and abdominal fur was removed by shaving. Following surgical procedures, the anesthetized mouse was euthanized with an overdose of pentobarbital via cardiac puncture.

Solutions.
Physiological salt solution (control PSS) was used to superfuse EC tubes [(in mmol/L): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 10 Glucose]. During SMC dissociation to produce EC tubes, PSS contained 0.1% Bovine Serum Albumin (USB Corp.; Cleveland, OH; USA). During dissection to prepare EC tubes, CaCl₂ was absent and 0.01 mmol/L sodium nitroprusside (SNP) was added to PSS (dissection PSS) to relax SMCs. During dissociation of SMCs, SNP was replaced with 0.1 mmol/L CaCl₂ (dissociation PSS). All reagents were obtained from Sigma-Aldrich (St. Louis, MO; USA) unless otherwise indicated.

Surgery and microdissection.
A ventral midline incision was made from the sternum to the pubis. While viewing through a stereo microscope (SMZ800, Nikon; Tokyo, Japan), fat and connective tissue superficial to the sternum were removed to expose the proximal ends of abdominal muscle feed arteries (superior epigastric artery) bilaterally. To maintain blood in the lumen and thereby facilitate visualization during dissection, each AFA was ligated along with its adjacent vein using 6-0 silk suture (Ethicon; Somerville, NJ; USA). Abdominal muscles were removed bilaterally and placed in chilled (4 °C) dissection PSS. A muscle was pinned onto transparent silicone rubber (Sylgard 184, Dow Corning; Midland, MI; USA) and the vessel segment (length: ~2 cm) was dissected free from surrounding tissue. Residual blood was flushed from the vessel lumen by cannulating one end with a pipette made from heat-polished borosilicate glass capillaries (G150T-4, Warner Instruments; Hamden, CT; USA) with an outer diameter of 50-80 μm and connected to a static column (height, ~10 cm) of dissection PSS.

Endothelial cell tube isolation and superfusion.
As described,¹,² feed arteries were cut into segments (length, ~3 - 5 mm) and placed into dissection PSS containing 0.62 mg/ml papain, 1.0 mg/ml dithioerythritol, 1.5 mg/ml collagenase then incubated for 30 min at 34 °C. Following partial digestion, the PSS containing enzymes was replaced with dissociation PSS and vessel segments were transferred to a 100 x 15 mm Petri dish and gently triturated to remove SMCs using borosilicate glass capillary tubes [1.0 mm outer diameter (OD)/ 0.58 mm ID; World Precision Instruments (WPI), Sarasota, FL; USA] that were pulled (P-97; Sutter Instruments; Novato, CA; USA) and heat-polished (tip ID: 80-120 μm). Following removal of SMCs (confirmed by visual inspection at 200X magnification), an EC tube was transferred to a tissue chamber (RC-27N, Warner Instruments; Hamden, CT; USA) with an outer diameter of 50-80 μm and connected to a static column (height, ~10 cm) of dissection PSS.
at 4 ml/min with PSS. The temperature of the chamber was regulated using an inline heater (SH-27B, Warner) and heating platform (PH6, Warner) coupled to a temperature controller (TC-344B, Warner). Temperature was increased over 30 min to 32 ºC where intact preparations were studied for up to 4 hr. Pharmacological agents were added to the superfusion solution, thereby exposing the entire EC tube to the treatment. Preliminary experiments ejecting blue dye from micropipettes positioned within the chamber confirmed that superfusion flow was laminar in the axial direction of EC tubes.

**Intracellular recording.** Membrane potential ($V_m$) in EC tubes was recorded with an Axoclamp amplifier (2B; Molecular Devices; Sunnyvale, CA; USA) using microelectrodes pulled (P-97; Sutter) from glass capillary tubes (GC100F-10, Warner) and backfilled with 2 mol/L KCl (tip resistance, ~150 MΩ). For experiments testing dye transfer between cells through gap junctions, microelectrodes were backfilled with 0.1% propidium iodide dissolved in 2 mol/L KCl. An Ag/AgCl pellet was placed in effluent PSS to serve as a reference electrode. The output of the amplifier was connected to an analog-to-digital converter (Digidata 1322A, Molecular Devices; Sunnyvale, CA; USA) with data recorded at 1000 Hz on a Dell personal computer using Axoscope 10.1 software (Molecular Devices). For dual simultaneous intracellular recordings, a second amplifier (IE-210, Warner) was integrated into the data acquisition system. Current ($\pm 0.1$-3 nA, 2 s) was delivered using the Axoclamp electrometer driven by a function generator (CFG253, Tektronix; Beaverton, OR; USA). For current injection, an EC was penetrated at a site located ~150 μm from the downstream (with respect to the direction of PSS superfusion) end of where EC tube was pinned (referred to as Site 1) while recording $V_m$ from an EC at Site 2, which was located at a defined separation distance (50-2000 μm) from Site 1 with reference to a calibrated eyepiece reticle while viewing at 200X magnification. For all experiments (except for length constant determinations), separation distance between microelectrodes was standardized at 500 μm, which corresponds to the distance of ~15 ECs placed end-to-end.

Successful impalements were indicated by sharp negative deflection of $V_m$, stable $V_m$ for >1 min, hyperpolarization ($\geq 20$ mV) to 1 μmol/L NS309 (IKCa/SKCa activator; Tocris; Bristol, UK), recovery to resting $V_m$ after NS309 washout and return to ~0 mV upon withdrawal from the cell. Correspondence between current injection at Site 1 and $V_m$ responses at Site 2 indicated simultaneous intracellular current injection and $V_m$ recording. Following dye microinjection, using a filter set for rhodamine with illumination provided from a 50W Hg lamp, images of propidium iodide fluorescence were acquired using a 40X objective (Nikon Fluor40; NA: 0.75) and focused onto a cooled CCD camera (MicroFire, Optronics; Goleta, CA; USA).

**Pharmacology.** NS309 and SKA-31 (Tocris; Bristol, UK) were used to evoke hyperpolarization in endothelial tubes by direct activation of SKCa/IKCa channels. Alternatively, the physiological agonist acetylcholine (ACh) chloride was used to indirectly activate SKCa/IKCa channels through Gq protein coupled muscarinic receptors. To evaluate the potential effects of nitric oxide synthesis during NS309 or ACh treatment, $N^\omega$-Nitro-L-arginine (L-NNa) was applied during select experiments alone (10 min pre-treatment) and subsequently in combination with either NS309 or ACh. To test for the presence of large-conductance Ca$^{2+}$-activated K+ channels (BKCa) and KATP channels within the endothelium, NS1619 and levocromakalim were applied, respectively. The compounds NS309, SKA-31, NS1619 and levocromakalim were dissolved in DMSO and diluted to final working concentrations in PSS on the day of an experiment. Final concentration of DMSO was < 1%. Vehicle controls with DMSO (≤ 2%) in PSS had no effect on $V_m$ or electrical coupling (n=3). Apamin (Alomone; Jerusalem, Israel) and charybotoxin (Alomone...
Jerusalem, Israel or Anaspec; Fremont, CA) were dissolved and diluted into superfusion PSS in combination to block $SK_{Ca/IK_{Ca}}$ channels.\textsuperscript{13}

Data analysis.

One EC tube was studied per mouse. Analyses included: 1) Resting $V_m$ (mV) under Control conditions; 2) Change in $V_m$ ($\Delta$ mV) = peak response $V_m$ – preceding baseline $V_m$; 3) Conduction Amplitude (CA, mV/nA) = $V_m$ at Site 2 / current injected at Site 1. With linearity of I-V relationships (see Results), -1 nA was used as a standard current for evaluating CA; 4) Fraction of Control CA = CA during treatment / preceding control CA; 5) Conduction Efficiency = CA at each separation distance / CA at 50 µm separation; 6) Length constant ($\lambda$) = distance over which the electrical signal decayed to 37\% \left(\frac{1}{e}\right) of the 'local' value. The shortest separation distance was 50 µm to ensure that both microelectrodes were not in the same EC while recording as close to the signal origin as possible; the length of individual ECs in these freshly-isolated tubes is typically 35-40 µm\textsuperscript{2}. Fraction of control CA was defined as the CA during NS309 or SKA-31 at respective concentrations divided by CA under control conditions. Linear regression ($\Delta V_m$ at Site 2 versus current injection at Site 1), curve fitting (estimates of $\lambda$) were performed using GraphPad Prism (GraphPad Software, Inc.; La Jolla, CA; USA). Statistical analyses included repeated measures Analysis of Variance with Tukey or Bonferroni post-hoc comparisons, linear regression and paired Student's -tests (GraphPad Prism). Differences between treatments were accepted as statistically significant with $P < 0.05$. Summary data are presented as means ± S.E.
References


Supplemental Table I.

<table>
<thead>
<tr>
<th>Distance (µm)</th>
<th>-1 nA</th>
<th>-1 nA</th>
<th>-2 nA</th>
<th>-3 nA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control $\Delta V_{m2}$ (mV)</td>
<td>NS309 $\Delta V_{m2}$ (mV)</td>
<td>NS309 $\Delta V_{m2}$ (mV)</td>
<td>NS309 $\Delta V_{m2}$ (mV)</td>
</tr>
<tr>
<td>50</td>
<td>-11.3 ± 0.3</td>
<td>-5.7 ± 0.3*</td>
<td>-11.4 ± 0.6</td>
<td>-17.1 ±0.9</td>
</tr>
<tr>
<td>500</td>
<td>-7.7 ± 0.5</td>
<td>-3.1 ± 0.3*</td>
<td>-6.3 ± 0.6*</td>
<td>-9.4 ±0.8</td>
</tr>
<tr>
<td>1000</td>
<td>-5.8 ± 0.4</td>
<td>-1.8 ± 0.2*</td>
<td>-3.6 ± 0.3*</td>
<td>-5.4 ±0.5</td>
</tr>
<tr>
<td>1500</td>
<td>-3.9 ± 0.3</td>
<td>-1.0 ± 0.2*</td>
<td>-2.1 ± 0.3*</td>
<td>-3.1 ±0.5</td>
</tr>
<tr>
<td>2000</td>
<td>-2.9 ± 0.3</td>
<td>-0.6 ± 0.1*</td>
<td>-1.2 ± 0.3*</td>
<td>-1.8 ±0.4*</td>
</tr>
</tbody>
</table>

Spatial decay of electrical conduction increases during $\text{SK}_{\text{Ca}}$/IK$_{\text{Ca}}$ activation with 1 µmol/l NS309. The standard current pulse microinjected at Site 1 to evaluate a change in membrane potential at Site 2 ($\Delta V_{m2}$) at distances of 50-2000 µm was -1 nA (Control, Column 2). Treatment with NS309 reduced $\Delta V_{m2}$ to -1 nA at each distance (Column 3). To achieve the same $\Delta V_{m2}$ at 50 µm in the presence of NS309 required twice the current (-2 nA; Column 4); note greater signal loss at 500-2000 µm vs. Control (these data are complementary to Figure 3C). Raising microinjection current to -3 nA during NS309 treatment exceeded Control $\Delta V_{m2}$ responses at 50 and 500 µm while further illustrating enhanced signal dissipation by 2000 µm during $\text{SK}_{\text{Ca}}$/IK$_{\text{Ca}}$ activation. *Significantly less than Control $V_{m2}$ responses to -1 nA for the respective distance indicated, P < 0.05 (n = 11 at 50 - 1,500 µm; n=7 at 2,000 µm).
### Supplemental Table II.

<table>
<thead>
<tr>
<th></th>
<th>-1 nA</th>
<th>-1 nA</th>
<th>-2 nA</th>
<th>-3 nA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Interventions</td>
<td>Treatment</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>ΔV_m2 (mV)</td>
<td>ΔV_m2 (mV)</td>
<td>ΔV_m2 (mV)</td>
<td>ΔV_m2 (mV)</td>
</tr>
<tr>
<td>-7.4 ± 0.5</td>
<td>NS309 (1 µmol/L)</td>
<td>-3.7 ± 0.3*</td>
<td>-7.4 ± 0.7</td>
<td>-11.1 ± 1.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>NS309 (3 µmol/L)</td>
<td>-1.6 ± 0.1*</td>
<td>-3.1 ± 0.2*</td>
<td>-4.7 ± 0.3*</td>
</tr>
<tr>
<td>&quot;</td>
<td>NS309 (10 µmol/L)</td>
<td>-0.3 ± 0.1*</td>
<td>-0.7 ± 0.2*</td>
<td>-1.0 ± 0.2*</td>
</tr>
<tr>
<td>-7.6 ± 0.6</td>
<td>SKA-31 (10 µmol/L)</td>
<td>-4.2 ± 0.6*</td>
<td>-8.3 ± 1.2</td>
<td>-12.5 ± 1.7</td>
</tr>
<tr>
<td>&quot;</td>
<td>SKA-31 (30 µmol/L)</td>
<td>-2.1 ± 0.4*</td>
<td>-4.3 ± 0.7*</td>
<td>-6.4 ± 1.1</td>
</tr>
<tr>
<td>&quot;</td>
<td>SKA-31 (100 µmol/L)</td>
<td>-1.3 ± 0.2*</td>
<td>-2.6 ± 0.3*</td>
<td>-3.9 ± 0.5*</td>
</tr>
<tr>
<td>-7.0 ± 0.4</td>
<td>NS309 (1 µmol/L) + KCl (60 mmol/L)</td>
<td>-3.5 ± 0.3*</td>
<td>-7.1 ± 0.7</td>
<td>-10.6 ± 1.0</td>
</tr>
<tr>
<td>-7.1 ± 0.5</td>
<td>ACh (3 µmol/L)</td>
<td>-2.0 ± 0.4*</td>
<td>-3.9 ± 0.9*</td>
<td>-5.9 ± 1.3</td>
</tr>
</tbody>
</table>

**Effect of SK_{Ca}/IK_{Ca} activation on electrical conduction at a constant reference distance.**

These experiments evaluated the change in V_m from rest (ΔV_m2 = resting V_m - peak response V_m) at a constant distance (500 µm) from the site of current microinjection and correspond to Figures 4, 6 and 7. Note that ΔV_m2 Treatment responses to -1 nA are significantly less (P < 0.05) than ΔV_m2 Control responses during each intervention. During 1 µmol/L NS309 (either alone or with 60 mmol/L KCl) and for 10 µmol/L SKA-31, doubling current to -2 nA increased ΔV_m2 responses to approximate Control values. During 30 µmol/L SKA-31 and 3 µmol/L ACh, tripling current to -3 nA increased ΔV_m2 to approximate Control values but still could not restore ΔV_m2 responses to 10 µmol/L NS309 or 100 µmol/L SKA-31. *Significantly less than Control ΔV_m2 in response to -1 nA, P < 0.05.

Note: During SK_{Ca}/IK_{Ca} blockade with apamin + charybdotoxin, ΔV_m2 responses to -1 nA increased (P < 0.05) from -6.5 ± 0.9 to -8.1 ± 0.9 mV, n=6; see Fig. 8B) and less current (-0.8 nA) was required to evoke ΔV_m2 responses (-6.5 ± 0.7) similar to those evoked by -1 nA under Control conditions.
Supplemental Figure I. Acetylcholine inhibits electrical conduction without hyperpolarization. Data are from continuous $V_m$ recordings at Site 2 located 500 µm from current microinjection. A, Representative recording illustrating responses to ±0.1-3 nA before and during 3 µmol/L ACh + 60 mmol/L [K+]o. Note loss of $V_m$2 responses during 3 µmol/L ACh + 60 mmol/L [K+]o. B, Summary data (means ± S.E.; n=4) for Conduction Amplitude to -1 nA. Plateau $V_m$ during ACh + 60 mmol/L [K+]o was -20 ± 1 mV. *Significantly different from Control, $P < 0.05$. Conduction Amplitude recovered to 7.5 ± 0.5 mV/nA (n=4) upon washout of ACh + 60 mmol/L [K+]o.
Supplemental Figure II. Impaired electrical conduction during SK$_{Ca}$/IK$_{Ca}$ activation is maintained during inhibition of nitric oxide synthase. Data represent continuous (paired) recordings of $V_m$ at 500 µm from site of current microinjection. A, Responses to ±0.1-3 nA before and during 1 µmol/L NS309. Note diminished responses during NS309. B, As in A with NS309 + 100 µmol/L L-N^o-Nitro-L-arginine (L-NNA). C, Summary data for Conduction Amplitude under the initial Control conditions (Control 1), during NS309 alone, following washout of NS309 (Control 2; note reversibility) and during NS309 + L-NNA. The reduction in $V_m$ responses to current injection during NS309 + L-NNA was not different from NS309 alone. *Significantly different from Control, P < 0.05 (n = 4).

In separate experiments (n=3), complementary results were obtained when comparing ACh (3 µM) alone or in combination with 100 µmol/L L-NNA (also paired continuous recordings) as follows: Conduction Amplitude (mV/nA) at 500 µm separation: Control 1, 7.9 ± 1.4; ACh, 1.9 ± 0.4; Control 2, 7.7 ± 0.7; ACh + L-NNA: 2.0 ± 0.3. Across experiments, treatment with 100 µM L-NNA to inhibit nitric oxide synthase began at least 10 min before addition of NS309 or ACh.