Cannon's Reply to Ruff

Slow Sodium Channel Inactivation Need Not Be Disrupted in the Pathogenesis of Myotonia and Periodic Paralysis

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A biophysically subtle defect in sodium channel inactivation has been implicated as the direct cause of both myotonia and paralysis for two related disorders, hyperkalemic periodic paralysis (Cannon et al., 1991) and paramyotonia congenita (Lerche et al., 1993). Unitary sodium currents recorded from affected muscle occasionally fail to inactivate: bursts of openings persist throughout maintained depolarizations of several hundred milliseconds, and the mean open time is prolonged. The failure of rapid inactivation occurs in clusters of successive trials, which implies a slow modal switch in gating. The functional defect arises from any one of several identified point mutations in the coding region of the adult skeletal muscle isoform of the sodium channel α subunit gene on chromosome 17 (reviewed in Ptacek et al., 1993). Mutant sodium channels gate in the noninactivating mode in 2–10% of trials, whereas in comparison, 0.1% of depolarizations show bursts of openings for wild type channels. We have sought to establish that this small proportion of noninactivating sodium channels (20- to 100-fold higher than normal) is sufficient to cause myotonia and paralysis. In a toxin-based model in rat skeletal muscle, sodium channels with a steady-state open probability of 0.01–0.02 caused myotonia (Cannon and Corey, 1993). This result was corroborated in a theoretical model and extended to show that a slightly higher proportion of noninactivating channels (Popen 0.02–0.07) produced paralysis by depolarization-induced block of action potential generation (Cannon et al., 1993).

Dr. Ruff points out that "slow" inactivation of sodium channels can be an important regulator of electrical excitability for skeletal muscle (Ruff et al., 1988). Slow inactivation occurs on a minute time scale, it occurs at more negative potentials than the more commonly studied Hodgkin-Huxley type fast inactivation, and it may drastically alter the distribution of channels between closed and slow-inactivated states for small shifts in the resting potential (Almers et al., 1984). Dr. Ruff contends that slow, as well as fast, inactivation must be disrupted in order to evoke paralysis by prolonged depolarization. The fidelity of slow inactivation has never been tested adequately in muscle from patients with myotonia or periodic paralysis, so an experimental basis for Dr. Ruff's claim remains unknown. I have unpublished records of bursts of openings in mutant channels that persist throughout an entire 1-second depolarization, but this is still a very short duration in comparison to the 2- to 5-minute time constant for slow inactivation. Thus, the premise can only be tested with modeling at present.

We did not include slow inactivation in our published theoretical model for several reasons. First, the time scale of slow inactivation is too slow to have any impact on the *development* of myotonia or paralysis. On the time scale of milliseconds or even seconds, slow inactivation is static and, therefore, is effectively incorporated already into the sodium peak conductance variable, $\bar{g}_{Na}$. Second, the steady-state behavior of the model reduces to the trivial solution of returning to the normal resting potential if a slow inactivation variable is included. All of the insight gleaned from the phase plane analysis, which is valid on the time scale of a few tens of seconds, would be lost. In one sense, Dr. Ruff is correct: an *infinitely* prolonged depolarization cannot occur if slow inactivation is intact.

To understand the effect of slow inactivation on a time scale of minutes, I modified our theoretical model (Cannon et al., 1993) to incorporate slow inactivation. The sodium conductance was multiplied by a slow inactivation parameter, $S(V,t)$. The steady-state voltage dependence of S was approximated with a single-barrier Boltzmann distribution

$$S_\infty(V) = \frac{1}{1 + e^{(V - V_s)/A_s}}$$

where V_s = -108 mV and A_s = 5.8 mV, as reported for rat fast twitch muscle by Ruff et al. (1988). The rate of slow inactivation varies by about twofold over the range −100 to −50 mV (Ruff et al., 1988; Almers et al., 1984). For convenience, a voltage-independent time constant of 120 s was chosen for the simulation. A holding current was applied during the simulation so that the resting potential was set to the value for rat muscle reported by Ruff (1988) of −109 mV. At this potential S = 0.53. To keep the baseline excitability of the fiber comparable to the previous model, $\bar{g}_{Na}$ was doubled. All other parameters assumed the values from our previous model.

We repeated the simulation presented in our Fig. 7 (Cannon et al., 1993). Slow inactivation was intact for all of the sodium channels. Fast inactivation was disrupted in 7% of sodium channels, "f" equaled 0.07, and a suprathreshold current pulse was applied for 2 ms. The transmembrane voltage for the sarcolemma and the slow inactivation parameter are plotted for a 500-s simulation in Fig. 1. The initial response is identical to the case with no slow inactivation: a single action potential is elicited, followed by an oscillation

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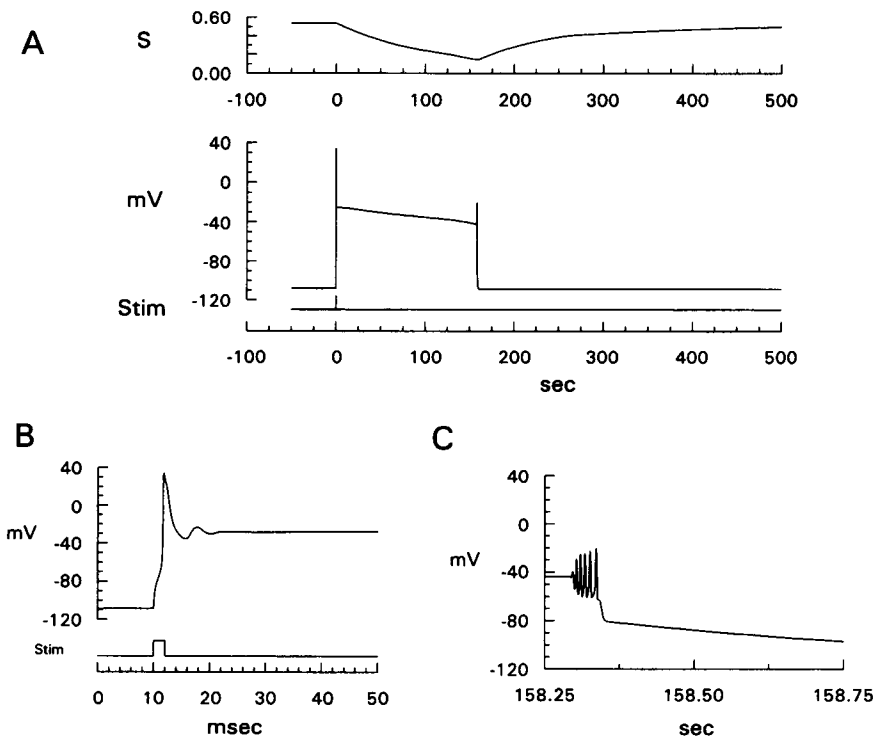

FIGURE 1 Model response to a brief suprathreshold stimulus for a simulated muscle fiber with partially disrupted fast inactivation and normal slow inactivation of sodium channels. (*A*) The slow inactivation parameter, *S*, and transmembrane voltage are plotted on a slow time scale. The response at the onset and termination of the prolonged depolarization are illustrated on expanded times scales in (*B*) and (*C*). Simulation was performed using the model presented by Cannon et al. (1993) with the following modifications. Slow inactivation was incorporated as described in the text. $\bar{g}_{Na}$ was increased to 300 mS/cm^2. Holding current was −55 μA/cm^2 and a stimulus current of 160 μA/cm^2 was applied for 2 ms. A variable time step was used for the numerical integration with a maximum set to 0.1 ms as in our previous computations.

and relaxation to a depolarized potential of −28 mV (Fig. 1 *B*). From this potential, 93% of the sodium channels have undergone rapid inactivation, the model is refractory to generating an action potential, and the fiber is paralyzed. As slow inactivation progresses, the membrane potential then slowly repolarizes. Finally, after 158 s, slow inactivation has reduced the persistent sodium current sufficiently for the system to repolarize to the normal resting potential. During repolarization, a train of attenuated spikes occurs as a brief myotonic run (Fig. 1 *C*). A recovery to normal excitability lagged far behind repolarization of the membrane because of the prolonged time required for recovery from slow inactivation. *S* must exceed 0.3 in order for the same stimulus to elicit an action potential of normal amplitude. The slow inactivation parameter reached a nadir of 0.14 and did not increase to 0.3 until 50 s after repolarization of the membrane. Thus, the simulated fiber would be paralyzed for 210 s in response to a single suprathreshold stimulus. In vivo, another action potential would occur within a few tens to hundreds of ms and start the cycle again: a duty cycle for which the fiber has normal excitability <0.001% of the time. The asynchronous activation of different motor units within a muscle would result in flaccid paralysis, even with intact slow inactivation of sodium channels.

Thus, slow inactivation does not prevent the paralytic depolarization of fibers, but it does return them to an excitable state after several minutes. This may be an important mechanism in the *recovery* from a plateau depolarization or a train of myotonic discharges, but the presence of slow inactivation will *not* prevent the development of these pathologic states. Although slow inactivation does eliminate the possibility of a stable depolarized potential as a *steady-state* solution for the model, it does not alter the physiological consequence of a disruption in fast inactivation of sodium channels. A loss of fast inactivation for a small proportion of sodium channels, without any defect in slow inactivation, is sufficient to produce both myotonia and paralysis.

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