The Journal of General Physiology

Generally Physiological

Eschewing ischemia or responding to it

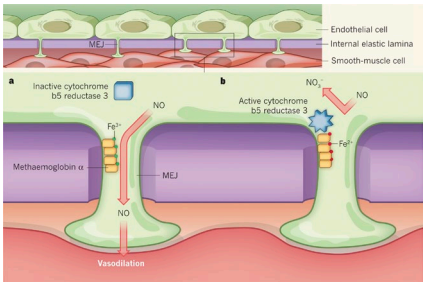
This month's installment of *Generally Physiological* considers the regulation of nitric oxide (NO) delivery to vascular smooth muscle cells, the effects of an increase in free intracellular magnesium (Mg^{2+}_i) on calcium current in ventricular myocytes during transient cardiac ischemia, and the silencing through SUMOylation of acid-sensitive potassium channels in the cerebellum.

Reducing NO signaling to smooth muscle

NO produced in endothelial cells acts on vascular smooth muscle cells to mediate vasodilation. Noting that stimulating NO production in ex vivo thoracodorsal arteries or endothelial cells co-cultured with smooth muscle cells failed to elicit its extracellular appearance (as would be expected with free diffusion of this gaseous molecule), Straub et al. (2012) explored mechanisms for regulating its delivery. NO binds to heme proteins, and Straub et al. found that hemoglobin α was abundant at myoendothelial junctions (structures that protrude through the internal elastic lamina that separates the vascular endothelium from smooth muscle, enabling the passage through gap junctions of various small molecules; see Gladwin and Kim-Shapiro, 2012). Hemoglobin α knockdown in endothelial cells of isolated arteries enhanced vasodilatation in response to acetylcholine, decreased vasoconstriction in response to phenylephrine, and promoted NO diffusion across the arterial wall. NO undergoes a rapid dioxygenation reaction with ferrous (Fe^{2+}) oxyhemoglobin to produce nitrate and ferric (Fe^{3+}) methemoglobin (to which NO binds less well). Straub et al. determined that hemoglobin α existed in both the Fe^{2+} and Fe^{3+} states and found that the methemoglobin reductase CYB5R3 (cytochrome b5 reductase 3) was present at myoendothelial junctions and formed a complex with hemoglobin α. CYB5R3 knockdown or inhibition, like hemoglobin α knockdown, decreased the response to phenylephrine, increased the response to acetylcholine, and promoted NO diffusion. The authors thus propose that regulation of the oxidative state of endothelial hemoglobin α provides a mechanism for controlling NO diffusion to vascular smooth muscle and thereby the arterial vasculature.

Decreasing cardiac calcium influx

Despite the existence of complex vascular regulatory mechanisms, the blood supply to a crucial tissue can be interrupted, and this will elicit various responses. For instance, transient cardiac ischemia leads to an increase in Mg^{2+}_i in ventricular myocytes and



Regulation of NO diffusion to vascular smooth muscle cells through the strategic location of hemoglobin α and cytochrome b5 reductase 3. MEJ, myoendothelial junction; NO_3^-, nitrate. (Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. M.T. Gladwin and D.B. Kim-Shapiro. 491:344–345, copyright 2012.)

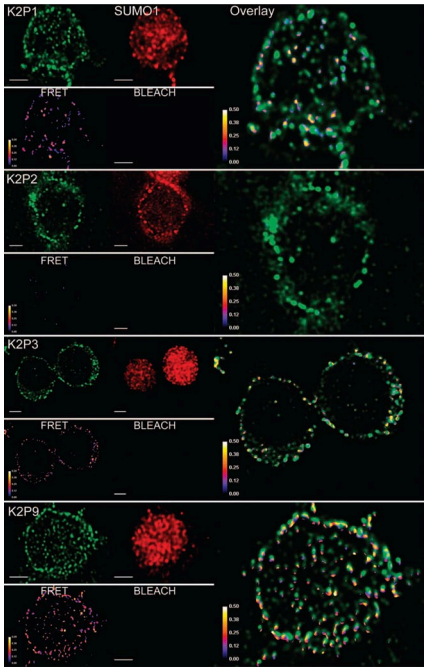
then to an increase in sympathetic tone. β-adrenergic receptor signaling increases L-type Ca^{2+} current ($I_{Ca,L}$) through activation of the protein kinase A (PKA) signaling cascade and the phosphorylation of the $Ca_v1.2$ Ca^{2+} channel. In the absence of β-adrenergic receptor signaling, increased Mg^{2+}_i decreases $I_{Ca,L}$; however, Mg^{2+} can interact with the PKA signaling cascade at various points and thus the effect on $I_{Ca,L}$ of a rise in Mg^{2+}_i in the context of increased β-adrenergic signaling is unclear. In this issue, Brunet et al. found that increasing Mg^{2+}_i decreased stimulation of $I_{Ca,L}$ in response to subsequent exposure to the β-adrenergic agonist isoproteronol in isolated adult mouse ventricular myocytes. A careful pharmacological analysis of the effects of increasing Mg^{2+}_i on $I_{Ca,L}$ in conjunction with activation of distinct steps in the PKA pathway or direct activation of $Ca_v1.2$ indicated that Mg^{2+} likely acts directly on $Ca_v1.2$, functioning downstream of the PKA pathway to inhibit Ca^{2+} influx. Brunet et al. (2013) propose that, during transient ischemia, the rise in Mg^{2+}_i—caused by hydrolysis of Mg^{2+}-ATP—provides a cell-autonomous mechanism for limiting calcium influx,

The regulation of NO delivery to vascular smooth muscle cells, the effects of an increase in Mg^{2+}_i on Ca^{2+} current in ventricular myocytes during transient cardiac ischemia, and the silencing through SUMOylation of acid-sensitive K^+ channels in the cerebellum

and thereby metabolic stress, even under conditions of increased sympathetic tone.

A silent partner

Ischemia can also lead to a decrease in pH, affecting the function of acid-sensitive ion channels such as the TASK channels proposed to mediate the acid-sensitive component of the standing outward K^+ current (IKso) in cerebellar granule cells. TASK channels are members of the two-pore domain K^+ (K2P) channel family that consist of homo- or heterodimers of the K2P3 and K2P9 channel subunits. Using a combination of confocal microscopy, mRNA-specific probes, and cyanine dye–based signal amplification, Plant et al. (2012) found that transcripts encoding K2P1 were present in the same cultured cerebellar granule cells as transcripts encoding K2P3 and K2P9. K2P1 forms a potassium-selective channel that is electrically silenced through SUMOylation (covalently modified by small ubiquitin–related modifier protein [SUMO]), and FRET analysis indicated that SUMO1 was in close proximity with K2P1 at the neuronal surface. Unexpectedly, however, FRET was also apparent between SUMO1 and K2P3 and K2P9, which lack the lysine residue SUMOylated in K2P1. The deSUMOylating enzyme SENP1 increased IKso in cerebellar granule cells, and analyses of CHO cells heterologously expressing individual subunits, combinations of subunits, or concatenated proteins indicated that K2P3 and K2P9 were not SUMOylated but formed heterodimers with K2P1, with a single SUMO required to silence such K2P1-bearing heterodimers. When deSUMOylated, K2P1-bearing heterodimers mediated acid-sensitive IKso and, like homo- and heteromeric K2P3 and K2P9 channels, were responsive to the volatile anesthetic halothane. The authors postulate that incorporation of K2P1 into heterodimeric channels in cerebellar granule cells will enable the regulation through SUMOylation of their response to changes in pH, volatile anesthetics, and the various other agents to which these channels are sensitive.



FRET occurs in cerebellar granule cells between SUMO1 and K2P1, K2P3, and K2P9, but not K2P2. (From Plant et al., 2012. *Science Signaling*. 5:ra84. Reprinted with permission from AAAS.)

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