Homocellular Conduction Along Endothelium and Smooth Muscle of Arterioles in Hamster Cheek Pouch

Unmasking an NO Wave

Stéphane Budel, Iain S. Bartlett, Steven S. Segal

Abstract—We investigated roles for homocellular (endothelium or smooth muscle) and heterocellular (myoendothelial) conduction pathways along hamster cheek pouch arterioles in vivo (n=64; diameter, 33±1 μm). Endothelium-dependent and -independent vasoactive agents were delivered from micropipettes (0.5 or 1 second pulse) onto an arteriole while observing diameter changes at defined distances along the vessel. Acetylcholine (ACh) produced maximal diameter (63±1 μm) locally and vasodilation conducted rapidly (~10 μm response at 2 mm, <1 second). Responses to bradykinin (BK) were similar, whereas sodium nitroprusside produced maximal dilation locally without conduction. KCl evoked biphasic conduction of vasoconstriction and vasodilation, whereas phenylephrine (PE) produced conducted vasoconstriction. Disrupting the integrity of endothelium as a conduction pathway using focal light-dye treatment (LDT) abolished conducted vasodilation to BK and to KCl but not to ACh. Disruption of smooth muscle integrity with LDT abolished conducted vasoconstriction with no effect on conducted vasodilation. After LDT of respective cell layers at sites 1 mm apart, vasodilation to ACh conducted past disrupted smooth muscle or disrupted endothelium, but not beyond both sites in series. The loss of conduction after selective LDT indicates a lack of effective myoendothelial coupling along the arteriolar wall. During NO synthase inhibition (L-NA, 100 μmol/L), conducted vasodilation was abolished to BK and to KCl yet remained intact to ACh. However, after LDT of smooth muscle, L-NA inhibited conduction to ACh by 60%. Thus, conduction of vasodilation entails a wave of NO release along arteriolar endothelium that is masked when smooth muscle provides a parallel conduction pathway. (Circ Res. 2003;93:1111-1119)

Key Words: microcirculation ∙ conduction ∙ endothelium ∙ smooth muscle ∙ nitric oxide

Coordination of vasomotor activity within and among resistance microvessels is integral to the local control of tissue blood flow. Vasodilation or vasoconstriction initiated at a local site can conduct along arterioles for several millimeters and manifests the transmission of electrical signals from cell-to-cell through gap junction channels. Outstanding goals have been to define the role of endothelium and smooth muscle in the initiation and the conduction of vasomotor responses, and to resolve the nature of heterocellular communication along the arteriolar wall. In the hamster cheek pouch, for example, arteriolar endothelium and smooth muscle can each provide a homocellular pathway for conduction. When cheek pouch arterioles are isolated and studied in vitro, heterocellular coupling between endothelium and smooth muscle is observed. Indeed, for isolated arterioles and feed arteries, electrical signals conduct readily between microvascular smooth muscle and endothelium. However, there is a paucity of evidence for myoendothelial coupling in the intact system. In the present study, we investigated whether myoendothelial coupling enables conduction of vasomotor responses past focal sites of cellular disruption within respective layers of arterioles controlling blood flow to the hamster cheek pouch.

The initiation and the conduction of vasodilation with acetylcholine (ACh) have consistently been shown to be independent of NO release. Throughout these studies, however, the integrity of both endothelium and smooth muscle layers has remained intact. Therefore, we also investigated whether redundancy in signaling pathways along the arteriolar wall may have obscured a role for NO in conducted vasodilation that has not previously been recognized. For this purpose, NO synthase (NOS) was inhibited in intact arterioles and after focal disruption of smooth muscle integrity. Our findings show that, in the absence of effective myoendothelial coupling, conduction along endothelium produces concomitant release of NO to relax arteriolar smooth muscle. However, this pathway is masked when smooth muscle provides a parallel conduction pathway, eg, with EDHF production in response to ACh.
Materials and Methods

Hamster Cheek Pouch Preparation

Procedures were approved by the Animal Care and Use Committee of the John B. Pierce Laboratory. Male golden hamsters (102±1 g; n=64) were anesthetized with pentobarbital sodium (65 mg/kg, intraperitoneal injection) and tracheotomized (PE 190 tubing) to ensure airway patency. Esophageal temperature was maintained at 37°C to 38°C. A cannula (PE 50) was secured in the left femoral vein for fluid replacement and maintenance of anesthesia (10 mg pentobarbital mL⁻²1 sterile saline, infused at 0.42 mL/h). At the end of the experiment, an overdose of pentobarbital was delivered intravenously.

The cheek pouch was everted, pinned onto a transparent Plexiglas board, and superficial connective tissue was removed using microdissection. The preparation was superfused continuously (5 mL/min) with bicarbonate-buffered physiological saline solution (PSS; pH 7.4, 37°C) of the following composition (in mmol/L): 137.0 NaCl, 4.7 KCl, 1.2 MgSO₄, 2.0 CaCl₂, 18.0 NaHCO₃ dissolved in deionized H₂O (dH₂O), and equilibrated with 5% CO₂/95% N₂. Salts were obtained from J.T. Baker or Sigma. The completed preparation was secured onto a fixed stage of an intravital microscope mounted on an X-Y translation platform and equilibrated at least 30 minutes. Arterioles were observed using video microscopy.

5,7,9 Internal diameter was measured continuously with a video micrometer (resolution, ≤2 μm). Data were recorded onto a personal computer at 40 Hz; an event marker coincided with stimulus delivery. One arteriole was studied per hamster; those selected for experiments were typically second-order branches located at least 1 cm from tissue edges, devoid of adjacent venules, had regions 1- to 2-mm long with minimal branching, and constricted when the superfusion solution was equilibrated with 5% or 10% O₂ (and 5% CO₂, balance N₂), which was sustained to promote vasomotor tone and the ability to study conducted vasodilation over several hours. Increasing arterial tone with oxygen does not affect conduction.2,17,18 Maximal diameter was measured during topical SNP (10 μmol/L). Preparations that displayed vasomotion (<10%) were not used due to the uncertainty in measuring conducted responses when baseline diameter changes continuously.

Vasomotor Stimuli and Responses

Glass micropipettes were filled, secured in a micromanipulator, and positioned with the tip adjacent and perpendicular to the arteriolar wall. Acetylcholine (1 mol/L) and PE (0.5 mol/L) were microiontophoresed [500 ms, 500 or 1000 nA; tip internal diameter (ID) ~1 μm]. Bradykinin (BK; 1 mmol/L) and SNP (1 mmol/L) were delivered as a pulse (typically 1 second, adjusted to produce maximal diameter at the site of delivery) using pressure ejection (28 to 35 kPa, tip ID, 2 to 3 μm); KCl (1 mol/L) was delivered similarly. The local response was evaluated at the micropipette tip (Figure 1) and conducted responses were evaluated at defined distances from the local stimulus.5,7 Vasomotor responses were quantified as follows: Diameter change=peak response diameter−baseline diameter. A separate pulse was delivered to observe each response; 2 to 3 minutes elapsed between each stimulus. Tachyphylaxis to respective stimuli was negligible.

Microejection of KCl readily produces conduction along the arteriolar wall that can be biphasic in nature,2,4,5 which may be attributable the transient increase and decline in [K⁺]o. The effect of defined [K⁺], on arteriolar diameter was evaluated by equimolar substitution of KCl for NaCl in the superfusion solution.19 This was achieved by switching between reservoirs, which required ~1 minute to reach a new steady state [K⁺]o over the tissue. For each [K⁺], diameter was recorded after 4 to 5 minutes of equilibration. Control [K⁺], (4.7 mmol/L) was restored to return to control diameter between each increment in [K⁺].

Figure 1. Local and conducted vasomotor responses in hamster cheek pouch arterioles. Illustration at top (depicted as longitudinal section through one edge of vessel wall) indicates distances (μm) along arteriole where diameter was observed. SMC indicates smooth muscle cells; EC, endothelial cells. Each stimulus (1-second pulse) was delivered at 0 μm by micropipette at each arrow. Records illustrate representative diameter changes in response to acetylcholine (ACh), bradykinin (BK), potassium chloride (KCl), and phenylephrine (PE) observed at 0 μm ("local") and at 500 and 1000 μm upstream (conducted). Note similarity of vasodilation in response to ACh and BK, vasoconstriction in response to PE, and biphasic vasoconstriction followed by vasodilation with KCl.
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(excitation, 450 to 490 nm) through an immersion objective (40 × 0.75, Zeiss) using a 75-watt Xenon lamp. The site was centered or loss of constriction to PE (with smooth muscle disruption).7,20 For treatment until loss of dilation to ACh (with endothelium disruption) responses to ACh and to PE were evaluated before and during addition of L-arginine (L-Arg, 1 mmol/L).2,9,15 The efficacy of NOS inhibition assessed by reversing the effects of L-NA after the treatment was then performed to disrupt smooth muscle at 500 m upstream from the original “local” site of stimulation. Local responses to ACh and to PE were evaluated before and during treatment until loss of dilation to ACh (with endothelium disruption) or loss of constriction to PE (with smooth muscle disruption).2,9 For dual LDT, arterioles with segments ≥2 mm long were first evaluated for conducted responses to ACh in both directions. Light-dye treatment was then performed to disrupt smooth muscle at 500 m and endothelium at 1500 μm. Responses to ACh were then re-evaluated in both directions. Selectivity of cellular disruption with LDT was confirmed by labeling with propidium iodide.7,20

Light-Dye Treatment (LDT)
Light-dye treatment was performed using fluorescein isothiocyanate (FITC) conjugated to bovine serum albumin (3% solution)7 or to dextran (70 kDa, 0.67% solution)20 with similar results. For disruption of endothelium, dye was injected into the femoral vein (1 mL/kg). For disruption of smooth muscle, dye was perfused across the segment from a micropipette (10 μm tip ID).7,20 As the site of LDT, the arteriolar segment (length, ≈250 μm) was illuminated (excitation, 450 to 490 nm) through an immersion objective (40×; NA=0.75, Zeiss) using a 75-watt Xenon lamp. The site was centered 500 μm upstream from the original “local” site of stimulation. Local responses to ACh and to PE were evaluated before and during treatment until loss of dilation to ACh (with endothelium disruption) or loss of constriction to PE (with smooth muscle disruption).2,9 For dual LDT, arteriolar segments ≥2 mm long were first evaluated for conducted responses to ACh in both directions. Light-dye treatment was then performed to disrupt smooth muscle at 500 m and endothelium at 1500 μm. Responses to ACh were then re-evaluated in both directions. Selectivity of cellular disruption with LDT was confirmed by labeling with propidium iodide.7,20

Figure 2. Local vasomotor responses to KCl. A, Ejection of 1 mol/L KCl from a micropipette at 14 to 21 kPa with increasing pulse duration (n=5). With 0.5 seconds, only dilation occurred. As pulse duration increased, a biphasic vasoconstriction (sustained for the duration of the pulse; not shown) was followed by vasodilation (Figure 1). Vasoconstriction was blocked by nifedipine (Nif, 1 μmol/L; filled bars). *P<0.05 vs 1-second stimulus. B, With incremental concentrations of KCl in the superfusate (control=4.7 mmol/L), dilation ensued with [K+]=10 or 20 mmol/L; vasoconstriction was manifest at 40 to 100 mmol/L (n=8).

Evaluating a Role for NO in Local and Conducted Vasodilation
To inhibit the activity of NOS, Nω-nitro-l-arginine (L-NA, 100 μmol/L) was equilibrated in the superfusate, with the specificity of inhibition assessed by reversing the effects of L-NA after the addition of l-arginine (L-Ang, 1 mmol/L).2,9,15 The efficacy of NOS inhibition over time was ascertained by evaluating local responses to ACh and to BK. To evaluate whether NO was integral to the conduction of vasomotor responses along intact arterioles, stimuli were applied at 0 μm and conduction was evaluated at sites 500 and 1000 μm upstream along arterioles under control conditions and after equilibration with L-NA. In additional experiments, conduction was triggered by ACh in the middle of a 2-mm segment of the arteriole and evaluated for 1000 μm in each direction. Smooth muscle LDT was then performed midway along one direction, with the integrity of both cell layers maintained in the opposite direction. L-NA was then added to test whether NO release from endothelium would be unmasked by disrupting the smooth muscle conduction pathway.

Data Analysis
Local and conducted responses were evaluated before and after LDT using paired t tests and analysis of variance with Tukey tests for post hoc comparisons. Differences were considered statistically significant with P<0.05. Summary data are mean±SEM; n indicates number of arterioles.

Results
Control Responses
Resting diameter after equilibration averaged 44±1 μm and elevating superfusate O2 constricted arterioles to 33±1 μm (P<0.01, n=61). Maximal diameter of arterioles during equilibration with SNP was 65±1 μm. When delivered from a micropipette, each stimulus (with the exception of SNP, which produced local but not conducted vasodilation) evoked reproducible and characteristic changes in diameter along the arteriolar wall (Figure 1). The amplitudes and durations of local responses were typically ≈2-fold greater than respective conducted responses (Figure 1). Irrespective of the stimulus, observation site, or experimental intervention, the interval between the onset of local and conducted responses was consistently <1 second. Both ACh and BK produced vasodilation and PE produced vasoconstriction. When KCl was ejected from a micropipette for more than 1 second, vasoconstriction occurred first, was sustained for the stimulus (up to 15 seconds; Figure 2A), and was followed by transient vasodilation. With a 0.5-second pulse, only vasodilation was observed. A 1-second pulse was used thereafter and both vasoconstriction and vasodilation conducted with high fidelity (Figure 1). Nifedipine (1 μmol/L; Sigma) inhibited the...
vasomotor tone and conducted vasodilation to ACh, prepared nature of each stimulus. Based on the maintenance of from an arteriole eliminated responses, confirming the local-

constrictor component (Figure 2B), whereas increasing rest-
ing diameter by 7±1 μm (n=5). During sustained elevations in [K+], arterioles dilated at 10 to 20 mmol/L and constricted at 40 to 100 mmol/L (Figure 2B). Stimuli delivered from micropipettes at 1000 μm (and at 500 μm before LDT) produced local responses at respective sites that were not different from those recorded at 0 μm before and after LDT (data not shown). Moving a micropipette >100 μm away from an arteriole eliminated responses, confirming the localized nature of each stimulus. Based on the maintenance of vasomotor tone and conducted vasodilation to ACh, preparations remained stable for ~5 hours.

Light-Dye Treatment of Endothelium

For stimuli delivered at 0 μm with responses observed at 1000 μm, disruption of endothelium at 500 μm had no effect on conducted vasoconstriction to either PE or to KCl. However, conducted vasodilation in response to BK and to KCl were abolished (Figure 3). In contrast, conducted vasodilation evoked by ACh remained intact at 1000 μm. The reduction in conducted vasodilation at 500 μm (Figure 3) was associated with partial loss of tone in the segment of LDT, where resting diameter increased from 26±2 to 46±2 μm (P<0.05, n=10).

For stimuli delivered at the site of LDT, vasodilation to ACh was abolished locally along with the conducted response at 1000 μm. Local constriction to PE increased from −21±2 μm (control) to −29±2 μm (P<0.05, n=6), whereas conducted vasoconstriction at 1000 μm remained intact (~8±2 μm). After LDT, the local response to BK converted from vasodilation (22±3 μm) to vasoconstriction (~13±3 μm; P<0.05, n=6) that did not conduct. KCl evoked vasoconstriction (local, −16±3 μm) that readily conducted (−14±1 μm; n=6), with no evidence of the vasodilatory component. Local dilation to SNP was reduced after LDT (20±1 versus 8±2 μm; P<0.05, n=10) in association with the partial loss of tone.

Light-Dye Treatment of Smooth Muscle

For stimuli delivered at 0 μm with responses observed at 1000 μm, disruption of smooth muscle at 500 μm had no effect on conducted vasodilation in response to ACh or BK, or to the dilatory component of the biphasic response to KCl (Figure 4). However, conduction of vasoconstriction in response to PE was abolished, along with the constrictor component of the biphasic response to KCl (Figure 4).

For stimuli delivered at 500 μm, the loss of local responses confirmed disruption of smooth muscle integrity by LDT. Observed at 1000 μm, conduction of vasoconstriction in response to PE before LDT (~11±2 μm, n=5) or to KCl (~14±2 μm, n=6) was also abolished. However, the conduction of vasodilation (pre- versus post-LDT, respectively) in response to ACh (14±2 versus 16±2 μm, n=6), to BK (13±2 versus 12±2 μm; n=5), or to KCl (10±3 versus 13±1 μm; n=6) remained intact.

Dual Light-Dye Treatment

Under control conditions, the conduction of vasodilation in response to ACh was equally effective in both directions for at least 2000 μm (Figure 5). After LDT, control diameter (30±2 μm; n=7) was maintained along arterioles except for constriction (to 21±3 μm; P<0.01) of the segment with

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smooth muscle disruption and dilation (to 54 ± 2 μm; \( P < 0.01 \)) of the segment with endothelium disruption. When ACh was applied at 0 or 2000 μm, conduction of vasodilation in the middle of the vessel (observed at 1000 μm) from either direction through the first site of LDT was not different from control (Figure 5). However, conduction was ineffective beyond the second site of LDT in both directions. In 5 of these experiments, conducted responses were abolished beyond the second site of LDT. In 2 remaining experiments, the conducted response (4 ± 1 μm) was diminished by 60%.

In additional control experiments (n = 4), conducted vasodilation through two sites of LDT in the same cell layer (i.e., at 500 and 1500 μm in endothelium or in smooth muscle) remained intact along 2000 μm. In one arteriole studied along a total distance of 4000 μm, conduction initiated in the middle of the arteriole was blocked at 2000 μm in the direction of dual LDT (as in Figure 5) yet remained intact for the same distance in the opposite (untreated) direction.

Figure 4. Effect of smooth muscle disruption on local and conducted vasomotor responses to ACh (n = 6), PE (n = 5), BK (n = 5), and KCl (n = 6). Data indicate the change in diameter at respective distances (0, 500, and 1000 μm) along arteriole, with stimuli applied at 0 μm and smooth muscle disruption (darkened segment in illustration at top) centered at 500 μm. Biphasic responses to KCl are shown as vasoconstriction followed by vasodilation at each site. Note abolition of conducted vasoconstriction to PE and KCl with preservation of conducted vasodilation to ACh, BK, and KCl. * \( P < 0.05 \), pre- vs post-LDT.

Figure 5. Dual light-dye treatment to test for myoendothelial coupling (n = 7). Illustrations depict arteriolar wall with reference to distances (μm) where diameter was observed. SMC indicates smooth muscle cells; EC, endothelial cells. Control responses to ACh delivered at 0 or 2000 μm were symmetrical in both directions. LDT disrupted smooth muscle at 500 μm and endothelium at 1500 μm (darkened segments in illustrations). When initiated from either end of the 2-mm segment, local responses and conduction through one site of disruption (observed at 1000 μm) remained intact. However, conducted vasodilation was abolished through both sites of disruption in 5 experiments. In 2 experiments, residual responses at 0 and 2000 μm may be attributed to incomplete disruption SMCs or ECs at respective sites of LDT; a detectable level of myoendothelial coupling may also have been present in these cases. Note loss of vasomotor responses at site of SMC disruption. Attenuation of conducted response at site of EC disruption is due to corresponding loss of tone. * \( P < 0.05 \) vs control.
Inhibition of NO Production

Under control conditions, ACh and BK increased local arteriolar diameter by 25 to 30 μm (Figure 1). Within 10 minutes of adding L-NA to the superfusate, arteriolar diameter decreased from 35±1 to 24±1 μm (n=17, P<0.01). Local dilation to ACh was reduced to 21±3 μm (P<0.05) after 25 minutes and stabilized thereafter. Local dilation to BK was attenuated progressively through 15 minutes of L-NA; by 25 minutes, the response reversed to vasoconstriction (−28±6 μm), which increased to −214±3 μm at 35 minutes. Remaining experiments equilibrated with L-NA for at least 30 minutes.

In the presence of L-NA, conducted vasodilation to ACh was maintained but was abolished to BK (Figure 6), and the ensuing vasoconstriction did not conduct. For KCl, conducted vasodilation was also abolished, with the local dilatory response attenuated by 47%. In contrast, conducted vasoconstriction to PE or to KCl remained intact. Attenuation of the local vasoconstriction to PE (Figure 6) was associated with the reduction in baseline diameter. Each effect of L-NA was reversed within 10 minutes of adding L-Arg. Maximal diameter with superfusion of SNP was unaffected by L-NA.

Inhibition of NO Production With Disruption of Smooth Muscle Conduction

After smooth muscle disruption at 1500 μm, the conduction of vasodilation in response to ACh delivered at 1000 μm was not different from control in either direction along the arteriole (ie, at 0 or 2000 μm; Figure 7). With L-NA, however, conducted vasodilation was attenuated by 60% beyond the site of smooth muscle LDT (eg, at 2000 μm) while being maintained in the direction that both cell layers remained intact (eg, at 0 μm; Figure 7). This effect of L-NA also reversed with L-Arg.

Discussion

The present study has investigated cellular signaling pathways underlying the conduction of vasomotor responses along arterioles controlling blood flow in the cheek pouch of anesthetized hamsters. Distinct sites of endothelium or smooth muscle were selectively disrupted alone or in combination with the inhibition of NOS activity. Conduction was initiated with endothelium-dependent vasodilators (ACh, BK), with a smooth muscle agonist (PE), and independent of membrane receptors (KCl). We show for the first time that conduction of hyperpolarization along arteriolar endothelium produces concomitant release of NO that relaxes smooth muscle cells along the vessel wall. This “wave” of NO is essential to conduction with BK but is masked when smooth muscle provides a parallel conduction pathway, which occurs when ACh is the stimulus (Figure 8). Remarkably, the biphasic response to KCl is explained by initiation and conduction of vasoconstriction along smooth muscle and of vasodilation along endothelium. These complementary signaling pathways illustrate both selectivity and redundancy in mechanisms by which cell-to-cell conduction can coordinate vasomotor responses in arteriolar resistance networks. Our findings indicate that myoendothelial coupling observed in vitro is not manifest under the conditions of the present experiments and substantiate that endothelium and smooth muscle can each provide an independent, homocellular pathway for conduction in vivo.

Bidirectional electrical coupling between smooth muscle and endothelium implies that respective cell layers comprise...
an electrical syncytium. The simultaneous intracellular recordings from endothelium and smooth muscle indicate that myoendothelial coupling enables hyperpolarization and depolarization to spread readily between cell layers and to thereby coordinate vasomotor responses along resistance microvessels. However, our finding that LDT at a single site could eliminate conducted vasodilation (with endothelium disruption; Figure 3) or conducted vasoconstriction (with smooth muscle disruption; Figure 4) is not consistent with effective electrical coupling between respective cell layers. Further, after dual LDT, the ability of vasodilation to conduct past either smooth muscle or endothelial disruption, but not beyond both sites in series (Figure 5), provides further evidence against effective heterocellular coupling along arterioles in the superfused cheek pouch. In light of differences reported for myoendothelial coupling between studies performed in vivo and those performed using isolated vessels, we suggest that heterocellular coupling in the arteriolar wall may be modulated by experimental conditions. It may also be possible to

**Figure 7.** Test for NO release after smooth muscle disruption (n=5). ACh was delivered at 1000 μm (indicated by micropipette), and conducted vasodilation was observed at 0 and 2000 μm after smooth muscle (SMC) was disrupted with LDT at 1500 μm (darkened segment in illustration). Inhibition of NOS reduced local dilation by 40%, yet had no effect on conduction along the intact region. At the same time, conduction was inhibited by 60% beyond the segment with smooth muscle disruption, recorded at 2000 μm. Addition of L-Arg (1 mmol/L) restored the conducted response at 2000 μm along with the local response. *P<0.05 vs control.

**Figure 8.** Summary of signaling pathways underlying conduction in hamster cheek pouch arterioles. A, Homocellular conduction pathways. Signal for vasodilation conducts along smooth muscle cells (SMC) and endothelial cells (EC) in response to ACh. For BK and KCl, ECs provides the conduction pathway. The signal for vasoconstriction conducts along smooth muscle alone. B, Model of redundant signaling pathways underlying conducted vasodilation in response to ACh. Along the intact arteriole, ACh elicits hyperpolarization of ECs along with release EDHF to hyperpolarize SMCs. Release of NO with conduction along ECs is masked by conduction along SMC. After SMC disruption (darkened segment), the contribution of NO with conduction along EC is unmasked.
regulate myoendothelial coupling and/or conduction in a stimulus-specific manner.23

The biphasic response to microejection of KCl is explained by the initiation and conduction of vasoconstriction along smooth muscle and of vasodilation along endothelium (Figures 3 and 4). Thus, the initial vasoconstriction reflects depolarization and activation of voltage-gated Ca\textsuperscript{2+} channels in smooth muscle, as predicted by a Nernst effect and confirmed here by the inhibition of vasoconstriction with nifedipine (Figure 2). However, with membrane potential of −30 to −35 mV,5,9 arteriolar dilation when raising \([K^{+}]_o\) to 10 to 20 mmol/L is not consistent with a passive effect on cellular excitability. In turn, we propose that as the local elevation in \([K^{+}]\), dissipates, vasoconstriction can give way to vasodilation on activation of K\textsubscript{IR}25,26 channels or Na\textsuperscript{+}/K\textsuperscript{+} ATPase activity.27 This interpretation is also consistent with observing only vasodilation in response to the shortest pulse of KCl (Figure 2). Although further experiments will be required to resolve the contributions of K\textsubscript{IR} channels or Na\textsuperscript{+}/K\textsuperscript{+} ATPase activity to arteriolar dilation in the cheek pouch, our finding that respective components of the biphasic response to KCl are conducted along distinct cellular pathways provides additional evidence that smooth muscle and endothelium are not directly coupled to each other in vivo.5,7,24 Thus, according to the integrity of respective cell layers and changes in local milieu, elevating \([K^{+}]\), can differentially activate arteriolar endothelium and smooth muscle to initiate the conduction of vasodilation and vasoconstriction, respectively.

**Unmasking a Role for NO in Conducted Vasodilation**

In response to ACh, a role for NO has been investigated through competitive inhibition of NOS activity using analogues of L-arginine.5,11,13,15,16 Consistent with these earlier studies, conducted vasodilation to ACh was maintained during treatment with 100 \(\mu\text{mol/L}\) L-NA, despite significant vasoconstriction and attenuation of the local response (Figure 6). At the same time, L-NA eliminated conducted vasodilation to BK and to KCl (Figure 6) and reversed the BK response to vasoconstriction that did not conduct, suggesting that smooth muscle contraction occurred without a significant change in membrane potential; eg, through release of Ca\textsuperscript{2+} from intracellular stores or changes in Ca\textsuperscript{2+} sensitivity of regulatory proteins. Our finding that the actions of L-NA were reversed with a 10-fold excess of L-arginine supports the specificity of NOS inhibition. Further, the differential inhibition of conducted vasodilation to BK and to KCl, but not to ACh, indicates that vasoconstriction itself is not the mechanism by which L-NA exerted its effect. Moreover, arteriolar conduction is maintained with similar levels of vasoconstriction during elevated superfusate PO\textsubscript{2},2,17,18 Thus, for two vasodilator responses triggered independent of muscarinic receptors, smooth muscle relaxation along arterioles is explained by NO release along endothelium. Indeed, the actions of L-NA mimic those seen after disruption of endothelium integrity (Figure 3). The observation that conducted vasoconstriction to PE or to KCl was unaffected by L-NA or by disruption of endothelium strengthens the conclusion that smooth muscle alone mediates this response.7

Arteriolar conduction is often studied along segments \(\approx 1\ \text{mm long.4,5,7,9,13}\) The present experiments establish that conduction is bidirectional and equivalent for distances of at least 2 mm (Figure 5). Thus, with ACh delivered at the center of a 2-mm segment and LDT performed midway along a 1-mm segment in one direction, the 1-mm segment in the opposite direction provided a corresponding control (Figure 7). In this manner, conducted responses to ACh were compared at equivalent distances along the same vessel in the presence of L-NA both with and without smooth muscle disruption. Our finding that the conducted response was inhibited (reversibly) by L-NA only beyond the segment of smooth muscle disruption lends further support to the conclusion that conduction along the endothelium generates the release of NO (Figure 8). In response to ACh, the endothelium can also release a signal that can initiate conducted vasodilation along smooth muscle, which we attribute to production of EDHF9,16 (Figure 8B) and the induction of hyperpolarization along this parallel conduction pathway.5,7 In contrast to ACh, neither BK nor KCl appears to initiate conduction along the smooth muscle layer.

The distinction between conducted vasodilation triggered by ACh as compared with BK may be explained by differences in respective signaling pathways28–30 and whether eNOS is activated alone or in conjunction with cytochrome P-450 enzymes to produce EDHF.9,16 During L-NA treatment, the residual conduction to ACh after smooth muscle disruption (Figure 5) may reflect incomplete inhibition of NOS, although the concentration of inhibitor used here (100 \(\mu\text{mol/L}\)) is 2- to 10-fold greater than used in previous studies.9,11,13,15,16 It is also possible that there is residual release of another autacoid along the arteriolar wall in response to ACh. Indeed, the combination of L-NA+17-octadecynoic (a cytochrome P-450 antagonist) suppressed conducted vasodilation to ACh by \(\approx 80\%\), which is greater than the 60% inhibition observed here with L-NA alone. Although indomethacin alone has no effect on conduction,9 prostaglandins may further contribute to the residual response.16 Nevertheless, and despite such redundancy in signaling pathways for endothelium-dependent vasodilation, the present experiments are the first to resolve a novel and integral role for NO in conducted vasodilation that has been masked in previous using ACh as the triggering stimulus.2,9,13–16 In turn, we propose that the role of endothelium or smooth muscle in providing a conduction pathway can be determined by whether NO and/or EDHF, respectively, are released in response to a vasodilator (Figure 8B).

Nitric oxide donors delivered as a brief local stimulus have proven to be ineffective in generating conducted responses,9,13,16,31 as confirmed here with SNP. In light of the present findings, we propose that eNOS is activated rapidly and transiently12 as hyperpolarization travels along the endothelium, eg, through promoting the driving force for calcium entry.33 This prediction is supported by the common requirement for integrity of the endothelium to conduct vasodilation in response to BK, to KCl, and to ACh after smooth muscle disruption.
In summary, this study has revealed an integral role for NO in mediating conducted vasodilation in arterioles of the hamster cheek pouch. With conduction along the endothelium in response to BK and to KCl, a “wave” of NO is released along the arteriolar wall that is integral to vasodilation. In response to ACh, conduction is triggered in parallel along the smooth muscle layer, masking the NO-dependent pathway that is resolved here by disrupting smooth muscle integrity in conjunction with NOS inhibition. Both the initiation and conduction of vasoconstriction are inherent to smooth muscle and independent of endothelium. In such fashion, respective cell layers can provide parallel pathways for conduction that are distinct as well as complementary. These findings imply that the consequences of vascular pathology on blood flow control in arteriolar networks will depend not only on stimulus specificity but also on the functional integrity of each cell layer.

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