Generally Physiological

Of cilia, titin, and neurosteroids

This month’s installment of Generally Physiological discusses a calcium compartment in primary cilia, how changes in titin splicing can lead to skeletal muscle myopathy, and a negative feedback loop whereby a neurosteroid protects against hyperactivation of type-1 cannabinoid (CB₁) receptors.

A ciliary calcium compartment

A pair of papers from the same group (DeCaen et al., 2013; Delling et al., 2013) has identified a heteromeric calcium-permeable TRP channel in primary cilia and defined these organelles as a specialized calcium signaling compartment. Solitary nonmotile structures known as primary cilia project from most vertebrate cells to act as sensory organelles; these specialized structures have been implicated in hedgehog signaling pathways, and ciliary defects are associated with various human disorders, including polycystic kidney disease. Patch-clamp analysis of primary cilia visualized with targeted, genetically encoded fluorophores revealed an outwardly rectifying noninactivating current ($I_{\text{cilia}}$), with current density substantially greater than that in the cell body; permeability to calcium of the ciliary channel was estimated as six times that of sodium or potassium (DeCaen et al., 2013). $I_{\text{cilia}}$ was activated by extracellular uridine or adenosine phosphates (presumably acting through a purinergic GPCR), and by cell-permeable calmodulin antagonists, but was inhibited by Gd³⁺ and ruthenium red. siRNA-mediated knockdown of the polycystin proteins PKD1L1 or PKD2L1 decreased $I_{\text{cilia}}$, and the much-diminished $I_{\text{cilia}}$ apparent in mice lacking PKD2L1 was linear and insensitive to a calmodulin antagonist. Heterologously expressed PKD1L1 and PKD2L1, which could be coimmunoprecipitated from HEK293 cells, yielded whole-cell currents with a single-channel conductance similar to that in primary cilia; moreover, like $I_{\text{cilia}}$, these currents were activated by calmodulin antagonists and blocked by Gd³⁺ and ruthenium red.

In the second paper, Delling et al. (2013) used a ratiometric calcium sensor targeted to cilia (SMO-mCherry-GCaMP3) to measure ciliary [$Ca^{2+}$] simultaneously with that in the cytoplasm (monitored with Fluo-4). Rupturing the membrane at the ciliary tip elicited a rapid increase in ciliary [$Ca^{2+}$] that traveled down the cillum, with little effect on cytoplasmic [$Ca^{2+}$], even at the cilia–cell body junction. $Ca^{2+}$ moved readily from the cytoplasm to the cillum, indicating that the lack of effect of an increase in ciliary [$Ca^{2+}$] on cytoplasmic [$Ca^{2+}$] did not result from diffusion barriers at the ciliary base, but rather from the difference in volume between the two compartments (a ratio of ∼1:30,000). Resting ciliary [$Ca^{2+}$] was substantially higher than resting cytoplasmic [$Ca^{2+}$] and ciliary membrane potential was substantially more positive than that in the cell body, indicating that cilia represent a functionally distinct ionic compartment. Mice lacking PKD2L1 showed defects in hedgehog signaling as well as intestinal malrotation (a phenotype consistent with defects in the hedgehog pathway). The authors thus conclude that the high [$Ca^{2+}$] in cilia is maintained through their small volume and density of $Ca^{2+}$ influx pathways, with PKD1L1-PKD2L1 acting as a ciliary $Ca^{2+}$ channel to modulate ciliary [$Ca^{2+}$] and thereby hedgehog signaling.

Triggering myopathy

The enormous muscle protein titin functions as a molecular spring, with isoforms of different size and resistance to stretch produced through alternative splicing of the extensible spring region (composed of repeating immunoglobulin [Ig]-like domains and the PEVK region). Noting that various skeletal muscle myopathies are associated with changes in titin’s elasticity, in this issue Buck et al. used a mouse model lacking nine titin Ig domains (IG KO) to investigate the effects of a small increase in...
titin stiffness. The mutant mice showed a slight curvature of the spine—consistent with skeletal muscle myopathy—a decrease in the sizes of the soleus and diaphragm, a shift in myosin isoform composition, and changes in muscle contractility. Soleus muscle showed an increase in passive stress greater than predicted from the loss of only nine Ig domains, and titin exon microarray analysis revealed additional changes in titin splicing that produced much smaller (and therefore much stiffer) isoforms than anticipated. Further investigation revealed that abundance of the splicing factor RBM20 was increased in the IG KO mice, and that IG KO mice crossed with a mouse with decreased RBM20 activity failed to show these additional changes in titin splicing. Thus, the authors conclude that RBM20 plays a crucial role in determining titin’s size and elasticity, and that increasing titin’s resistance to stretch can lead to pathological changes in skeletal muscle.

Proposed mechanism whereby THC increases pregnenolone synthesis from cholesterol through a rapid pathway involving an increase in P450scC that depends on Erk1/2MAPK phosphorylation, and slower pathways involving an increase in P450scC independent of Erk1/2MAPK phosphorylation, and phosphorylation of hormone-sensitive lipase (HSL). (From Vallée et al. 2014. Science, 343:94–98. Reprinted with permission from AAAS.)

Blocking the effects of THC
Although most familiar as peripheral hormones that act through nuclear receptors, steroids, which can also act through nongenomic mechanisms, are also synthesized in the brain (neurosteroids), where they are thought to influence mood and behavior. Vallée et al. (2014) found that intraperitoneal injection of Δ⁶-tetrahydrocannabinol (THC; the main active ingredient in marijuana) produced a large increase in pregnenolone (3β-hydroxypregn-5-en-20-one), generally viewed as an inactive precursor to functionally active steroids, in various regions of rodent brain. In the brain, THC acts mainly through CB₁ receptors, and injection of CB₁ agonists increased pregnenolone in the nucleus accumbens (a region showing a marked increase in response to injection of THC), whereas a CB₁ antagonist suppressed the THC-dependent increase. THC increased the abundance of cytochrome P450scC, the mitochondrial enzyme that converts cholesterol to pregnenolone, in a manner that depended partially on phosphorylation of Erk1/2MAPK, and stimulated a slower increase in the phosphorylation of hormone-sensitive lipase, which hydrolyzes cytoplasmic cholesterol esters. Conversely, pregnenolone inhibited CB₁-dependent synaptic, behavioral, and somatic effects of THC in rodents, and attenuated a THC-dependent increase in Erk1/2MAPK phosphorylation and mitochondrial respiration in cultured cells expressing human CB₁, although it did not attenuate a CB₁-dependent decrease in cAMP. Thus, the authors propose that pregnenolone inhibits a CB₁ signaling pathway to act in a negative feedback loop and protect against CB₁ receptor hyperactivation.

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REFERENCES