Generally Physiological

Stretching and setting boundaries

This month's installment of *Generally Physiological* considers a channel in skeletal muscle and a cardiac G protein–coupled receptor (GPCR) that are sensitive to mechanical stress as well as their respective ligands, and how the geometry of subcellular domains influences GPCR dynamics.

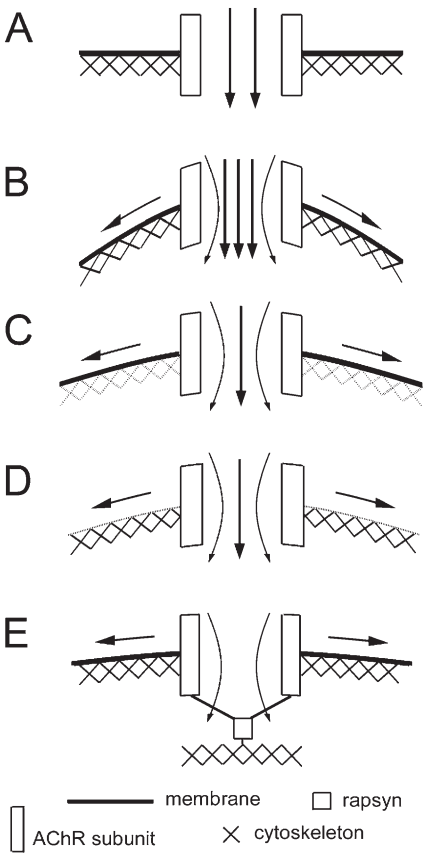
Stretching the boundaries of nAChR gating

Although ion channels are often broadly classified as "voltage-gated" or "ligand-gated," they can also respond to various other stimuli—such as light or stretch—and gating of a particular channel can be influenced by more than one modality. The nicotinic acetylcholine receptor (nAChR) that mediates excitatory transmission at the vertebrate neuromuscular junction is among the most familiar of the ligand-gated channels. Noting that mechanosensitive channels are abundant in skeletal muscle, and that the nAChR is subject to stretch associated with muscle contraction, Pan et al. (2012) explored the possibility that gating of this prototypical "ligand-gated channel" might be sensitive to mechanical stress. Patch-clamp analysis of endogenous nAChRs in cultured *Xenopus laevis* muscle cells or C2C12 myotubes, or heterologously expressed in HEK293T cells, indicated that membrane stretch enhanced ACh-dependent nAChR activity without affecting the amplitude of single-channel currents. Further analyses of nAChRs expressed in HEK293T cells implicated both the membrane microenvironment and the cytoskeleton in nAChR mechanosensitivity. nAChR activity was less sensitive to mechanical forces when membrane cholesterol was depleted, and more sensitive after exposure to lysophosphatidylcholine, suggesting a role for the lipid bilayer; disruption of the cytoskeleton with latrunculin A or cytochalasin D decreased nAChR mechanosensitivity, suggesting a role for the cytoskeleton. The mechanosensitivity of heterologously expressed nAChRs was greater than that of endogenous nAChRs and, intriguingly, coexpression of rapsyn (a protein essential for nAChR clustering at the neuromuscular junction) decreased nAChR mechanosensitivity, an effect that involved both the rapsyn nAChR-binding domain and the cytoskeleton. The authors thus concluded that both the membrane lipid environment and the actin cytoskeleton participate in regulation of nAChR mechanosensitivity and hypothesized that rapsyn—by linking nAChR to the cytoskeleton—may limit its mechanosensitivity and thereby protect muscle fiber integrity under conditions of high mechanical stress.

A channel and a GPCR sensitive to both ligands and mechanical stress and how subcellular geometry influences GPCR dynamics

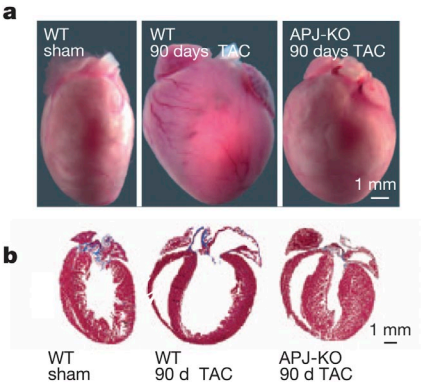
A bifunctional GPCR

Scimia et al. (2012) describe another stretch-sensitive ligand-activated receptor—in this case, not a channel but a GPCR. The apelin receptor APJ, like various other GPCRs, has been implicated in the regulation of cardiac function, suggesting that APJ—or its peptide ligand apelin—might provide therapeutic targets. Surprisingly, Scimia et al. (2012) found that, whereas mice lacking APJ were resistant to pathological cardiac hypertrophy in response to sustained pressure overload resulting from transaortic constriction, mice lacking apelin were not. Further analysis revealed that APJ mediated apelin-independent effects of stretch, and identified distinct physiological, pathophysiological, and biochemical APJ-mediated



Model of how nAChR mechanosensitivity could be influenced by the membrane microenvironment and by the actin cytoskeleton (© Pan et al. 2012. *Pflugers Arch.* 464: 193–203).

interactions between the two stimuli. In freshly isolated adult cardiomyocytes, APJ mediated a physiological stretch response that was decreased by apelin. Moreover, stretch acted through APJ to promote hypertrophy of cultured neonatal cardiomyocytes, and apelin attenuated this response. The distinct physiological and pathophysiological effects of stretch and apelin were associated

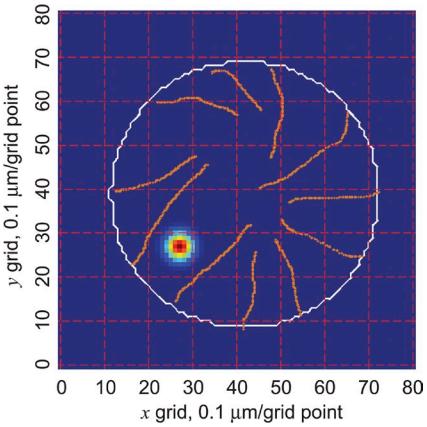


Loss of APJ protects mice from pathological cardiac hypertrophy secondary to transaortic constriction. (a) Hearts and (b) histological sections of hearts from mice subjected to sham surgery or transaortic constriction. WT, wild-type; APJ-KO, APJ knocked-out; TAC, transaortic constriction. Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. 488:394–398. 2012.

with differential activation of signaling pathways downstream of APJ: stretch decreased apelin-mediated G protein signaling but enhanced the recruitment of β-arrestin. Indeed, β-arrestin knockdown diminished the ability of stretch to promote hypertrophy, whereas the $G\alpha_i$ inhibitor pertussis toxin blocked apelin-mediated protection against stretch-induced hypertrophy. Thus, APJ appears to act as a bifunctional receptor for mechanical stretch and the peptide apelin, integrating the response to the two stimuli to influence the cardiac response to sustained overload.

Setting boundaries on GPCR diffusion

The downstream sequelae of GPCR activation can depend not only on how they are triggered but also on where they are located and what molecules are nearby. In living cells, GPCRs are typically found in specialized microcompartments, and it has been difficult to study how the geometry of such subcellular domains could affect GPCR signaling. Taking a careful and quantitative approach to this question, Najafi et al. (2012) used high-resolution multiphoton fluorescence recovery after photoconversion to monitor diffusion of fluorescently labeled GPCRs (constructs in which green fluorescent protein variants were fused to rhodopsin) in the subcellular domains formed by rod discs and their incisures in live *Xenopus* photoreceptors. Rod outer segments are filled with stacks of membranous discs, which are split into discrete segments by clefts (the incisures) that extend radially from the edge of the disc toward its center. Rhodopsin, the light-activated GPCR found in retinal photoreceptors, diffuses along the disc membrane, sequentially encountering and activating multiple G proteins to initiate downstream cascades so that, remarkably, activation of a single molecule of rhodopsin—activated by a single photon of light—can generate a photoreceptor response. Najafi et al. (2012) found that rhodopsin movement across the surface of disc membranes was markedly heterogeneous, but not within the microcompartments defined by the disc incisures. Using a model of molecular diffusion that explicitly took disc geometry into account, they determined that microcompartment geometry alone was sufficient to explain the heterogeneity of rhodopsin diffusion, providing a quantitative approach with which to begin to understand the effects of compartment geometry on GPCR signaling.



Grid used for modeling diffusion, with tracings from the image of a rod outer segment indicating disc perimeter (white) and incisures (orange) (from Najafi et al., 2012).

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