This month’s installment of *Generally Physiological* concerns the identification of a previously unknown type of purinergic receptor in plants, differential regulation of calcium channels by two different AKAP proteins, and sodium regulation of opioid receptors.

### Plant perception of extracellular ATP

Initially greeted with skepticism, the notion that ATP plays a role in mammalian extracellular signaling is now generally accepted, an acceptance facilitated by identification of plasma membrane P2-type purinergic receptors, which recognize and respond to extracellular ATP. Various lines of evidence suggest that ATP also acts as an extracellular signal in plants; however, attempts to identify plant ATP receptors similar to either P2X receptors (a family of ligand-gated channels) or P2Y receptors (a family of GPCRs) have been unsuccessful. Choi et al. (2014) screened 50,000 mutagenized Arabidopsis seedlings and identified two allelic mutants, which they named *Does not Respond to Nucleotides 1-1* (*dorn1-1*) and *dorn1-2*, which failed to show the increase in intracellular calcium elicited by ATP in wild-type plants. Unlike wild-type plants, *dorn1-1* mutants failed to phosphorylate mitogen-activated protein kinase (MPK3) and MPK6 in response to ATP, and microarray analysis identified more than 500 genes that responded to ATP in wild-type plants but not in the *dorn1-1* mutant. The *dorn1* mutations occurred in the kinase domain of a gene that encoded a protein with an extracellular lectin domain, a single transmembrane domain, and an intracellular kinase domain known to be localized to the plasma membrane. Ectopic expression of the wild-type protein restored the calcium response to ATP to *dorn1-2* mutants; moreover, recombinant DORN1 protein bound ATP with a dissociation constant of 45.7 ± 3.1 nM. The authors thus propose that DORN1 is essential for Arabidopsis perception of extracellular ATP, and may be the founding member of a family of plant-specific purinergic receptors.

### Competing AKAPs

Calcium entry through the voltage-gated calcium channels Cav1.1 and Cav1.2 initiates various cellular processes, including excitation–contraction coupling in muscle and excitation–transcription coupling in neurons. β-adrenergic signaling leads to activation of protein kinase A (PKA), which phosphorylates Cav1.1 and Cav1.2, thereby increasing their activity (and consequently muscle contractile force) during the sympathetic fight-or-flight response. A kinase anchoring protein 15 (AKAP15), which binds to the distal C-terminal domain of Cav1.1 and Cav1.2, enables their phosphorylation by recruiting PKA. In this issue, noting that AKAP79, which binds the protein phosphatase calcineurin, binds to the same site as AKAP15 on the distal C-terminal domain (DCT), Fuller et al. used a reconstructed system to explore the possible role of AKAP79 in modulating Cav1.2 activity. Unlike AKAP15, AKAP79 failed to support the PKA-dependent increase in Cav1.2 activity; indeed, its coexpression with AKAP15 antagonized the latter’s ability to do so. Deletion of the AKAP79 calcineurin-binding site, however, enabled it to mediate the PKA-dependent increase in Cav1.2 activity, leading Fuller et al. (2014) to propose that recruitment of calcineurin leads to rapid dephosphorylation of Cav1.2, thereby blocking the effects of PKA. Thus, competitive binding of different AKAPs that interact with distinct signaling partners to the Cav1.2 intracellular C-terminal domain may act to fine-tune channel activity and downstream calcium-mediated processes.
Sodium regulation of opioid receptor signaling

The opioid GPCRs mediate the response to both opiates and endogenous opioid peptides, signaling primarily through $\text{Go}_{i/o}$. Although it has long been known that physiological concentrations of sodium allosterically alter opiate binding and opioid receptor signaling, the underlying mechanisms have been unclear. Fenalti et al. (2014) obtained the 1.8-Å crystal structure of the human $\delta$-opioid receptor (residues 36–338 with an amino terminal $\beta_{60}$RIL [BRIL] fusion protein [BRIL-$\delta$OR($\Delta$N/$\Delta$C)]) in a complex with the $\delta$-opioid receptor antagonist naltrindole and used it to elucidate the mechanisms underlying sodium’s allosteric effects. This high resolution structure enabled characterization of the allosteric sodium site in a polar interaction network in the BRIL-$\delta$OR($\Delta$N/$\Delta$C)-naltrindole seven-transmembrane bundle core. In addition to the side chains of two highly conserved residues (Asp 95 and Ser 135) and two structurally conserved waters, the sodium ion was directly coordinated by the side chain of Asn 131, a residue conserved among opioid receptors but not other class A GPCRs. Substituting Asn 131 with alanine or valine increased basal $\beta$-arrestin signaling (a noncanonical pathway), but not that of $\text{Go}_i$. Asn 131 mutants showed increased affinity for a peptide agonist, and the Asn 131 Ala mutation abolished sodium’s reduction of agonist binding and also $\text{Go}_i$ activity. Mutation of Asp 95 also abolished the effect of sodium on agonist binding and, like mutation of two other sodium site residues (Asn 310 and Asn 314), converted $\delta$-opioid receptor ligands that fail to activate $\beta$-arrestin signaling in wild-type receptors into $\beta$-arrestin-biased agonists. Thus, the authors conclude that sodium-coordinating residues not only influence ligand binding affinity but also act as “efficacy switches” for downstream pathways of opioid GPCR signaling.

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