On the opening of voltage-gated ion channels

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Abstract

Voltage-gated ion channels are key players in fast neuronal signalling. Detailed knowledge about channel gating is essential for our understanding of channel function in general and of drug action of channels in particular. Despite a number of recent atomic channel structures, the opening of voltage-gated channels is the subject of heated debates. Here we will discuss two of the controversies: one concerning the mechanism of opening and closing the pore, and the other concerning the location and movement of the voltage sensor. The channels were originally suggested to open at a conserved proline rich sequence (PVP) at the intracellular end of the transmembrane segment 6 (S6). The crystallization of a channel in the open state instead suggested an opening involving a conserved glycine hinge located in the middle portion of S6. Based on pharmacological studies, autodocking and molecular dynamics simulations we have found support for the PVP-bend model. The voltage sensor, transmembrane segment 4 (S4), was originally suggested to be buried in the channel protein, undergoing a helical-screw-like motion to open the channel. A recent crystallographic study suggested that S4 is located in the periphery, facing lipid, and undergoing a paddle-like motion to open the channel. We have found experimental evidence for a novel helical-screw model; with the voltage sensor moving in a screw-like fashion but being located in the periphery of the channel. This model opens up for understanding how lipophilic drugs and toxins directly affect the voltage sensor.

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1. Voltage-gated ion channels

Ion channels are proteins, forming transmembrane pores in biological cell membranes. Opening and closing of the pores regulate the ion currents through the membrane, and consequently vital bodily functions from hormonal homeostasis to cognition. Dysfunctional channels in many cases cause disease [1], and a large number of medical drugs (1/3 of all marketed medicines), and animal and plant toxins target ion channels [2]. Stimuli activating channels are multifarious; channels are opened chemically by ligands such as neurotransmitters, Ca ions, and cAMP, mechan-

ically by stretching the membrane, or electrically by changing the transmembrane voltage. The detailed molecular mechanisms of these opening processes are poorly understood. In the present work we will review and discuss some recent controversies in this area.

We will focus on voltage-gated ion channels — key players of the nervous and muscle system function, forming the basis for conduction of nervous impulses, muscular contraction, and synaptic transmission. These channels (together with closely related voltage-insensitive channels) form the third largest superfamily of signal-transduction proteins, only outnumbered by G protein-coupled receptors and protein kinases, comprising 143 members in the human genome [3]. Voltage-gated channels include the classical Na, K and Ca channels (studied by Hodgkin and Huxley and followers already in the 50s) as well as Ca2+-activated, cyclic nucleotide-gated and hyperpolarisation-activated channels (see Ref. [2] for a review).

At present, the opening of voltage-gated channels is the subject of heated debates. Here we will discuss two of the controversies, one concerning (1) the mechanism of opening and closing the pore, and the other concerning (2) the location and movement of the voltage sensor. Both controversies are issues of great physiological
and therapeutic interest. And in both cases we will present our own views, clashing with some of the mainstream ideas.

1.1. Molecular structure and evolution

Voltage-gated ion channels are composed of four similar or identical subunits or domains, symmetrically arranged around a central ion-conducting pore. Na and Ca channels are single polypeptides, organized in four linked domains, while K channels are formed by four separate subunits. These subunits consist of assemblies of transmembrane \(\alpha\) helices. For K channels they are essentially of two types: two transmembrane helices (2TM) or six (6TM). The \(\alpha\) helices are labelled M1 and M2 in the 2TM channels, and S1 to S6 in the 6TM channels. M1 and M2 are homologous to S5 and S6. Fig. 1A shows the transmembrane topology of a voltage-gated 6TM channel, separated in an ion-conducting pore domain and a voltage-sensing domain.

The ion-conducting domain is formed by S5 and S6. The linker between S5 and S6 forms a selectivity filter—a narrow pathway that determines which ion will pass the pore. At the intracellular half of the pore, the four S6s form an inner vestibule, at the internal end, limited by a narrow passage (a gate) that can open up when the channel is activated. Armstrong and Hille elegantly summarized the pre-atomic view of voltage-gated ion channels in 1998 [4]. At about the same time the first atomic structure of an ion channel appeared [5]. The channel structure met almost all of our expectations. This and later studies will be discussed in more detail below. Fig. 1B shows how the gate is assumed to open at depolarisation according to the prevailing view (but see below). The bundle-crossing region is thus forming the internal gate, regulating the passage of ions through the conducting pathway.

The voltage-sensing domain is formed by S1 to S4, where S4, comprising highly conserved positive charges in every third position, has been identified as the voltage sensor (reviewed in [6,7]). As depicted in Fig. 1B, S4 is assumed to move in outward direction at depolarisation, in some way leading to the opening of the internal gate. This is the case for most channels studied. Some channels, however, are activated by negative voltages. The gate is opened at hyperpolarisation. How is this accomplished? Is the voltage sensor moving in opposite direction compared to the case of depolarisation-activated channels? Recent experiments in our laboratory show that this is not the case. Instead the difference is

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Fig. 1. Structure and kinetics of voltage-gated ion channels. (A) Transmembrane topology of one subunit of a 6TM channel. The transmembrane segments from left to right are S1 to S6. Four subunits make an ion channel. (B) Structural working hypothesis of voltage-gated 6TM ion channels in closed and open configurations. The pore domain in the closed channel is KcsA [5], and in the open channel MthK [11]. For clarity only two subunits are shown in the side view (upper part). Views from the extracellular side (lower part). (C) Ion currents (thin line) and gating currents (thick line) at the voltages indicated. Holding potential is \(-100\) mV. The cut-open oocyte voltage-clamp technique is used. Ion currents are measured from wild-type Shaker K channels and gating currents are measured from non-conducting Y434F Shaker K channels. Note that 250 times more RNA is used for the expression of Y434F than for wild-type. (D) Integrated gating current vs. voltage, \(Q(V)\), and conductance vs. voltage, \(G(V)\).
caused by different coupling mechanisms between the voltage sensor and the gate in depolarisation- and hyperpolarisation-activated channels [8].

The basic features of the evolution of voltage-gated ion channels [3] are now beginning to appear, mainly due to the dramatic developments in molecular biology techniques. K channels are evolutionary older than Ca and Na channels [2]. Presumably, the K protochannel was structurally similar to the pore-forming S5-P-S6 motif (2TM) in extant K channels. Such 2TM channels are found among prokaryotes, and presumably originated before 1400 million years ago, but are also present in higher organisms in the shape of the inward rectifiers (Kir). 6TM channels probably originated in protists between 1400 and 700 millions years ago. In one scenario, a 2TM subunit was associated with highly charged voltage-sensing 4TM protein (corresponding to S1–S4). This scenario is supported by the recent discovery of a voltage-dependent phosphatase that has an intracellular enzymatic domain connected to a voltage-sensor domain homologue [9].

1.2. Ion and gating currents

Voltage-gated ion channels are steeply dependent on membrane voltage. Fig. 1C shows the ion current (thin line) through a voltage-gated K channel associated with three different voltage steps from a holding potential of −100 mV: At −40 mV no channels open, at −20 mV the channels open after a delay of about 1.5 ms, at 0 mV the channels open faster and almost all channels are opened at the end of the stimulation pulse. The conductance vs. voltage curve, $G(V)$, is shown in Fig. 1D. This steep voltage dependence suggests that around 12 elementary charges are required to move through the membrane electric field to open the channel [6,7]. Thus, there are three charges per subunit in the tetramer. When these charges are moving, they should cause a tiny gating current. This was realized already by Hodgkin and Huxley in their classical studies from 1952. However, due to its smallness and the consequent contamination by ion currents it withstood measurements for a long time. The first measurements were possible only after development of efficient signal averaging techniques and were published 30 years ago [6]. With the development of expression systems for cloned ion channels and corresponding improvements in electrical recording techniques it is now possible to record gating currents with relative ease. Fig. 1C shows the result of such recordings together with associated ion currents. Even though there is no channel opening at −40, there is a large gating current (thick line). Larger voltage steps (−20 mV) move more charges and open the channel. The major gating current component precedes the opening of the channel. Still higher voltage steps (0 mV) speeds the movement but do not increase the total gating charge. The integrated gating current vs. voltage, $Q(V)$, is plotted in Fig. 1D. The $Q(V)$ curve is typically to the left of the $G(V)$ curve. This means that the voltage sensors have to move before the channel can open.

2. Opening the internal gate

2.1. The glycine-bend model

The dramatically increased knowledge of channel structure opened up for a serious attack on the long standing problem how channels gate. The first crystallized channel, the 2TM channel KcsA from the eubacterium Streptomyces lividans, was crystallized in a closed state [5] (see Fig. 1B, left). It was natural to speculate that the gate was located at the bundle crossing and that the process of opening was caused by rotational and outward movements of relatively rigid S6 helices, increasing the diameter at the bundle crossing (see Fig. 1B). Consequent studies, including electron paramagnetic resonance measurements, confirmed and extended this view [10]. The result of the next step in channel crystallization, therefore, came as a surprise. The 2TM channel MthK from Methanobacterium thermoautotrophicum showed a channel in an open state [11], but not in an open state with only a marginally opened entrance to the internally vestibule. It showed a channel with a large entrance, caused by bent S6 structures, where the pivot point was the strongly conserved glycine close to the selectivity filter (Fig. 1B, right). This finding led to the view, soon almost generally accepted, that all voltage-gated K channels open by a centrifugal swinging movement around the conserved glycine hinge. The glycine-hinge hypothesis was further corroborated in the X-ray analysis of the first 6TM voltage-gated K channel to be crystallized, KvAP from the archae bacterium Aeropyrum pernix [12,13], showing a glycine-associated curvature of the S6 helix.

2.2. The PVP-bend model and the recent controversy

However, the glycine-hinge or glycine-bend hypothesis for the gating of voltage-gated K channels, was not unchallenged. Studying the accessibility of the internal vestibule to thiol reagents after blocking the pore, Yellen and collaborators [14] found that S6 in Shaker K channels seemed bent outwards at the bundle crossing. This portion of S6 comprises in Shaker a proline sequence (Pro–Val–Pro, PVP) strongly conserved in eukaryotes (but not in prokaryotes). Studies using critical cysteine replacements and consequent metal-bridge formation further refined this PVP-bend
model, suggesting that the PVP-bend existed both in open and closed states [15]. Follow-up studies, specifically scrutinizing the glycine-bend model derived from the MthK study [11], made it clear that this model has difficulties in explaining a number of new findings. For instance it has difficulties in explaining the action of metals on Kv channels with cysteines introduced at specified locations in the PVP region [16]. The estimated lengths of the formed metal bridges are too short to fit the glycine-bend model. The consequent conclusion was that the Shaker K channel opens by relatively small movements in the PVP region. This also means that the opening of the pore in this model is much smaller than that predicted by the glycine-bend model.

We have come to similar conclusions on other grounds. On the basis of a number of investigations, local anaesthetics have been assumed to block voltage-gated channels by binding to the internal vestibule [2]. To investigate the molecular binding mechanisms in more detail, we analyzed how the local anaesthetic bupivacaine blocks different types of wild-type and critically mutated voltage-gated K channels [17]. We found that different Kv channel types differ in their capacity to close when bupivacaine is bound, suggesting that the binding site is located in the PVP region and that the internal vestibule volume differs between channel types (with Kv1.5 and other Kv1 channels being most narrow and least probable to close in bound state). Following up these findings with autodocking and molecular dynamics simulations we unexpectedly found that the simulations predicted that bupivacaine can bind to the internal vestibule of a PVP-bend Kv1.5 homology model, but not to the vestibule in a corresponding glycine-bend model (see Fig. 2) [18]. The strongest binding affinity was found for the PVP region (of the PVP-bend model), in agreement with the predictions of the experimental finding that bupivacaine binding to Kv1.5 prevents the closure of the channel [17]. Another interesting finding was that a PVP-bend of the S6 helix appears much more flexible and more likely to be formed than a glycine-bend. Also other recent molecular dynamics simulations (e.g., concerning the coupling of the voltage-sensor and the pore domain under the influence of voltage) seem to support a PVP-bend model [19]. The recent crystallization of an eukaryotic 6TM voltage-gated K channel (Kv1.2; in complex with an auxiliary Kvβ2 subunit) and the following X-ray analysis [20,21] strengthened a PVP-bend hypothesis further. The channel was crystallized in an open state and the S6 helices showed a curved shape, with a bend located at the PVP sequence.

In summary, accumulating evidence suggest that a PVP-hinge opening is involved in the gating of voltage-gated eukaryotic K channels. The reason for the conflicting opening hypotheses might be simple. The mechanism of opening in prokaryotic (MthK and KvAP) and eukaryotic channels (Shaker and Kv1.2) might differ, reflecting different evolutionary stages. In this scenario a restricted eukaryotic PVP-hinge opening process evolved from a more extensive prokaryotic glycine-hinge opening motion, also explaining the ten-fold smaller single channel conductance in Shaker than in the prokaryotic equivalents [16]. Perhaps we also can find intermediate stages; both the glycine and the PVP regions are strongly conserved in eukaryote voltage-gated K channels. The gating of some eukaryotic voltage-gated K channels might involve both a PVP and a glycine-bending site [16,18].

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Fig. 3. Molecular models for location and movement of the voltage sensor S4. (A) Three proposed models for the S4 movement upon activation of the channel. Modified after Ref. [38]. (B) Proposed location for S4. KcsA is shown in the centre. The unsigned circles are S1–S3. S4 is facing both lipids and proteins. Modified after Ref. [27]. (C) Proposed location of S1–S4 and proposed movement of S4. The pore domain is KcsA [5]. The S1–S4 structure is the crystallographic structure of the isolated domain [12]. Note that only one S1–S4 domain is shown. S1 (blue), S2 (green), S3 (light blue), and S4 (dark red). The arrow indicates the S4 motion upon activation of the channel. Modified after Ref. [29]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3. The voltage-sensor movement

3.1. Early helical-screw and helical-twist models

Already when the first voltage-gated ion channel was cloned 20 years ago, the remarkable pattern of several positively charged residues in every third position of the fourth transmembrane segment (S4) was recognized as being involved in voltage sensing. S4 was generally accepted as the voltage sensor, but the exact location and movement in the 3D channel structure are still surrounded by controversy. Sparked by the first crystallization of a voltage-gated K channel, KvAP [12,13], the controversy has even escalated [22].

The first model to describe the S4 movement was the helical-screw model [23,24]. As depicted in Fig. 3A the positive charges are lined up in a spiral around S4. They are supposed to pair with conserved negative counter charges in S2 and S3. Upon activation, S4 moves to new stable positions by rotating 60° and translating 4.5 Å per step. Three steps, thus a rotation by 180° and a translational movement of 13.5 Å, are required to transfer three charges per subunit to open the channel [6]. This relatively large-scale movement of S4 was almost generally accepted as charge-transfer mechanism in voltage-gated channels, until it was challenged by the helical-twist model, involving a much smaller movement, as shown in Fig. 3A — a 180° rotation with no translational movement [25]. Narrow, water-filled crevices make it possible to transfer the charges during gating.

3.2. Electrostatic interactions suggest a novel helical-screw model

The location of S4 within the channel has also been a matter of debate. Most investigators have placed S4 buried in the channel protein, in direct contact with the pore-forming domain (Fig. 1A), and shielded from the lipid bilayer by surrounding S1 to S3 (e.g., [19,26]). In contrast we have suggested a lipid-facing position in the periphery of the channel [27] (Fig. 3B). We reasoned that a charge on the extracellular surface of the channel, close to S4, would electrostatically interfere with the movement of the positively charged S4 — a position close to S4 would have a larger effect than a charge further away. A positive charge would decrease and a negative charge would increase the outward movement. We therefore mutated several residues, located on the perimeter of the pore domain of the Shaker K channel, to cysteines, and applied cysteine specific methanethiosulfonate (MTS) reagents. The reagents, either negatively or positively charged, irreversibly bind to the cysteine. We thus had the possibility to measure the effect on the G(V) curve (see Fig. 1C) when introducing extra charges on the channel’s surface at different distances from S4. A position at the extracellular end of S5 (residue 419) showed the largest effect on the G(V) curve compared to the other investigated positions, shifting the curve to ±8.2 mV, the sign depending on the sign of the reagent. Calculations positioned the charges of S4 8 Å outside S5 — not in the crevice between two adjacent subunits as previously assumed (Fig. 3B). The other segments, S1–S3, were placed to satisfy electrostatic interactions and to fill in structural gaps in the molecule. With this arrangement charges of S4 face S1–S3 and the hydrophobic side of S4 face the lipid bilayer. We suggested that such an unorthodox solution allows large-scale movements of S4 with little friction from the fluid lipid bilayer.

Next we investigated the magnitude of the S4 movement [28]. Also here we used an electrostatic strategy. We reasoned that a voltage sensor close to our mutated residue would attract negatively charged reagents, and increase the rate of reaction, while it would repel positively charged reagents, and decrease the rate of reaction. Depending on the position of S4 in open and closed states we would expect different reaction rates. We found that S4 increased the local potential with 35 mV at the extracellular end of S5 when the channel was in open state. The top charge (residue 362) alone contributed with >50% (19 mV). Electrostatic calculations suggested that the top of S4 moved from a distant position (>20 Å) to a position close to S5 (8 Å) upon activation. These results are compatible with a helical-screw motion with the helix located as suggested in Fig. 3B.

3.3. The paddle model and the recent controversy

The voltage sensor resisted crystallographic determination many years after the first crystallization of the pore domain. However, when at last the results of the successful crystallization of the voltage-gated KvAP channel were presented [12,13], the society of channelologists was shaken. While the structure of the pore domain (S5–S6) was similar to previously determined pore structures, the structure of the voltage-sensing domain (S1–S4) deviated from all predictions. S4 was found to be tightly bound to the C-terminal end of S3 (called S3b), forming a “paddle”, and seemed located at the perimeter of the channel. The S3b/S4 “paddle” was suggested to move through the lipid bilayer as a hydrophobic cation during gating (see the paddle model in Fig. 3A).

This novel model immediately met strong criticism [22]. For instance, it seems difficult to generalize the paddle model to a model for all voltage-gated K channels, since in some channel types there are as many negative charges in S3b as there are positive charges in S4, making the S3b–S4 paddle electroneutral [29]. This electroneutrality would make the paddle unusable as a voltage sensor. Furthermore, the paddle model is incompatible with a number of experimental findings from other voltage-gated K channels. The model assumes S4 to be at a considerable distance from the pore domain in open state, which stands in sharp contrast to the conclusions from our electrostatic experiments [28] and to the conclusions from experiments showing that a cysteine at the N-terminus of S4 can make a disulfide bond with a cysteine at the C-terminus of S5 [26,29–31].

Recently, as mentioned above, MacKinnon and colleagues solved the X-ray crystal structure of the Kv1.2 channel [20,21]. In contrast to the KvAP structure, the new Kv1.2 structure was almost generally accepted. The voltage-sensing domain was again found to be loosely connected to the pore domain and S4 to partially face the lipid bilayer, but the overall transmembrane topology was now compatible with experimental data, and S4 was interacting with S5 in a neighbouring subunit (as we and others had suggested before [26–29]). However, whether the movement was of paddle, helical-
twist or helical-screw type was not clarified. Other recent studies put further constraints of the voltage-sensor movement. By tethering large biotin molecules to S4 and by using avidin binding, the S4 was assessed to move ∼20 Å relative the lipid bilayer [32]. By converting the voltage-sensor pathway to an ion-conducting channel, or by studying charges tethered to S4 by linkers of different lengths, the transmembrane barrier for the membrane voltage drop was found to be short, ∼5 Å [33–35]. Thus, the translational voltage-sensor movement is large but the transmembrane barrier is thin. Together with the electroneutrality argument mentioned above, ruling out the weakly charged paddle as a candidate for the voltage sensor, we conclude that a helical-screw model, assuming a peripherally located voltage sensor in contact with deep water-filled crevices [27,29], best explains the voltage-sensor movement in voltage-gated ion channels.

4. Concluding remarks

In conclusion, the questions about the gate and voltage-sensor movements are still not settled. We will continue our efforts to clarify aspects of these problems. The reason is that detailed knowledge about the gating is essential for our understanding of channel function in general and essential for understanding of drug action of channels in particular. Knowledge about the internal gate structure is fundamental for understanding the action of pore blocking compounds, including the widely used local anesthetics, antiarrhythmics, and antiepileptics, and is thereby essential for the attempts to construct new drugs with better effects and less negative side effects [36]. Knowledge about the voltage-sensor location and movement is fundamental for understanding complicated ion channel kinetics [37], and for understanding effects of compounds that modify channel kinetics, exemplified by metal ions [38] and certain toxins. Especially, the proposed lipid-facing position of the voltage sensor [12,13,20,21,27,29] opens up for understanding how lipophilic substances directly affect the voltage sensor. For instance, the dramatic effect on the voltage sensitivity by the lipophilic batrachotoxin (BTX) from the arrow poison frogs has been hard to understand, using prevailing channel models. Likewise it has been hard to understand how the highly lipophilic intravenuous general anaesthetics propofol affects the voltage sensitivity of both Na and K channels as well as if the channels’ voltage sensitivity has been affected [39]. It now seems reasonable that it binds to the lipid bilayer and directly affects the lipid-facing voltage sensor.

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