Long Distance Conduction of Vasodilation: A Passive or Regenerative Process?

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ABSTRACT

The mechanism enabling coordination of the resistance of feed arteries with microcirculatory arterioles to rapidly regulate tissue blood flow in line with changes in metabolic demand has preoccupied scientists for a quarter of a century. As experiments uncovered the underlying electrical events, it was frequently questioned how vasodilation could conduct over long distances without appreciable attenuation. This perspective reviews the data pertinent to this phenomenon and provides evidence that this remarkable response could be made possible by a simple mechanism based on the steep relationship between membrane

potential and calcium entry demonstrated by the voltage-dependent calcium channels which mediate the control of vascular tone *in vivo*.

Key words: vasodilation, conduction, mechanism, voltage-dependent calcium channels, threshold

Abbreviations used: 17-ODYA, 17-octadecynoic acid; ACh, acetylcholine; CGRP, calcitonin gene-related peptide; EDHF, endothelium-derived hyperpolarizing factor; E_K , equilibrium potential for potassium; $[K^+]_o$, extracellular potassium concentration; K_{Ca} channels, calcium-dependent potassium channels; K_{IR} channel, inwardly rectifying potassium channel

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INTRODUCTION

Rapid and substantial changes in blood flow, which finely tune nutrient supply of tissues to fluctuations in metabolic demand, require the integration of vasomotor responses in local microvessels with those of upstream supply arteries. This response is particularly prominent in skeletal muscle where changes in blood flow during intense exercise can approach nearly two orders of magnitude [54]. The mechanism by which this remarkable coordination of the resistance of feed arteries and microvessels takes place has thus fascinated scientists for the past 25 years, and continues to be the focus of review [1]. Although conduction of both local vasodilator and vasoconstrictor responses has been reported, the rapid and long distance spread of the former has particularly attracted attention. Since conduction of vasodilation is underpinned by hyperpolarization-mediated inhibition of voltage-dependent calcium channels [44], the observed long distance spread of responses has prompted speculation concerning the involvement of an underlying electrical event which could regenerate the hyperpolarization as it travels along the vascular wall. However, little direct evidence has been presented to support such a

hypothesis. This perspective critically reviews these data and discusses the case for an alternative and simpler mechanism, based around the narrow voltage window underlying activation of the voltage-dependent calcium channels responsible for the maintenance of vascular tone *in vivo*.

MECHANISM UNDERLYING THE CONDUCTION OF VASOMOTOR RESPONSES

The rapid spread of vasomotor responses from the sites of local mechanical or chemical stimulation was described in the frog lingual and cutaneous microcirculations some 90 years ago [37,38]. However, local nerve networks were assumed to be responsible for these responses, even though external nerves had been severed during the experiments [38]. Indeed, substantial progress in our understanding of the underlying mechanism in the microcirculation did not occur until an experimental paradigm was established to facilitate more controlled investigation [13,56]. Under this protocol, the vasodilator, ACh, was applied focally from a micropipette to a downstream arteriole of the hamster cheek pouch, cremaster or gracilis muscle microcirculation

and the magnitude of the ensuing vasodilation quantified at this local site and at sites up to 2 mm upstream of the stimulus, a distance some 50-fold greater than the diameter of the conducting arterioles.

Studies employing this experimental regime initially probed the role of the vascular innervation and cell coupling in the conduction mechanism, and extended description of the phenomenon to vasoconstriction in response to norepinephrine [57]. In contrast to the conclusion of Krogh *et al.* [38], these experiments demonstrated that conduction of both vasodilation and vasoconstriction was insensitive to inhibition of voltage-dependent sodium channels with tetrodotoxin, and hence independent of vascular innervation [57,71]. Importantly, conducted vasodilation also persisted in occluded arteriolar segments, demonstrating its independence from local changes in blood flow or transmural pressure [56]. In contrast, the conducted responses were attenuated or abolished by putative antagonists of gap junctions [57], leading the authors to hypothesize “that propagation of vasomotor responses along arterioles is initiated via a local change in membrane potential secondary to receptor occupation and that changes in potential spread electrotonically through gap junctions coupling smooth muscle cells, endothelial cells, or both.” Consistent with this proposal, later studies confirmed considerable heterogeneity in the ability of different agonists to initiate conducted responses; the authors concluding again that this variability might be linked to an ability to induce a change in membrane potential [10], although electrical measurements were not made in these studies. A definitive role for endothelial gap junctions was later established with the significant attenuation of conducted vasodilation along cremaster arterioles of anaesthetized mice lacking the prominent endothelial gap junctional protein, connexin40 [9].

EVIDENCE THAT VOLTAGE-DEPENDENT EVENTS ARE REQUIRED FOR CONDUCTION

Direct demonstration of a critical role for voltage-dependent events in the conduction of vasomotor responses was provided some years later, with simultaneous measurement of membrane potential, with fine intracellular microelectrodes, and of vessel diameter. These studies, conducted in microcirculatory vessels *in vivo* and pressurized arteries and unpressurized arterioles *in vitro*, observed that ACh and phenylephrine could evoke changes in membrane potential and diameter at both local and remote sites; ACh elicited hyperpolarization and vasodilation, whereas phenylephrine evoked depolarization and constriction [16,17,67,71]. Importantly, the endothelial and smooth muscle cells were shown to be electrically coupled, since either positive or negative current injected into endothelial or smooth muscle

cells caused depolarization or hyperpolarization, respectively, in cells of the other layer [17].

In the case of ACh, the effects were not mediated by nitric oxide or prostaglandins. Not only did the nitric oxide donor, sodium nitroprusside, produce only a local vasodilation without any conducted response [10], but inhibition of endogenous nitric oxide or prostaglandin synthesis had no effect on conducted responses [17,18,27,54]. Consistent with a contribution of these vasodilators to pharmacomechanical, but not electromechanical coupling, their inhibition also failed to alter resting membrane potential of either smooth muscle cells or endothelial cells, in spite of eliciting significant increases in resting vascular tone [69]. Recently, the calcium signal evoked at the local site by ACh has been shown to also spread along the endothelium [63,64]. However, this calcium wave has a slow conduction velocity and does not contribute to the long distance conducted vasodilation beyond 0.5 mm [63,64]. In contrast, long distance conducted vasodilations were dependent on an electrical response generated by the opening at the local site of calcium-dependent potassium channels (K_{Ca}) [27], specifically small and intermediate conductance subtypes, genetic deletion of which attenuated both local and conducted responses [4,70].

In summary, focal application of ACh induces a local endothelial hyperpolarization through activation of small and intermediate conductance K_{Ca} channels. This hyperpolarization causes smooth muscle relaxation, either via direct current transfer through myoendothelial gap junctions [17,23] and/or via generation of a diffusible factor that activates potassium channels in smooth muscle cells [14]. Either or both these events produce smooth muscle hyperpolarization and closure of voltage-dependent calcium channels [44], lowering smooth muscle calcium concentration to induce vasodilation [31,57,71]. Spread of the hyperpolarization over distance induces a synchronous relaxation as voltage-dependent calcium channels in the smooth muscle close along the vessel wall.

WHICH CELL LAYER IS RESPONSIBLE FOR CONDUCTION OF VASODILATION AND VASOCONSTRICTION?

Localized disruption of the smooth muscle cell layer (~0.3 mm long) of cheek pouch arterioles *in vivo* with light dye damage had no effect on conducted vasodilation, nor, surprisingly, did disruption of the endothelial cell layer, leading the authors to conclude that vasodilation could conduct through either cell layer [2]. In contrast, in microcirculatory arterioles of the cremaster muscle *in vivo* [41,58,69] and in isolated, pressurized feed arteries, which supply the retractor muscle of the hamster cheek pouch [16,18] and in larger vessels of the mesenteric circulation

in vitro [22,63], selective disruption of the endothelial cell layer, either mechanically or with light dye damage, completely abolished conduction of vasodilation. In these vessels, then, vasodilation was conducted upstream only through the endothelium.

Results of investigations into the conduction of vasoconstriction have been more controversial. Early studies in the microcirculation of the hamster cheek pouch *in vivo* demonstrated that vasoconstriction could propagate over several millimeters, in a similar manner to vasodilation [57]. However, in contrast to the long distance spread of vasodilation, the conduction of vasoconstriction was reported to only occur in 50% of preparations [57]. This conduction occurred selectively in the smooth muscle cell layer, since it was abolished by light dye damage to the smooth muscle, but not the endothelial cell layer [2]. Membrane potential recordings in cheek pouch arterioles showed that large depolarizations in smooth muscle cells were evoked by application of either norepinephrine or phenylephrine and these membrane potential responses also conducted to sites 0.5 mm upstream [67].

However, *in vivo* studies of feed arteries and arterioles of the hamster retractor muscle controlling the cheek pouch and of the cremaster skeletal muscle produced diametrically opposite results; vasoconstrictor responses to either norepinephrine or a depolarizing high KCl solution were confined to the local site of application, without any evidence of upstream spread [29,59]. In contrast, vasodilation in these same vessels conducted upstream, as previously described [29,59]. The basis for this differential ability to conduct vasoconstriction and vasodilation in feed arteries has subsequently been attributed to differential conduction of responses in the smooth muscle and endothelial cell layers, respectively [11,65]. Using a computational model of the vascular wall, previous studies have shown that focal depolarization of smooth muscle cells in the vascular wall does not conduct effectively in the smooth muscle layer, due to limited smooth muscle cell coupling [26,72] and the transverse orientation of the smooth muscle cells; both factors acting to increase the longitudinal coupling resistance of this layer [11,65]. Moreover, due to the mismatch in input resistance of the highly coupled endothelial cell layer and the relatively uncoupled smooth muscle cell layer [72], the amount of current required locally to depolarize the smooth muscle cells may well be insufficient to produce appreciable change in the membrane potential of the low resistance endothelial cell layer, after spread through myoendothelial gap junctions, even if the two cell layers are well coupled [11,33]. Interestingly, subsequent experiments showed that the apparent depolarization produced by focal application of phenylephrine in pressurized feed arteries *in vitro* was in fact a movement artifact and that this agonist produced a constriction which was solely dependent on

release of calcium from intracellular stores [65]. Such voltage-independent constrictions would understandably fail to result in any conducted responses.

Why localized vasoconstriction of cheek pouch arterioles did propagate over distance and whether movement artifacts also contributed to the large depolarizations recorded in these vessels [67] remains unknown. Since the light dye damage experiments showed that vasodilation could conduct through a localized region of endothelial damage [2], perhaps the coupling of smooth muscle cells in these arterioles is significantly higher than that in other microcirculatory beds, thereby enabling transfer of sufficient current from the endothelium to smooth muscle, and back. Certainly, myoendothelial gap junctions are abundant in these arterioles [52] and the current source, after endothelial stimulation and transfer into the smooth muscle layer, may still be sufficiently high to produce an endothelial voltage change beyond the site of cell damage. However, computational modeling has shown that the transverse orientation of the smooth muscle cells would still prevent conduction occurring through the smooth muscle over distances equivalent to those achieved through the longitudinally oriented endothelial cells, even if coupling resistances were equally low in both layers [11].

Taken together, these studies underscore the crucial role of the endothelium in the long distance conduction of electrical responses, whether depolarization or hyperpolarization. Any membrane potential response initiated focally in the smooth muscle layer would fail to propagate substantially within this layer, due to cell orientation and the high coupling resistance amongst the muscle cells. Although conduction of depolarization could occur through the endothelium following current transfer through myoendothelial gap junctions, this would only happen if a sufficiently large enough number of smooth muscle cells were activated and sufficient myoendothelial coupling existed, so as to generate the larger current required to change the membrane potential of the well-coupled endothelial cells [11,33,65]. By the same argument, a large endothelial-initiated voltage response would only be generated by activating a substantial number of endothelial cells in this low resistance pathway, although an equivalent voltage response could be generated in the poorly coupled smooth muscle cells with a substantially smaller endothelial stimulus.

ARE MYOENDOTHELIAL GAP JUNCTIONS FUNCTIONAL *IN VIVO*?

Coupling of endothelial and smooth muscle cells via myoendothelial gap junctions has been demonstrated ultrastructurally in many different vascular beds [15,26,50–52] and electrical conductivity confirmed in isolated vessels *in vitro* [17,53]. However, experiments

conducted *in vivo* have suggested that these junctions may not normally be functional.

In hamster cheek pouch arterioles, the time course of ACh-induced hyperpolarization recorded in smooth muscle was reported to be longer than that recorded in endothelial cells, although resting membrane potentials of cells in the two layers did not vary [67]. However, this observation may not be definitive proof of a lack of myoendothelial coupling since the differential form of the potentials would be expected to result from the differential input resistance of the two cell layers [11]. In contrast, *in vivo* studies in the same cheek pouch arterioles, under conditions of vasomotion, showed similar oscillatory membrane potentials in dye identified smooth muscle and endothelial cells, suggestive of electrical coupling [55].

A lack of functional myoendothelial coupling has also been suggested to occur in mouse cremaster arterioles *in vivo*, since both the resting membrane potential and ion channels contributing to ACh-induced hyperpolarization of smooth muscle and endothelial cells differed [60]. However, in these studies, diameter and membrane potential were not recorded in the same vessels and endothelial cell coupling, in addition to myoendothelial coupling, appeared to be impaired, as dye was not reported to spread between adjacent endothelial cells [60]. This observation contrasts markedly with the rapid spread of dye along the endothelium found previously in many studies of arteries and arterioles, both *in vivo* and *in vitro* [16,17,55,67], as well as the essential role of the endothelium for conduction of electrical signals, discussed above. Thus, the techniques used to facilitate electrode recordings in this study may have led to an artifactual electrical isolation of impaled smooth muscle and endothelial cells. Indeed, recent studies in the same murine cremaster preparation *in vivo*, have provided evidence in favor of electrotonic coupling of the two cell layers. Not only were membrane potentials of both smooth muscle and endothelial cells always in register, but the hyperpolarizing responses to both focal and bath applied ACh were also identical in both cell types, although recordings in the two cell types were not made simultaneously [69]. In these cremaster arterioles, the two layers were confirmed at the ultrastructural level to be highly coupled by myoendothelial gap junctions [69].

Nevertheless, if the two cell layers of vessels were not electrically coupled, it would be important to show that the transfer of endothelial hyperpolarization into the smooth muscle layer could result from release from the endothelium of a chemical endothelium derived hyperpolarizing factor or EDHF. One proposal for such a chemical EDHF involves the epoxyeicosatrienoic acid metabolites of the cytochrome P450 pathway [20]. In pressurized hamster feed arteries *in vitro*, in which the two cell layers are electrically coupled, 17-ODYA was without effect on conducted

vasodilation to ACh [17], consistent with a role for electrotonic coupling. In contrast, in cheek pouch and cremaster arterioles *in vivo*, 17-ODYA attenuated vasodilation to ACh both locally and at remote sites [27,29,68], although reduction in the local stimulus might be expected to result in diminished conduction, even in the presence of functional myoendothelial coupling [69]. 17-ODYA was also reported to inhibit ACh-induced hyperpolarization of smooth muscle cells, but not endothelial cells, and this was taken as proof that myoendothelial coupling was absent in these *in vivo* preparations [68]. However, it should be noted that in these studies, 17-ODYA was applied for 45 minutes in the presence of elevated oxygen in the superfusate, a condition previously reported to lead to uncoupling of the two cell layers [66]. Therefore, in these *in vivo* experiments, the two cell layers may have been spuriously uncoupled by the pharmacological incubation procedure.

From the foregoing discussion, we conclude that the evidence to date does not support the conclusion that conduction of vasodilation can occur if the two cell layers *in vivo* are uncoupled. Admittedly, there is limited data comparing conduction in vessels from the same vascular bed, measured *in vivo* and *in vitro*, however, it is difficult to see what the stimulus for release of a chemical at the distal sites could be. It cannot be calcium spread from the local site, since the calcium wave does not conduct over long distance [63,64], and there is no evidence that the conducted hyperpolarization itself can increase intracellular calcium levels in endothelial cells to promote the distal release of an endothelial vasodilator [61]. We therefore favor the conclusion that, where myoendothelial gap junctions can be demonstrated anatomically, they appear to be functionally open *in vivo*.

THE PROBLEM

To summarize, the mechanism underlying the coordination of resistance of upstream feed arteries with microvessels in metabolically active tissues relies on the initiation and electrotonic conduction of hyperpolarization along the endothelium. Electrical coupling via myoendothelial gap junctions, in turn, enables the rapid transfer of this hyperpolarization into the smooth muscle layer, resulting in closure of voltage-dependent calcium channels, decrease in intracellular calcium and synchronous relaxation along the vessel wall.

However, the problem arises that such an electrical signal would be expected to decay with distance from the stimulation site, due to loss of current through open ion channels in the membranes of both endothelial and smooth muscle cells, and also current loss at the sites of cell to cell contact along the axial conduction pathway. Consequently, if vasodilation is directly proportional to the underlying

hyperpolarization, one would expect the associated vasodilation to also decay with distance from the site of stimulation. As this clearly does not occur [9,16,29,68], it has been suggested that a regenerative electrical event acts to boost the underlying hyperpolarization as it is conducted along the vascular wall. Only one ion channel has been described to be activated by hyperpolarization; the inwardly rectifying potassium channel, K_{IR} [48]. The possible involvement of this channel in a regenerative electrical event will be discussed in the next section.

An alternative regenerative mechanism, based on the activity of voltage-dependent sodium and calcium channels located in the endothelium, has also been proposed. This latter mechanism requires that regeneration of hyperpolarization occurs as a result of stretch-dependent activation of voltage-dependent sodium channels, calcium influx through voltage-dependent calcium channels expressed in the endothelium and concomitant activation of K_{Ca} channels [19]. Although an interesting concept, there is little evidence in the literature for the expression and function of voltage-dependent sodium channels in endothelial cells of resistance arteries under physiological conditions [46], and no electrophysiological evidence for an initial rapid depolarization preceding the hyperpolarization, either in this study or any other study. An additional important failing of this study lies in the dismissal of the involvement of perivascular nerves in the conducted responses measured therein. This conclusion was based solely on the lack of effect of the α_1 -adrenoceptor antagonist, prazosin [19], and completely overlooked the vast literature on the multiplicity of neurotransmitters released by perivascular sympathetic nerves and their ability to elicit vasodilation, in addition to vasoconstriction [5,21,26]. It is also likely that transmural electrical stimulation could evoke vasodilation through release of Substance P and CGRP from sensory motor nerves [21,24,26]. Indeed, the finding in this study that tetrodotoxin blocked the conducted responses would support the conclusion that a neural pathway was involved, since insensitivity to tetrodotoxin has been used previously as the gold standard to eliminate a role for perivascular nerves in conducted responses [57,71]. Taken together, the data provide little convincing evidence that this regenerative mechanism operates in the microcirculation.

CAN K_{IR} CHANNELS PROVIDE THE ELECTRICAL REGENERATION NECESSARY FOR LONG DISTANCE CONDUCTION OF VASODILATION?

The most plausible mechanism suggested to provide electrical regeneration of the conducted hyperpolarization involves K_{IR} channels. These channels are blocked by micromolar concentrations of barium ions, activated by

hyperpolarization and most readily pass inward currents at potentials more negative than the equilibrium potential for potassium (E_K), giving rise to the channel name. However, at membrane potentials more depolarized than E_K (~ -90 mV), K_{IR} channels sustain small outward currents [25]. Since the resting membrane potential of smooth muscle and endothelial cells measured in vessels *in vivo* lies between ~ -30 and -40 mV [67,69], within the range of potentials over which K_{IR} channels show negative slope conductance, these channels have the potential to facilitate hyperpolarization. However, this effect is maximal around -50 mV, beyond which the slope is positive; hyperpolarization leading to a decrease in K_{IR} current [33].

The first suggestion that K_{IR} channels could contribute to conduction of vasodilation came from a study of pressurized coronary arterioles *in vitro* [49]. Previous studies in coronary and cerebral arteries had demonstrated that K_{IR} channels were responsible for hyperpolarization and vasodilation in response to small increases in $[K^+]_o$ and that K_{IR} currents were expressed in isolated coronary smooth muscle cells [35,47]. Although barium significantly reduced both local and remote vasodilation to bradykinin and KCl in pressurized coronary arterioles, a role for K_{IR} channels in the conduction process was concluded from the attenuation by barium of remote, but not local responses to adenosine [49].

However, other studies have suggested that the role of K_{IR} channels in vasodilatory responses varies with vessel size and cellular site of expression of the channels themselves. Thus, in the renal, retinal and coronary circulations, K_{IR} current density was greater in myocytes from smaller, more distal, vessels [6,42,47]. Other studies have provided evidence that the expression of these channels can vary between the endothelium and the smooth muscle. In cremaster arterioles *in vivo*, where K_{IR} channels are expressed abundantly in the endothelium (Figure 1), barium had no effect on conduction of vasodilation to ACh, but did attenuate conducted responses to adenosine [8]. Similarly, in mesenteric arteries with endothelial K_{IR} channel expression [7,12,22], the effect of barium in reducing conducted responses to ACh resulted from an action at the local site, without effect on the rate of decay of the conducted membrane potential and diameter changes [22], although membrane potential and diameter measurements were not made in the same vessels in this study. In contrast, K_{IR} channels are expressed in the smooth muscle of retractor feed arteries, where conducted, but not local vasodilatory responses were significantly attenuated following treatment with barium [32].

A subsequent study in comparably sized coronary, mesenteric and cerebral arteries showed that if K_{IR} channels were expressed in the smooth muscle, as in the coronary and cerebral arteries, but not in the endothelium

Figure 1. Expression of Kir2.1 channels in cremaster arterioles and mesenteric arteries. **(A)** Transverse sections show staining in the endothelium (EC) of cremaster arterioles. **(B)** Staining reduced by preincubation with the antigenic peptide. **(C,D)** Whole mount preparations of the cremaster circulation, showing uniform expression of Kir channels throughout the endothelium bounded by the internal elastic lamina (IEL; red), but not in the smooth muscle cells (SMCs), lying immediately outside the IEL. **(E,F)** Transverse sections of mesenteric arteries showing K_{IR} staining in ECs and abolition of staining following preincubation in antigen.

(mesenteric arteries), then they could act as amplifiers of vasodilatory responses initiated in either cell layer [62]. The selective effects of barium on responses to adenosine, but not ACh or bradykinin, in cremaster and coronary arterioles are more difficult to explain. Even in the event that K_{IR} channels are also expressed in the smooth muscle layer in cremaster arterioles; below the level of detection of the light microscope (Figure 1); any endothelial derived hyperpolarization, whether conducted chemically or electrically into the smooth muscle, should also be amplified by K_{IR} channels expressed in the smooth muscle cells of these vessels.

To summarize, K_{IR} channels are heterogeneously expressed amongst arteries and arterioles and their component cell layers. Although these channels have the potential to augment hyperpolarization initiated in the endothelium, and this could assist in extending the distance over which vasodilation can spread, vasodilations can still occur over

long distances through the endothelium in microcirculatory arterioles, even in the absence of any contribution from these channels [8]. Thus, it is unlikely that K_{IR} channels could provide a unifying mechanism to account for the un-attenuated long distance endothelial conduction of vasodilation across vascular beds.

WHAT OTHER MECHANISMS EXIST TO PROMOTE CONDUCTION OF VASODILATION?

A clue to a more generalized mechanism which could impact on responses in different vessels was provided from studies of membrane potential changes underlying the myogenic response which controls vascular tone of resistance sized vessels [36]. In pressurized skeletal muscle arterioles, the entire myogenic response to elevation of intraluminal pressure was accompanied by a depolarization

of only ~ 10 mV [36]. This remarkably tight relationship between membrane potential and vascular tone suggested that a voltage-dependent vasodilation could also be elicited by a correspondingly small hyperpolarization of the membrane potential. Since the long distance conduction of vasodilation depended on conduction of hyperpolarization along the vessel wall and closure of voltage-dependent calcium channels, it seemed reasonable to assume that maximal dilation would occur at membrane potentials corresponding to the activation potential of the underlying voltage-dependent calcium channels.

We therefore hypothesized that the unimpaired spread of vasodilation in microcirculatory networks resulted from restriction in the linear relationship between membrane potential and diameter to a narrow voltage window, beyond which additional hyperpolarization did not produce further vasodilation (Figure 2). The aim of our studies was to test this hypothesis by making simultaneous recordings of electrical and mechanical responses in fully functional arterioles *in vivo*, at both the local site and at 1500 μm upstream in response to focal application of ACh (Figure 3A) [69]. Results showed that hyperpolarizations decayed passively with distance, whereas vasodilation spread upstream without attenuation (Figure 3B–D) [69]. When all simultaneous recordings of membrane potential and vessel tone were analyzed, we discovered that these two variables were only linearly related over a restricted voltage range, from the resting membrane potential at -28 mV, to -35 mV, beyond which dilation was maximal [69].

In contrast to our data, previous studies had described a linear relationship between these two parameters [17,67]. However, in one of these studies, the relationship was investigated over a very narrow voltage window of 10 mV

Figure 2. Model to explain the long distance conduction of vasodilation. Hyperpolarization attenuates electrotonically with distance from the focal site of stimulation with ACh. Due to the nonlinear relationship of membrane potential and vasodilation, no attenuation of dilation is seen until the amplitude of hyperpolarization is reduced below the threshold potential for activation of voltage-dependent calcium channels. Reproduced from [69].

near rest [17], where we would expect the relationship to be linear. In the other study, the conclusion was compromised by three important issues. At the local site, pharmacomechanical effects of nitric oxide and prostaglandins were not inhibited, thereby distorting the relationship between membrane potential and dilation. Of course, this membrane potential independent effect at the local site would not contribute to responses at the distal site, where a linear relationship between membrane potential and change in diameter was still apparent (open symbols, figure 6 in [67]). However, the data at the remote site was obtained from recordings to stimulus durations ranging from 150 to 2000 ms (Figure 6 in [67]). Since the amplitude of dilation also varies with stimulus duration [69] and it is not clear what stimulation durations are represented in Figure 6 [67], nor which points represent which durations, it is impossible to draw a clear conclusion in regard to the relationship between membrane potential and dilation at the distal site. Furthermore, the changes in diameter in Figure 6 are expressed in terms of micrometers and hence do not take into account the fact that vessels in and between preparations vary in diameter and also in the degree of resting tone. Therefore, we do not know how many of the points represent maximal dilation. The very large spread in diameter change for membrane potential changes >10 mV in Figure 6 [67] may well reflect the impact of these various issues.

To interrogate whether our experimental data could be explained by electrotonic conduction of hyperpolarization, we developed a computational model of the vascular wall. In this model the two cell layers were represented by parallel electrical cables connected via myoendothelial gap junctions. The current–voltage relationships of the smooth muscle and endothelial cells were taken from the literature, as were estimates of coupling resistances both within and between the two cell layers [69]. Anatomical parameters of the cells and the vessel wall were obtained from direct measurements in the vessels under study. Using this model, it was found that the observed decay of the hyperpolarization with distance from the site of stimulation could be simulated by allowing the initial response to decay electrotonically with distance (Figure 3C,E) [69]. In contrast, the unattenuated conduction of vasodilation could only be mimicked by applying the experimentally determined threshold potential (Figure 3D,F) [69]. Proof of principle of this model was provided by reducing the amplitude of the local hyperpolarization through change in the duration of the focal stimulus [69]. Under these conditions, it was expected that the threshold value would be reached closer to the local site and the conducted vasodilation would therefore show attenuation. As predicted, the hyperpolarization decayed with similar slope to the remote site, but entered the linear voltage range closer to the focal

Figure 3. Nonlinear relationship between membrane potential and vessel tone explains the unattenuated conduction of dilation. **(A)** Experimental setup for paired recordings of simultaneous membrane potential and diameter measurements which were made at the site of ACh application and 1500 μm upstream in the same vessel after movement of the intracellular microelectrode. **(B)** Representative traces of paired recordings at the local site (0) and at 1500 μm in the same vessel show decrement in the amplitude of hyperpolarization but not dilation with distance. **(C,D)** Summary data showing that hyperpolarization, but not dilation, decays over 1500 μm ($n = 7$ cell pairs/7 animals). Mean $\pm$ SEM, * $p < 0.05$ vs. local. **(E,F)** Membrane potential data could be modeled by the electrotonic decay of the local hyperpolarization with distance **(E)**, whereas the unattenuated dilation could only be mimicked by application of a threshold. (-35 mV), but not if a threshold was not applied (NT; Panel **F**). ECs open triangles; SMCs closed triangles. Reproduced from [69].

stimulation site, and the vasodilation was significantly reduced at the remote site (Figure 4A,B) [69]. This experimental situation demonstrated that a decrease in the amplitude of the hyperpolarization at the local site could result in attenuation of the conducted vasodilation.

Additional experiments demonstrated that a nonlinear relationship between diameter and membrane potential also existed under steady state conditions in which vasodilation induced by ACh was maximal [69]. In this case the threshold potential was shifted to slightly more negative

Figure 4. Attenuation of conducted vasodilation following reduction in the local stimulus can be predicted by application of a threshold. **(A)** After reduction of stimulation duration from 1 to 0.5 seconds, the evoked hyperpolarization decayed over 1500 μm to values which were more depolarized than the threshold potential of -35 mV. **(B)** This resulted in a decay of vasodilation over the same distance ($n = 4$ cell pairs/4 mice). Mean $\pm$ SEM, $\#p < 0.05$ vs. 1 second stimulus. Reproduced from [69].

potentials to account for the increase in amplitude of the relaxation. This simple nonlinear mechanism could also explain the decay of vasodilation with distance observed in multilayered mesenteric arteries when the anatomical parameters of the model were changed to those measured in these larger vessels [22,63,69]. Taken together, the data showed that conducted dilations spread without attenuation, until the underlying hyperpolarization had decayed to a threshold value, beyond which dilation decayed linearly with hyperpolarization.

WHAT DETERMINES THE THRESHOLD POTENTIAL?

The contribution of L-type voltage-dependent calcium channels to myogenic tone has long been acknowledged in both cerebral and systemic circulations [34,43]. These channels show a steep relationship between membrane potential and calcium entry with threshold potential for activation around -40 mV and peak current at 0 mV [28]. However, other members of the voltage-dependent calcium channel family, including the high voltage activated P/Q- and N-subtypes and the low voltage activated T-types, have recently been shown to be expressed in renal, mesenteric and cerebral circulations and implicated in vasomotor responses, particularly in small arteries and arterioles [39]. A contribution to vascular tone from non L-type channels has also been demonstrated in cremaster arterioles *in vivo* and *in vitro* [36,69]. The involvement of multiple voltage dependent calcium channel subtypes, with different activation properties, might explain the previously reported heterogeneity between cerebral and skeletal muscle vessels in

the voltage range over which active myogenic tone develops [34,36]. In order to probe the impact of this potential variability on the ability of vascular networks to conduct vasodilation over distance, future studies need to identify and characterise the voltage dependency of the calcium channel subtypes contributing to vascular tone in different vascular beds and correlate this data with experimentally determined threshold potentials for conducted vasodilation.

CONCLUSION

Vasodilations initiated locally in the microcirculation of metabolically active tissues spread rapidly upstream with relatively little attenuation to larger feed arteries, to coordinate resistance and facilitate increases in blood flow. Review of the literature confirms that this phenomenon is underpinned by conduction of hyperpolarization along the endothelium and electrotonic transmission through myoendothelial gap junctions into the smooth muscle (see also [1]). Analysis of the relationship between membrane potential and vasodilation measured in cremaster microcirculatory arterioles *in vivo* showed that a linear relationship between these two parameters only existed to a threshold potential, which equated to the activation potential for the underlying voltage-dependent calcium channels. Beyond this threshold potential, vasodilation was maximal. This mechanism thus minimizes the impact of the electrotonic decay of voltage with distance, since any increase in the amplitude of the local hyperpolarization beyond the threshold would increase the distance over which vasodilation could spread.

PERSPECTIVE

The ability of microcirculatory networks to adjust blood flow rapidly to match changes in local metabolic demand is essential for the maintenance of optimal organ function. If the mechanism described above is important, then changes in expression of the subtypes of voltage dependent calcium channels underlying vasoconstriction, or reduction in cell coupling in the endothelium, could impact on the efficacy of this vital response and hence compromise organ function. Such changes in expression of calcium channel subtypes and endothelial coupling have indeed been reported to occur during vasospastic conditions [30,45] and sepsis [3,40], respectively. Future strategies aimed at targeting these changes may assist in improving organ function in these and a range of other pathophysiological conditions.

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REFERENCES

1. Bagher P, Segal SS. Regulation of blood flow in the microcirculation: role of conducted vasodilation. *Acta Physiol (Oxford, England)* 202: 271–284, 2011.
2. Bartlett IS, Segal SS. Resolution of smooth muscle and endothelial pathways for conduction along hamster cheek pouch arterioles. *Am J Physiol* 278: H604–H612, 2000.
3. Bolon ML, Peng T, Kidder GM, Tymk K. Lipopolysaccharide plus hypoxia and reoxygenation synergistically reduce electrical coupling between microvascular endothelial cells by dephosphorylating connexin40. *J Cell Physiol* 217: 350–359, 2008.
4. Brahler S, Kaistha A, Schmidt VJ, Wolfle SE, Busch C, Kaistha BP, Kacik M, Hase-nau AL, Grgic I, Si H, Bond CT, Adelman JP, Wulff H, de Wit C, Hoyer J, Kohler R. Genetic deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation* 119: 2323–2332, 2009.
5. Burnstock G, Ralevic V. New insights into the local regulation of blood flow by perivascular nerves and endothelium. *Br J Plastic Surg* 47: 527–543, 1994.
6. Chilton L, Smirnov SV, Loutzenhiser K, Wang X, Loutzenhiser R. Segment-specific differences in the inward rectifier K⁺ current along the renal interlobular artery. *Cardiovasc Res* 92: 169–177, 2011.
7. Crane GJ, Walker SD, Dora KA, Garland CJ. Evidence for a differential cellular distribution of inward rectifier K channels in the rat isolated mesenteric artery. *J Vasc Res* 40: 159–168, 2003.
8. de Wit C. Different pathways with distinct properties conduct dilations in the microcirculation *in vivo*. *Cardiovasc Res* 85: 604–613, 2010.
9. de Wit C, Roos F, Bolz SS, Kirchhoff S, Kruger O, Willecke K, Pohl U. Impaired conduction of vasodilation along arterioles in connexin40-deficient mice. *Circ Res* 86: 649–655, 2000.
10. Delashaw JB, Duling BR. Heterogeneity in conducted arteriolar vasomotor response is agonist dependent. *Am J Physiol* 260: H1276–H1282, 1991.
11. Diep HK, Vigmond EJ, Segal SS, Welsh DG. Defining electrical communication in skeletal muscle resistance arteries: a computational approach. *J Physiol* 568: 267–281, 2005.
12. Doughty JM, Boyle JP, Langton PD. Blockade of chloride channels reveals relaxations of rat small mesenteric arteries to raised potassium. *Br J Pharmacol* 132: 293–301, 2001.
13. Duling BR, Berne RM. Propagated vasodilation in the microcirculation of the hamster cheek pouch. *Circ Res* 26: 163–170, 1970.
14. Edwards G, Feletou M, Weston AH. Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. *Pflugers Arch* 459: 863–879, 2010.
15. Ellis A, Goto K, Chaston DJ, Brackenbury TD, Meaney KR, Falck JR, Wojcikiewicz RJ, Hill CE. Enalapril treatment alters the contribution of epoxyeicosatrienoic acids but not gap junctions to endothelium-derived hyperpolarizing factor activity in mesenteric arteries of spontaneously hypertensive rats. *J Pharmacol Exp Ther* 330: 413–422, 2009.
16. Emerson GG, Segal SS. Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery. *Circ Res* 86: 94–100, 2000.
17. Emerson GG, Segal SS. Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. *Circ Res* 87: 474–479, 2000.
18. Emerson GG, Segal SS. Electrical activation of endothelium evokes vasodilation and hyperpolarization along hamster feed arteries. *Am J Physiol* 280: H160–H167, 2001.
19. Figueroa XF, Chen CC, Campbell KP, Damon DN, Day KH, Ramos S, Duling BR. Are voltage-dependent ion channels involved in the endothelial cell control of vasomotor tone? *Am J Physiol* 293: H1371–H1383, 2007.
20. Fleming I. Cytochrome P450 epoxygenases as EDHF synthase(s). *Pharmacol Res* 49: 525–533, 2004.
21. Gibbins IL, Morris JL. Pathway specific expression of neuropeptides and autonomic control of the vasculature. *Regul Peptides* 93: 93–107, 2000.
22. Goto K, Rummery NM, Grayson TH, Hill CE. Attenuation of conducted vasodilation in rat mesenteric arteries during hypertension: role of inwardly rectifying potassium channels. *J Physiol* 561: 215–231, 2004.
23. Griffith TM. Endothelium-dependent smooth muscle hyperpolarization: do gap junctions provide a unifying hypothesis? *Br J Pharmacol* 141: 881–903, 2004.
24. Haddock RE, Hill CE. Sympathetic overdrive in obesity involves purinergic hyperactivity in the resistance vasculature. *J Physiol* 589: 3289–3307, 2011.
25. Hill CE. Inward rectification and vascular function: as it was in the beginning. *J Physiol* 586: 1465–1467, 2008.
26. Hill CE, Phillips JK, Sandow SL. Heterogeneous control of blood flow amongst different vascular beds. *Med Res Rev* 21: 1–60, 2001.
27. Hoepfl B, Rodenwaldt B, Pohl U, De Wit C. EDHF, but not NO or prostaglandins, is critical to evoke a conducted dilation upon ACh in hamster arterioles. *Am J Physiol* 283: H996–H1004, 2002.
28. Hu H, Marban E. Isoform-specific inhibition of L-type calcium channels by dihydropyridines is independent of isoform-specific gating properties. *Mol Pharmacol* 53: 902–907, 1998.
29. Hungerford JE, Sessa WC, Segal SS. Vasomotor control in arterioles of the mouse cremaster muscle. *Faseb J* 14: 197–207, 2000.
30. Ishiguro M, Wellman TL, Honda A, Russell SR, Tranmer BJ, Wellman GC. Emergence of a R-type Ca²⁺ channel (Ca_v 2.3) contributes to cerebral artery constriction after subarachnoid hemorrhage. *Circ Res* 96: 419–426, 2005.
31. Jackson WF. Potassium channels in the peripheral microcirculation. *Microcirculation* 12: 113–127, 2005.
32. Jantzi MC, Brett SE, Jackson WF, Corteling R, Vigmond EJ, Welsh DG. Inward rectifying potassium channels facilitate cell-to-cell communication in hamster retractor muscle feed arteries. *Am J Physiol* 291: H1319–H1328, 2006.
33. Kapela A, Nagaraja S, Tsoukias NM. A mathematical model of vasoreactivity in rat mesenteric arterioles. II. Conducted vasoreactivity. *Am J Physiol* 298: H52–H65, 2010.
34. Knot HJ, Nelson MT. Regulation of arterial diameter and wall [Ca²⁺] in cerebral arteries of rat by membrane potential and intravascular pressure. *J Physiol* 508: 199–209, 1998.
35. Knot HJ, Zimmermann PA, Nelson MT. Extracellular K⁺-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K⁺ channels. *J Physiol* 492: 419–430, 1996.

36. Kotecha N, Hill MA. Myogenic contraction in rat skeletal muscle arterioles: smooth muscle membrane potential and Ca(2+) signaling. *Am J Physiol* 89: H1326–H1334, 2005.
37. Krogh A. Studies on the capillariometer mechanism: I. The reaction to stimuli and the innervation of the blood vessels in the tongue of the frog. *J Physiol* 53: 399–419, 1920.
38. Krogh A, Harrop GA, Rehberg PB. Studies on the physiology of capillaries: III. The innervation of the blood vessels in the hind legs of the frog. *J Physiol* 56: 179–189, 1922.
39. Kuo IY, Wolfle SE, Hill CE. T-type calcium channels and vascular function: the new kid on the block? *J Physiol* 589: 783–795, 2011.
40. Lidington D, Ouellette Y, Tyml K. Endotoxin increases intercellular resistance in microvascular endothelial cells by a tyrosine kinase pathway. *J Cell Physiol* 185: 117–125, 2000.
41. Looft-Wilson RC, Payne GW, Segal SS. Connexin expression and conducted vasodilation along arteriolar endothelium in mouse skeletal muscle. *J Appl Physiol* 97: 1152–1158, 2004.
42. Matsushita K, Puro DG. Topographical heterogeneity of K(IR) currents in pericyte-containing microvessels of the rat retina: effect of diabetes. *J Physiol* 573: 483–495, 2006.
43. Moosmang S, Schulla V, Welling A, Feil R, Feil S, Wegener JW, Hofmann F, Klugbauer N. Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. *EMBO J* 22: 6027–6034, 2003.
44. Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 259: C3–C18, 1990.
45. Nikitina E, Kawashima A, Takahashi M, Zhang ZD, Shang X, Ai J, Macdonald RL. Alteration in voltage-dependent calcium channels in dog basilar artery after subarachnoid hemorrhage. Laboratory investigation. *J Neurosurg* 113: 870–880, 2010.
46. Nilius B, Droogmans G. Ion channels and their functional role in vascular endothelium. *Physiol Rev* 81: 1415–1459, 2001.
47. Quayle JM, Dart C, Standen NB. The properties and distribution of inward rectifier potassium currents in pig coronary arterial smooth muscle. *J Physiol* 494: 715–726, 1996.
48. Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev* 77: 1165–1232, 1997.
49. Rivers RJ, Hein TW, Zhang C, Kuo L. Activation of barium-sensitive inward rectifier potassium channels mediates remote dilation of coronary arterioles. *Circulation* 104: 1749–1753, 2001.
50. Sandow SL, Bramich NJ, Bandi HP, Rummary NM, Hill CE. Structure, function, and endothelium-derived hyperpolarizing factor in the caudal artery of the SHR and WKY rat. *Arterioscler Thromb Vasc Biol* 23: 822–828, 2003.
51. Sandow SL, Haddock RE, Hill CE, Chadha PS, Kerr PM, Welsh DG, Plane F. What's where and why at a vascular myoendothelial microdomain signalling complex. *Clin Exp Pharmacol Physiol* 36: 67–76, 2009.
52. Sandow SL, Looft-Wilson R, Doran B, Grayson TH, Segal SS, Hill CE. Expression of homocellular and heterocellular gap junctions in hamster arterioles and feed arteries. *Cardiovasc Res* 60: 643–653, 2003.
53. Sandow SL, Tare M, Coleman HA, Hill CE, Parkinson HC. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ Res* 90: 1108–1113, 2002.
54. Segal SS. Regulation of blood flow in the microcirculation. *Microcirculation* 12: 33–45, 2005.
55. Segal SS, Beny JL. Intracellular recording and dye transfer in arterioles during blood flow control. *Am J Physiol* 263: H1–H7, 1992.
56. Segal SS, Duling BR. Flow control among microvessels coordinated by intercellular conduction. *Science (New York)* 234: 868–870, 1986.
57. Segal SS, Duling BR. Conduction of vasomotor responses in arterioles: a role for cell-to-cell coupling? *Am J Physiol* 256: H838–H845, 1989.
58. Segal SS, Jacobs TL. Role for endothelial cell conduction in ascending vasodilation and exercise hyperaemia in hamster skeletal muscle. *J Physiol* 536: 937–946, 2001.
59. Segal SS, Welsh DG, Kurjiaka DT. Spread of vasodilatation and vasoconstriction along feed arteries and arterioles of hamster skeletal muscle. *J Physiol* 516: 283–291, 1999.
60. Siegl D, Koeppen M, Wolfle SE, Pohl U, de Wit C. Myoendothelial coupling is not prominent in arterioles within the mouse cremaster microcirculation *in vivo*. *Circ Res* 97: 781–788, 2005.
61. Silva HS, Kapela A, Tsoukias NM. A mathematical model of plasma membrane electrophysiology and calcium dynamics in vascular endothelial cells. *Am J Physiol* 293: C277–C293, 2007.
62. Smith PD, Brett SE, Luykenaar KD, Sandow SL, Marrelli SP, Vigmond EJ, Welsh DG. KIR channels function as electrical amplifiers in rat vascular smooth muscle. *J Physiol* 586: 1147–1160, 2008.
63. Takano H, Dora KA, Spitaler MM, Garland CJ. Spreading dilatation in rat mesenteric arteries associated with calcium-independent endothelial cell hyperpolarization. *J Physiol* 556: 887–903, 2004.
64. Tallini YN, Brekke JF, Shui B, Doran R, Hwang SM, Nakai J, Salama G, Segal SS, Kotlikoff MI. Propagated endothelial Ca²⁺ waves and arteriolar dilation *in vivo*: measurements in Cx40BAC GCaMP2 transgenic mice. *Circ Res* 101: 1300–1309, 2007.
65. Tran CH, Vigmond EJ, Plane F, Welsh DG. Mechanistic basis of differential conduction in skeletal muscle arteries. *J Physiol* 587: 1301–1318, 2009.
66. Welsh DG, Jackson WF, Segal SS. Oxygen induces electromechanical coupling in arteriolar smooth muscle cells: a role for L-type Ca²⁺ channels. *Am J* 274: H2018–H2024, 1998.
67. Welsh DG, Segal SS. Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. *Am J Physiol* 274: H178–H186, 1998.
68. Welsh DG, Segal SS. Role of EDHF in conduction of vasodilation along hamster cheek pouch arterioles *in vivo*. *Am J Physiol Heart Circ Physiol* 278: H1832–H1839, 2000.
69. Wolfle SE, Chaston DJ, Goto K, Sandow SL, Edwards FR, Hill CE. Non-linear relationship between hyperpolarisation and relaxation enables long distance propagation of vasodilatation. *J Physiol* 589: 2607–2623, 2011.
70. Wolfle SE, Schmidt VJ, Hoyer J, Kohler R, de Wit C. Prominent role of KCa3.1 in endothelium-derived hyperpolarizing factor-type dilations and conducted responses in the microcirculation *in vivo*. *Cardiovasc Res* 82: 476–483, 2009.
71. Xia J, Duling BR. Electromechanical coupling and the conducted vasomotor response. *Am J Physiol* 269: H2022–H2030, 1995.
72. Yamamoto Y, Klemm MF, Edwards FR, Suzuki H. Intercellular electrical communication among smooth muscle and endothelial cells in guinea-pig mesenteric arterioles. *J Physiol* 535: 181–195, 2001.

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