This month’s installment of Generally Physiological focuses on ion channels, considering the photoreceptor cascade that leads to transient receptor potential (TRP) channel activation by light, mechanisms of gating in channels that open in response to hyperpolarization, and a class of snake venom toxins that inhibit acid-sensing ion channels to block pain.

Illuminating changes in membrane tension

In the tightly packed microvilli of the fly photoreceptor, transformation of light into an electrical signal is initiated by photoisomerization of rhodopsin and the consequent activation of Gq and thereby phospholipase C (PLC). PLC hydrolyzes the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP₂), generating the soluble second messenger inositol 1,4,5-trisphosphate (InsP₃), diacylglycerol (DAG; which is retained in the inner leaflet of the membrane bilayer), and a proton. Acidification, in combination with PIP₂ depletion, can activate the light-sensitive TRP and TRPL channels to initiate an electrical signal; however, the underlying mechanism has been unclear (see Liman, 2012). Noting that members of the TRP family may be sensitive to stretch, Hardie and Franze (2012) explored the hypothesis that loss of the PIP₂ inositol headgroup from the inner leaflet changes membrane tension, generating a mechanical force that promotes TRP channel gating. Flashes of light elicited visible contractions in fly photoreceptors; atomic force microscopy revealed that PLC-dependent contractions could be elicited repeatedly in excised retinas and that the latency of contractions induced by the brightest stimuli was shorter than that of the electrical response. Furthermore, light activated mechanosensitive channels (gramicidin) introduced into dissociated photoreceptors, and osmotic manipulation enhanced or decreased light-induced TRP and TRPL currents in a manner consistent with the predicted effects on membrane tension and phospholipid crowding. Similarly, structurally distinct cationic amphipaths—predicted to increase membrane crowding and decrease membrane stiffness—reduced light-induced current without affecting PLC activity. The authors thus propose that PIP₂ depletion promotes TRP channel activation (and thereby phototransduction) through changes in the physical properties of the bilayer, a mechanism that—given the broad involvement of TRP channels in numerous processes—could have wide-ranging implications.

Exploring mechanisms of HCN gating

In another set of articles focused on mechanisms of channel activation, Yellen and colleagues (Kwan et al., 2012; Ryu and Yellen 2012) explored gating of hyperpolarization-activated and cyclic nucleotide–modified (HCN) channels. Structurally similar to voltage-gated K⁺ channels (Kv channels, which contain four subunits, each with six transmembrane domains), HCN channels, unlike most voltage-gated channels, open in response to hyperpolarization rather than depolarization, playing a crucial role in regulating the activity of cardiac pacemaker cells and spontaneously firing neurons (see Trudeau, 2012). Kwan et al. (2012) substituted pairs of cysteines for specific residues in the S4–S5 linker (the region that links the fourth transmembrane domain with the fifth). This modification destabilized the pore region and reduced TRP and TRPL currents in a manner consistent with the predicted effects on membrane tension and phospholipid crowding. Similarly, structurally distinct cationic amphipaths—predicted to increase membrane crowding and decrease membrane stiffness—reduced light-induced current without affecting PLC activity. The authors thus propose that PIP₂ depletion promotes TRP channel activation (and thereby phototransduction) through changes in the physical properties of the bilayer, a mechanism that—given the broad involvement of TRP channels in numerous processes—could have wide-ranging implications.
[S4] to S5) and post-S6/C-linker region of the sea urchin HCN channel to explore the mechanism whereby the voltage sensor couples to the gating mechanism. When Cd²⁺ was added to bridge nearby cysteines, locking the channel into a particular conformational state, some bridged pairs locked the channel open, whereas others locked it closed, indicating that these regions could interact in both open and closed states. The pattern of “lock-open” and “lock-close” effects observed indicated that these regions moved relative to each other during gating and—together with analyses of concatenated dimers—suggested that the S4–S5 linker of one subunit can interact with the post-S6/C-linker not only in the same but also in neighboring subunits. In a second study, Ryu and Yellen (2012) measured gating currents in lock-open and lock-close mutants. Their data, which was consistent with easier activation of the voltage sensor when the gate is open and more difficult activation when it is closed, indicated that, unlike Kv channels, the coupling between voltage-sensor movement and gating in HCN channels is weak. The authors speculated that such weak voltage coupling could facilitate the effects on gating of such modulatory factors as cyclic nucleotide binding or channel phosphorylation.

**Mamba venom peptides inhibit ASIC channels to block pain**

Turning from channel activation to channel inhibition, the last article I consider in this month’s installment of *Generally Physiological* concerns the identification of a class of peptides that abolish pain by inhibiting acid-sensing ion channels (ASICs). ASICs, proton-activated cation channels found in both central and peripheral neurons, have been implicated in pain perception. Diochot et al. (2012) identified venom of the black mamba (*Dendroaspis polylepis*) as a reversible ASIC inhibitor in a *Xenopus laevis* oocyte expression screen for unknown ASIC inhibitors. Purification of the active fractions enabled them to identify two 57–amino acid isopeptides belonging to the three-finger toxin family, which they named mambalin-1 and mambalin-2. The mambalgins potently inhibited heterologously expressed homomeric and heteromeric ASIC subtypes found in the central nervous system (homomeric ASIC1a, heteromeric ASIC1a + ASIC 2a and ASIC1a + ASIC 2b) and sensory neurons (homomeric ASIC1b, heteromeric ASIC1a + ASIC 1b) as well as native ASIC currents in sensory, spinal, and hippocampal neurons. Central injections in mice produced analgesic effects as strong as those produced by morphine, but elicited less tolerance than morphine and no respiratory depression, and were resistant to the opioid antagonist naloxone. These central effects were lost in mice lacking ASIC1a, and central ASIC2a knockdown induced a naloxone-resistant analgesia with decreased sensitivity to mambalin-1, implicating both ASIC1a and ASIC2a in the mambalin’s central analgesic effects. In contrast, the peripheral analgesic effects of mambalin-1 appeared to be independent of ASIC1a but to instead depend on inhibition of ASIC1b. Thus, the mambalgins appear to represent a previously unknown class of analgesic agents that block pain through different ASIC subclasses in central neurons and peripheral nociceptors.

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