Does TRPC3 macrodominate the myoendothelial gap junction microdomain?

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In 1998, Griffith and colleagues proposed that myoendothelial gap junctions (MEGs) play a central role in endothelium-dependent hyperpolarization (EDH), the key mechanism coupling the activation of the endothelium by shear stress and blood borne-stimuli to vasodilation in the resistance vasculature. They envisioned that MEGs, which are located at the ends of endothelial cell (EC) projections that protrude through holes in the internal elastic lamina of arteries and arterioles and link the EC to adjacent vascular smooth muscle cells (VSMCs), enabled EDH by acting as low-resistance pathways allowing the flow of hyperpolarizing current from the endothelium to the adjacent smooth muscle. Subsequently, however, it has become apparent that far from merely providing a locus for MEGJ, myoendothelial projections (MEPs) are unique microdomains that are capable of generating localized Ca2+ signals. This may enable them to influence the flow of information between ECs and VSMCs and also probably allows them to play a pivotal active role in triggering EDH (Figure 1).

In the current issue of Cardiovascular Research, Senadheera et al. provide direct and novel evidence that MEP Ca2+ signalling is crucially involved in EDH and also, at least potentially, in myoendothelial feedback, the process by which the stimulation of VSMCs leads to EC activation that functions to limit constriction. They demonstrate that the non-selective cation channel TRPC3 is present on the EC (but not the VSMC) plasmalemma, where it is mainly localized to the MEP. Remarkably, the blockade of TRPC3 with the drug Pyr3, the selectivity of which they meticulously validate, largely ablates EDH, and the associated vasodilation.

1. EDH, K+ channels, and MEGJ

An increase in [Ca2+] within the EC evokes a vasodilation caused by multiple mechanisms, including most famously the release of nitric oxide. However, the most important mechanism of vasodilation in arterioles is the EDH of the VSMCs which closes their L-type Ca2+ channels, thereby attenuating Ca2+ influx. Although many pathways influence EDH-associated vasodilation, the core response in most vascular beds results from the opening of EC Ca2+-activated K+ channels. This raises the extracellular [K+] within the vascular wall, leading to the activation of Na+ pumps and inwardly rectifying K+ channels (KIR) on the VSMC; this in turn hyperpolarizes them. It also results in EC hyperpolarization that is directly transmitted to adjacent VSMCs via the MEGJs.

Two types of Ca2+-activated K+ channels are central to EDH. Small conductance Ca2+-activated channels (SKCa or KCa2.3) are widely distributed over the EC plasmalemma and are particularly important for EC hyperpolarization. Intermediate conductance Ca2+-activated K+ channels (IKCa or KCa3.1), which probably provide for most of the increase in [K+]ext, are mainly located in the MEGJs.

Chaytor et al. found that blockade of the MEGJ by a peptide designed to block the connexons spanning the MEGJ strongly antagonized EDH-associated relaxation of the rat superior mesenteric artery, and they proposed that EDH was due to the flow of hyperpolarizing current from the EC to the VSMC. This concept subsequently received much support from diverse observations by this and other laboratories. However, in 2005 Mather et al. demonstrated that Cx40, which they localized to the MEP, is responsible for EDH-associated vasodilation in rat small mesenteric arteries, but that this is the case only when arteries are maximally stimulated. Together with reports that the EC-dependent rise in [K+] within the myoendothelial space is mainly responsible for causing EDH during submaximal (i.e. physiological) VSMC excitation, these observations challenged the idea that MEGJ-mediated hyperpolarization was important in EDH under physiological conditions.

However, work by Dora et al. then showed that blocking the MEGJ, while not preventing EDH-associated vasodilation at submaximal levels of VSMC excitation, rendered this relaxation exquisitely sensitive to IKCa channel antagonism. They proposed that the activation of these channels contributed to EDH at normal levels of VSMC stimulation by causing a local rise in extracellular [K+] surrounding the MEP. This acted on Na+ pumps clustered in the VSMC membrane...
adjacent to the MEP, thus hyperpolarizing the VSMC. Thus, the MEPs might contribute to EDH not only (or primarily) by transmitting EC hyperpolarization to the VSMCs, but also by acting as releasable ‘K⁺ stores’.

2. Ca²⁺ signalling in myoendothelial projections

It now appears that at least two types of Ca²⁺-‘events’ may cause the opening of IKCa channels in MEPs. Very recently, it was reported that the cooperative opening of several TRPV4 channels in ECs causes localized [Ca²⁺]ᵢ elevations, christened ‘sparklets’, which activate IKCa and SKCa channels and are responsible for much of the EDH response to acetylcholine, at least in mouse small mesenteric arteries. Although sparklets are not exclusive to MEPs, another type of Ca²⁺ signal, the ‘pulsar’, seems to be generated mainly within the MEP. Pulsars are transient elevations of [Ca²⁺]ᵢ caused by the opening of clusters of inositol trisphosphate (IP₃) receptors on elements of the endoplasmic reticulum (ER) present in the junctions. Their frequency is enhanced by EC activation, and they are probably involved in IKCa activation during EDH. Pulsars are particularly interesting because, theoretically at least, they might be triggered by IP₃ entering the MEP from either the EC or the VSMC. The former might contribute to EDH, whereas the latter could be important for what might be termed ‘myo-MEP’ feedback, by which smooth muscle excitation could limit itself by causing K⁺ efflux from the MEP.

As one would expect, the study by Senadheera et al. raises many fascinating questions. Their observations imply that TRPC3 may be largely responsible for enabling pulsars, either by supplying the ER with Ca²⁺ to support IP₃-mediated release or by mediating Ca²⁺ influx, which directly activates KCa channels in the MEP. However, this remains to be demonstrated—a task which should be facilitated by their demonstration that low concentrations of Pyr3 selectively block TRPC3. In addition, one now wonders whether it is TRPV4 or TRPC3 that is more important for controlling IKCa in the MEP microdomain. What about other potential MEP Ca²⁺ influx pathways such as TRPA1? Are TRPC3 channels also required for ‘wavelets’, another IP₃-dependent EC Ca²⁺ signal that has been shown to be important in myoendothelial feedback? And, more generally, will it turn out that the MEP microdomain macrodominates blood flow to the microcirculation?

References