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Visualizing Proton Conductance in the Gramicidin Channel

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In this month's *Biophysical Journal*, Pomes and Roux (1996) provide a molecular dynamics simulation of protonic conductance along a single strand of hydrogen-bonded water in the gramicidin channel. This result complements an earlier report by Sagnella and Voth (1996) and is significant because protons do not depend on diffusion alone to move through aqueous media. Instead a proton can hop along chains of hydrogen-bonded water molecules, so that the measured ionic mobility of protons in water is several times higher than that of sodium or potassium ions. Proton conduction by hopping along water chains was first proposed by Hladky and Haydon (1972) to explain the anomalously high proton conductance through the gramicidin channel, which is approximately 15 times that of potassium ions.

Although proton conductance through the gramicidin channel is intrinsically interesting, it may also provide a useful model system for biological systems of more general interest. The term "proton wire" was coined by Nagle and Morowitz (1978) to describe possible proton conductance pathways in proteins in which hydrogen-bonded side chains of amino acids could provide pathways for protons. Although a proton wire composed purely of amino acid side chains has not yet been discovered, there are now a number of proposed protonic conductance processes in which water molecules are likely to be involved. For instance, proton conductance along water chains in proteins has been proposed for bacteriorhodopsin and bacterial reaction centers (see Pomes and Roux, this issue, for references) and the F_1Fo ATP synthase (Schulten and Schulten, 1985; Akeson and Deamer, 1992). It is here that the new molecular dynamics simulations will be most useful in guiding further research. Pomes and Roux show that protons in the channel are present as $O_2H_5^+$, rather than OH_3^+. Furthermore, a proton strongly orients the water in the channel and moves within the water by a semi-collective transfer mechanism, rather than by random diffusion or a highly coordinated process. Proton translocation is limited by the hydrogen bonds between water molecules and polar groups on the sides of the channels. If the hydrogen bonding forces are turned off in the simulation, proton mobility increases dramatically. Taken together, these results provide a new conceptual framework to investigate mechanisms by which proteins conduct protons as part of their function.

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Slow Inactivation of Sodium Channels: More Than Just a Laboratory Curiosity

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Sodium currents are usually regarded as fast transients. In response to depolarization, channels open but then rapidly close within a millisecond to an inactive state. Repolarization of the membrane is necessary to reprime channels for subsequent depolarization-induced opening. The recovery from inactivation is voltage dependent and, after brief depolarizations, occurs within 10 ms or so at the resting potential of excitable cells (−70 to −90 mV). This orchestrated sequence of rapid voltage-dependent changes in sodium channel conformation produces many of the salient features of an action potential: fast upstroke of the depolarizing phase (activation), termination of depolarization (inactivation), and the refractory period (recovery from inactivation), as originally described so elegantly by Hodgkin and Huxley.

In addition to these rapid gating transitions, sodium channels undergo very slow voltage-dependent shifts in availability for opening, on a time scale of seconds to minutes. In this issue of the *Biophysical Journal*, Cummins and Sigworth (1996) show that slow inactivation is impaired in a mutant form of the skeletal muscle sodium channel that causes the hereditary muscle disorder hyperkalemic periodic paralysis (HyperPP). This result may be the key to a previously unanswered question about the mechanism of the prolonged episodes of weakness in HyperPP (Ruff, 1994) and provides the first direct evidence for a potential physiological role of slow inactivation.

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Episodic weakness in HyperPP typically lasts for hours during which muscles are flaccid, depolarized, and unable to generate action potentials. The aberrant depolarization has been attributed to defects observed in the rapid gating of sodium channels (reviewed in Cannon, 1996). Persistent Na^+ currents, slowed rates or shifted voltage dependence of fast inactivation, or negative shifts in the voltage dependence of activation have been observed for each of the 15 or so missense mutations of the skeletal muscle α subunit that cause periodic paralysis. Furthermore, even a small persistent Na^+ current is sufficient to provoke the repetitive myotonic discharges that often occur in HyperPP; and activity-driven accumulation of K^+ in the transverse tubule may exacerbate a mild depolarization to produce electrical inexcitability and weakness.

These defects of fast inactivation account for the development of episodic weakness, but why does an attack persist for hours? Ruff (1994) proposed that slow inactivation must also be impaired; otherwise over a matter of minutes slow inactivation would shut off the aberrant Na^+ current, the muscle would repolarize, and excitability would be restored. Previous voltage-clamp studies were limited to depolarizations of 50 or 100 ms, long compared to fast inactivation but too brief to assess slow inactivation. Interestingly, on theoretical grounds it is not necessary to include a defect of slow inactivation (Cannon, 1994). Model simulations that incorporated slow inactivation showed prolonged periods of membrane inexcitability and weakness are interspersed with only very brief moments (<0.001% of the duration of inexcitability) of restored excitability due to the sluggish entry to and recovery from normal slow inactivation. Hence, there were no data nor theoretical bases to confirm or refute Ruff's hypothesis.

In this issue, Cummins and Sigworth (1996) provide the first evidence that slow inactivation is impaired by a mutation linked to HyperPP. The distinction between slow and fast inactivation is made on the basis of recovery from inactivation. Sodium channels recover quickly, within milliseconds at −100 mV, from the inactivation that occurs during brief depolarizations lasting up to a few hundred milliseconds. After depolarization of several minutes duration, however, sodium channels enter slow inactivated state(s) from which recovery at −100 mV requires seconds to minutes. When the rat α subunit homolog of the human HyperPP mutant T704 M was expressed in human embryonic kidney (HEK) cells, Cummins and Sigworth (1996) found that even after a 5 min conditioning pulse to −20 mV, ~25% of the maximal Na^+ current recovered within 10 ms at −100 mV. For wild-type channels, <3% of the Na^+ current had recovered by the same voltage protocol. The magnitude of this defect implies that the anomalous persistent Na^+ current, observed in previous studies at the end of a 50 ms depolarization, will be slowly reduced by only a factor of 4, instead of the greater than 40-fold reduction if slow inactivation had been intact. A direct measure of the slow inactivation defect on the persistent current was not possible, however, because in Cummins and Sigworth's studies (1996) the persistent current was small and washed out over minutes. Another surprise was that the voltage dependence of slow inactivation for wild-type Na^+ channels expressed in HEK cells was different from the behavior observed for native channels in skeletal muscle. In loose-patch measurements from mammalian muscle (Almers et al., 1984), the steady-state voltage dependence of slow inactivation is shifted −20 mV relative to that of fast inactivation (h_∞), whereas in HEK cells the voltage dependence was nearly identical. Cummins and Sigworth (1996) reported that this difference was not because of to their use of internal fluoride, and we have observed that cotransfection of HEK cells with the Na^+ channel α and β_1 cDNA does not restore the normal voltage dependence of slow inactivation (Hayward and Cannon, unpublished results). Despite these loose ends, the slow inactivation defect reported by Cummins and Sigworth (1996) is clearly different from the behavior of wild-type channels and is likely to contribute to the attacks of weakness in HyperPP.

The functional and structural relations between fast and slow inactivation of Na^+ channels are very poorly understood. Fast inactivation is distinct from, and yet possibly coupled to, slow inactivation (Rudy, 1978). The states are distinct in that slow inactivation is preserved even after a complete loss of fast inactivation by internal application of proteolytic enzymes. Evidence for coupling of the two processes is supported by the observation that internal protease exposure hastened the development of slow inactivation, by removing competition from fast inactivation. In contrast, the rate of recovery from slow inactivation was unaffected by the presence or absence of fast inactivation. Cummins and Sigworth (1996) followed up on these previous observations by using a more precise lesion of fast inactivation, the F1304Q mutation in the III-IV interdomain linker. Although fast inactivation was severely disrupted, F1304Q mutants were slow inactivated to the same extent as wild-type channels and recovered with the same slow kinetics. A reciprocal defect has been reported by Balser et al. (1996). Substitution of a single residue that lies near the outer mouth of the pore in domain I, W402C, almost totally prevents slow inactivation while fast inactivation remains intact. Impediment of slow inactivation at this external site may be an additional mechanism by which raised extracellular K^+ aggravates the weakness in HyperPP.

Slow inactivation of Na^+ channels was originally observed in preparations that had been chronically depolarized by internal perfusion of nonphysiological salt solutions. Subsequently, detailed study of this process required complex voltage protocols with long conditioning pulses, brief hyperpolarized gaps, and a depolarizing test pulse. What then, is the physiological role of slow inactivation? When would the natural activity of a cell ever reveal a distinction between fast and slow inactivation? One possibility is that slow inac-

tivation (which for Na^+ channels in skeletal muscle is shifted by -20 mV relative to rapid inactivation) regulates basal excitability (Almers et al., 1984.) The study by Cummins and Sigworth (1996) provides another clear example: an impairment of slow inactivation may predispose a cell to prolonged periods of depolarization and failure of impulse generation. Some mutations of the human skeletal muscle Na^+ channel α subunit disrupt fast inactivation and are associated with myotonia but do not cause weakness. It is tempting to speculate that the presence or absence of a slow inactivation defect will determine which mutations lead to prolonged episodes of weakness. Regardless of the answer, slow inactivation will no longer be viewed as a laboratory curiosity with uncertain physiological significance.

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