THE ROYAL SOCIETY

The screw-helical voltage gating of ion channels

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In the voltage-gated ion channels of every animal, whether they are selective for K^+, Na^+ or Ca^{2+}, the voltage sensors are the S4 transmembrane segments carrying four to eight positive charges always separated by two uncharged residues. It is proposed that they move across the membrane in a screw-helical fashion in a series of three or more steps that each transfer a single electronic charge. The unit steps are stabilized by ion pairing between the mobile positive charges and fixed negative charges, of which there are invariably two located near the inner ends of segments S2 and S3 and a third near the outer end of either S2 or S3. Opening of the channel involves three such steps in each domain.

Keywords: screw helix; sodium channel; potassium channel; calcium channel; voltage gating

1. INTRODUCTION

Important advances have recently been reported by Doyle *et al.* (1998) and Perozo *et al.* (1998) concerning the structure at the atomic level in bacterial K^+ channels of the potassium filter in the pore region, and of the activation gate at the cross-over point of the M1 and M2 transmembrane segments. In voltage-gated K^+ channels the selectivity filter in the pore region and the activation gate near the cytoplasmic end of segments S5 and S6 have closely similar structures (Holmgren *et al.* 1998), and although voltage-gated Na^+ and Ca^{2+} channels must necessarily have different selectivity filters, it is very probable that the activation gate is a feature common to all such channels. However, this impressive new evidence does not bear directly on the manner in which the opening of the activation gate is coupled with the membrane potential or with the factors that govern the opening and closing of other members of the potassium channel family. The purpose of this article, following up those of Keynes (1994) and Keynes & Elinder (1998*a*,*b*), is to present a unified picture of the mechanism by which segments S2, S3 and S4 are jointly responsible for the voltage gating of K^+, Na^+ and Ca^{2+} channels.

2. THE SCREW-HELICAL HYPOTHESIS

After the initial cDNA sequencing in Numa's laboratory of the sodium channel protein from the electric organ of *Electrophorus* and from rat brain (Noda *et al.* 1984, 1986), the sodium channels in *Drosophila* (Salkoff *et al.* 1987) as well as their potassium channels (Kamb *et al.* 1987; Timpe *et al.* 1988) were soon added. It quickly became evident that the most closely conserved feature of the polypeptide chain was the one in three spacing of the positively charged residues carried by the S4 transmembrane segments. An attractive hypothesis to explain the manner in which these charges could move outwards across the membrane on depolarization was then put forward by Guy & Seetharamulu (1986) and Catterall (1986), who proposed that they were arranged on an α-helix as a spiral ribbon of positive charges, and in the resting state were paired with fixed negative charges situated on neighbouring helices. When a depolarization of the membrane increased the outward force acting on the positive charges, the S4 helix would be free to undergo a screw-like motion by rotating through 60° and moving 0.45 nm outwards so that each movable positive charge could proceed to pair up with the next fixed negative charge. Such a movement would bring an unpaired positive charge to the outer surface of the membrane, and at the same time would create an unpaired negative charge at the inner surface, so transferring approximately one electronic charge outwards. By twisting three times in succession through 60°, each S4 segment could therefore bring about the transfer of *ca.* $3\,e_0$.

The screw-helical theory provided a good explanation for the regularity of spacing of the positive charges, but doubts have nevertheless been expressed concerning the α-helical nature of the transmembrane segments, although Yang *et al.* (1997) confirmed it for at least part of IVS4, as did Doyle *et al.* (1998) for the segments neighbouring the pore. It has been questioned by Armstrong & Hille (1998) whether segments other than S4 carry the counter-charges necessary for stabilizing this amphipathic helix in the membrane, but we shall show that this suspicion is unwarranted. It was also hard to believe that opening of the channel should involve a projection of the outermost arginine residues by as much as 1.35 nm into the external aqueous region, until it was shown by Sammar *et al.* (1992), Chahine *et al.* (1994) and Yang & Horn (1995) that such an exposure does indeed take place. The screw-helical theory did not provide an immediate explanation for the generation of single shots of gating current carrying about $2.4\,e_0$ that were reported by Conti & Stühmer (1989) and Sigg *et al.* (1994). Moreover, in its original form it assumed that the hydrophobic pore extended across the whole of the low dielectric

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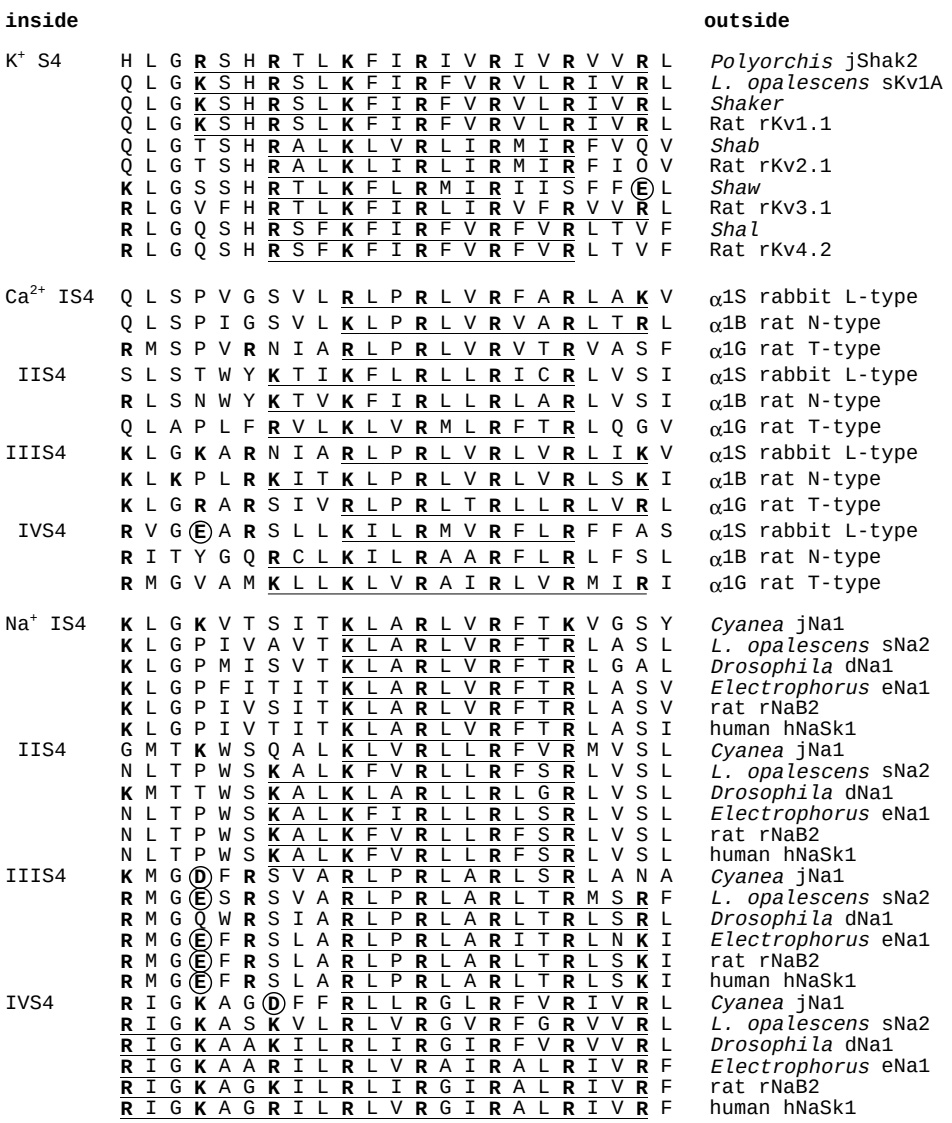

Figure 1. Sequences of amino-acid residues in the S4 transmembrane segments in all four domains of some typical tetrameric K^+ channels and in domains I–IV of homomeric Ca^{2+} and Na^+ channels, plotted in reverse order from C-terminal to N-terminal. Positively charged arginine residues (**R**) and lysine residues (**K**) are in bold type and the stretches over which they are separated by two uncharged residues are underlined. Negatively charged residues of aspartate (**D**) and glutamate (**E**) are ringed. Data for K^+ channels from Chandy & Gutmann (1995), Jegla *et al.* (1995) and Rosenthal *et al.* (1996), for Na^+ channels from Goldin (1995) and for Ca^{2+} channels from Stea *et al.* (1995) and Perez-Reyes *et al.* (1998).

constant portion of the membrane, a belief now seen to be contrary to the evidence of Yang & Horn (1995) and Yang *et al.* (1996, 1997) for sodium channels and of Larsson *et al.* (1996) and Baker *et al.* (1998) for potassium channels on the accessibility of the S4 charges to hydrophilic reagents on both sides of the membrane, which has established that the central hydrophobic pore must be appreciably shorter than was previously supposed.

Nevertheless, it was pointed out by Larsson *et al.* (1996) and Baker *et al.* (1998) that the acidic residues in segments S2 and S3 might suffice to stabilize the position of S4 in the membrane, and Keynes & Elinder (1998*b*) accordingly proposed that a modified version of the screw-helical theory, tailored to a substantial reduction in the length of the central hydrophobic pore and to the actual positions of the fixed negative charges in voltage-gated ion channels, could account for the experimentally observed behaviour of the gating system in a convincing fashion.

The next step in the argument is therefore to examine the published sequences of segments S1, S2, S3 and S4 of various members of the family of voltage-gated ion channels. It should be noted that, since the normal numbering of the residues runs from N-terminal to C-terminal, the numbers in α-helices S1, S3 and S5 run from inside to outside, while for S2, S4 and S6 they run in the opposite direction. Hence, in order to show the charges in S2 and S4 in their correct position relative to those in S1 and S3, the sequences of amino-acid residues in S2 and S4 have been plotted backwards in figures 1 and 3.

3. THE MOBILE POSITIVE CHARGES

The principal voltage sensors in all voltage-gated ion channels, whether they are selective for Na^+, K^+ or Ca^{2+}, are the transmembrane S4 α-helices in each domain which carry a number of positively charged arginine or

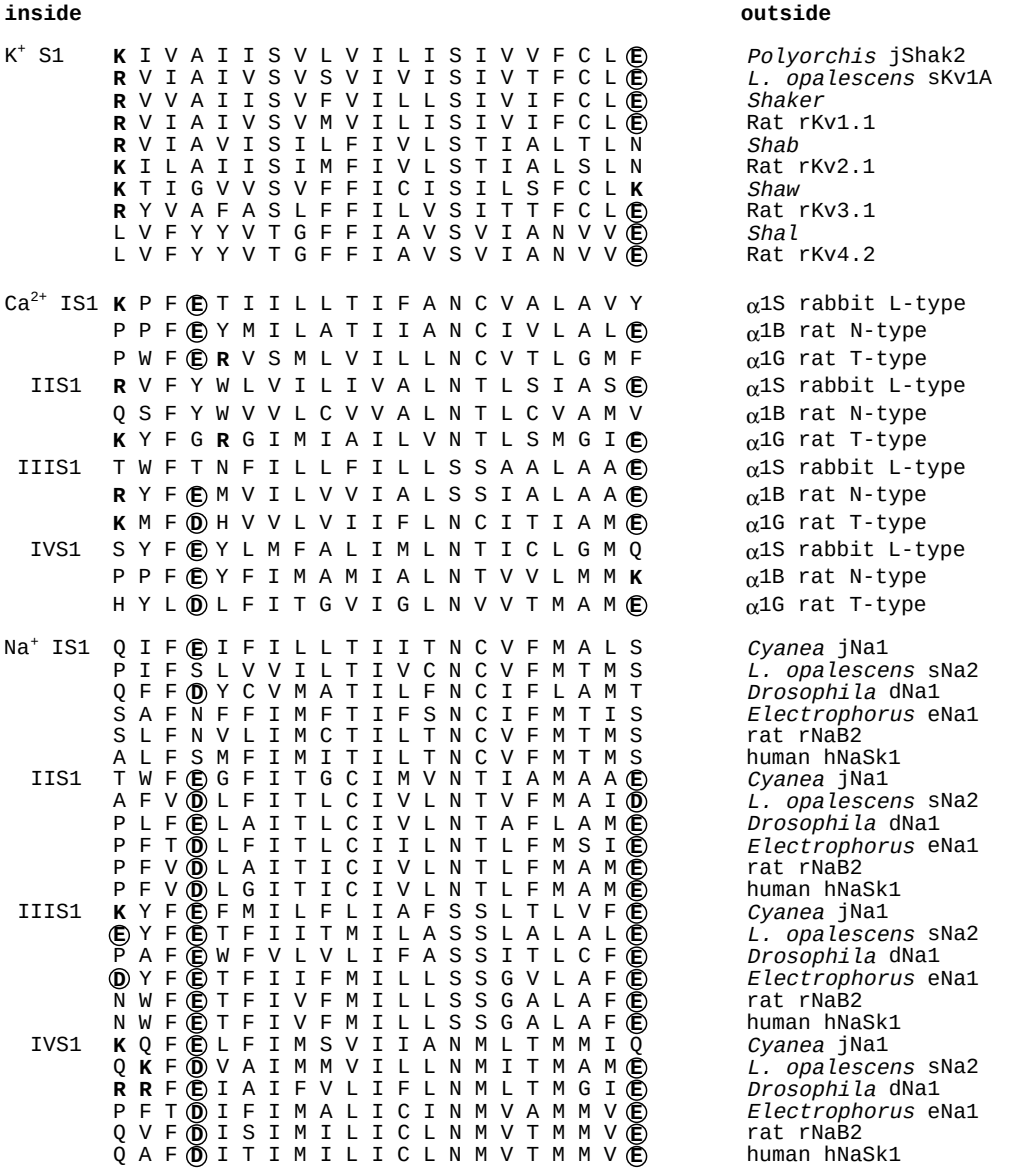

Figure 2. Sequences of residues in the S1 transmembrane segments of the four domains in the selection of voltage-gated ion channels also chosen for figure 1, plotted in the normal order. Sources of data and other details as for figure 1.

lysine residues that are characteristically spaced out to occupy every third position along the helix, separated by hydrophobic residues.

As may be seen in figure 1, in the S4 voltage sensors of tetrameric voltage-gated potassium channels, where all four transmembrane domains have the same sequence, the total number of positive charges varies appreciably within the family of channels. Thus the *Shaker* gene of *Drosophila*, together with its mammalian Kv1 homologues and genes in some other invertebrates, carries seven positive charges on S4. All Kv2 channels, which are homologues of *Shab*, possess only five positive charges. *Shaw* is reduced to four, though its Kv3 homologues have six. *Shal* and its Kv4 homologues have five, with a sixth at twice the normal spacing. The various homologues have widely different properties as far as the kinetics of their inactivation are concerned.

In the homomeric calcium and sodium channels, the sequences differ in the four domains. The majority of S4 segments in the ω-conotoxin-sensitive N-type Ca^{2+} channels of rabbit brain, the DHP-sensitive L-type Ca^{2+} channels of mammalian muscle and brain, and the neuronal low-voltage-activated T-type Ca^{2+} channels in mammals carry five positive charges with one in three spacing, though a few carry four or six. The structure of the S4 segments in sodium channels is seen to be very closely conserved in a wide range of both vertebrates and invertebrates, including jellyfish, *Drosophila*, *Loligo opalescens*, *Electrophorus*, rat brain and human muscle. However, the number of positive charges in each domain varies, for there are typically four such charges with one in three spacing in domain I, five in domains II and III and eight in domain IV, most commonly making a total of 22 for the whole channel. In IIIS4 of both sodium and calcium channels, there is an additional well-conserved positive charge close to the cytoplasmic inner end that has a different spacing.

4. THE FIXED NEGATIVE CHARGES

A structural feature of crucial importance in all voltage-gated ion channels is the presence of a small

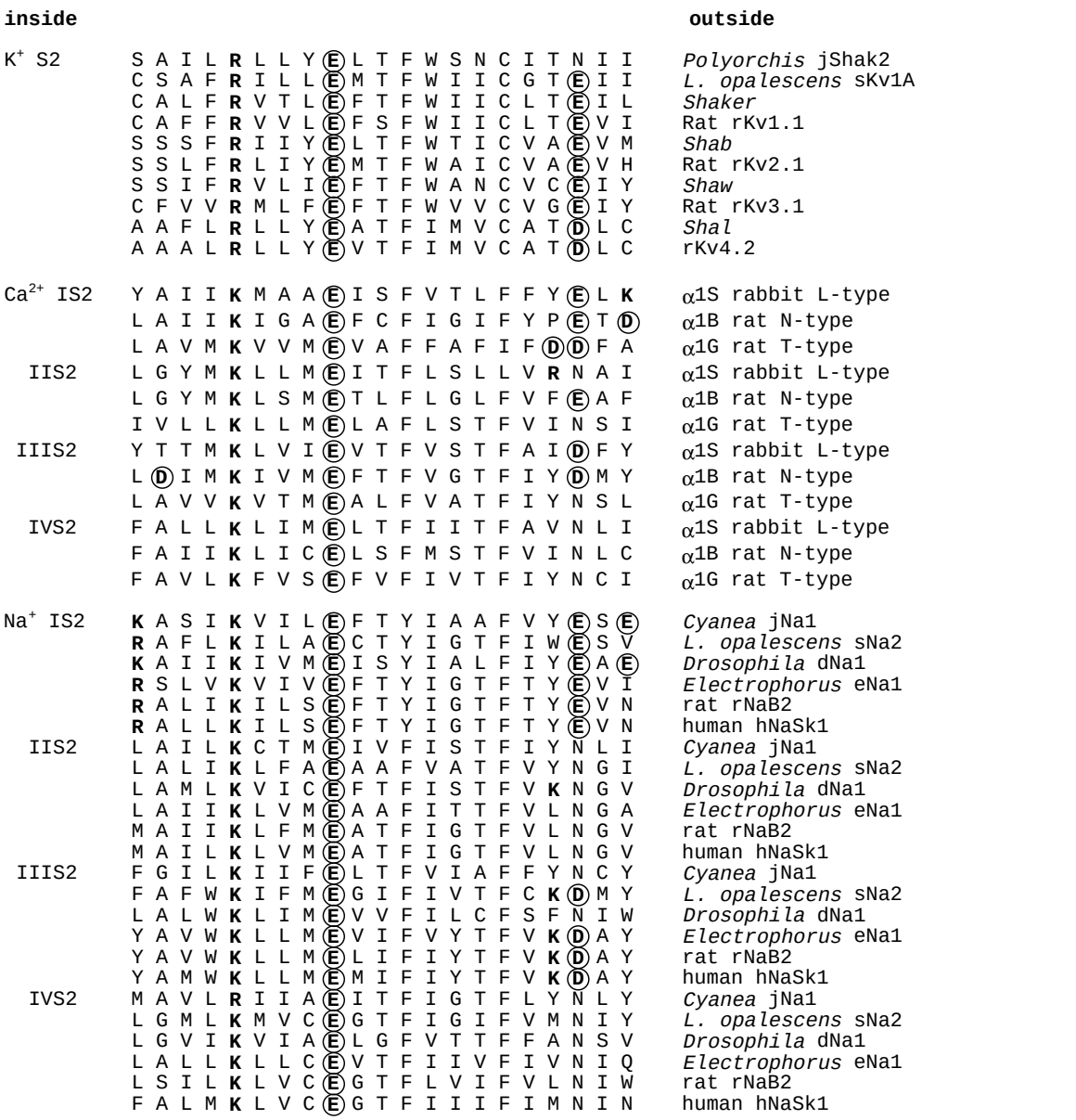

Figure 3. Sequences of residues in the S2 transmembrane segments of the four domains in the selection of voltage-gated ion channels also chosen for figure 1, plotted in reverse order from C-terminal to N-terminal, after alignment of the positive charges near the inner end of the segment. Sources of data as for figure 1.

number of fixed negative charges at well-conserved locations in transmembrane segments S2 and S3. There is inevitably some difference in opinion in the published sequences concerning the exact starting points of the hydrophobic transmembrane regions, but after choosing a consensus for the inner ends of the segments and lining up the negative charges that are perfectly conserved close by, their relative positions in the two segments could be decided with reasonable confidence.

Figures 2–4 show the negative charges in transmembrane segments Sl, S2 and S3 of the set of channels for which the positive charges in S4 are shown in figure l. The picture is simplest for Sl, where the great majority of channels have a single well-conserved negative charge at the outermost end. The potassium channels usually have a positive rather than a negative charge at the inner end, but most though not all calcium and sodium channels have a negative charge four residues from their inner ends. Although these well-conserved negative charges are likely to have a significant functional role, it would not appear to be in voltage gating.

In S2 of all four domains there is, in every channel without exception, a positively charged arginine or lysine residue five places from the inner end and a negatively charged glutamate at place nine. Nineteen places from the inner end, there are also well-conserved negative charges in the majority of potassium and calcium channels and in domains I and III of sodium channels, while further out in domain I of sodium channels there are a few more. In S3 of all four domains there is invariably an aspartate six residues from the inner end, and an aspartate or a glutamate at place l6 in a few potassium channels, in approximately half the calcium channels and in domain II of sodium channels, with a number of others scattered further out.

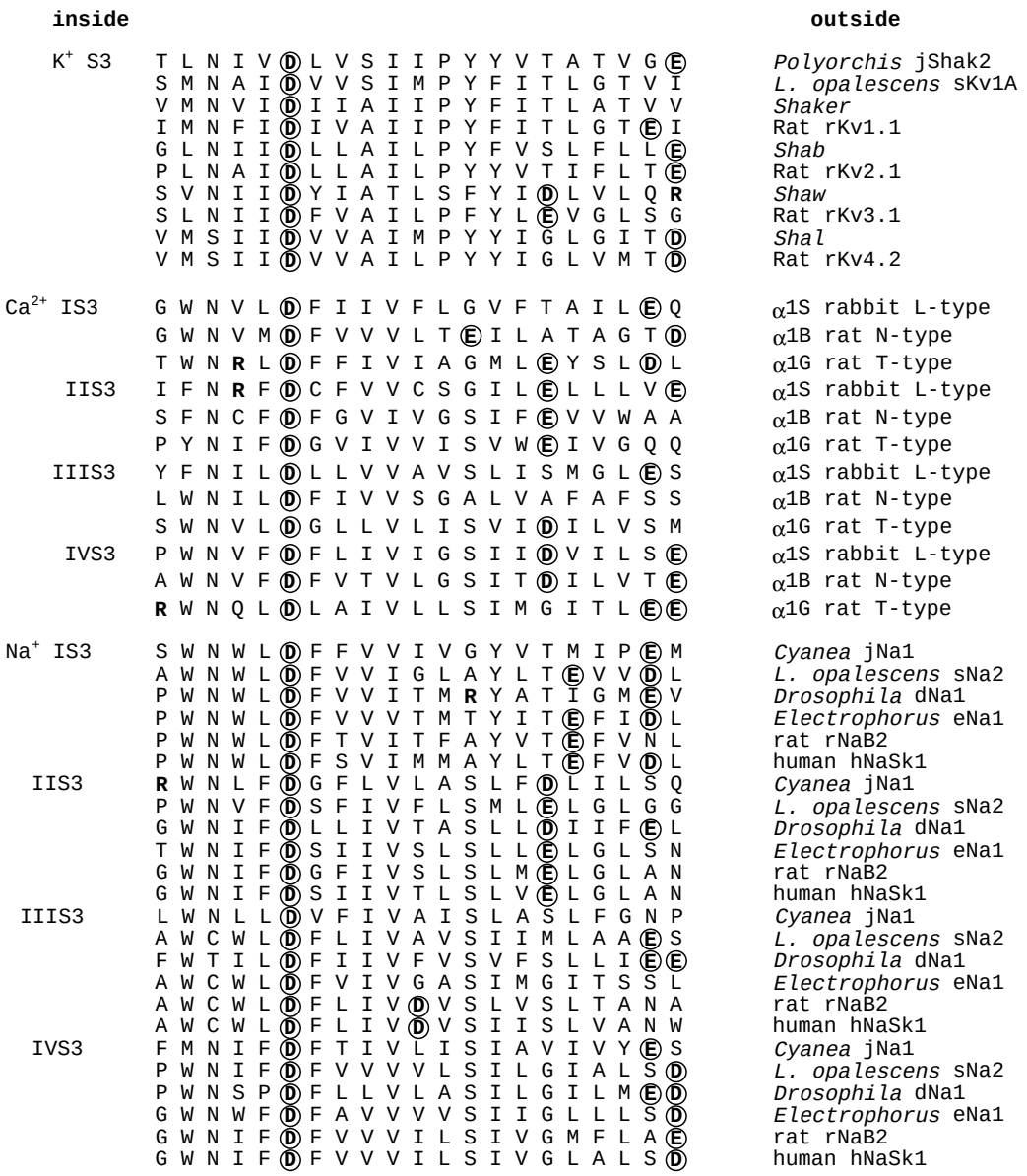

Figure 4. Sequences of residues in the S3 transmembrane segments of the four domains in the selection of voltage-gated ion channels also chosen for figure 1, plotted in the normal order, after alignment of the aspartate residues near the inner end of the segments. Sources of data and other details as for figure 1.

Hence, in every kind of voltage-gated ion channel, all the way from cnidarians to man, to match the positive charges on each movable S4 segment there are two fixed negative charges, one in S2 and the other in S3, located near the inner end of the segment. A third negative charge is generally found near the outer end of S2 in potassium channels, and in domains I and III of S2 or II and IV of S3 in sodium and calcium channels, with a few others in more scattered positions. The locations of these charges are effectively the same in the tetrameric potassium channels as they are in the homomeric sodium and calcium channels. However, there are fewer negative charges with a different pattern of locations in Sl and, in data not shown here, none in S5 and S6 except in the case of *Cyanea*. The very nearly 100% conservation of these strategically placed negative charges and of the one in three spacing of the positive charges carried by the S4 segments, ever since the first evolution more than 550 million years ago of voltage-gated potassium channels, followed in due course by that of the calcium and sodium channels needed for nerve conduction in primitive nervous systems (Hille 1992), argues very strongly that all such channels incorporate an identical voltage-gating mechanism.

5. THE TRANSFER OF CHARGE FOR ACTIVATION AND OPENING OF CHANNELS

In a series of experiments on squid giant axons described by Keynes *et al.* (1990), Keynes (1994) and Keynes & Elinder (1998*a*), the total gating charge transferred between −150 and +80 mV averaged 31 nC cm^{-2}. The channel density in cut-open squid axons had been determined by fluctuation analysis as around 180 mm^{-2} (Bekkers *et al.* 1986), so that the charge for opening a single sodium channel in a squid axon was of the order of 11 e_0. More accurate determinations of the total charge

displacement for the opening of wild-type and mutant *Shaker* potassium channels by Schoppa *et al.* (1992) and Aggarwal & MacKinnon (1996) gave values of 12.3 and 13.6 e_0, while from a careful determination of the voltage dependence of the steady-state open probability in non-inactivating sodium channels expressed in oocytes, it was concluded by Hirschberg *et al.* (1995) that the total transfer of charge to open a single channel was not less than 12 e_0. The best figure for the total transfer of charge needed to open a voltage-gated ion channel is therefore now agreed to be 12 e_0 or slightly more.

A set of semi-logarithmic plots of the voltage-dependence of the time constants of the rapidly and slowly relaxing components of the sodium gating current of squid giant axons were shown by Keynes & Elinder (1998*a*) to fall on straight lines with slopes at 5 and 10 °C, respectively, for an *e*-fold change of 53 ± 1 (s.e.m.) or 58 ± 3 mV for the fast component and 49 ± 3 or 51 ± 4 mV for the slow component corresponding, for a symmetrical electric field, to charges slightly below 1 e_0. The fits began 120 μs after the start of the test pulse, so that the current was contributed mainly by the final opening step of the channels. The studies of Keynes & Meves (1993) on the properties of the voltage sensor responsible for the late reopenings of the squid sodium channel in the inactivated steady state suggested that it carried a charge of *ca.* 0.8 e_0. In the long series of experiments on the squid giant axon discussed by Keynes (1994) there was, therefore, no good evidence for the existence of a voltage sensor carrying more than a single electronic charge.

In the two readily distinguishable phases of gating current in *Shaker* K^+ channels, termed Q_1 and Q_2 by Bezanilla *et al.* (1994), Q_1 was suggested by Papazian & Bezanilla (1997) to be generated by a series of steps each carrying a small charge, while Q_2 resulted from one or a few steps carrying *ca.* 2.4 e_0. Baker *et al.* (1998) estimated values of 1.93 e_0 for Q_1 and 1.50 e_0 for Q_2, generated first by the outward transfer of two positive charges to arrive at a relatively stable intermediate state and then a second 'concerted' step transferring two more as shown in their figure 7*A*. Further analysis (O. S. Baker, personal communication) indicates that their data would be equally consistent with the view that the opening of potassium as well as sodium channels depends basically on three transitions in the S4 segment, each involving the transfer of 1 e_0, and it may be suggested that a fourth transition might take place in only half of the S4 subunits to account for the extra 0.5 e_0.

The question consequently arises as to the origin in gating current noise of the single 'shots' reported to carry 2.3 e_0 in rat brain sodium channels (Conti & Stühmer 1989) or 2.4 e_0 in *Shaker* potassium channels (Sigg *et al.* 1994). It has, however, been pointed out by Crouzy & Sigworth (1993), in an analysis of the variance and auto-covariance of non-stationary shot noise fluctuations, that the figure of 2.3 e_0 obtained for the two-state model of Conti & Stühmer (1989), under recording conditions with an unavoidably restricted bandwidth, might in fact represent the sum of smaller charge movements that occur relatively rapidly in the gating process; and Sigg *et al.* (1994) conceded that one event apparently carrying 2.4 e_0 might actually be composed of smaller events provided that they occurred almost simultaneously. In the multistate model of Keynes & Elinder (1998*b*), it was accordingly assumed that the parallel A→B transitions responsible for opening the channel involved positive cooperativity between the four S4 segments, which bunched their movements together to an appreciable extent. The voltage-independent cooperativity factors used for this purpose were very similar in magnitude to those found necessary by Tytgat & Hess (1992) in order to obtain a good fit of the kinetics of the rKv1.1 *Shaker* homologue K^+ channel. It would hence appear that the large single shots in the gating current noise may be more plausibly attributed to a bunching together of unit charges in a strongly cooperative transition, than to the transfer of 2.3 or 2.4 e_0 by a single voltage sensor.

6. SCREW-HELICAL ION PAIRING

Any explanation for the movement of positive charges in discrete steps across the hydrophobic portion of the membrane under the influence of the electric field presupposes that, in order to stabilize their intermediate positions, salt bridges to suitably located negative charges are established. The energy for making and breaking the bonds in such bridges would be large, but it should not be forgotten that the electric field acting on the movable charges is of the order of $10^6\,\mathrm{V\,cm^{-1}}$, which is also large. The evidence that ion pairing between the movable positive charges in S4 and the fixed negative charges in S2 and S3 plays a central role in voltage-gating must therefore be examined.

It was shown by Papazian *et al.* (1995), in experiments in which the initial folding of the *Shaker* K^+ channel protein that had been blocked by S4 neutralization mutations K374Q and R377Q and then rescued by second-site mutations E293Q and D316N, that the structure of the channel was stabilized by a network of strong, local, electrostatic interactions between E293, D316 and K374. From some measurements of total gating charge in *Shaker* K^+ channels after specific neutralization mutations, Seoh *et al.* (1996) then concluded that the main contributions were made by E293 in S2 and R365, R368 and R371 in S4, but did not decide on their precise roles in the voltage sensor. Tiwari-Woodruff *et al.* (1997) carried out further rescue studies on the folding of the membrane protein in *Shaker* K^+ channels, confirming that K374 in S4 interacts electrostatically with E293 in S2 and D316 in S3, while similarly R368 and R371 interact with E283 in S2. They concluded that these interactions could be explained by supposing that S2 and S3 are parallel α-helices, while S4 is another α-helix tilted to cross them at an angle. This is close to our conclusions and those of Larsson *et al.* (1996) about the ion pairing that takes place specifically in the open state, which is satisfactory because rescue studies of this kind presumably relate strictly to the folding of the protein when it is unconstrained by any electric field, as would be the case in a functional channel when the membrane potential is close to zero, though not to the rather different pairing when the membrane is hyperpolarized. Tiwari-Woodruff *et al.* (1997) and Papazian & Bezanilla (1997) were evidently searching for a voltage sensor capable of accounting for the 2.4 e_0 single shots observed in gating current noise by Sigg *et al.* (1994). In place of their proposal that R368, R371, K374, E283,

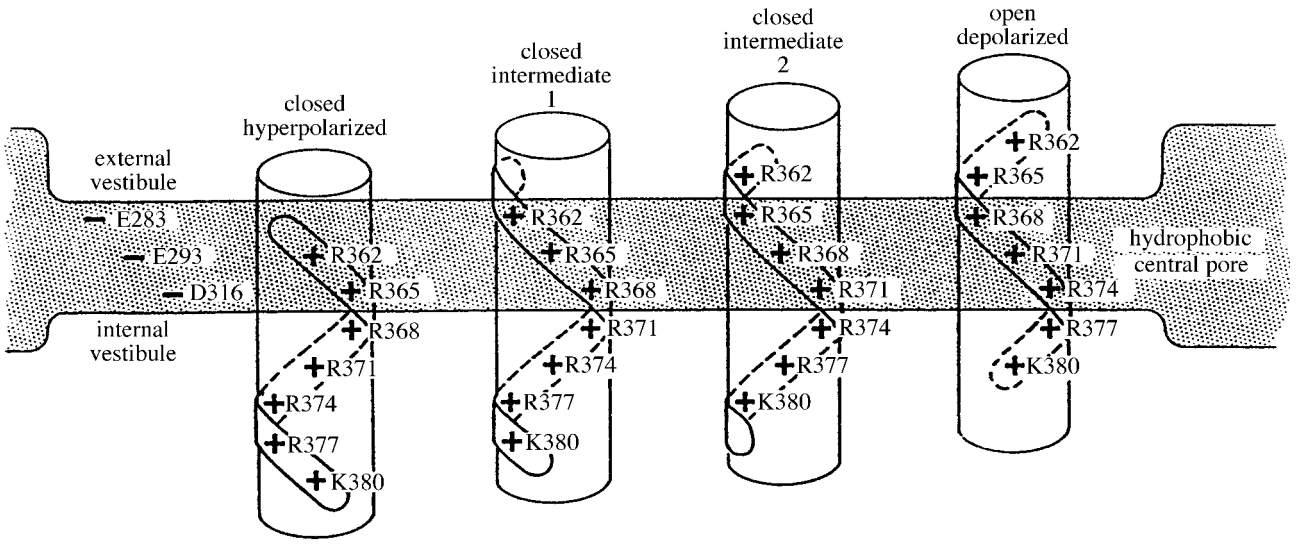

Figure 5. A diagrammatic representation of the screw-helical outward movement of the positive charges carried by S4 in a *Shaker* K^+ channel. The charges are represented as close to the surface of the α-helix, but in fact project on flexible connections *ca.* 0.6 nm in length. The three negative charges shown at the left occupy fixed positions on S2 and S3 and salt bridges are formed with two of the positive charges in the closed hyperpolarized state and three in the closed, intermediate and open states. The reported accessibility from the inside of R365 in *Shaker* or R2 in Na^+ channels may be related to a difference of size between the uncharged cysteine residues and the arginines that they replace in the labelling experiments.

E293 and D316 operate in a combined group as such a sensor, assisted by solvent intrusion in the vicinity, the more straightforward view seems preferable that the movements of S4 are in fact strictly α-helical and that the single shots are generated as proposed above by the bunching together of the unit charges involved in the cooperative opening step.

An instructive study of the function of the negative charges in segments S2 and S3 has been carried out by Planells-Cases *et al.* (1995) by systematic mutations in mouse brain Kv1.1 channels. They showed that all mutations of the aspartate residue on S3, whether conservative (D258E) or the charge substitutions D258N, D258K or D258T, knocked out activation altogether. The corresponding glutamate E235 on S2 was less sensitive, since E235D had no significant effect on activation of the channnel, while E235Q and E235K shifted the midpoint of the activation curve *ca.* 20 mV negatively. The second perfectly conserved negative charge at the outer end of S2, E225, showed a 20 mV positive shift of activation for the conservative mutation E225D, but both E225Q and E225K abrogated channel activity. The effects on the energies required for making and breaking the electrostatic bonds at three points along the S4 segment must be expected to be additive and may interact in a complex fashion. Thus it seems possible that ion pairing at D258 dominated that at the other two sites, so that if it was intact even the insertion of a positive fixed charge, E235K, did not prevent the channel from opening. This line of approach is a promising one that deserves further exploitation.

It was suggested by Keynes & Elinder (1998*b*) that S4 might operate in a screw-helical fashion with a short hydrophobic section of the pore across which the whole of the electric field is developed, but which is nevertheless long enough to accommodate two or three of the fixed negative charges on S2 and S3. The next question is to ask how well this proposition fits with the crucial findings of Yang & Horn (1995), Yang *et al.* (1996, 1997), Larsson *et al.* (1996) and Baker *et al.* (1998), using cysteine mutagenesis in conjunction with the membrane-impermeant thiol reagent MTSET, on the accessibility of positively charged and also some uncharged residues in the S4 segments from either side of the membrane, in the closed, intermediate and open states of both Na^+ and K^+ channels. The system being a dynamic one, in which the S4 segments are constantly jumping in a Markovian fashion between alternative metastable states, it is hard to determine with great precision whether at any moment a given positive charge is paired with one of the fixed negative charges buried in the membrane or is temporarily in the external or internal aqueous phase. Labelling experiments are therefore liable to make the hydrophobic pore appear to be rather shorter than in fact it is. However, the diagram shown in figure 5 presents the best consensus at which we can arrive from the MTSET accessibility studies of Larsson *et al.* (1996) and Baker *et al.* (1998) and the gating current titrations of Starace *et al.* (1997) after replacing arginine in R365 and R368 by histidine, for the positions in relation to the central hydrophobic pore in successive states of the seven positive charges between R362 and K380 in *Shaker* K^+ channels. A similar picture emerges from the studies of Yang & Horn (1995) and Yang *et al.* (1996, 1997) on the corresponding charges between R1448 and R1469 in IVS4 of human skeletal muscle hNaSk1, with due allowance for the effect mentioned above that makes the length of the hydrophobic pore appear to be less than is shown in figure 5.

This evidence provides invaluable support for the screw-helical gating hypothesis, but although it is easy to see how the two perfectly conserved negative charges at the inner ends of S2 and S3, E293 and D316 in *Shaker* K^+ channels and E1399 and D1422 in IVS4 of hNaSk1 would be suitably placed to form salt bridges with two neighbouring positive charges carried by S4, it is less obvious how pairing could simultaneously be achieved between a third positive charge on S4 and one or more of the

negative charges at the outer ends of S2 or S3. However, the construction of a model of S2, S3 and S4 as solid α-helical rods carrying charges at appropriate positions along the rods and at the correct distances from their central axes, shows that it is possible, by keeping S2 and S3 parallel to one another and then tilting S4 at an angle of *ca*. 30° to them, for the positive charges to move outwards in a screw-helical fashion while remaining close enough to the fixed negative charges for salt bridges to be formed. A similar crossing of S2 and S3 at an angle by S4 was arrived at by Tiwari-Woodruff *et al.* (1997) in their rescue studies, and their figure 7 shows interactions taking place between precisely the same charges as in the open state of our figure 5. The principle has also been followed in the tilting helix model of the *Shaker* K^+ channel proposed by Durell *et al.* (1998). As pointed out by Lecar & Larsson (1997), simultaneous interactions may take place between several charges and at long range, which perhaps helps to explain why the negative charges that interact with the outermost of the S4 charges passing through the hydrophobic central pore are somewhat scattered across the outer ends of S2 and S3 in the four domains of Na^+ channels, though less so in Ca^{2+} channels.

7. KINETIC MODELLING OF THE SODIUM CHANNEL

Any definitive kinetic models of the voltage-gating systems of Na^+, K^+ and Ca^{2+} channels have to take proper account of the structural evidence. The first necessity is to treat the channels as multistate systems with four voltage sensors operating in parallel, as was proposed by Zagotta *et al.* (1994) for the *Shaker* K^+ channel in a model where each sensor made two voltage-dependent transitions in succession. For Na^+ channels, Keynes (1994) suggested that the channels were first activated in a similar way, and were then opened by a further step that was later identified by Keynes & Elinder (1998*b*) as consisting of a third highly cooperative transition in the four S4 segments. Since the location of the negative charges is so well conserved in segments S2 and S3, they must have an important functional role, in all probability as essential components of the voltage-gating mechanism, while the one in three spacing of the positive charges carried by the S4 segments is another invariant feature. In addition to the fact that the K^+ channels are tetrameric, while the Na^+ and Ca^{2+} channels are homomeric, the one characteristic that varies between and within the three families is the total number of positive charges in the individual S4 segments. Precisely what purpose is served by this variation between four and eight charges in the S4 subunits remains to be decided, except that an extra charge in IVS4 is needed to account for the voltage dependence of inactivation in sodium channels, and another for the reopening in the inactivated steady state.

In this connection, there are similarities between sodium channels in which opening is always followed by a rapid inactivation and those potassium channels homologous with *Shaker* that display an equally fast N-type or A-type inactivation, but there are also K^+ channels involved, as in the classical squid giant axon (Hodgkin & Huxley 1952; Rosenthal *et al.* 1996), in the conduction of nerve impulses that behave as delayed rectifiers and inactivate very slowly. In *Shaker* K^+ channels and their mammalian homologues, neither N-type nor C-type inactivation is voltage dependent, and while N-type inactivation involves a 'ball and chain' blockage of the internal mouth of the channel by a particle tethered near the N-terminus that is similar in structure to the triplet at the centre of the III-IV linker in sodium channels, C-type inactivation involves interactions with residues in the walls of the external aqueous vestibule, as may also the slow inactivation of sodium channels (Wang & Wang 1997). In the fast inactivation of sodium channels, however, it is now clear from kinetic studies (Keynes 1994; Keynes & Elinder 1998*b*) and from the observations of Chen *et al.* (1996) and Mitrovic *et al.* (1998) which directly implicate voltage-sensor IVS4, that the process must be regarded as voltage dependent. In kinetic modelling of the Na^+ channel, it was therefore proposed by Keynes & Elinder (1998*b*) that inactivation was controlled by a third transition of IVS4, labelled A→I in their figure 1*a*, which had the effect of dehydrating and closing the channel. Although, using rate constants deduced from the gating current data, this model was able to simulate the complex kinetics of both the ionic and the gating currents in a satisfactory manner, it needs slight revision in order to take account of the fact that IS4 carries only four positive charges. It is therefore proposed in figure 6 that IS4 is capable of proceeding only as far as state $AAAA^*$ and that the channel is opened by the concerted A→B transitions of IIS4, IIIS4 and IVS4. The fourth transition of IVS4, labelled B→I, initiates the dehydration of the channel that takes it to the inactivated steady state, it being noted that this can take place independently of the A→B steps of IIS4 and IIIS4, so that the channel can be inactivated without prior opening. The fifth transition, I→C, as before briefly reopens the channel in the inactivated state. It remains, however, to submit the revised model to a rigorous quantitative examination.

A number of difficult problems still remain to be solved. In modelling the Na^+ channel, Keynes & Elinder (1998*b*) found it necessary to assume that completion of the P→A step in all four domains was followed by the voltage-independent rearrangement, shown as $AAAA \rightarrow AAAA^*$, after which the concerted A→B steps in domains II, III and IV and then hydration cause the channel to open. The analyses of Larsson *et al.* (1996) and Baker *et al.* (1998) would not appear to be inconsistent with a similar behaviour of K^+ channels, but in neither class of channel is there any indication as to the precise nature of the conformational change prior to the opening steps, nor of their mode of coupling with the hydration pathway and the activation gate itself. Better understanding is also required of the physics underlying the cooperative interactions between subunits in the different domains, which may be separated from one another by an appreciable distance. These are responsible for generating not only the slowly rising phase of the sodium gating current in the squid giant axon (Keynes & Elinder 1998*a*), but also the phenomena described by Smith-Maxwell *et al.* (1998*a*,*b*) primarily involving a late step in the opening of *Shaker* K^+ channels, where it emerged that small steric changes resulting from mutations of uncharged residues in the S4 segments could give rise to large changes in cooperativity. Since steric changes could

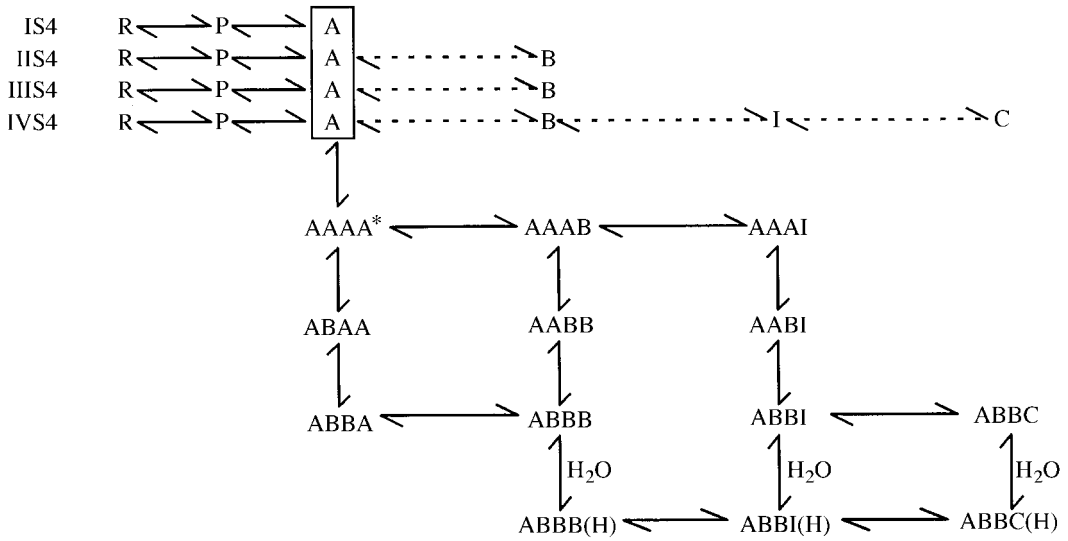

Figure 6. Revised version of the outline state diagram of the series-parallel model of a voltage-gated sodium channel proposed by Keynes & Elinder (1998*b*). As soon as state A has been reached in all four domains, but not before, there is a voltage-independent rearrangement of the channel taking it to state AAAA*, followed by the cooperative A→B transitions and hydration to arrive at the initial open state ABBB(H). Inactivation is controlled by the B→I transition of IVS4, taking the channel to the second open state ABBI(H) and after dehydration to the closed inactivated steady state ABBI. Reopening is caused by the I→C transition of IVS4 taking the channel to the third open state ABBC(H).

readily affect the closeness of approach between the positive charges on S4 and the negative charges on S2 and S3, perhaps at long range they might conceivably modulate the electrostatic interactions in screw-helical voltage gating to bring about cooperativity in the manner considered by Lecar (1998).

8. CONCLUSIONS

Two of the best conserved structural features in all voltage-gated ion channels, whatever their selectivity and across the entire animal kingdom, are shown to be the constancy of spacing of the positive charges carried by the S4 segments and the precision of location of the negative charges on S2 and S3. This is most readily explained by concluding that all such channels depend in common on a screw-helical mechanism of voltage gating, stabilized by ion pairing between the mobile positive charges and the fixed negative charges. The opening of the channels is brought about by a sequence of three transitions in each of the S4 segments of the four domains, operating in parallel and carrying single electronic charges. Recent evidence on the accessibility of the S4 charges in both Na^+ and K^+ channels is shown to be fully consistent with this proposal.

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