Modelling the activation, opening, inactivation and reopening of the voltage-gated sodium channel

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A model of the voltage-gated sodium channel is put forward suggesting that the four S4 voltage-sensors behave as screw-helices making a series of discrete transitions that carry one elementary charge for each notch of the screw helix. After the channel has been activated by the first two steps $R \rightleftharpoons P \rightleftharpoons A$ in all four domains, followed by a voltage-independent rearrangement, it is opened by a third cooperative step $A \rightleftharpoons B$ in domains I, II and III in conjunction with hydration. Inactivation is a voltage-dependent process controlled by the third step $A \rightleftharpoons I$ in sensor IVS4, and the closing of the channel is brought about its dehydration. From the inactivated steady state the channel may be reopened by a fourth step, $I \rightleftharpoons C$ in sensor IVS4 and rehydration. The computed kinetics of the model are shown to conform closely with those observed experimentally.

Keywords: sodium channel; voltage gating; model of ion channels

1. INTRODUCTION

It was proposed by Keynes (1992, 1994) that the sodium channel is first activated by two successive steps $R \rightleftharpoons P \rightleftharpoons A$ in the four S4 voltage sensors, each carrying a charge of the order of $0.9 e_0$, and that its opening then involves a further voltage-driven step and its voltage-independent hydration. However, no suggestion was made as to the identification and location of the voltage-sensor responsible for opening the channel. The purpose of this paper, following up that of Keynes & Elinder (1998) presenting new evidence on the kinetics of the $R \rightleftharpoons P \rightleftharpoons A$ steps, is to propose a unified series-parallel model based on the concept that all the voltage-dependent steps in the activation, opening, inactivation and reopening of sodium channels involve unit transitions in the S4 α -helices of the four domains carrying a single elementary charge, and that their opening and closing follows their voltage-independent hydration and dehydration.

2. THE STRUCTURE AND FUNCTION OF THE S4 VOLTAGE-SENSORS

The S4 α -helices are characterized by the precise spacing of the positively charged arginine or lysine residues that occupy every third position, separated by hydrophobic residues. As may be seen in figure 2 of Keynes (1994), there are four closely conserved positive charges thus spaced in the S4 unit of domain I, five in domains II and III, and eight in domain IV, making a total of 22 for the whole channel, with additional positive charges close to the inner ends of IS4 and IIIS4 that have different spacings. It was proposed by Guy & Seetharamulu (1986) and Catterall (1986) that each S4 unit was

thus enabled to operate as a screw helix, which was stabilized by the formation of ion pairs between the positive charges and fixed negative charges in neighbouring parts of the channel. Under the influence of the electric field, the S4 α -helix can twist through 60° and move 0.45 nm inwards or outwards so that each positive charge finds itself automatically in the right position to pair up with the next fixed negative charge, this movement being accompanied by the creation of unpaired charges at either end, and hence a transfer across the membrane of about $1 e_0$, the exact figure depending on the position of the α -helix and the fraction of the field that was involved. As ion pair formation between movable positive charges and fixed negative ones is central to the screw-helical theory, it should be noted that analysis of the published data for the location of negative charges shows that for the six species covered by fig. 2 of Keynes (1994), there are 13 just as closely conserved negative charges grouped in a zone not far from the inner ends of helices S1, S2 and S3, and four more outside this zone, together with a further seven in the SS1-SS2 β -hairpins connecting S5 and S6, though none in S5 and S6 themselves. Towards the inner ends of the S2 and S3 segments of *Shaker* and other K^+ channels there are two similarly placed negative charges (Wei *et al.* 1990).

The extremely close conservation of both sets of charges in Na^+ and K^+ channels right across the animal kingdom strongly implies that the one in three spacing of the movable positive charges, which enables some of them to pair up with a fixed set of negative charges, must be fundamentally essential for their functioning. Although the elegant evidence of Yang *et al.* (1996) on Na^+ channels, and of Larsson *et al.* (1996) on K^+ channels, on the accessibility of the S4 charges to hydrophilic reagents on both

sides of the membrane, indicates that the central hydrophobic pore is appreciably shorter than had previously been supposed, it could nevertheless be long enough for eight or even 12 of the 16 negative charges in S1, S2 and S3 to be located in its walls, so enabling a screw-helical pairing to take place with two or three of the positive charges carried by each S4 segment. The additional mobile charges carried by the S4 units would accordingly emerge into the aqueous phases on either side, where they would be balanced by anions in solution. A modified version of the screw-helical hypothesis conforming with the new evidence on the short length of the hydrophobic pore, could in this way account neatly for the transfer of precisely $1 e_0$ across the membrane for each notch of the screw helix.

As has been shown by Hirschberg *et al.* (1995) for the sodium channels of skeletal muscle, and confirmed for squid axons by Keynes & Elinder (1998), the total transfer of charge needed to open a channel is $12 e_0$. The structural evidence therefore fits with the view that each of the four S4 voltage-sensors is capable of occupying a series of four and in one case five discrete states, between all of which there are transitions involving a transfer of $1 e_0$. In the outline state diagram shown in figure 1a, and the extended version shown in figure 1b, the individual states are labelled R, P, A and B in domains I, II and III, while in domain IV they are labelled R, P, A, I and C.

After all four of the S4 segments have first reached their state A in the manner described by Keynes & Elinder (1998), the channel must be supposed to undergo a voltage-independent structural rearrangement taking it to the state labelled AAAA*, in which the individual voltage-sensors are rendered free to proceed with their third voltage-dependent A→B transitions. The intervention of such a conformational change, which is rate-limiting at large potentials, is necessary to delay to the experimentally observed extent both the opening of the channel and the generation of the gating current that accompanies it. In the state diagrams shown in figure 1a,b the transition from AAAA to AAAA* cannot be identified as the voltage-independent hydration step that must precede opening at some point (Rayner *et al.* 1993), because this would be incompatible with the evidence on the effect of D₂O on the gating system. It was shown by Conti & Palmieri (1968) that in heavy water the kinetics of both opening and closing in the squid giant axon were slowed by a factor of 1.4, but Meves (1974) subsequently found that D₂O had little or no effect on the sodium gating current, as was confirmed for *Myxicola* axons by Schauf & Bullock (1979), and most recently for crayfish axons by Alicata *et al.* (1990), leading inescapably to the conclusion that the main steps which generate the gating current must precede the principal D₂O-sensitive transition. It is consequently proposed that hydration mainly takes place after arrival of the channel at the state shown as BBBA, taking it to the initial open state BBBC, though a subsidiary route via a closed hydrated state AAAA has been included. When voltage-sensor IVS4 has moved to its fourth state I, the channel is postulated to remain open in state BBBI, but after a voltage-independent dehydration, it finally reaches the closed inactivated state BBBI. When IVS4 is in its fifth state C

and the channel has been rehydrated, it briefly reaches the third open state BBBC. The hydration and dehydration rate constants are subject to appropriate modification in these three configurations of the channel.

3. OPENING OF THE CHANNEL

Although, as described by Keynes & Elinder (1998), the initial transitions R⇌P and P⇌A and the late phases of the gating current for potentials from +20 to +100 mV do carry $1 e_0$, there are contrary indications that the transfer of charge involved in the actual opening of the channel might be appreciably greater. Thus, the studies of Conti & Stühmer (1989) on gating current fluctuations in rat-brain-II Na⁺ channels expressed in *Xenopus* oocytes showed that single shots of about $2.3 e_0$ were present, and in similar experiments on *Shaker* K⁺ channels Sigg *et al.* (1994) showed that the final stage of channel opening was accompanied by movements of at least $2.4 e_0$ in discrete blocks. There is also evidence for the transfer of more than $1 e_0$ in studies of the voltage dependence of the tail currents flowing on closure of Na⁺ channels by deactivation, this being the backward step closest to the open state. Thus, for the experiment illustrated in figure 16a of Keynes (1994) the charge for deactivation Q_d on stepping back from the initial peak for a pulse to 0 mV was estimated as $3.4 e_0$ for an electrically symmetrical system, in contrast to the inactivation charge Q_i of $0.9 e_0$, whereas the average of Q_d from seven similar experiments done by Keynes & Meves (1993) was $1.8 e_0$. Comparably large values have also been reported in the studies of the open times of single Na⁺ channels from other sources (Aldrich & Stevens 1987; Yue *et al.* 1989; Berman *et al.* 1989; Benndorf & Koopman 1993).

To explain these and other observations within the framework of the S4 gating system shown in figure 1b, we propose that the A⇌B transitions in domains I, II and III operate with a degree of positive cooperativity that results in their behaving almost like a single transition, whereas the corresponding A⇌I transition in domain IV has the function of controlling inactivation. The properties of the resulting system may be explained by the simplified state diagram shown in (1) below:

where the three-letter states denote different combinations of states A and B of IS4, IIS4 and IIIS4, and k and l are forward and backward rate constants expressed in the conventional way as in equations (6) and (7).

The need to introduce sufficient auto-correlation between the AAA→AAB, AAB→ABB and ABB→BBB transitions to make them look like single shots of $2.3 e_0$ in the gating current noise could not be met by the type of voltage-dependent cooperativity factor used by Keynes & Elinder (1998) to account for the slowly rising phase of the sodium gating current for the R⇌P⇌A transitions, because the cooperativity factor κ had to be of the order of 10 for this purpose. Had κ been voltage-dependent the existence of at least six extra charges would be implied, for which there was no evidence. If κ is large, the overall rate from AAA to BBB can be written as

$$k_{AAA \rightarrow BBB} = 6\kappa k^3 / ((2k + \kappa l)(\kappa k + 2l)), \quad (2)$$

and the backward rate as

$$k_{BBB \rightarrow AAA} = 6\kappa l^3 / ((2k + \kappa l)(\kappa k + 2l)), \quad (3)$$

so that the time constant is

$$\tau = 1 / (k_{AAA \rightarrow BBB} + k_{BBB \rightarrow AAA}) = (2k + \kappa l)(\kappa k + 2l) / 6\kappa(k^3 + l^3). \quad (4)$$

The resulting τ (V) curve can be approximated to the sum of two components

$$\tau = 1 / (3(k + l) + (\kappa + 2) / (6(k^3 + l^3))), \quad (5)$$

where the first and second terms denote, respectively, singly and triply charged voltage-sensors.

To check the validity of this treatment of the kinetics of the transition between the resting state RRRR and the open states, we used fitted data for the $R \rightleftharpoons P \rightleftharpoons A$ steps from Keynes & Elinder (1998), and for the overall opening from figs 12a and 15 of Keynes (1994). The compound rate constant from AAAA to the open states was found manually, by changing its value until the opening time-course of the model was in close agreement with the experimental data. The values plotted as filled circles in figure 2 are the reciprocals of the resulting rate constants, and it will be noted that they flatten off markedly above +30 mV. This would indicate that there is a rate-limiting step in the system that makes itself apparent at large potentials, and that prevents the rate from rising above about 7 ms⁻¹. We propose that this is the voltage-independent rearrangement step $AAAA \rightleftharpoons AAAA^*$ in figure 1b.

For the $A \rightleftharpoons B$ transitions, experimental values for deactivation were taken from fig. 16 of Keynes (1994), shown in figure 2 as unfilled circles, and for the opening step between 0 and +30 mV from the filled circles. If κ was set at 10, then V_{eq} was found to be -28 mV, and k_{eq} to

Figure 1. Outline (a) and extended (b) state diagrams of the proposed model. In (b), the values of the forward and backward rate constants shown in lower case italics were taken from table 1, and were multiplied by appropriate powers of the cooperativity factors shown in Greek characters. The voltage-dependent cooperativity factor α in the upper part of the extended diagram corresponds to the product of the cooperativity factors shown in figure 6 of Keynes & Elinder (1998). In the lower part of the diagram the cooperativity factor κ was voltage-independent, and the various multiplying factors were introduced to ensure microscopic reversibility within the closed loops. Closed hydrated states are singly underlined, and open states are doubly underlined.

Figure 2. Kinetics of the cooperative $A \rightleftharpoons B$ steps in domains I, II and III that, followed by hydration, open the channel. Open circles show time constants observed experimentally for the deactivation, and the filled circles show the time constants between state AAAA and the open states determined as described in the text. The continuous line shows the sum and the dotted lines show the two components given by equations (5), (6) and (7) for $\kappa=10$, $k_{eq}=1.7\text{ ms}^{-1}$, $z=1$, $\beta=0.5$ and $V_{eq}=-28\text{ mV}$. The dashed line indicates a level of $140\text{ }\mu\text{s}$.

be 1.7 ms^{-1} . Even though the expressions in equations (2)–(5) apply strictly to much larger values of κ , simulations showed that deviations were small for cooperativities down to 10. It may consequently be concluded that the occurrence of this degree of cooperativity between the $A \rightleftharpoons B$ transitions of voltage-sensors IS4, IIS4 and IIIS4 each carrying $1\text{ }e_0$ is capable of accounting not only for the apparently larger single shots observed by Conti & Stühmer (1989) and Sigg *et al.* (1994) in the gating current noise, but also for the raised voltage-dependence of deactivation.

4. INACTIVATION OF THE CHANNEL

The modelling of inactivation has long been complicated by arguments as to whether or not its voltage-dependence is intrinsic, or is derived wholly from its coupling with activation. That it is truly voltage-dependent has been argued by Keynes (1991, 1992, 1994) on the grounds that as the process of recovery from inactivation at large negative potentials, as plotted in figs 20 and 45a of Keynes (1994), must be agreed to carry a charge of not less than $1\text{ }e_0$, the same applies to inactivation itself. It was therefore postulated that inactivation is controlled by the third transition, shown as $A \rightleftharpoons I$ in figure 1, of voltage-sensor IVS4. This proposition has recently received strong support from the demonstrations by Chen *et al.* (1996) and Kontis & Goldin (1997) that the voltage-dependence of fast inactivation time constants is markedly decreased by mutations solely in IVS4, and not in the S4 segments of the other domains. The argument against the voltage-dependence of inactivation originally put forward by Armstrong & Bezanilla (1977) was the apparent absence of a component of the gating current that had an appropriate time-course; but because inactivation is appreciably slower than activation, and is now seen to account for only one-twelfth of the total charge required

for opening of the channel, the inactivation charge is inevitably rather hard to separate from the slow components of the activation charge, although Keynes *et al.* (1990) did succeed in recording a τ_h component by raising the temperature to 15°C . A difficulty with the proposition that inactivation is voltage-dependent is the tendency, as confirmed in figs 19 and 45a of Keynes (1994), for a semi-logarithmic plot of τ_h invariably to flatten off markedly at large potentials. This could possibly be explained by supposing that the electric field seen by IVS4 for the transition $A \rightleftharpoons I$ is very asymmetrical, which would have the effect of making the apparent voltage-dependence of the transition much smaller at the positive end of the potential range than during recovery at large negative potentials. However, the symmetry factor β has been shown by Keynes & Elinder (1998) to be not far from 0.5 for the $R \rightleftharpoons P \rightleftharpoons A$ transitions in all four domains, and also for the $A \rightleftharpoons B$ transitions in domains I, II and III, and the field acting on IVS4 seems unlikely to be any less symmetrical. Another way of accounting for the flattening off of the τ_h curve has therefore to be sought.

The simplest alternative would be the existence of a rate-limiting step that prevented the rate of inactivation from increasing indefinitely with a rise of pulse potential. The evidence summarized by Rayner *et al.* (1993), that osmotic stress and D_2O substitution slow down activation and inactivation without affecting the gating current, indicates that the solvent-sensitive steps must succeed the voltage-dependent ones, and suggests that the voltage-independent hydration and dehydration of the channel which accompany both their opening and their closing are rate-limiting. In the lower part of the model shown in figure 1b, it is postulated that the channel opens on its arrival at the fully hydrated state BBBA. The voltage-dependent inactivation transition $A \rightarrow I$ in IVS4 then drives the channel to another open state BBBI, after which it is closed by its dehydration to the inactivated state BBBI. The dehydration rate n from BBBI to BBBI is estimated as 1 ms^{-1} at 5°C , this being the asymptotic limiting rate of inactivation at large positive potentials as seen in fig. 17 of Keynes (1994).

It is thus not strictly a gate that opens and closes the channel, but its hydration and dehydration, and the initial opening encompasses state BBBI as well as BBBA. With two open states the current peaks would be more rounded than with only one, as is in fact observed. The rate constant $10m$ for the initial hydration must be assumed to be large enough (70 ms^{-1}) not to have a rate-limiting effect on channel opening, and a suitable value for m is taken as 7 ms^{-1} . The main configurational change brought about by the $A \rightarrow I$ transition of voltage-sensor IVS4 is an interference with the hydration path, slowing down the rate of the backward transition BBBI $\rightarrow$ BBBI to 0.035 ms^{-1} ($=0.005m$). The BBBA $\rightleftharpoons$ BBBA transition therefore strongly favours the initial open state, whereas the BBBI $\rightleftharpoons$ BBBI transition strongly favours the closed state. According to this concept, the central hydrophobic cluster of three non-polar residues in the short chain linking domains III and IV, which has been shown to play an important part in fast inactivation (Patton *et al.* 1992; Catterall 1993), is probably tucked into the aqueous vestibule at the internal mouth of the channel, and is not caused by the $A \rightarrow I$ transition of IVS4 simply to act as a

plug that occludes entrance to the channel, but instead has the more subtle role of shifting the hydration equilibrium for the channel in the direction of dehydration.

5. REOPENING OF THE CHANNEL

An interesting phenomenon first reported by Chandler & Meves (1970) was the existence in the squid giant axon of a second open state that resulted in a small flow of current during the inactivated steady state. This has been shown by Correa & Bezanilla (1994) to be caused by a relatively infrequent reopening of the channel in the steady state. The properties of the voltage sensor responsible for this reopening were investigated by Keynes & Meves (1993), who showed that it carried a charge of about $0.8 e_0$ and had an equilibrium potential of about +50 mV. It is accordingly postulated that reopening is controlled by a fourth transition of IVS4 labelled $I \rightleftharpoons C$ which takes the channel from BBBI to BBBC, and after hydration to the third open state BBBC. As the BBBI $\rightarrow$ BBBI transition is forced to BBBI 200 times more than is predicted from the 'ground' rates $m=7$ and $n=1$, the loop BBBI $\rightarrow$ BBBC $\rightarrow$ BBBC $\rightarrow$ BBBI must be forced to the same extent to maintain microreversibility, which is achieved by dividing m by 10, and speeding up h from BBBC to BBBI by a factor of 20. The halving of the initial current peak and doubling of the steady state current that is observed when the axons are dialysed with TMA instead of Cs (Keynes *et al.* 1992) suggests that although TMA has no direct action on the voltage sensors, it halves the single-channel conductance for outward ionic current in all three open states, while simultaneously quadrupling the probability of arriving at state BBBC by an appropriate action on the hydration and dehydration coefficients.

6. COMPUTER SIMULATION OF THE MODEL

The kinetics of the ionic and gating currents for the system of transitions shown in figure 1b were computed in the manner described by Keynes & Elinder (1998) using a simple Euler integration with an integration step of 10 ns, which was found to be sufficient. The forward and backward rate constants were written as

$$k_f(V) = k_{eq} \exp(z\beta(V - V_{eq})FR^{-1}T^{-1}) \quad (6)$$

and

$$k_b(V) = k_{eq} \exp(-z(1-\beta)(V - V_{eq})FR^{-1}T^{-1}), \quad (7)$$

where k_{eq} is the rate constant at equilibrium, when $k_f=k_b$, z is the charge of the gate that traverses the entire membrane potential, β is the symmetry factor (the fraction of the total drop of potential across the membrane that the gate traverses before reaching the activation energy peak), V is the absolute membrane potential, and V_{eq} is the membrane potential at equilibrium. F , R and T have their usual thermodynamic significances. The total gating current for all transitions is given by

$$I_g(t) = \sum z_{ij}(k_{ij}P_i(t) - k_{ji}P_j(t)), \quad (8)$$

where z_{ij} is the charge for the transition, $P_i(t)$ and $P_j(t)$ are the probabilities that the channel is in states i or j , and k_{ij} and k_{ji} are the rate constants from equations (6) and (7),

multiplied by appropriate cooperativity factors. In the upper part of figure 1b the voltage-dependent factors, α , were given by

$$\alpha = \exp((V - V_{eq})/s), \quad (9)$$

where s was the slope in mV. In the lower part of figure 1b the voltage-independent factor κ is a dimensionless number.

The rate constants at 5 °C were taken as shown in table 1 from the experimental evidence on the currents described in previous sections of this paper and by Keynes & Elinder (1998). The coefficients by which they were multiplied as indicated in figure 1b were chosen to achieve microreversibility within the various closed loops.

As may be seen in figure 3a,b, the time-courses of opening and inactivation, and the steady state sodium currents computed for an axon dialysed with TMA, agreed well with those recorded experimentally. The simulation could readily have been further improved by adjusting the parameters to those of the particular axon for which figure 3a was recorded, rather than the average for a large group of axons. The deactivation time-course from the initial open state BBBA was also closely simulated by the model, though less well from the open states BBBI and BBBC, probably because the model should have included direct pathways for their closure by the $B \rightarrow A$ transitions.

Assuming that, on a slow time-scale, IVS4 can move to its fourth position (I) independently of the positions of the other S4 segments, the normalized steady-state inactivation (h_∞) curve described by Keynes (1994) is shown in figure 4 to be well-predicted by the model.

Figure 5a shows a computed family of gating currents, in which the traces for the largest pulses cross over those for the smaller ones just as they do in the records reproduced in figs 1a and 2 of Keynes & Elinder (1998). The timing of the gating current generated by the separate transitions $R \rightarrow P$, $P \rightarrow A$, $A \rightarrow B$ and $A \rightarrow I$ is shown in figure 5b, and it can be appreciated why inclusion of the $AAAA \rightarrow AAAA^*$ transition was essential to simulate correctly the delay in the rise of the $A \rightarrow B$ component and consequently in the opening of the channel. The 'off' gating current observed experimentally at the end of a pulse is, as seen in figs 28a,b of Keynes (1994), the sum of two or three exponentials, with the very slowly relaxing or immobilized fraction comprising about 90% of the total charge. This behaviour was reproduced reasonably well by the model.

Lastly, the time-course of recovery of the gating current after an inactivating prepulse shown in fig. 3c of Keynes & Elinder (1998) may be seen to be reasonably well described in figure 6, except that the model makes no provision for simulating the rapidly relaxing I_{g1} component of the gating current that generates the initial blip in experimental records.

7. DISCUSSION

The multi-state model of the sodium channel illustrated in the state diagram shown in figure 1 is based strictly on the most recent experimental evidence supporting the view that the four S4 voltage-sensors behave as screw-helices

Table 1. *Values for the parameters that determined the rate constants used for computation of the ionic and gating currents*

transition	k_{eq} ms^{-1}	V_{eq} mV	β	cooperativity
$R \rightleftharpoons P$ <i>a/b</i>	5	-40	0.5	slope = 320 mV
$P \rightleftharpoons A$ <i>c/d</i>	5	-40	0.5	slope = 320 mV
$A \rightleftharpoons I$ <i>e/f</i>	0.2	-60	0.5	
$I \rightleftharpoons C$ <i>g/h</i>	10	+50	0.5	
$AAAA \rightleftharpoons AAAA^* i/j$	7/1			
$A \rightleftharpoons B$ <i>k/l</i>	1.7	-28	0.5	$\kappa = 10$
$H_2O m/n$	7/1

Figure 3. (a) I_{Na} family recorded at 5°C for a squid axon dialysed with TMAF on application of test pulses rising from -41 to 74 mV in steps of 9.6 mV. For other details see figure 7b of Keynes (1994). (b) I_{Na} family computed from the model for the corresponding set of pulses. Taking the peak channel conductance as 35 mS cm⁻² (Bekkers *et al.* 1986), the computed currents are seen to agree reasonably well in magnitude with the experimental ones.

operating in parallel and making a series of discrete transitions each carrying $1e_0$. The slowly rising phase of the sodium gating current described by Keynes & Elinder (1998) is then well explained by the incorporation of an appropriate degree of voltage-dependent cooperativity between the S4 units in all four domains for the $R \rightleftharpoons P$ and $P \rightleftharpoons A$ transitions. The occurrence of positive but voltage-independent cooperativity between IS4, IIS4 and IIIS4 in the $A \rightleftharpoons B$ transition involved in the opening of the channel is also invoked to account for the observation of larger shots in the gating current noise. The channel opens after arriving at state BBBA thanks to a voltage-independent hydration. As far as the activation, opening, and reopening of the sodium channel are concerned, the model is therefore nicely consistent with the experimental findings.

Our arguments should be equally relevant to the modelling of K^+ channels, but the rather different scheme recently put forward by Papazian & Bezanilla (1997)

Figure 4. Normalized steady-state inactivation data. The circles show values recorded at 5°C from figure 22 of Keynes (1994). The solid line is computed for the model.

Figure 5. (a) Gating current family computed for pulses from -40 to +80 mV, with holding potential -80 mV and temperature 5°C. (b) Computations of the time course of the total gating current (heavy line), and the currents generated by transitions $R \rightarrow P$ and $P \rightarrow A$ in domains I, II, III and IV, $A \rightarrow B$ in domains I, II and III, and $A \rightarrow I$ in domain IV.

differs from ours in several ways. Thus, the strength of the case in favour of the modified screw-helical hypothesis for the functioning of the S4 segments is not taken into account, despite the fact that in both vertebrates and invertebrates their structure is just as well conserved, not only with regard to the one in three spacing of the positive charges, and to the location of the negative charges involved in ion-pairing, but also in other respects. The consequence is that although some of the transitions in

Figure 6. Computations showing the recovery of the gating currents after a long pulse to +60 mV on returning to a holding potential of -80 mV at 5°C . The recovery intervals are 0.25, 0.5, 1, 2, 4 and 8 ms, and the heavy line is for full recovery.

the system are agreed to carry a single charge, an unidentified voltage-sensor carrying $2.4 e_0$ is nevertheless required to account for the single shots in the gating current noise observed by Sigg *et al.* (1992). On this issue it may be noted that Tytgat & Hess (1992) have provided experimental evidence for the occurrence of cooperative interactions in K^+ channel gating very similar to those that we have now invoked to explain the single shots of gating current in Na^+ channels. Moreover, evidence is quoted by Papazian & Bezanilla (1997) that positive charges R368, R371 and K374 have electrostatic interactions with negative charges E293 and D316, as would indeed be predicted for screw-helical ion-pairing.

It is further postulated that inactivation is controlled by the third transition labelled $\text{A} \rightleftharpoons \text{I}$ in voltage-sensor IVS4, and involves an allosteric change in the structure of the III–IV link that leads to the dehydration and consequent closing of the channel. The proposition that the channel is opened by its hydration and closed by its dehydration, which is the rate-limiting step that explains the flattening off of the τ_h curve, goes an appreciable way towards providing a straightforward explanation for the kinetics of inactivation, and might also help to account for the anomalous effects of permeant ions discussed by Yellen (1997) and Townsend *et al.* (1997). A feature whose importance has been widely recognized is the immobilization of some 90% of the gating current in the inactivated state, the gating charge being rather slowly restored to the resting state. This behaviour is satisfactorily simulated by the model.

It remains to consider the variations often reported in the fast inactivation time constant τ_h , such as those observed in the single-site mutations responsible for the channelopathies described by Lehmann-Horn & Rüdel (1997), and for the speeding up of fast inactivation resulting from the presence of β_1 as well as α_1 -subunits (Isom *et al.* 1994). The factor most likely to affect the forward rate constant of the $\text{A} \rightleftharpoons \text{I}$ transition of voltage-sensor IVS4 would be a change in the height of the energy barriers for ion-pairing between the movable positive charges in IVS4 and the fixed negative charges in trans-membrane segments S1, S2 and S3, which might well result from slight alterations in the packing of neighbouring α -helices caused by single-site

mutations of the uncharged residues in IVS4, or from small displacements of the III–IV linker. The closeness of packing between IVS4 and the neighbouring S1/3 segments could also be influenced by single-site mutations in S5 and S6, so explaining channelopathies arising from segments not previously thought to have a role in channel gating. However, no attempt will be made here to explain the much slower type of inactivation that has often been reported, and which has not always been clearly distinguished from the reopening of the channels described by Chandler & Meves (1970).

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