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Superresolution Subspace Signaling

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Exquisitely local signaling provides robust, precise, and rapid communication in single cells. Nanometer-wide regions constitute the signaling conduit that separates interacting ion channels, organelles, and sensor and effector proteins. Such signaling domains are primary features of many systems, including those responsible for excitation-contraction coupling in the heart (1, 2), smooth muscle tone in the vasculature (3, 4), and Ca^{2+} signaling in neurons (5). These intimate pathways, also known as “fuzzy spaces” (1), “subspaces” (6), or “nanoscopic spaces,” permit efficient signaling with rapid and reliable information transfer, yet are too small to be seen with a standard optical microscope. Such superresolution domains may become experimentally visible with the implementation of optical superresolution microscopy (7–9). On page 597 in this issue, Sonkusare et al. (10) identify a specific and broadly important local signaling organization in small mesenteric arteries (~100 μm in diameter) that uses nano meter-wide communication regions to regulate local vascular blood flow.

Endothelial cells form intimate spot-welded contacts with vascular smooth muscle cells at “holes” in the internal elastic lamina that are micrometers in diameter (see the figure). These regions of close contact can include gap junctions that create an electrical conduit from the endothelial cells to the effector smooth muscle cells. Sonkusare et al. suggest that this specific organization is used by endothelial cells in two signaling pathways. In one pathway, inositol tris phosphate receptor (IP3R)–dependent Ca^{2+} release from endoplasmic reticulum (ER) in endothelial cells can produce regions with very high local concentrations of intracellular free calcium ($[\text{Ca}^{2+}]_i$) called “pulsars” (4). In the other pathway, clustered transient receptor potential vanilloid 4 (TRPV4) channels in the plasmalemma can produce regions with locally very high $[\text{Ca}^{2+}]_i$ called “sparklets” (10–12). In both cases, local increase of $[\text{Ca}^{2+}]_i$ activates nearby Ca^{2+} -activated K^+ channels [intermediate-conductance (IK; $\text{K}_{\text{Ca}3.1}$) and small-conductance (SK; $\text{K}_{\text{Ca}2.3}$)] to hyperpolarize endothelial cells. These two signaling pathways converge on the same functional target—the K^+ channels. The efficacy of this activation depends in part on the proximity of the K^+ channels to the Ca^{2+} source, hence the importance of subspaces or fuzzy spaces (1, 3). As endothelial cells hyperpolarize relative to smooth muscle cells, current flows from smooth muscle cells to hyperpolarize and thus relax the smooth muscle cells (3). The effect is to increase blood flow. Sonkusare et al. point out that there may also be a role for the increase in nearby extracellular $[\text{K}^+]$ in the restricted extracellular spaces between the cells. If so, they can increase inward-rectifier K^+ channel (Kir) efflux of K^+ to augment smooth muscle cell hyperpolarization and relaxation (4, 13). Perhaps this is also how the restricted but larger

internal elastic lamina (up to several micrometers) between endothelial cells and smooth muscle cells, of presumed varying thickness (14), contributes to this process.

Important to the study by Sonkusare et al. and other investigations was the development of a critical tool: the expression of the genetically encoded Ca^{2+} indicator protein GCaMP2 specifically in endothelial cells in mice (4, 10), which provides a Ca^{2+} sensor tool with high signal to noise specific only to endothelial cells. This tool allows the systematic identification of signals that depend on fuzzy-space Ca^{2+} that would otherwise be masked by neighboring cell signals if standard Ca^{2+} probes were used.

The principle demonstrated by Sonkusare et al. is critically important for the rapid regulation of tone in small mesenteric arteries. This finding is likely to be broadly relevant to all tissues where local factors control blood flow. For example, smooth muscle cells encircle the endothelial cells in an end arteriole from rat heart. This 15- to 20- μm -diameter arteriole has little or no apparent internal elastic lamina, consistent with its smaller size (14). The functional consequences of the finding of Sonkusare et al. in regulating cardiac blood flow are clear and consistent with the speculative hypothesis of electrotonic blood flow regulation in heart. Here, the electrical consequences of cardiac myocyte metabolism are linked to smooth muscle cell tone in precapillary sphincters via endothelial cells (15). The energetic demand of gut smooth muscle would appear to be equally important for mesenteric vascular flow and the regulation of flow in other important vascular beds. Understanding the intricate signaling of various vascular beds will allow for identification of potential therapeutic targets—especially important in diseases that result in vascular remodeling such as diabetes and hypertension.

Acknowledgments

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Figure. Arterial relaxation

The superresolution signaling system in vascular endothelial cells may be organized with respect to smooth muscle cells. Sonkusare et al. examined 100- μm -diameter arterioles in mice. Cooperative gating of clustered TRPV4 channels of a vascular endothelial cell (EC) allows Ca^{2+} influx. This localized increase in $[\text{Ca}^{2+}]_i$ activates local K^+ channels, hyperpolarizes endothelial cells, and increases nearby $[\text{K}^+]$ in the internal elastic lamina (IEL). Activated IP_3 Rs in the endothelial cell ER produce locally high $[\text{Ca}^{2+}]_i$ signals. Locally elevated $[\text{K}^+]$ increases inward-rectifier K^+ channel (Kir) current in the smooth muscle cell (SMC) to hyperpolarize the cells, which underlies vascular relaxation and increased blood flow. ACh, acetylcholine; PIP_2 , phosphatidylinositol 4,5-bisphosphate. “CREDIT: C. BICKEL/SCIENCE”