Bridging the Gap between Anomalous Sodium Channel Molecules and Aberrant Physiology

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Ours is a scientific era captivated by molecular reductionism. We often succumb to a belief that the key to understanding complex organisms resides in discovering the molecules and molecular properties that undergird macroscopic phenomena. Once the relevant molecular secrets are unlocked, the implications of their properties for macroscopic behavior is believed to be obvious and direct, at least in-the-large. The track record for this philosophical viewpoint can be quite convincing. Mutations in cone visual pigments explain differences in color perception (Merbs and Nathans, 1992). Long-term potentiation at synapses explains spatial learning (Silva et al., 1992a, 1992b). A chloride channel defect gives rise to cystic fibrosis (Rommens et al., 1989). This issue's paper by Cannon and Corey concerns the inherited disease of hyperkalemic periodic paralysis (HPP (Gamstorp, 1956)), one of the most beautiful examples of pathophysiological disorders explainable by the molecular reductionist approach. Patients with this affliction suffer attacks of muscular rigidity or flaccidity following modest elevations of serum [K^+]. The molecular culprit appears to be a mutant voltage-gated sodium channel in skeletal muscle, which fails to inactivate with maintained depolarization. Yet, there has been a worrisome gap between the marked sequelae of the disease and the admittedly small defect found in sodium channel function. Could there be more to the story? Cannon and Corey do not add here to the molecular details of the picture; rather, their contribution is to elaborate mathematically the consequences of a very slight defect in sodium channel inactivation upon skeletal muscle excitability. Their analysis shows that very modest perturbations in sodium channel gating lead quite naturally to devastating disturbances in skeletal muscle function. Beyond clarifying the molecular pathogenesis of HPP, Cannon and Corey's paper serves as an elegant reminder of the limitations of molecular reductionism, and the crucial need for other scientific approaches. To appreciate the full impact of their work, it is worth summarizing the quickening pace of research on HPP.

Physiological studies of muscle derived from HPP patients began to implicate faulty sodium channels as the root cause of the disease. In the mid 1980s, Lehmann-Horn and others provided preliminary evidence that an increase in extracellular [K] produced excess depolarization in muscle fibers biopsied from HPP patients. Coarse voltage clamp measurements showed that modest depolarization activated a small, noninactivating inward current that was blockable by tetrodotoxin. They surmized that sodium channels defective in inactivation could be the cause of HPP. Cannon et al. (1991) provided the single-channel evidence that something was indeed awry with sodium channels of myotubes cultured from HPP patients. Not only did a significant fraction of sodium channels fail to inactivate, but the unusual lack of inactivation was observed only when extracellular [K] was raised. This provided a most satisfying molecular explanation for the K^+ dependence of HPP attacks.

Work from several laboratories used genetic approaches to show tight linkage of HPP inheritance to the adult skeletal muscle sodium channel gene on chromosome 17. Two specific mutations were later found in the coding region of the skeletal muscle sodium channel, and both were very unlikely to represent benign polymorphisms. Finally, the sodium channel mutations identified in HPP patients were introduced into wild-type sodium channels, expressed in mammalian cells. These channels failed to inactivate to the extent observed in wild-type channels (Cannon and Strittmatter, 1993), thereby building an apparently air-tight case for the causative role of mutant sodium channels in HPP.

Nevertheless, there remained some ill-fitting pieces to the puzzle. The failure of sodium channels to inactivate occurred in only a few percent of the sodium channels (Cannon et al., 1991). It seemed a leap of faith to expect that this miniscule alteration in sodium channel gating could produce the broad panorama of conspicuous electrophysiological disturbances in HPP. Moreover, when the HPP mutations were introduced into wild-type sodium channels (Cannon and Strittmatter, 1993), the slight failure to inactivate was not sensitive to external [K], as were the sodium channels in myotubes cultured from HPP patients. How, then, could the potassium dependence of HPP attacks be explained? Were the sodium channels in cultured myotubes not representative of sodium channels in vivo? Could the sodium channel gating defects represent only minute epiphenomena of a more fundamental molecular defect?

Two approaches were used to address these concerns: one physiological, and the other theoretical. The physiological approach (Cannon and Corey, 1993) aimed to create an HPP model by using a pharmacological agent to slow inactivation in a small fraction of sodium channels in normal skeletal muscle fibers. This is rather like a quick-and-dirty version of a transgenic animal model for HPP. The empirical result is that HPP-like behavior is created, including the potassium dependence of "attacks." Thus, inactivation need fail in only a small action of sodium channels to create the disease, and the potassium sensitivity of HPP can arise as an emergent system property of the muscle. While this empirical approach demonstrates the sufficiency of modest sodium channel gating defects in producing HPP, it doesn't tell us how this comes about. That's where this issue's theoretical analysis by Cannon and Corey makes an enormous contribution.

The first half of their paper describes numerical integration of a system of nonlinear Hodgkin-Huxley-type equations that describe skeletal muscle excitability. The surface membrane and *t*-tubule compartments are treated as distinct entities, and the essential spirit of the analysis is not much different than the model of Adrian and Marshall (1976). The distinction of the Cannon

and Corey model is that the analysis aims specifically to evaluate the ability of a small defect in sodium channel inactivation to produce HPP-like behavior, and it does so completely. However, the numerical analysis is no more than a theoretical version of the physiological validation described above; the system of nonlinear differential equations are sufficiently complex that we still don't understand how HPP behavior arises from such a minor defect in sodium channel gating. The major advance comes in the second half of the paper. Here Cannon and Corey reduce the full-scale model into a two-variable approximation, amenable to classical phase-plane analysis. Although this sort of reduction of Hodgkin-Huxley models is not new (FitzHugh, 1961, 1969; Nagumuo et al., 1962;), never has the approach been more elegantly and aptly applied to a critically important problem. The phase-plane analysis represents the entire repertoire of behaviors of which the system is capable, all in terms of a two-dimensional graph. The impact of elevated [K] and sluggish sodium channel inactivation become apparent through "a rapid geometric intuition." Hence, the reduced-model analysis allows us to know why just a few bad sodium channels and elevated external [K] can conspire to create myotonia or paralysis.

This initial chapter in HPP research opens up exciting possibilities for the future. Certainly other specific sodium channel mutations will be found in different patients diagnosed under the broad umbrella of HPP. Elaborating the functional consequences of these will contribute to our understanding of structure-function relationships in sodium channels, as well as to explain, perhaps, the discrepant findings regarding the potassium sensitivity of sodium channels (Cannon et al. (1991) versus Cannon and Strittmatter (1993)). Different mutational variants may have been involved. The pace of discovery of defective channels in related inherited disorders is likely to quicken, and quantitative analysis of the functional consequences of these will be required. Ultimately, gene therapy involving knockout and/or replacement of misbehaving components will follow.

Of more general import, the reduced model of Cannon and Corey points to a type of analysis that will become increasingly important. It is certainly essential to identify the aberrant molecules involved in disease. But molecules interact with each other in gigantic dynamical systems to produce physiological function. So the transduction from molecular properties to organismic function cannot always be obvious and direct, but is more often subtle and complex. Huge numerical integrations will help in linking up these two domains, just as brute-force molecular dynamic simulations help elucidate structure-function relations of proteins. However, what is critically required beyond these computer-intensive approaches is this: an understanding of how certain molecular properties translate into unexpected macroscopic behavior. The Cannon and Corey strategy succeeds royally in the particular setting of HPP, but there will no doubt be instances where problems can't be boiled down into two state variables, and alternate analytical strategies and approaches will need to be developed and applied. Answering this need constitutes an ongoing challenge for biophysics.

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