The resistance network that controls blood flow to skeletal muscle comprises terminal arterioles, as well as larger arterioles and the small (feeder) arteries from which they derive. For example, in a contracting skeletal muscle, low P0₂ and vasoactive metabolites elicit a local response and initiate a conducted vasodilation that “ascends” the vascular tree to induce the simultaneous vasodilation of the feed arteries as well as branch arteries and thus increase blood flow. This coordinated longitudinal transmission of vasomotor responses is essential to achieve optimal organ perfusion.¹

Experimentally, ascending dilatation can be studied in vivo and in vitro by assessing the response to a vasoactive substance at the point of application (local response) as well as at a remote site, usually 0.5 to 2 mm upstream of the local site. This conducted vasodilatation can travel bidirectionally, but upstream sites are generally chosen since the vasoactive compounds applied in superfused systems are unable to directly affect upstream sites. The amplitude of the conducted vasodilatation is generally smaller than that of the local response and although there is a gradual decline in the conducted vasodilatation along some arterioles, there is no obvious decay of the conducted response along feed arteries.

Conducted responses have been intensively investigated and although the exact mechanism remains to be clarified, most researchers agree that nitric oxide (NO) does not play a major role in this response. Indeed, conducted vasodilatation in response to a number of stimuli is not affected by NO synthase (NOS) inhibitors,²–⁴ and the response is apparently intact in eNOS-deficient mice.⁵ Changes in membrane potential appear to be central to the phenomenon of ascending dilatation, and responses are generally attributed to the propagation of a hyperpolarization along the vascular wall,⁶ which is either linked to the actions of an endothelium-derived hyperpolarizing factor (EDHF)³,⁴ or to the direct transmission of an electrical signal between vascular cells.⁷–¹⁰ Over the past few years, evidence has accumulated to suggest that homocellular¹¹ as well as heterocellular¹²–¹⁴ gap junctional communication are involved in the phenomenon of conducted dilatation.

In this issue of Circulation Research, Budel et al¹⁵ report that acetylcholine and bradykinin elicit a rapid conducted vasodilatation along hamster cheek pouch arterioles that the authors attribute to a wave of NO release. Redundancy in the vasodilator mechanisms that regulate the acetylcholine-induced, endothelium-dependent dilator response is suggested to be the reason this phenomenon has not previously come to light. The study by Budel et al also reiterates a previous conclusion from this group that the conducted vasodilatation observed after the application of acetylcholine simultaneously stimulates two cellular pathways, one involving conduction along smooth muscle cells and the other along endothelial cells.¹⁶ The authors attribute the signal conducted along the smooth muscle cells to the actions of an EDHF. The evidence suggesting that the endothelial pathway is linked to the generation of NO was obtained from a set of experiments using a combination of specific cell layer damage and an NOS inhibitor and demonstrates that when the smooth muscle conductance pathway is blocked by light dye treatment, an NOS inhibitor attenuates the conducted response by 30% to 60%. The authors suggest that this finding indicates that the supposedly homocellular conduction along the endothelium can be attributed to a “wave” of NO release (Figure). This is not the first report of a role for NO in conducted vasodilatation since this autacoid was previously proposed to play a role in the maintenance of sustained conducted responses, although on its own appeared insufficient to initiate conducted responses.¹⁷ The data presented by Budel et al confirm this hypothesis, since the effect of the NOS inhibitor on the local response to acetylcholine was less pronounced than its effect on the conducted dilatation. Unfortunately, exactly how this wave of NO is thought to be generated was not addressed.

The authors speculate that acetylcholine and bradykinin initiate the hyperpolarization of endothelial cells and that this response is propagated from one cell to another. However, in order for the endothelial cell hyperpolarization to activate eNOS, there needs to be an increase in the endothelial cell Ca²⁺ concentration ([Ca²⁺]), and the authors propose that the conducted hyperpolarization triggers Ca²⁺ transients of sufficient amplitude to activate eNOS. This issue was not addressed experimentally but is perhaps the most critical point in the hypothesis. Indeed, although endothelial cell hyperpolarization has been reported to increase the driving force for Ca²⁺ into endothelial cells stimulated by a receptor-dependent agonist and to increase endothelial cell [Ca²⁺],¹⁸,¹⁹ there is no evidence to suggest that membrane hyperpolarization on its own can elicit an adequate Ca²⁺ transient.
The authors’ interpretation of their data, while provocative, is also not so easy to reconcile with the fact that both the NO wave and the EDHF response are apparently dependent on endothelial cell hyperpolarization. As mentioned, in the case of NO, the hyperpolarization is suggested to modulate $[\text{Ca}^{2+}]_i$, while the majority of EDHF-mediated responses described to date are preceded by the activation of $[\text{Ca}^{2+}]_i$-dependent K$^+$ channels in endothelial cells. It is therefore necessary to address this point, in particular the mechanisms responsible for endothelial cell hyperpolarization, in more detail.

A further intriguing observation was the differential effect of acetylcholine and bradykinin. Budel et al compared the effects of both agonists and report that the conducted response to bradykinin can be attributed entirely to an NO wave as it was blocked by damaging the endothelial cell layer and was abolished by NOS inhibition, ie, a very different response to the parallel activation of two cellular pathways by acetylcholine. These data are also difficult to reconcile with reports that bradykinin is a potent stimulus for the generation of EDHF in numerous arterial beds. Although the ability of bradykinin to induce conducted responses is highly variable, the inability of bradykinin to elicit NO-independent vasodilatation may be a phenomenon that is specific to hamsters since bradykinin stimulates pronounced EDHF-mediated responses in mice. Moreover, expanding the above discussion on hyperpolarization, it would appear that bradykinin elicits a hyperpolarization that is conducted only along endothelial cells whereas acetylcholine elicits a hyperpolarization that can be conducted along two cellular pathways. Again, a more detailed analysis, preferably simultaneously assessing changes in membrane potential and vessel diameter, is required to address these issues.

What about the role of gap junctional communication in the phenomenon of ascending dilation? The authors assume that the hyperpolarization that travels by both cellular pathways is conducted via gap junctions, although by homocellular gap junctions and not the myoendothelial gap junctions that several groups have reported to couple endothelial cells with smooth muscle cells in vitro. There is certainly evidence for distinct conduction pathways in different cell layers. For example, connexin40 is selectively expressed in mouse cremaster muscle artery endothelial cells and its removal (ie, in the connexin40 knockout mouse) significantly affected the conducted vasodilatation elicited in vivo by electrical stimulation as well as by acetylcholine and bradykinin, without affecting conducted vasoconstriction along vascular smooth muscle cells. Although hyperpolarization along the endothelial cell layer can be regarded as the initiating signal for the generation of NO, additional experiments are required to determine whether inhibitors of gap junctional communication attenuate the wave of NO generation or whether the NO generated affects the propagation of the hyperpolarization.

In summary, the hypothesis that ascending dilatation can be linked to a wave of NO release is interesting but currently supported only by pharmacological data. Moreover, the mechanisms underlying this response need to be investigated in more detail and linked to changes in endothelial cell $[\text{Ca}^{2+}]_i$, and membrane potential. The differential response to acetylcholine and bradykinin is intriguing, but it would be more important to determine the role of the NO wave in regulating ascending dilatation in response to a physiological stimulus, such as in a contracting muscle.

References

KEY WORDS: gap junctional communication ■ microcirculation ■ endothelium-derived hyperpolarizing factor ■ Ca²⁺ signaling
Bobbing Along on the Crest of a Wave: NO Ascends Hamster Cheek Pouch Arterioles
Ingrid Fleming

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