On Mutations that Uncouple Sodium Channel Activation from Inactivation

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ABSTRACT Computations on sodium channel gating were conducted using a closed–open-inactivated coupled kinetic scheme. The time constant of inactivation (τ_h) derives a voltage dependency from coupling to voltage-dependent activation even when rate constants between inactivated and other states are strictly voltage independent. The derived voltage dependency does not require any physical, molecular link between the structures responsible for inactivation and the charges producing voltage-dependent activation. The only requirement is that the closed to inactivated rate constant (k_{CI}) differs from the open to inactivated (k_{OI}), consistent with experimental results. A number of mutations and other treatments uncouple sodium channel activation and inactivation in that the voltage dependency of τ_h is substantially reduced while voltage-dependent activation persists. However, a clear basis for uncoupling has not been described. A variety of experimental results are accounted for just by changes in the difference between k_{OI} and k_{CI}. In wild type channels, $k_{OI} > k_{CI}$ and inactivation develops with a delay whose time constant is just that for channel opening. Mutations that reduce the $k_{OI} - k_{CI}$ difference reduce the amplitude of the delay process and the derived voltage dependency of τ_h. If $k_{OI} = k_{CI}$, inactivation develops as a single exponential (no matter what the number of closed states), activation and inactivation become independent, parallel processes, and any voltage dependency of τ_h is then entirely intrinsic to inactivation. If $k_{OI} < k_{CI}$, inactivation develops as the sum of exponentials, τ_h at negative potentials speeds and then slows with more positive potentials. These predicted $k_{OI} < k_{CI}$ effects have all been seen experimentally (O'Leary, M.E., L.-Q. Chen, R.G. Kallen, and R. Horn. 1995. *J. Gen. Physiol*. 106: 641–658). An open to closed rate constant of zero also removes the derived voltage dependency of τ_h, but activation and inactivation are still coupled and the inactivation delay remains.

INTRODUCTION

In native sodium channels, the sodium conductance (g_{Na}) activation and inactivation processes are coupled together. One important observation, among a number, that establishes this result is gating charge immobilization (Armstrong and Bezanilla, 1977), in which voltage clamp protocols that promote the entry of channels into the inactivated state affect the gating current (I_g). As I_g has substantially the kinetics of the activation process, such findings are incompatible with independent, parallel activation and inactivation. Another important observation is that inactivation develops with a delay, indicating a precursor process (Goldman and Schauf, 1972; Gillespie and Meves, 1980; Bean, 1981; Goldman and Kenyon, 1982; Goldman, 1989; O'Leary et al., 1995; Mitsuiye and Noma, 1995). In *Myxicola* axons, the time constant of the inactivation-delaying process was found to be essentially identical to that governing the rise in g_{Na} (Goldman, 1989). This finding provides a particularly clear demonstration of coupling; the process delaying inactivation development is channel opening.

The delay in inactivation development is actually diagnostic of coupling independently of the identification of the delaying process as channel opening. Strictly independent, parallel activation and inactivation requires that the probability of a transition into the inactivated state (the transition rate constant given that the state is occupied) is identical for all states. In that case, the inactivation time course is independent of the relative occupancies of all other states. Should even one of these rate constants significantly differ from the others, then activation and inactivation are coupled. Any delay in inactivation development means that one or more early states have an appreciably different (in fact smaller, see below) inactivation rate constant from later states. A delay cannot be attributed solely to an independent inactivation process. This is the case even if inactivation is itself multistate, and can be easily seen by considering the progress of inactivation during a depolarizing step in potential when all channels are initially in the closed state furthest away from the open. The first transition in a multistep inactivation process will then be to a state from which a subsequent net transit to the open state (either directly or through intermediate transitions) either is or is not possible. If it is not possible, then that first transition has been to an inactivated state, and a delay occurs only if the rate constants for transition into this state are not identical for all states, i.e., activation and inactivation are coupled. If it is possible, then there will be a delay in inactivation (whose time constant need not be that of channel opening) and activation and inactivation are coupled because there is at least one conformational transition of the channel protein that is common to both activation and inactivation development. The early time course of the development of inactivation is particularly informative regarding the channel state diagram.

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One consequence of activation–inactivation coupling is that the observed voltage dependence of inactivation, e.g., of the time constant of the decay of the current during a step in potential (τ_h), will then necessarily derive, at least in part, from that of activation, i.e., from the voltage dependency of closed–closed and closed–open transition rate constants. This derived voltage dependency will be in addition to any that may be intrinsic to open-inactivated or closed-inactivated conformational changes. However, there have been a number of recent reports, using several different sodium channel isoforms, of site-directed mutations that uncouple activation from inactivation in that the voltage dependence of τ_h was substantially reduced while voltage dependent activation persisted, with any changes in activation properties showing no consistent pattern among the various mutations. Mutations that produce uncoupling have all been localized to one of three regions: 1) positively charged residues of putative transmembrane segment S4 of repeat domain 4 (D4; Chahine et al, 1994; Chen et al., 1996); 2) residues in the D3–D4 linker (O'Leary et al., 1995; Kellenberger et al., 1997a,b) which is a central component of the inactivation gate (Vassilev, et al., 1988; Stuhmer et al., 1989; Patton et al., 1992; West et al., 1992); or 3) residues in the S4–S5 cytoplasmic loop of D4 (Tang et al., 1996, 1998; Filatov et al., 1998), which is believed to contribute to the site that the inactivation gate associates with on closure in both potassium and sodium channels (Isacoff et al., 1991; Holmgren et al., 1996; Mitrovich et al., 1996; Smith and Goldin, 1997). A similar uncoupling was seen in cardiac channels modified by the sea anemone toxin, Anthopleurin A (Hanck and Sheets, 1995). A question of interest, then, is how uncoupling might be produced.

To address this question, most of the mutagenesis studies attributed the derived voltage dependency of τ_h seen in wild type (WT) channels to some physical, molecular link between the structures responsible for inactivation and the core of the channel protein. The sodium channel protein core includes the positively charged residues of the S4 segments, which function as activation voltage sensors (Stuhmer et al., 1989; Auld et al., 1990; Fleig et al., 1994; Yang and Horn, 1995; Yang et al., 1996). This proposed physical linkage between structures was expected to, in some way, allow a small component of the charge displacement that accompanies activation transitions and so provides activation voltage dependency (Yang and Horn, 1995; Yang et al., 1996) to also accompany transitions into the inactivated state, thus conferring a voltage dependency on inactivation. As the detailed nature of the proposed physical linkage is not known, the actual mechanism by which the various mutations produced uncoupling could not be specified. In no case has a clear basis for uncoupling been described.

I present here computations with a simple kinetic scheme that do provide reasonable explanations for the experimental uncoupling results. The nature of the proposed physical linkage need not be specified, nor is a physical linkage necessarily implied by the derived voltage dependency of inactivation. The derived voltage dependency arises just from the state diagram alone. An array of complex kinetic effects seen experimentally (O'Leary et al., 1995) are predicted by the computations, and it is noted that comparisons of the early time course of inactivation development in WT and mutant channels can be an effective method for identifying the basis of uncoupling.

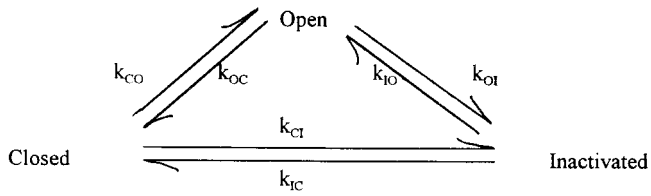
A preliminary communication of some of these results has been made (Goldman, 1999).

RESULTS AND DISCUSSION

Origin of the derived voltage dependency of τ_h

The proposed physical linkage between the structures responsible for inactivation and the core of the channel protein is a mechanism by which an intrinsic voltage dependency is conferred on inactivation transitions. In this case, regardless of where they may be located, there would still be a displacement of charges during the conformational changes associated with inactivation, and the inactivation rate constants would then be voltage dependent. While there might actually be an intrinsic voltage dependency of inactivation produced by such a physical, molecular link, inactivation will derive a voltage dependency from coupling to activation even when all inactivation rate constants are strictly voltage independent. This is because activation–inactivation coupling arises entirely from the fact that the rate constants for transitions into the inactivated state are not the same for all states. Hence, the progress of inactivation depends on the time course of the *relative* occupancies of the various states, and therefore, on all the rate constants that determine these relative occupancies. *Constant* τ_h is determined by the net draining of the conducting state and, in general, depends on the rate constants connecting open and inactivated, those connecting open and closed, and those connecting closed and inactivated states, because these latter determine an open–closed-inactivated pathway for draining the open state. Any voltage dependency of any of these rate constants contributes to the observed voltage dependency of τ_h.

This is illustrated with a closed–open-inactivated scheme:



SCHEME

This scheme is quantitatively analyzed in the Appendix. Expressions are presented for the time courses of changes in the probability of occupancy of the open state ($Y(t)$; Eq. A3) and the inactivated state ($Z(t)$; Eq. A8), during a step in

potential, entirely in terms of the transition rate constants defined in the scheme and the initial state occupancies at the start of the step. This scheme is considerably simplified compared to any that might be an accurate representation of a real sodium channel. For example, a number of closed states have been lumped into just one, and there must be more than one inactivated state because recovery from inactivation develops with a delay (Chandler and Meves, 1970; Schauf, 1974; Kuo and Bean, 1994). However, some insights can still be gained.

$Y(t)$ (Eq. A3) and $Z(t)$ (Eq. A8) are each given by a steady state and two exponential terms. The relaxation rate constants (a and b) for the two exponential terms are identical for both $Y(t)$ and $Z(t)$, and are described in terms of the transition rate constants of the scheme by Eqs. A4 and A5. The term $1/b$ corresponds to the experimental τ_h. As is shown in Eq. A5, τ_h depends on both k_{CO} and k_{OC}. Numerical computations readily establish that a more positive test potential (simulated by increasing k_{CO} and decreasing k_{OC}) will speed τ_h (provided that $k_{OI} > k_{CI}$) even when all other rate constants remain fixed, i.e., are voltage independent. It is not necessary, then, for inactivation to be intrinsically voltage dependent or for there to be some physical link between the structures mediating inactivation and the positively charged residues of the S4 segments for inactivation to derive a voltage dependency from activation.

A mechanism for uncoupling

Goldman (1995), in a single channel study, directly measured the time course of sodium channel inactivation just from closed states in neuroblastoma N1E 115 cells and concluded that all closed states directly inactivate and do so with about the same closed to inactivated rate constant. This provides some justification for the lumped closed states of the above scheme, and also suggests a mechanism for uncoupling. If other sodium channels behave like those in neuroblastoma, then a sufficient condition for activation–inactivation coupling is that the rate constant from open to inactivated states (k_{OI}) differs from those from closed to inactivated (k_{CI}). Any mutation or treatment that eliminates this difference (e.g., by lowering the free energy level of the open state and so raising the open-inactivated energy barrier) will fully uncouple activation and inactivation leaving independent, parallel processes. This is readily demonstrated with the above scheme.

By setting $k_{OI} = k_{CI}$, Eq. A2 becomes

$$dZ/dt = -(k_{IC} + k_{IO} + k_{OI})Z + k_{OI}, \tag{1}$$

and $Z(t)$ is independent of $Y(t)$ and $X(t)$. A solution to Eq. 1 for a step in potential is given by

$$Z(t) = Z(\infty) + (Z(0) - Z(\infty))\exp(-t/\tau_h), \tag{2}$$

with

$$\tau_h = (k_{IC} + k_{IO} + k_{OI})^{-1} \quad \text{and} \quad Z(\infty) = \frac{k_{OI}}{k_{IC} + k_{IO} + k_{OI}}.$$

The identical result is, of course, obtained from the second order solution for $Z(t)$ (Eq. A8). The condition $k_{OI} = k_{CI}$ requires that the coefficient on $\exp(-at)$ will vanish, because a and b are then given by

$$a = k_{CO} + k_{OC} + k_{OI} \quad \text{and} \quad b = k_{IO} + k_{IC} + k_{OI}.$$

Under this $k_{OI} = k_{CI}$ condition, inactivation develops as a single exponential (Eq. 2). This same simple exponential time course is also obtained if the above scheme is expanded to include any number of closed states, given the finding (Goldman, 1995) that all closed states directly inactivate with about the same rate constant, because the progress of inactivation is then independent of the relative occupancies of the various states. Similarly, under these conditions, inactivation displays a simple exponential time course even if there is more than one inactivated state, for example, one with activation gates open and one with activation gates closed (Kuo and Bean, 1994), when the various inactivated states, in aggregate, are absorbing. The $k_{OI} = k_{CI}$ condition, then, is a plausible uncoupling mechanism even for considerably more complex schemes than the one illustrated.

The occupancy of the conducting state ($Y(t)$) is evaluated for this same $k_{OI} = k_{CI}$ condition in the Appendix (Eq. A12). Over the potential range where inactivation goes to completion, $k_{IO} = k_{IC} = 0$, $Y(\infty) = 0$, and b becomes just k_{OI}. Evaluating Eq. A12 over this range, we have

$$\begin{aligned} Y(t) = {} & Y(0)[\exp(-(k_{CO} + k_{OC})t))(\exp(-k_{OI}t)] \\ & + \frac{k_{CO}}{k_{CO} + k_{OC}}(1 - Z(0)) \\ & \cdot [\exp(-k_{OI}t) - (\exp(-(k_{CO} + k_{OC})t))(\exp(-k_{OI}t))]. \end{aligned} \tag{3}$$

Equation 3 is just mh (Hodgkin and Huxley, 1952) as expected, with

$$Y(t) = m(t)h(t), \qquad h(t) = 1 - Z(t),$$

$$\tau_m = (k_{CO} + k_{OC})^{-1}, \qquad m(\infty) = k_{CO}/(k_{CO} + k_{OC}),$$

$$\tau_h = 1/k_{OI} \quad \text{and} \quad h_\infty = 0.$$

There is a power of one on m because only a single closed state has been assumed. It requires inactivation to go to completion to demonstrate this identity because, otherwise, a four-state scheme would be needed to reduce to mh (one inactivated state with activation gates open and another with them closed). The condition $k_{OI} = k_{CI}$, then, fully uncouples activation and inactivation.

The result expressed in Eq. 3 and following demonstrates the essential continuity between the views developed here and the analysis of Hodgkin and Huxley (1952). It is shown below that, with regard to activation–inactivation coupling, Hodgkin–Huxley kinetics corresponds to one point on a continuum of behaviors determined by the relative values of k_{CI} and k_{OI}.

Agreement with experimental observations

Changes in the relative values of k_{OI} and k_{CI} can account for a wide array of experimental uncoupling observations. In WT channels, there is a clear delay in the development of inactivation. In the above scheme, a delay in inactivation is produced when $k_{OI} > k_{CI}$. This can be easily seen by inspection of Eqs. A5 and A8 under the conditions at which the experimental $\tau_h(V)$ observations were made, i.e., with inactivation going to completion ($k_{IC} = k_{IO} = 0$; $Z(\infty) = 1$) and negligible occupancy of the inactivated state at the start of the step. From Eq. A5, when $k_{OI} = k_{CI}$, then $b = k_{CI}$. When $k_{OI} > k_{CI}$, then $b > k_{CI}$, and $b < k_{CI}$ when $k_{CI} > k_{OI}$. The effect of these relations on the early time course of inactivation can be assessed using Eq. A8. For simplicity, Eq. A8 can be evaluated for the condition that all channels are in the closed state at the start of the step which, is a reasonable approximation to the experimental conditions. We have

$$Z(t) = 1 - [(k_{CI} - b)/(a - b)]\exp(-at) + [(k_{CI} - a)/(a - b)]\exp(-bt). \quad (4)$$

As a will always be greater than k_{CI}, when $b > k_{CI}$, Eq. 4 is the difference of exponentials. When $b = k_{CI}$ then $Z(t)$ is described by a single exponential, and when $b < k_{CI}$, Eq. 4 is the sum of exponentials.

In WT channels, then, $k_{OI} > k_{CI}$, and there is a delay in inactivation development whose time constant in the above scheme is identical to that governing the rise in g_{Na} (Eq. A8). This is just the result seen experimentally in *Myxicola* axons (Goldman, 1989), suggesting that the close similarity between the various k_{CI} values found in neuroblastoma may also be the case for other sodium channels. Inactivation just from closed states develops as a simple exponential with no detectable delay (Goldman, 1995). Delays in inactivation development have only been seen when that from open states is also included. During the delay interval, then, channels are still inactivating from closed states (Bean, 1981; Aldrich and Stevens, 1983; Goldman, 1995). The rate of inactivation is just slower than it will be later in the step. It is only when channels have transited to the open state that the rate of inactivation significantly increases yielding a delay process whose kinetics are the same as channel opening. Additional delaying process arising from transitions between closed states not included in the above scheme will be too rapid relative to the other relaxations and so too small in relative amplitude to be detected over the potential ranges used experimentally for determination of the inactivation delay (positive to about −35 mV; Goldman and Kenyon, 1982). Mutations or other channel modifications that reduce the difference between k_{OI} and k_{CI} will reduce the relative amplitude of the delay process. The weight of the voltage-dependent k_{CO} and k_{OC} terms in the determination of b will also be reduced. Hence, the voltage dependency of τ_h will be reduced. If the $k_{OI} - k_{CI}$ difference is reduced as a result of a decrease in k_{OI}, then τ_h should both slow and display a reduced voltage dependency. This result, a slowed τ_h accompanying a reduced voltage dependency, has been seen experimentally (e.g., Chahine et al., 1994; Tang et al., 1996; Chen et al., 1996). When k_{OI} and k_{CI} no longer display any appreciable difference, inactivation is described by a single exponential, b no longer depends on k_{CO} or k_{OC}, and any observed voltage dependency of τ_h will then be entirely intrinsic to the inactivation process. If $k_{CI} > k_{OI}$, inactivation develops as the sum of two exponentials (with, again, any additional relaxations arising from closed–closed transitions often being too small to detect experimentally). Hence, the early time course of inactivation can be particularly informative as regards the nature of the effects produced by treatments that uncouple activation from inactivation.

The condition that $k_{CI} > k_{OI}$ has additional consequences. First, increasing k_{CI} so that it is greater than k_{OI} with no other changes can, itself, speed τ_h (Eq. A5). Second, under this condition, a positive step in potential (simulated by increasing k_{CO} and decreasing k_{OC}) will actually increase, i.e., slow, τ_h. This is also evident from inspection of Eq. A5 and readily demonstrated with numerical computations. These effects have all been seen experimentally.

O'Leary et al. (1995) studied a number of mutations in the D3–D4 linker of human H1 (SkM2) cardiac sodium channels. One of these, a double mutation of two tyrosines at positions 1494 and 1495 to two glutamines (YY/QQ), abolished the normal voltage dependence of τ_h. The time constant of inactivation over negative potential ranges as determined with two pulses (τ_c) was considerably speeded in the mutant. These τ_c values primarily reflect inactivation from closed states (Goldman, 1995), suggesting that k_{CI} was considerably increased. Correspondingly, the clear delay in inactivation development seen in WT channels was not seen in the mutant. Rather, inactivation in the YY/QQ mutant developed as the sum of two exponentials (Fig. 1), τ_h at more negative potentials was speeded over that in WT, and τ_h increased, i.e., slowed, with more positive potentials (O'Leary et al., 1995), all in striking agreement with the predictions of the above scheme when $k_{CI} > k_{OI}$. The ability to predict this complex array of experimental kinetic effects is encouraging for the uncoupling mechanism suggested here and the underlying ideas on which it is based: that all closed states directly inactivate with about the same closed to inactivated rate constant, and that the origin of coupling in native sodium channels is that the probability of inactivating from the open state is greater than that from closed states. Some similar effects (a speeding of τ_h over that in WT at negative potentials and a slowing of τ_h with more positive potentials) were seen following substitution of glutamine for alanine at position 1649 of the S4-S5 cytoplasmic loop of D4, again in human H1 (Tang et al., 1998). τ_h also slowed with more positive potentials following substitution of methionine for threonine at 1491 in the in the D3–D4 linker of rat brain type IIA sodium channels (Kellenberger et al., 1997a). However, in neither of these latter two cases were effects on the early time course of

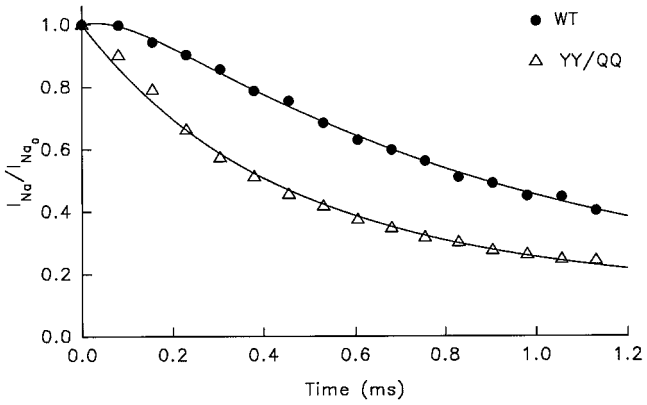


FIGURE 1 Time course of two-pulse inactivation in wild type (WT; *filled circles*) and mutant (YY/QQ; *open triangles*) human H1 sodium channels expressed heterologously. Data are from O'Leary et al. (1995) and represent the means of five WT and six YY/QQ cells. The YY/QQ mutant has two glutamines replacing two tyrosines at positions 1494 and 1495 of the D3–D4 linker. Holding potential was −120 mV, conditioning potential was −30 mV, and test potential was 0 mV. A 1-ms step back to −100 mV was included between each conditioning and test pulse pair. Smooth curves are a best fit two exponential description in each case. Fits were to a more extended set of values than those illustrated (See O'Leary et al., 1995; Fig. 3). WT data were fitted with the difference of exponentials with a τ_{delay} of 0.097 and a τ_C of 0.937 ms. QQ/YY data are described by the sum of two exponentials with $\tau_{C\ Fast}$ of 0.359 and a $\tau_{C\ Slow}$ of 1.035 ms. Hence, the mutation converts a delay in the development of inactivation to inactivation that develops as the sum of exponentials. The fitted values reported by O'Leary et al. (1995) were based on a single exponential raised to a power. (Data kindly provided by Drs. R. Horn and M. E. O'Leary.)

inactivation reported, and it is not clear if a similar $k_{CI} > k_{OI}$ mechanism applies.

Another mechanism for uncoupling

Uncoupling of the voltage dependency of τ_h from that of activation can also be produced by setting the back, open to closed rate constant (k_{OC}) to zero. If the steady state of the above scheme is to be a true thermodynamic equilibrium, i.e., the scheme is not coupled to an energy source, the following relation is required (detailed balance):

$$k_{CO}k_{OI}k_{IC} = k_{OC}k_{CI}k_{IO}. \tag{5}$$

If k_{OC} is zero, Eq. 5 requires that at least one other rate constant also be zero. This can be satisfied with the condition that inactivation proceeds to completion. In that case,

$$a = k_{CO} + k_{CI} \quad \text{and} \quad b = k_{OI}.$$

Activation and inactivation actually remain coupled. $Z(t)$, in the above scheme, is still described by two exponentials. However, b no longer depends on the closed–open transition rate constants, and so, the voltage dependency of τ_h will be reduced. Activation rate constants will affect only the early time course of inactivation, again producing a delay if $k_{OI} > k_{CI}$ and the sum of two exponentials if $k_{CI} > k_{OI}$. The loss of the derived voltage dependency of b is because the net draining of the conducting state is now determined solely by k_{OI}. Note that, just as for the effects produced by reducing the $k_{OI} - k_{CI}$ difference, τ_h becomes ever less dependent on the voltage dependency of k_{CO} and k_{OC} as k_{OC} approaches zero (Eq. A5).

At more positive potentials, k_{OC} may approximate zero in native sodium channels. If so, then any voltage dependency of τ_h observed over very positive potential ranges could be intrinsic to inactivation.

Effects on $h_\infty(V)$

The experimental voltage dependency of steady-state fast inactivation ($h_\infty(V)$) is just $1 - Z(\infty)$ in the above scheme. State $Z(\infty)$ (Eq. A9) can be written as

$$\begin{aligned} Z(\infty) = {} & k_{CI}(k_{CO} + k_{OC} + k_{OI}) + k_{CO}(k_{OI} - k_{CI}) \\ & \div k_{IC}(k_{CO} + k_{OC} + k_{OI}) + k_{IO}(k_{CO} + k_{OC} + k_{CI}) \\ & + k_{CI}(k_{CO} + k_{OC} + k_{OI}) + k_{CO}(k_{OI} - k_{CI}). \end{aligned} \tag{6}$$

Numerical computations with Eq. 6 demonstrate that $Z(\infty)$ is voltage dependent even when only k_{CO} and k_{OC} are voltage dependent with no intrinsic inactivation voltage dependency.

As for τ_h, treatments that reduce the difference between k_{OI} and k_{CI} will reduce the voltage dependency of $Z(\infty)$, because $Z(\infty)$ will then be weighted less by k_{CO} and k_{OC} (Eq. 6). In sixteen experimental treatments that both produced a clear reduction in the voltage dependency of τ_h (see references in the Introduction) and for which steady-state inactivation data were available, the steepness of the $h_\infty(V)$ curve was reduced in twelve and unchanged in two. In two cases, the voltage dependency of steady state inactivation increased somewhat even though that of τ_h decreased. Effects on the midpoint of the $h_\infty(V)$ curve varied widely and showed no consistent pattern among the various experimental treatments. In the case in which $k_{OI} = k_{CI}$, Eq. 6 reduces to the result obtained directly from Eq. 2 for this condition, and any voltage dependency of the steady state occupancy of the inactivated state will then be entirely intrinsic to inactivation.

While activation–inactivation coupling means that the voltage dependency of k_{CO} and k_{OC} will contribute to the voltage dependency of steady-state inactivation, Eq. 6 cannot account for the experimental $h_\infty(V)$ curve if there is no intrinsic inactivation voltage dependency, i.e., with k_{OI}, k_{IO}, k_{CI} and k_{IC} all voltage independent. Over potential ranges for which inactivation goes to completion ($Z(\infty) = 1$), Eq. 6 requires that the quantity $(k_{CO}(V_2)(k_{IO} + k_{IC}) + k_{OC}(V_2)(k_{IO} + k_{IC}) + k_{IC}k_{OI} + k_{IO}k_{CI})$ be negligible. As $Z(\infty)$ will be unity over very positive potential ranges where k_{CO} can be very large, this condition can only be satisfied when the quantity $(k_{IO} + k_{IC})$ is negligible. However, over very negative potentials where there is no occupancy of the inactivated state ($Z(\infty) = 0$), Eq. 6 requires that

$$\begin{aligned} & k_{CO}(V_1)k_{OI} + k_{OC}(V_1)k_{CI} + k_{OI}k_{CI} \\ & \ll k_{CO}(V_1)(k_{IO} + k_{IC}) + k_{OC}(V_1)(k_{IO} + k_{IC}) + k_{IC}k_{OI} + k_{IO}k_{CI}, \end{aligned}$$

which is incompatible with the requirements that ($k_{IO} + k_{IC}$) is fixed and negligible. If there is no intrinsic voltage dependency to inactivation, $Z(\infty)$, as computed from Eq. 6, will saturate over negative potentials at $Z(\infty) > 0$ and over positive potentials at $Z(\infty) < 1$, and inactivation will never go to completion, suggesting that there is some intrinsic voltage dependency to inactivation.

CONCLUSIONS

The voltage dependency that sodium channel inactivation derives owing to its coupling to activation does not require a physical, molecular link between the structures responsible for inactivation and the core of the channel protein. A derived voltage dependency is produced even when all rate constants for transitions between inactivated and other states are strictly voltage independent. All that is required is that the rate constants for transit into the inactivated state are not the same for all states.

Experiments on neuroblastoma (Goldman, 1995) suggest a simple basis for activation–inactivation coupling: the rate constants for transitions from each of the closed to the inactivated state are all very similar in value, but are significantly smaller than that between open and inactivated. An implication is that inactivation will develop with a delay whose time constant is the same as that governing channel opening, as is seen experimentally (Goldman, 1989). A second implication is that the voltage dependency of the rate constant of inactivation from closed states (*e*-fold for 22 mV; Goldman, 1995) will be entirely intrinsic to inactivation, because the progress of closed state inactivation is then independent of the relative occupancies of the various closed states. A third implication is that a simple basis for uncoupling is provided. It is only necessary, in that case, to eliminate any significant disparity in the values of the closed to inactivated and the open to inactivated rate constants to uncouple.

Although changes in the relative values of closed to inactivated and open to inactivated rate constants can reasonably account for some of the experimental mutagenesis findings (O'Leary et al., 1995), a number of other mechanisms are also possible, and the actual mechanism must be experimentally identified. The central conclusion of this work is that comparisons of the early time course of inactivation development in WT and mutant channels can be helpful in identifying a mechanism for uncoupling.

APPENDIX

Analysis of a closed–open-inactivated kinetic scheme

The scheme presented in Results and Discussion is described, in the usual way, by three coupled first-order differential equations, giving the rate of change of the probabilities of occupancy of the closed (dX/dt), open (dY/dt), and inactivated (dZ/dt) states. As the sum of the probabilities of occupancy of the three states is unity at all times, X can be eliminated yielding the pair of coupled first-order differential equations

$$dY/dt = -(k_{OC} + k_{OI} + k_{CO})Y + (k_{IO} - k_{CO})Z + k_{CO}, \quad \text{(A1)}$$

$$dZ/dt = -(k_{IC} + k_{IO} + k_{CI})Z + (k_{OI} - k_{CI})Y + k_{CI}. \quad \text{(A2)}$$

Equations A1 and A2 can be combined into a single second order differential equation for either X or Y, with the solutions for a step in potential

$$Y(t) = Y(\infty) - \left[\frac{\dot{Y}(0) + b(Y(0) - Y(\infty))}{a - b}\right]\exp(-at) + \left[\frac{\dot{Y}(0) + a(Y(0) - Y(\infty))}{a - b}\right]\exp(-bt), \quad \text{(A3)}$$

where

$$a = 1/2(k_{CO} + k_{OC} + k_{OI} + k_{IO} + k_{CI} + k_{IC}) + [1/4(k_{CO} + k_{OC} + k_{OI} - k_{IO} - k_{IC} - k_{CI})^2 + (k_{IO} - k_{CO})(k_{OI} - k_{CI})]^{1/2} \quad \text{(A4)}$$

$$b = 1/2(k_{CO} + k_{OC} + k_{OI} + k_{IO} + k_{CI} + k_{IC}) - [1/4(k_{CO} + k_{OC} + k_{OI} - k_{IO} - k_{IC} - k_{CI})^2 + (k_{IO} - k_{CO})(k_{OI} - k_{CI})]^{1/2} \quad \text{(A5)}$$

$$Y(\infty) = (k_{IC}k_{CO} + k_{IO}k_{CO} + k_{CI}k_{IO}) \div (k_{IC}k_{OC} + k_{IC}k_{OI} + k_{IC}k_{CO} + k_{IO}k_{OC} + k_{IO}k_{CO} + k_{CI}k_{OC} + k_{CI}k_{OI} + k_{CO}k_{OI} + k_{CI}k_{IO}) \quad \text{(A6)}$$

$$\dot{Y}(0) = -(k_{OC} + k_{OI} + k_{CO})Y(0) + (k_{IO} - k_{CO})Z(0) + k_{CO}, \quad \text{(A7)}$$

with $Y(0)$ and $Z(0)$ indicating the probabilities of occupancy at the start of the step, and

$$Z(t) = Z(\infty) - \left[\frac{Z(0) + b(Z(0) - Z(\infty))}{a - b}\right]\exp(-at) + \left[\frac{Z(0) + a(Z(0) - Z(\infty))}{a - b}\right]\exp(-bt), \quad \text{(A8)}$$

where

$$Z(\infty) = (k_{CI}k_{OC} + k_{CI}k_{OI} + k_{CO}k_{OI}) \div (k_{IC}k_{OC} + k_{IC}k_{OI} + k_{IC}k_{CO} + k_{IO}k_{OC} + k_{IO}k_{CO} + k_{CI}k_{OC} + k_{CI}k_{OI} + k_{CO}k_{OI} + k_{CI}k_{IO}), \quad \text{(A9)}$$

$$Z(0) = -(k_{IC} + k_{IO} + k_{CI})Z(0) + (k_{OI} - k_{CI})Y(0) + k_{CI}. \quad \text{(A10)}$$

Behavior when $k_{OI} = k_{CI}$

$Z(t)$, for this condition, is given in Results and Discussion. Evaluating $Y(t)$ for $k_{OI} = k_{CI}$ yields

$$Y(t) = Y(\infty) - \left[\frac{-aY(0) + (k_{IO} - k_{CO})Z(0) + k_{CO} + b(Y(0) - Y(\infty))}{k_{CO} + k_{OC} - k_{IO} - k_{IC}}\right] \cdot exp(-at) + \left[\frac{(k_{IO} - k_{CO})Z(0) + k_{CO} - aY(\infty)}{k_{CO} + k_{OC} - k_{IO} - k_{IC}}\right]\exp(-bt). \quad \text{(A11)}$$

This can be written as

$$Y(t) = Y(\infty) - \left[\frac{-aY(0) + b(Y(0) - Y(\infty))}{k_{CO} + k_{OC} - k_{IO} - k_{IC}}\right]\exp(-at) - \left[\frac{aY(\infty)}{k_{CO} + k_{OC} - k_{IO} - k_{IC}}\right]\exp(-bt) + \left[\frac{(k_{IO} - k_{CO})Z(0) + k_{CO}}{k_{CO} + k_{OC} - k_{IO} - k_{IC}}\right](\exp(-bt) - \exp(-at)), \quad \text{(A12)}$$

which can be shown to be just mh when inactivation goes to completion.

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