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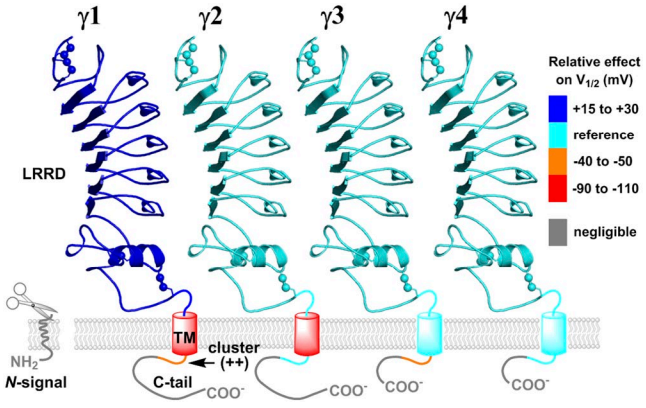
Generally Physiological

Of BK regulation, painful connections, and versatile neuropeptide signaling

This month's installment of *Generally Physiological* considers BK channel regulation by its γ subunits, how a cation channel and an anion channel work together to increase pain, and a new pathway for signaling through the melanocortin-4 receptor.

Defining the crucial regions

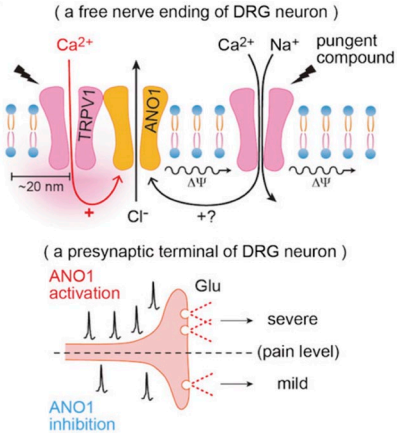
Large conductance Ca^{2+}- and voltage-activated potassium (BK) channels are made up of pore-forming α subunits and regulatory β or γ auxiliary subunits. The four BK γ subunits, proteins with a large extracellular LRR domain, a single transmembrane domain, and a short intracellular C-terminal tail, have similar overall sequences, and all of them shift the voltage dependence of BK activation in the hyperpolarizing direction. Intriguingly, however, the different subunits, which show tissue-specific patterns of expression, show remarkably different efficacy in shifting BK activation, with γ1 shifting activation ∼140 mV, γ4 shifting activation ∼20 mV, and γ2 and γ3 having intermediate effects. In this issue, Li et al. performed mutational analysis, using domain swapping as well as deletion and substitution, to identify the transmembrane segment, together with a neighboring intracellular cluster of positively charged amino acids, as playing a crucial role in determining the efficacy of the different γ subunits in modulating BK activation.



Structures of the four BK γ subunits, color coded to indicate the relative contribution of different structural elements to the γ subunit–mediated shift in the voltage dependence of BK activation. See Li et al. (2015).

A painful connection

Takayama et al. (2015) have uncovered an intriguing interaction between the TRPV1 cation channel and the calcium-activated chloride channel anoctamin 1 (ANO1) that enhances nociception in primary sensory neurons. TRPV1 activation by various noxious stimuli (including noxious heat, acid, and capsaicin [an active component of chili peppers]) has been thought to stimulate the firing of primary sensory neurons (thereby mediating pain) through the activation of voltage-sensitive sodium channels secondary to the depolarization inherent with cation influx. Noting that TRPV1 is permeable to calcium, and that ANO1 has been implicated in nociception, and having confirmed their coexpression in mouse dorsal root ganglion (DRG) neurons, Takayama et al. (2015) used whole-cell patch-clamp analysis of HEK293T cells expressing the two channels alone or together to investigate their interaction. Cells expressing both TRPV1 and ANO1 showed calcium-dependent chloride currents in response to capsaicin. The coexpressed channels coimmunoprecipitated, indicating a physical interaction, and, in DRG neurons, the ANO1 inhibitor A01 reduced capsaicin-activated currents in the presence of the slow calcium chelator EGTA (in the pipette) but not of the fast chelator BAPTA, indicating that the TRPV1–ANO1 interaction involves a local calcium nanodomain. A01 inhibited capsaicin-dependent generation of action potentials in DRG neurons and capsaicin-induced pain-related behaviors in mice; moreover, it attenuated capsaicin-induced facilitation of the frequency of spontaneous excitatory postsynaptic potentials. The authors thus conclude that capsaicin-evoked depolarization



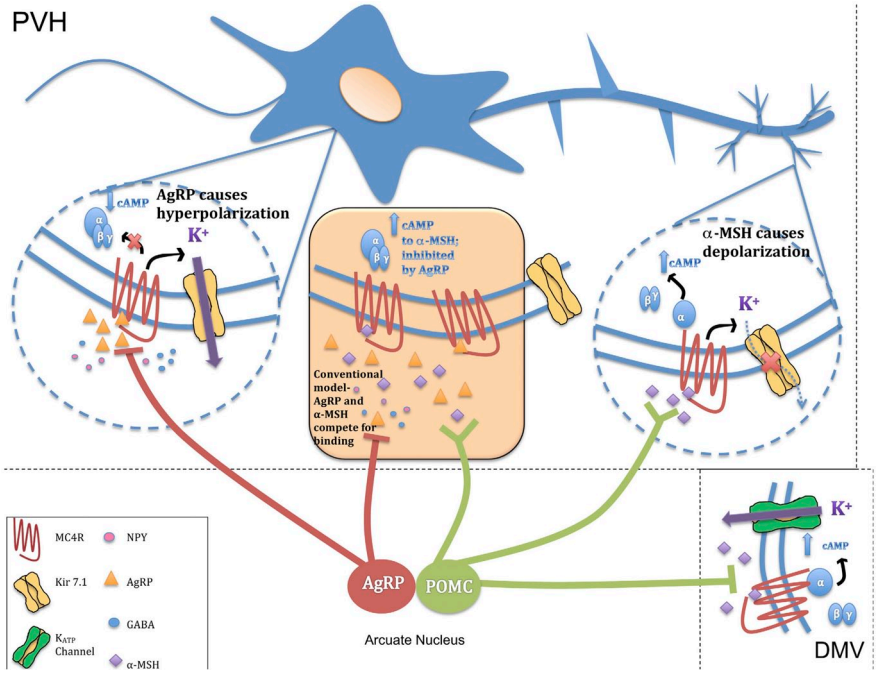
(Top) TRPV1 activation leads to cation influx, depolarizing the neuron; in addition, calcium entering the cell activates ANO1 to stimulate chloride efflux, causing further depolarization. (Bottom) The increased depolarization produced by ANO1 activation leads to an increase in glutamate release at the nerve terminal and enhanced perception of pain. (From Takayama et al., 2015.)

depends not only on cation influx through TRPV1 channels but also on a secondary depolarization caused by chloride efflux through associated ANO1 channels to enhance action potential generation and thereby the perception of pain.

Not just an antagonist

Ghamari-Langroudi et al. (2015) have uncovered an intriguing new pathway for melanocortin signaling in mice. The neuropeptides α-melanocyte–stimulating hormone (α-MSH) and Agouti-related peptide (AgRP) have opposing effects on appetite, with α-MSH stimulating feeding and AgRP inhibiting it. Both α-MSH and AgRP bind to the melanocortin-4 receptor (MC4R), with α-MSH acting as an agonist to stimulate Gαs signaling, and AgRP acting as a competitive antagonist that blocks α-MSH binding and an inverse agonist that blocks constitutive activation by a ligand-mimetic amino-terminal domain. Ghamari-Langroudi et al. (2015) found that α-MSH depolarized MC4R neurons in the paraventricular nucleus of the hypothalamus (PVN) in a mouse hypothalamic slice preparation, thereby increasing the frequency of action potential firing, whereas AgRP hyperpolarized PVN MC4 neurons, decreasing their firing rate. Surprisingly, however, pharmacological analysis indicated that the α-MSH–mediated depolarization, which depended on inhibition of a steady-state K^+ current, was independent of G protein signaling, and further investigation revealed that both the α-MSH–mediated depolarization and the AgRP-mediated hyperpolarization involved regulation of the Kir7.1 K^+ channel. Analyses of HEK293 cells transfected with MC4R and a Kir7.1 variant confirmed that MC4R signaling modulated Kir7.1 function, with assays of Tl^+ flux indicating that α-MSH acted through MC4R to close Kir7.1, with an IC50 of $10^{-7.5}$ M, whereas AgRP stimulated an MC4R-dependent increase in Tl^+ flux with an EC50 of $10^{-8.6}$ M. An AgRP analogue with normal potency in inhibiting Gαs signaling but decreased ability to stimulate feeding in rats had decreased potency in stimulating Tl^+ flux, whereas an α-MSH analogue that preferentially coupled MCR4 to Kir7.1 over Gαs was comparable to other α-MSH analogues at inhibiting feeding in mice. The authors thus conclude that MC4R signals through a G protein–independent pathway involving Kir7.1 that can be modulated by AgRP independent of its inhibition of α-MSH binding, and which may play a critical role in melanocortin regulation of energy homeostasis in the PVN.

Elizabeth M. Adler
Executive Editor, *JGP*
eadler@rockefeller.edu



In the conventional model of α-MSH/AgRP signaling at PVN MC4R neurons, AgRP acts as a competitive inhibitor to block α-MSH stimulation of Gαs and as an inverse agonist to block constitutive signaling through this pathway (central orange box). Ghamari-Langroudi et al. (2015) propose that AgRP also acts as an agonist at MC4R to activate Kir7.1, and thereby hyperpolarize the cell independently of α-MSH and the Gαs pathway (left circle). At some sites, α-MSH may act independently of AgRP to stimulate Gαs signaling and also to close Kir7.1 (right circle). In the dorsal motor nucleus of the vagus (DMV), α-MSH activates K_{ATP} channels through a Gαs pathway (lower right). (Reprinted by permission from Macmillan Publishers, Ltd. M. Ghamari-Langroudi et al. *Nature*. http://dx.doi.org/10.1038/nature14051, copyright 2015.)

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