Inward rectifying potassium channels facilitate cell-to-cell communication in hamster retractor muscle feed arteries

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Jantzi, Micaela C., Suzanne E. Brett, William F. Jackson, Randolph Corteling, Edward J. Vigmond, and Donald G. Welsh. Inward rectifying potassium channels facilitate cell-to-cell communication in hamster retractor muscle feed arteries. Am J Physiol Heart Circ Physiol 291: H1319–H1328, 2006. First published April 14, 2006; doi:10.1152/ajpheart.00217.2006.—This study examined whether inward rectifying K+ (KIR) channels facilitate cell-to-cell communication along skeletal muscle resistance arteries. With the use of feedback arteries from the hamster retractor muscle, experiments examined whether KIR channels were functionally expressed and whether channel blockade attenuated the conduction of acetylcholine-induced vasodilation, an index of cell-to-cell communication. Consistent with KIR channel expression, this study observed the following: 1) a sustained Ba2+-sensitive, K+–induced dilation in preconstricted arteries; 2) a Ba2+-sensitive inwardly rectifying K+ current in arterial smooth muscle cells; and 3) KIR2.1 and KIR2.2 expression in the smooth muscle layer of these arteries. It was subsequently shown that the discrete application of acetylcholine elicits a vasodilation that conducts with limited decay along the feed artery wall. In the presence of 100 μM Ba2+, the local and conducted response to acetylcholine was attenuated, a finding consistent with a role for KIR in facilitating cell-to-cell communication. A computational model of vascular communication accurately predicted these observations. Control experiments revealed that in contrast to Ba2+, ATP-sensitive- and large-conductance Ca2+-activated-K+ channel inhibitors had no effect on the local or conducted vasodilatory response to acetylcholine. We conclude that smooth muscle KIR channels play a key role in facilitating cell-to-cell communication along skeletal muscle resistance arteries. We attribute this facilitation to the intrinsic property of negative slope conductance, a biophysical feature common to KIR2.1- and 2.2-containing channels, which enables them to increase their activity as a cell hyperpolarizes.

gap junctions; intercellular conductions; K+ channels; skeletal muscle arteries

RESISTANCE ARTERIES dilate or constrict in a coordinated fashion to effectively modulate blood flow to metabolically active tissue (20, 29–31). For resistance arteries to respond in an integrated manner, vascular cells must communicate electrically with one another (5, 6, 33, 34). Direct electrical communication within the vessel wall is governed by gap junctions, intercellular channels that mediate the spread of ions and second messengers between neighboring cells (17, 26, 27). Gap junctions are composed of two homomeric, each containing six connexin (Cx) proteins. The Cx gene family is composed of at least 19 members with the most predominate subtypes being Cx37, Cx40, Cx43, and Cx45 in vascular tissue (9, 10, 16, 17).

Cell-to-cell communication is typically assessed in vascular studies by applying vasoactive agents to a small portion of a resistance artery (30, 32, 33). This discrete application initiates a localized change in smooth muscle or endothelial cell membrane potential that, with the aid of gap junctions, conducts to neighboring vascular cells (5, 6, 33, 34). The extent to which the electrical or the conducted vasomotor response conducts longitudinally reveals the nature of vascular communication. With this approach, microrciculatory investigations (5, 6, 32) have reported a range of vascular behaviors, including the ability of the endothelium to initiate hyperpolarizations that conduct with little or no electronic decay. This absence of decay has fostered the view that there is an ionic conductance within the vessel wall that facilitates the conduction of a hyperpolarizing phenomenon (4, 11).

For facilitation to occur in a resistance artery, a hyperpolarizing response must, in a voltage-dependent manner, augment an outward K+ conductance or inhibit a depolarizing inward current. Whereas a variety of ionic currents are present in resistance arteries (11, 21), one of the few conductances of which the biophysical properties fit the preceding criteria is the inward rectifying K+ (KIR) current. KIR channels are tetrameric structures, which in vascular tissue, are composed of KIR2.1 X subunits, including KIR2.1 and KIR2.2 (1, 13). When expressed in human embryonic kidney cells, these subunits give rise to macroscopic currents of which the outward component paradoxically increases as a cell hyperpolarizes toward the K+ equilibrium potential (EK) (2, 28). This so-called “negative slope conductance” arises from the known ability of intracellular Mg2+-polyamines to block KIR2.1- and KIR2.2-containing channels in a voltage-dependent manner (8, 18).

Given the intrinsic biophysical property of negative slope conductance, this study sought to ascertain whether KIR channels facilitate cell-to-cell communication along skeletal muscle resistance arteries. With the use of a full range of methodologies, initial experiments confirmed the expression of KIR2.1- and KIR2.2-containing channels in the smooth muscle cell layer of hamster retractor muscle feed arteries. Consistent with KIR facilitating cell-to-cell communication, micromolar Ba2+ attenuated the conduction of acetylcholine-induced dilation along the arterial wall. Computational modeling accurately...
predicted this attenuation along with the ability of Ba$_{2+}$ to attenuate the local and direct vasodilatory effects of acetylcholine. Other K$^+$ channel inhibitors did not attenuate conduction, a finding that indicates a unique role for K$_{IR}$ in the facilitation process. We propose that by facilitating cell-to-cell communication, K$_{IR}$ channels could augment the dynamic range of blood flow responses in skeletal muscle.

METHODS

Animal handling procedures were approved by the Animal Care and Use Committees at the University of Calgary or Michigan State University. Briefly, male golden Syrian hamsters (10–12 wk of age) were euthanized with pentobarbital sodium (90 mg/kg ip) or CO$_2$ asphyxiation, and an incision was made through the skin. Retractor muscles with attached feed arteries were rapidly removed and placed in ice-cold (pH = 7.4) phosphate-[whole vessel experiments (19)] or HEPES- [electrophysiology experiments (12)]-buffered solution. Feed arteries were then dissected free from fat cells, connective tissue, and muscle cells.

Vessel myography. An isolated feed artery was placed in an arteriograph and cannulated with glass micropipettes. The arteriograph was then positioned on an inverted microscope where the vessel was slowly pressurized to 60 mmHg (in vivo pressure) and superfused with physiological saline solution (PSS; 5% CO$_2$, 21% O$_2$; 36°C) containing the following (in mM): 119 NaCl, 4.7 KCl, 1.7 KH$_2$PO$_4$, 1.2 MgSO$_4$, 1.6 CaCl$_2$, 5 glucose, and 20 NaHCO$_3$. All vessels developed myogenic tone (response range ~20–40 μm) during the 60-min equilibration period. After equilibration, vessel tone was further increased by 1) elevating intravascular pressure (80 mmHg); or 2) superfusing the preparation with 0.1–1 μM phenylephrine. This was undertaken to strongly depolarize feed arteries and thus enhance the voltage-dependent block of K$_{IR}$ channels by intracellular Mg$_{2+}$ and polyamines (8, 18). Before experimentation, contractile status was ascertained by briefly superfusing the feed arteries with 60 mM KCl and 1 μM acetylcholine. Arterial diameter was monitored by using a ×10 objective and an automated diameter tracking system (IonOptix, Milton, MA).

Preconstricted feed arteries were exposed to one of two experimental protocols. First, to ascertain the functional presence of K$_{IR}$ channels, preconstricted feed arteries were superfused with elevated K$^+$ PSS (15.4 mM) and then exposed to an increasing concentration of Ba$_{2+}$ (1–300 μM). Second, to monitor cell-to-cell communication, a micropipette filled with acetylcholine (200 mM) was positioned with the tip adjacent to the one end of a feed artery (Fig. 1). With the use of iontophoresis (0.25–5.0 s pulse; ejection current 0.5 μA; retain current 0.2 μA), acetylcholine was discretely applied to preconstricted arteries in the absence and presence of Ba$_{2+}$ (30 or 100 μM), glybenclamide (10 μM; an ATP-sensitive K$^+$ (K$_{ATP}$) channel inhibitor (23)) or glybenclamide ± iberiotoxin [50 nM; a large-conductance Ca$^{2+}$-activated K$^+$ (BK) channel inhibitor (21)]. Peak dilatory responses were evaluated 0-, 900-, and 1,800-μm distal to the site of agent application.

Patch-clamp electrophysiology. Feed arterial myocytes were isolated as described previously for hamster arterioles with minor modifications (12). Artery segments were placed in dissociation media (22°C, pH = 7.4) containing (in mM): 140 NaCl, 5 KCl, 1 MgCl$_2$, 0.1 mM CaCl$_2$, 10 HEPES, 10 glucose, 0.1 sodium nitroprusside, 0.1 diltiazem, and 1% albumin for 10 min. Vessels were then exposed to a two-step digestion process that involved the following: 1) a 35-min incubation in dissociation media (37°C) containing 1.5 mg/ml papain and 1 mg/ml dithioerythritol; and 2) a 17- to 19-min incubation in dissociation media (37°C) containing 1.5 mg/ml collagenase, 1 mg/ml elastase, 1 mg/ml hyaluronidase, and 2 mg/ml soybean trypsin inhibitor. After enzyme treatment, arteries were washed three to four times with dissociation media (22°C) and gently triturated. Cells were stored in this solution (22°C) for up to 4 h.

Macroscopic K$^+$ currents were measured by using conventional whole cell patch clamp. Briefly, an aliquot of cells was placed in the recording chamber and allowed to settle before being superfused with a HEPES-buffered solution (22°C, pH = 7.4) containing (in mM) 140 NaCl, 5 KCl, 1 MgCl$_2$, 2 CaCl$_2$, 10 HEPES, and 10 glucose. Fire-polished patch pipettes (tip resistance 2–4 MΩ) were backfilled with solution (pH = 7.7–2.2) containing (in mM) 100 K-aspartate, 43 KCl, 1 MgCl$_2$, 10 HEPES, 10 glucose, and 0.5 EGTA. These pipettes were gently lowered onto a smooth muscle cell, and whole cell electrical access (<15 MΩ) was achieved by applying a sharp pulse.

![Fig. 1](http://ajpheart.physiology.org/)  
Diagram illustrating measurement of cell-to-cell communication. A feed artery is cannulated, and a stimulus pipette containing acetylcholine (0.2 M) is placed close to the vessel wall. Acetylcholine is liberated from the pipette by microiontophoresis (500 nA ejection current, 0.25–5.0 s duration), and arterial diameter is measured every 900 μm along the longitudinal axis of the artery.
of negative pressure. Smooth muscle cells were held at −60 mV with the aid of an Axopatch 200A patch-clamp amplifier coupled to a personal computer running Clampex software. Whole cell currents were filtered at 1 kHz, sampled at 5 kHz, and then normalized to cell capacitance (18 ± 1 pF; n = 20).

To monitor the Ba²⁺-sensitive KIR current, smooth muscle cells were first superfused with solutions containing 100 mM K⁺ (equimolar substitution of KCl for NaCl). Voltage was then stepped to −140 mV for 200 ms and then ramped to +20 mV at a rate of 0.8 mV/ms. The currents from three trials (5 s between trials) were subsequently averaged. Ba²⁺ sensitivity was assessed by applying the preceding protocol to cells superfused with solutions containing an increasing concentration of this KIR channel inhibitor for 2–3 min.

**Western blotting.** Feed arteries from four hamsters were placed in 100 µl of lysis buffer (pH 7.4) containing (in mM) 150 NaCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, 0.5% Tween, 10% mammalian protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Samples were mechanically disrupted, exposed to three freeze-thaw cycles, and then centrifuged (10 min; 13,000 rpm). Supernatant was placed in a clean tube, assayed for total protein, and stored at −20°C for up to 1 wk. Samples were prepared for electrophoresis by placing 15 µl of supernatant in 5 µl of 4× sample buffer, and 2 µl of DTT. After heating (10 min; 90°C) was completed, 20 mg of protein were loaded to run on a 10% polyacrylamide gel. Protein was transferred to a polyvinylidene fluoride membrane, blocked for 2 h (5% nonfat milk in Tris-buffered saline), and then incubated overnight (4°C) with a primary antibody (KIR2.1 or KIR2.2; 1:200 dilution; Sigma-Aldrich) diluted in milk-Tris-buffered saline. The PVDF membrane was subsequently washed and incubated with a horseradish peroxidase-conjugated secondary antibody (1:1,000; Jackson Laboratories) diluted in milk-Tris-buffered saline (1 h; 20 –22°C). Washing was repeated and the blot developed using chemiluminescent substrate (Pierce Biochemicals, Rockford, IL).

**Immunohistochemistry.** Feed arteries were arranged in cryoprotective media and rapidly frozen in liquid nitrogen. Tissue blocks were mounted in a cryostat and vessels cut in cross section (8–10 µm thickness). Cross sections were air-dried on poly-L-lysine-coated slides and washed with Tris-buffered saline (pH 7.4) containing 50 mM Tris, 0.9% NaCl, 0.1% BSA, and 0.01% Triton X. Sections were lightly fixed in 2% paraformaldehyde-Tris-buffered saline (20 min) and incubated (4 h) with a quench solution containing 100 mM Tris, 1.8% NaCl, 5% goat serum, 2% BSA, and 0.2% Triton X. Sections were subsequently incubated overnight (4°C) with a polyclonal (KIR2.1 or KIR2.2; 1:200) and a monoclonal (eNOS; 1:2,500; Chemicon, Ternecula, CA) antibody diluted in quench solution. Slides were then washed with Tris-buffered saline and incubated for 4 h (20–22°C) with an anti-rabbit-Cy3 and an anti-mouse-Cy5 IgG-specific secondary antibody. After further washing was completed, slides were air-dried and mounted in antifade media (Molecular Probes, Eugene, OR). Arterial cross sections were viewed and photographed by using a Zeiss fluorescent microscope coupled to a ×63 oil immersion lens.

**Computational modeling.** To complement our experimental approach, a computational model developed by Diep et al. (3) determined whether the elimination of a smooth muscle KIR-like current could attenuate vascular communication. Computational theory and base parameters are identical to the original publication with two...
notable exceptions. First, a second layer of smooth muscle cells was added to our virtual artery, a change consistent with the electron microscopy findings of Sandow et al. (27). Second, the nonlinear resistor representing the smooth muscle ionic conductance was divided into two components, one for KIR and the second for all other conductances. This study assumed that KIR was minimally active at −40 mV (0 pAs), maximally active at −60 mV (1.5 pAs), and reversed at −80 mV. A sigmoidal and exponential function fixed the data points between −40 to −60 mV and −60 to −80 mV, respectively. At 1.5 pAs, peak outward current would be below the resolution limit of whole cell patch-clamp electrophysiology. The nonlinear resistor representing all other smooth muscle conductances was comparable to Diep et al. (3) except that its relative magnitude (below −40 mV) was increased by 20%. Consequently, net inward current at −60 mV was 1.5 pAs greater than our previous publication (3).

To initiate current flow in our virtual artery, hyperpolarizing current was injected into one arterial segment of endothelium (50 μm in length and consisting of 48 endothelial cells) for 400 ms. Voltage responses were subsequently monitored along the entire length of the virtual artery (2,200 μm).

Statistical analysis. Data are expressed as means ± SE and n indicates feed artery number. Repeated measures ANOVAs compared feed artery responses in the absence and presence of Ba2+ or glybenclamide ± iberiotoxin. Where appropriate, a Bonferroni comparison was used for post hoc analysis. P values ≤ 0.05 were considered statistically significant.

Chemicals. Buffer chemicals, acetylcholine, phenylephrine, BaCl2, iberiotoxin, and glybenclamide were purchased from Sigma-Aldrich. All other reagents are listed with source.

RESULTS

KIR channel expression in retractor muscle feed arteries. The functional expression of KIR channels was assessed in initial experiments by examining the responsiveness of preconstricted feed arteries to elevated extracellular [K+]. Consistent with their presence, feed arteries dilated in a sustained and near-maximal manner as extracellular [K+] was elevated from 5.4 to 15.4 mM (Fig. 2). In this dilated, and presumably hyperpolarized state, the application of micromolar Ba2+ [a KIR channel inhibitor (23)] initiated feed artery constriction. This increase in tone was concentration dependent with 300 μM Ba2+ required to restore feed artery diameter to control levels. The method of vessel preconstriction had no qualitative influence on feed artery responsiveness to elevated extracellular K+ or Ba2+ (Fig. 2).

Whole cell patch-clamp electrophysiology was employed to ascertain whether KIR channels were present in feed artery smooth muscle cells. In the presence of high extracellular K+ (100 mM), this study observed an inward rectifying current that was Ba2+ sensitive and that reversed near the K+ equilibrium potential (Fig. 3). The IC50 of Ba2+ block (~140 mV) was 3.7 μM, a value slightly higher than that observed in rat arterial smooth muscle cells (1, 22, 25). Ba2+ block is voltage dependent, and although not examined in this study, IC50 would be expected to rise at physiological membrane potentials (1, 28). With the use of physiological bath and pipette solutions, this study did attempt to monitor outward KIR current. However, the magnitude was small and below the resolution limit (~2 pAs) of whole cell patch-clamp electrophysiology. Western analysis performed on whole arteries confirmed the presence of KIR2.1 and KIR2.2 (Fig. 4), two subunits that, when expressed alone or in combination with other KIR2.X subunits, give rise to currents with measurable negative slope conductance (2, 28).

Effect of KIR channel inhibition on cell-to-cell communication. Cell-to-cell communication was functionally assessed by applying acetylcholine to a small portion of a preconstricted feed artery while monitoring vasodilation along the vessel wall (Fig. 1). Irrespective of the method of preconstriction, discrete acetylcholine application elicited a vasodilation that conducted robustly and with modest decay (Figs. 5 and 6). The bath application of 30 μM Ba2+, a concentration sufficient to elicit a small constriction in K+–dilated feed arteries, tended to increase vasodilatory decay, but this did not reach statistical significance. In contrast, 100 μM Ba2+ enhanced conduction decay, and this attenuation was independent of the preconstriction method. In addition to impairing arterial conduction, Ba2+...
superfusion also attenuated vasodilation at the local site of acetylcholine application (Fig. 7). This attenuation was not apparent in Figs. 5 and 6 because the acetylcholine pulse duration was lengthened (approximately two- to threefold) so as to match the local response among the two experimental conditions. Note that Ba$^{2+}$ superfusion attenuated dilation at local site of acetylcholine application (see Fig. 7 for details). Local responses were, however, matched by increasing the acetylcholine pulse duration by two- to threefold in the presence of Ba$^{2+}$. A: vasodilatory responses in the absence and presence of 30 μM Ba$^{2+}$ ($n = 6; 60 \pm 5.56 \pm 5$, and $108 \pm 6 \mu m$ for preconstricted, preconstricted + Ba$^{2+}$, and maximal diameters, respectively). B: vasodilatory responses in the absence and presence of 100 μM Ba$^{2+}$ ($n = 6; 67 \pm 7, 60 \pm 5$, and $129 \pm 2 \mu m$ for preconstricted, preconstricted + Ba$^{2+}$, and maximal diameters, respectively). C: effects of Ba$^{2+}$ on conduction decay. Conduction decay was calculated as change in vasodilation between the 900- and 1,800-μm measurement sites. *Significant difference from control.

**Effect of KATP or BK channel inhibition on cell-to-cell communication.** Control experiments tested whether other K$^+$ channel inhibitors also attenuate cell-to-cell communication in retractor muscle feed arteries. In brief, acetylcholine was focally applied to preconstricted feed arteries, and vasomotor responses were monitored in the absence and presence of glybenclamide (10 μM; KATP channel inhibitor) or iberiotoxin (50 nM; BK channel inhibitor). In contrast to Ba$^{2+}$, neither the local or conducted responses to acetylcholine were affected by the bath application of glybenclamide or iberiotoxin (Fig. 8). The efficacy of glybenclamide as a KATP channel inhibitor was confirmed by the loss of pinacidil-induced vasodilation ($n = 2$; data not shown). Likewise, the bath application of iberiotoxin consistently elicited a subtle arterial constriction, a response consistent with BK channel inhibition (data not shown).

**Computational modeling of cell-to-cell communication.** Using the known structural, ionic, and gap junctional properties of resistance arteries, Diep et al. (3) recently developed a computational model that accurately predicts the nature of electrical communication in skeletal muscle feed arteries. With the use of this computational platform, a final set of experiments explored whether the elimination of a KIR-like current from the smooth muscle ionic conductance could attenuate the conduction of endothelium-initiated hyperpolarization, the electrical response that underlies the spread of vasodilation. The KIR-like current was minimally active at $-40 \text{ mV}$ (0 pA), maximally active at $-60 \text{ mV}$ (1.5 pAs), and retained the property of negative slope conductance. As noted in Fig. 9, A and B, the injection of 35 pAs of current into each of the 48 endothelial cells (i.e., one arterial segment $50 \mu m$ in length) initiated a hyperpolarization that conducted robustly along the endothelium and to the over-
lying smooth muscle cells via myoendothelial gap junctions. Consistent with our vasomotor observations in Figs. 5 and 6, the removal of the Kir-like current from the smooth muscle ionic representation enhanced electrical decay. Note that without Kir, more current had to be injected per endothelial cell to elicit an equivalent hyperpolarization in the smooth muscle cells overlying the endothelial stimulation site. If the current injection was maintained, hyperpolarization in the

Fig. 6. Kir channels facilitate conduction of endothelium-dependent vasodilation along arteries preconstricted to intravascular pressure and phenylephrine. Feed arteries preconstricted to 60 mmHg intravascular pressure and 0.1–1 μM phenylephrine were stimulated with an acetylcholine pipette while vessel diameter was monitored 0-, 900-, and 1,800-μm distal to the point of agent application. Note that Ba²⁺ superfusion attenuated dilation at the local site of acetylcholine application (see Fig. 7 for details). Local responses were, however, matched by increasing the acetylcholine pulse duration by two- to threefold in the presence of Ba²⁺. A: vasodilatory responses in the absence and presence of 30 μM Ba²⁺ (n = 6; 45 ± 6, 36 ± 5, and 97 ± 6 μm for preconstricted, preconstricted + Ba²⁺, and maximal diameters, respectively). B: vasodilatory responses in the absence and presence of 100 μM Ba²⁺ (n = 6; 50 ± 3, 38 ± 2, and 103 ± 7 μm for preconstricted, preconstricted + Ba²⁺, and maximal diameters, respectively). C: effects of Ba²⁺ on conduction decay. Conduction decay was calculated as change in vasodilation between the 900- and 1,800-μm measurement sites. *Significant difference from control.

Fig. 7. Ba²⁺ attenuates feed arteries dilation at the local site of acetylcholine application. In contrast to Figs. 5 and 6, an identical acetylcholine pulse was applied via micropipette to preconstricted feed arteries under control conditions or in the presence of 30 or 100 μM Ba²⁺. Vasomotor responses were monitored at the local site of agent application. A: feed arteries were preconstricted to 80 mmHg intravascular pressure (n = 6; diameter values in Fig. 5). B: feed arteries were preconstricted to 60 mmHg intravascular pressure + 0.1–1 μM phenylephrine (n = 6; diameter values in Fig. 6). *Significant difference from control.
overlying KIR-deficient smooth muscle cells was depressed (Fig. 8C).

**DISCUSSION**

This study examined whether KIR channels facilitate electrical communication along hamster retractor muscle feed arteries. Reflecting functional KIR expression in these vessels, a range of techniques revealed the following: 1) a sustained Ba²⁺-sensitive, K⁺-induced dilation in whole arteries; 2) the presence of a Ba²⁺-sensitive inward rectifying K⁺ conductance in isolated arterial myocytes; and 3) the expression of KIR2.1 and KIR2.2 in the smooth muscle cell layer of the retractor muscle feed arteries. Barium superfusion attenuated the conduction of acetylcholine-induced vasodilation, a finding that supports a facilitative role for KIR channels in cell-to-cell communication. Computational modeling predicted the preceding observations, along with the ability of barium to attenuate the direct vasodilatory effects of acetylcholine. Unlike Ba²⁺, agents that block ATP-sensitive and BK channels do not alter the local or conducted vasodilation to acetylcholine. We attribute this facilitation to the intrinsic property of negative slope conductance, a biophysical feature common to KIR2.1- and KIR2.2-containing channels that enables these integral membrane proteins to increase their activity as a cell hyperpolarizes.

**Cell-to-cell communication in small skeletal muscle arteries.** Cell-to-cell communication is typically assessed in small skeletal muscle arteries by applying vasoactive agents to a small portion of the vessel (30, 32, 33). This discrete application initiates local changes in endothelial or smooth muscle membrane potentials, which, with the aid of gap junctions, spread to neighboring vascular cells (5, 6, 33). With this approach, the nature of cell-to-cell communication is revealed by the extent to which the electrical or the corresponding vasomotor response conducts longitudinally along the arterial wall. Past studies have observed that hyperpolarizing responses initiated in endothelial cells conduct robustly along most skeletal muscle resistance vessels (5, 6, 32, 33). In contrast, agents that depolarize and constrict smooth muscle cells elicit a decidedly varied response, ranging from little to strong conduction (14, 15, 32, 35). This variability in cell-to-cell communication has been a

Fig. 8. ATP-sensitive K⁺ (K_ATP) and large conductance Ca²⁺ (BKCa) channels do not facilitate conducted vasodilation. Feed arteries preconstricted to 60 mmHg intravascular pressure + 0.1–1 μM phenylephrine were stimulated with an acetylcholine pipette while vessel diameter was monitored 0-, 900-, and 1,800-μm distal to the point of agent application. Experiments were performed in the absence and presence of glybenclamide (10 μM) ± iberiotoxin (50 nM). A: effects of glybenclamide (n = 6; 49 ± 2, 48 ± 2, 106 ± 2 μm for preconstricted, preconstricted + glybenclamide, and maximal diameters, respectively) on the conduction of acetylcholine-induced vasodilation. B: effects of glybenclamide and iberiotoxin (n = 5; 47 ± 2, 38 ± 2, and 106 ± 2 μm for preconstricted, preconstricted + glybenclamide-iberiotoxin, and maximal diameters, respectively). C: conduction decay was calculated as change in vasodilation between 900- and 1,800-μm sites.
KIR channels and conducted vasodilation

Fig. 9. Computational modeling predicts that smooth muscle KIR channels facilitate the conduction of endothelium-dependent hyperpolarization. Simulations: 35 or 85 pA of hyperpolarizing current was injected for 400 ms into each of 48 endothelial cells located within one 50-μm segment of endothelium (general location denoted by arrow). Voltage responses (VM) were monitored in outer smooth muscle cell layer in the presence and absence of a smooth muscle KIR-like current. A and B: smooth muscle VM response at 400 ms as color mapped along the vessel wall (A) or represented in a two-dimensional voltage plot (B). Note that in the absence of KIR, current injection per endothelial cell had to be increased to elicit a comparable response in smooth muscle cells overlying the stimulus site. C: current injection was maintained at 35 pA per endothelial cell, and VM response (at 400 ms) was monitored in the smooth muscle cells overlying stimulus site.

Facilitation of cell-to-cell communication. To examine whether KIR channels actively facilitate cell-to-cell communication, isolated retractor muscle feed arteries were strongly preconstricted and exposed to a brief acetylcholine pulse to elicit a vasodilation that conducts. In theory, strong preconstriction should depolarize smooth muscle cells and minimize KIR activity through the blocking effects of intracellular Mg2⁺ polyamines to block these channels in a voltage-dependent manner (8, 18).

KIR expression in retractor muscle feed arteries. Initial experiments focused on confirming the presence of KIR in retractor muscle feed arteries. Characteristic of arteries expressing this K⁺ conductance, elevated extracellular K⁺ (from 5.4 to 15.4 mM) elicited a sustained and near-maximal dilation (Fig. 2). In this dilated-hyperpolarized state where KIR is the dominant conductance setting resting membrane potential, the KIR channel inhibitor Ba2⁺ elicited a progressive concentration-dependent constriction. Consistent with these functional observations, patch-clamp electrophysiology revealed a Ba2⁺-sensitive KIR current in feed arterial smooth muscle cells (Fig. 3). The IC₅₀ of Ba2⁺ was subtly higher than that for rat arterial smooth muscle cells (1, 22, 25), a finding indicative perhaps of a species-dependent difference in the affinity of barium for KIR2.1 or KIR2.2. At physiological membrane potential, this value will be substantially higher given the known voltage dependency of Ba2⁺ block (1, 28). Western analysis confirmed the expression of KIR2.1 and KIR2.2 (Fig. 4A) in whole feed arteries, whereas immunohistochemistry analysis revealed their presence in the smooth muscle layer (Fig. 4B). KIR subunit expression was not apparent in the endothelium, although these findings should be interpreted cautiously given that the thin cross-sectional profile of endothelial cells makes identification of a modestly expressed protein difficult.

Facilitation of cell-to-cell communication. To examine whether KIR channels actively facilitate cell-to-cell communication, isolated retractor muscle feed arteries were strongly preconstricted and exposed to a brief acetylcholine pulse to elicit a vasodilation that conducts. In theory, strong preconstriction should depolarize smooth muscle cells and minimize KIR activity through the blocking effects of intracellular Mg2⁺ polyamines (8, 18). Under this basal condition, KIR activity would be expected to rise as an endothelium-dependent hyperpolarization spreads to smooth muscle cells via myoendothelial gap junctions. Such an increase would promote smooth muscle cell hyperpolarization and limit the voltage differential between the two cell types. This, in turn, would minimize radial charge loss to the smooth muscle cell layer and facilitate charge conduction along the endothelium. Consistent with the theory, our findings demonstrate that the discrete application of acetylcholine elicits a robust conducted response in which vasodilatory decay was enhanced by Ba2⁺ (Figs. 5 and 6). This enhanced decay was particularly evident at 100 μM Ba2⁺, a concentration that I constrains feed arteries when KIR activity is intrinsically high (Fig. 2), and 2) should substantively inhibit KIR channels even at depolarized physiological voltages (Fig.
A similar attenuation was absent in feed arteries treated with K
ATP and BK channel inhibitors (Fig. 8). Whereas this study is the first to reveal a role for KIR in facilitating cell-to-cell communication, it is not the first to address this question (7, 24). Indeed, similar to this study where decay was measured over a length of vessel distal to the point of agent application, Goto et al. (7) noted that while Ba
2+ altered the ability of acetylcholine to initiate hyperpolarization, it did not consistently enhance vasomotor or electronic decay. Species or tissue differences in the type and number of expressed KIR could partially account for this discrepancy. Furthermore, the Ba
2+ concentration (30 μM) employed by Goto et al. (7) may not have blocked a sufficient portion of KIR channels to augment decay. We highlight this issue given that in this study, 30 μM Ba
2+ only partially blocked sustained K+-induced dilation (Fig. 2) and did not significantly augment conduction decay (Figs. 5 and 6).

In addition to the preceding experiments, a supplemental approach was undertaken to reinforce the importance of KIR channels in facilitating cell-to-cell communication. Using a computational model based on the properties of resistance arteries (3), we explored on a theoretical level whether electrical conduction could be attenuated by eliminating a KIR-like current from the smooth muscle ionic representation. Our KIR-like current retained the intrinsic property of “negative slope conductance” and as such it was designed to be minimally and maximally active at ~40 mV and ~60 mV, respectively. Peak outward current was set to 1.5 pA/s, a subtle value generally below the detection limit of whole cell patch-clamp electrophysiology. Consistent with our vasomotor observations, simulations revealed that the elimination of the smooth muscle KIR current did indeed enhance the decay of electrical conduction (Fig. 9, A and B). Interestingly, in the absence of KIR, more current had to be injected per endothelial cell to elicit a comparable hyperpolarization in the overlying smooth muscle cells. If, however, the electrical stimulus was maintained (Fig. 9C), hyperpolarization in the overlying smooth muscle cells was reduced. These latter predictions are surprisingly consistent with our experimental observations and the need to lengthen the acetylcholine pulse duration during Ba
2+ superfusion to elicit a local vasomotor response that was comparable with control data (Figs. 5–7).

Functional implications. Blood flow to skeletal muscle is controlled by a network of resistance arteries linked in series and parallel with one another. Under high metabolic demand, these resistance arteries dilate in a coordinated manner to dramatically increase tissue blood flow (20, 30, 31). This coordinated dilation is intriguing in the retractor muscle where feed arteries lie external to the tissue. As such, these important resistors are not directly exposed to metabolic stimuli and thus rely on other mechanisms to initiate dilation. Using a light-dye ablation technique to focially damage endothelial cells, Segal and Jacobs (31) reported that cell-to-cell communication likely plays a key role in coordinating proximal vessel dilation. In detail, it was proposed that feed artery dilation arises as vasoactive stimuli produced by contracting muscle fibers initiate a hyperpolarization that conducts from distal arterioles into proximal feed arteries (31). For this mechanism to be effective, hyperpolarization must conduct over significant distances and across branch points with little electrotonic decay. It is enticing to suggest that KIR channels with their intrinsic property of negative slope conductance play a role in limiting this electrotonic decay and thus promote proximal vessel dilation and muscle blood flow.

In summary, this study employed a range of experimental approaches to reveal the importance of KIR channels in facilitating cell-to-cell communication along retractor muscle feed arteries. We attribute this facilitation to the intrinsic property of negative slope conductance, a biophysical feature common to KIR2.1- and KIR2.2-containing channels, which enables these integral membrane proteins to increase their activity as a cell hyperpolarizes. Facilitation could enable vasoactive stimuli produced by skeletal muscle fibers to more effectively dilate resistance arteries that lie external to the tissue. This would likely augment the dynamic range of blood flow responses to skeletal muscle.

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