Life Among the Axons

Clay M. Armstrong

Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104; email: carmstro@mail.med.upenn.edu

Abstract

A blink in history’s eye has brought us an understanding of electricity, and with it a revolution in human life. From the frog leg twitch experiments of Galvani and the batteries of Volta, we have progressed to telegraphs, motors, telephones, computers, and the Internet. In the same period, the ubiquitous role of electricity in animal and plant life has become clear. A great milestone in this journey was the elucidation of electrical signaling by Hodgkin & Huxley in 1952. This chapter gives a personal account of a small part of this story, the transformation of the rather abstract electrical conductances of Hodgkin & Huxley into the more tangible gated ion channel.
INTRODUCTION: MY LIFE AS AN ASPIRING SCIENTIST

My early years were in some respects quite tragic. I grew up a male in Texas too small to play football. Perhaps this fact more than any other destined me to be a scientist. To compensate, I struggled for a time on the tennis team, without notable success. Helpful in taking my mind off these failures were my high school English teachers, both spinsters, one shrunken but vital and witty, the other romantic and in thrall to Beowulf. Together they gave me a permanent love for literature. Math, chemistry, and physics scarcely existed in the curriculum, leaving me with deficits I am still struggling to overcome. All in all, it was not a promising start for a scientific career.

Things changed when I got to Rice, where my heart belongs, educationally speaking. At the time Rice was tough enough that a third of the freshman class did not return the second year. The idea was to whip those Texas country boys into shape (football players were exempt), and for the survivors it seemed to work quite well. My love of literature persisted and grew; in literature I found a continual awakening. And at Rice I got an excellent grounding in chemistry and physics but demonstrated no great talent.

What does a middle-class boy with a good memory and no particular aptitudes do with himself? He goes to medical school, in my case Washington University. It is and was a fine school, but after the first year I was not a fine student. Fatefully, in physiology class I was exposed to the Hodgkin & Huxley (1) formulation and was permanently imprinted by its mysteries. I was also fascinated by brain electrophysiology, and under the supervision of Bill Landau, I worked in the lab of the legendary George H. Bishop, using his handmade equipment. It was in his darkened, electrically shielded room that I discovered just how remote and specialized science is and learned many things that I could never explain to my mother. All the big questions about my place in the universe were at least temporarily shunted aside. In compensation, I found it exciting to walk mentally in obscure places where there were few footprints. One of my favorite pieces of work was done in Bishop’s lab, deciphering the electrical waves elicited in the visual cortex by lateral geniculate stimulation. The pieces of this puzzle all fell together one memorable day as I walked in Forest Park. Alas, only single-cell recordings were considered significant in those days, and my technically unfashionable work sank without a trace (2, 3).

Life, which is a series of chances, changed direction when the threat of the draft pushed me from Washington University to the NIH, where I worked in the lab of the famous K.S. (Kacy) Cole. Kacy’s lab had a mind-hand duality, and I signed on as a pair of unskilled hands. I learned an enormous amount there, studying, as my aunt Roberta called it, little bags of salt (cells). The focus in Kacy’s lab was, of course, nerve conduction, and as I saw it when I got my license to think a bit, there were three questions remaining after Hodgkin & Huxley’s (1) great work. (a) How do ions get through membranes? (b) How does the membrane distinguish between Na⁺ and K⁺? (c) How does the membrane change its electrical resistance and ion selectivity in a fraction of a millisecond? The dominant view was that carriers, one carrier for Na⁺ and one for K⁺ ions, ferry ions across membranes. Channels were a less popular idea because selectivity seemed harder to explain for a channel. Some guesses were pretty wild. For a time there was widespread intoxication with lipids and phase transitions and even a proposal that the conducting path in a membrane is formed of lipid, with protein as the insulator. One prominent biochemically oriented lab was certain that axon propagation was mediated by acetylcholine. Others, certain that ions were bound in cytoplasm, denied the importance of membranes. In Kacy’s physically oriented lab I was safe from many of these turbulent currents and had excellent exposure to electricity, feedback, mathematics, and physics.
In closing this short personal section, I have many debts to mentors, postdocs, and students. None is greater than my debt to A.F. Huxley, in whose lab I struggled unsuccessfully, after my NIH years, to become a worthy student of muscle. My failure in this regard in no way dampens my admiration and respect for this great and imaginative scientist.

TEA\(^+\) AND THE GROSS ARCHITECTURE OF THE K CHANNEL

My first (and perhaps only) good research idea came as a result of a short visit to A.F. Huxley’s lab, where a talented student, Denis Noble, was modifying the Hodgkin-Huxley equations to fit cardiac action potentials (4). These equations describe an action potential that is sharply depolarized to a peak by an increase of Na conductance and then repolarized by an increase of K conductance. One of Noble’s tasks was to explain why the peak of a cardiac action potential is followed by a long plateau rather than repolarizing promptly, as in an axon. For this purpose, he invoked the “anomalous rectifier,” recently discovered by Katz (5) in muscle fibers: anomalous in the sense that K permeability decreases with voltage, rather than increasing like the normal K permeability in axon membrane. On returning to the NIH I found that Tasaki & Hagiwara (6) had recently published a paper showing that internal TEA\(^+\) causes a cardiac-like plateau action potential when injected into squid axons. Their data suggested that total conductance during the plateau was lower than at rest, as though TEA\(^+\) somehow produced anomalous rectification. This was good enough to get started, so Leonard Binstock and I spent a summer at Woods Hole injecting squid axons with TEA\(^+\) and examining the membrane currents under voltage clamp. Neither of us was very good with axons, but luck eventually smiled on us, and we saw that with ∼40-mM TEA\(^+\) inside, the action potential indeed had a long plateau and that the outward I\(_K\) measured in voltage clamp was completely suppressed. In contrast, external TEA\(^+\) had almost no effect. Most excitingly, when we raised the extracellular [K\(^+\)], we found that inward K\(^+\) current was near normal even though there was no outward current! With intracellular TEA\(^+\), K conductance became a K-anomalous rectifier. Activation of the rectifier on depolarization (in present terms, the gating) seemed to occur normally; i.e., the conducting structures, whatever they were, activated normally but allowed only influx of K\(^+\) ions and no efflux (7).

Further work on the very giant axons of Chilean squid showed that with low concentration, when only half of I\(_K\) was blocked in the steady state, the time course of block was slow enough to be directly measurable (8). This made it possible to estimate the rate of K\(^+\) movement through the conductor simply by multiplying the TEA\(^+\) entry rate by the ratio of [K\(^+\)] to [TEA\(^+\)], yielding an intriguing number, approximately 600 K\(^+\) ions ms\(^{-1}\).

How does TEA\(^+\) work? I studied TEA\(^+\) with molecular models and found it to be roughly tetrahedral in shape, with little flexibility. Moreover, it is approximately the size of a K\(^+\) ion with one hydration shell (∼8 Å). The simplicity of the TEA\(^+\) molecule seemed to require an equally simple model, and the idea grew irresistibly in my mind that TEA\(^+\) was blocking a channel through the membrane that had a wide inner vestibule and a narrower outer section too small for TEA\(^+\) to pass through. Furthermore, it seemed that a gate at the inner end of the channel prevented TEA\(^+\) entry when it was closed (at rest). To investigate the features of TEA\(^+\) that affected its entry rate into the channels, I had Eastman Kodak synthesize derivatives of TEA\(^+\) with one of its ethyl arms replaced by a progressively longer hydrocarbon chain. The experiments were performed in Chile, where the ocean was inspiring, the facilities inspiring primitive, and the companionship wonderful. Much to my surprise, the blocking potency increased with chain length. Kinetically, the main effect was not on the entry but on the exit rate, roughly consistent with the idea that each

Anomalous or inward rectifier: prechannel names for K\(_R\) channels

Rest: resting V\(_m\).
Loosely, V\(_m\) < −60 mV

I\(_K\) or I\(_Na\): current through K or Na channels, respectively. Inward current is plotted down. In prechannel days, one spoke of Na conductance or permeability rather than Na channels.

Depolarization: driving V\(_m\) positive relative to rest.

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additional CH₂ group added ~600 cal mol⁻¹ to the binding energy. When TEA⁺ was applied at low concentration there was a dramatic “inactivation” of the current, shown in Figure 1a for C⁹⁺ (nonyltriethylammonium ion) (9). In Figure 1a, the top trace is control I₉, and below this are traces of two families of I₉ taken at low and higher internal C⁹⁺ concentrations. Each family was elicited by a series of voltage steps to −30 through +90 mV, with 2-s recovery intervals between steps. The inactivation rate is faster with high C⁹⁺ and changes little or not at all as the voltage is driven more positive. This implies that C⁹⁺ simply diffuses into the channel at a rate proportional to its concentration and that voltage has little effect on the entry rate.

The cartoons in Figure 1 depict what is occurring during C⁹⁺ block of I₉. At rest a gate at the inner end of the channel is closed, and the vestibule just above it is empty. After depolarization, several steps lead to the all-or-none opening of the gate, and internal K⁺ ions move into the vestibule, with their hydration shell in place. To pass through the channel’s narrow selectivity filter (a term coined

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**Figure 1**

C⁹⁺ block of I₉ resembles inactivation. The cartoons underneath parts a and b depict what is occurring at corresponding stages. (a) I₉ (in squid axon) normally increases, with a lag after a step depolarization (arrow), to a fixed level (trace labeled control). With 60 μM C⁹⁺ in the axon, I₉ increases normally and then “inactivates.” At rest (~60 mV) a gate at the inner end of the channel protects a relatively wide vestibule. When the gate is opened by depolarization, K⁺ ions enter the vestibule, dehydrate as they go through a narrow tunnel (filter), and rehydrate at the outer end of the tunnel. C⁹⁺, present at low concentration, takes time to find the channel but then enters the vestibule and gets stuck, with its hydrophobic nonyl chain bound to a hydrophobic region in the vestibule wall. “Inactivation” is faster at higher C⁹⁺ concentrations. A family of depolarizations is shown at each concentration for steps to −30 though +90 mV. (b) At the end of a short depolarization, I₉ is inward (external [K⁺] is high) but decays rapidly as the channels deactivate. Deactivation is much slower after a long step because the gate is unlikely to close when the vestibule contains C⁹⁺. In this case, I₉ initially increases as C⁹⁺ moves into the vestibule by inward-moving K⁺, and the gate then closes. (c) The gate can be slammed shut owing to repolarization to −100 mV, trapping C⁹⁺ in the vestibule. Figure modified from Reference 9.
by Bert Hille), they must dehydrate and then rehydrate as they leave the filter. After a time that depends on concentration, a \( \text{C}_9^+ \) ion diffuses into the vestibule and gets stuck because it cannot move through the filter. Its hydrocarbon nonyl chain increases affinity by binding to a hydrophobic portion of the vestibule’s lining.

On repolarization \( \text{C}_9 \) comes out of the channels, and there are several points of interest, illustrated in Figure 1b. The traces show \( I_K \) during a depolarizing step, with repolarization to \(-60 \text{ mV}\), for a \( \text{C}_9^+ \)-containing axon bathed in high external K\(^+\). When the step is short, there is little block, and the channel gates close relatively quickly, with a simple monotonic time course. After the longer step, when block is complete, \( I_K \) on repolarization is at first quite small but increases with time as external K\(^+\) ions move inward through the channel and push out the \( \text{C}_9^+ \) ions. In low external K\(^+\) (not shown in Figure 1), this knock-off effect does not occur, and recovery is much slower. Even with high K\(^+\), closing of a blocked channel is greatly prolonged: \( \text{C}_9 \) has a “foot-in-the-door” effect (an apt phrase from Hodgkin) that hinders the gate from closing. However, if voltage is made very negative it is possible to close the gate and trap \( \text{C}_9^+ \) ions in some of the channels for many seconds (Figure 1c).

All these phenomena were so easy to explain with a channel model that I accepted it and never looked back. At approximately the same time, Hille (10) was finding compelling reasons to believe that Na\(^+\) also traveled through channels. The main features of K channel architecture seemed as follows. (a) A gate at the inner end was, following Hodgkin and Huxley, fully open or fully closed and for this reason is portrayed as an open-or-closed trap door in Figure 1. How voltage operated this door remained to be determined. (b) The gate opened into a vestibule large enough for TEA\(^+\) or a hydrated K\(^+\) ion. Part of the lining was hydrophobic. (c) Above the vestibule was a narrow tunnel, permeable to a dehydrated or partially dehydrated K\(^+\) ion but not to TEA\(^+\), with its covalently bound side chains.

TEA\(^+\) sites are at both ends of K channels in frog myelinated axons. The inner site is similar to the one just described for squid channels, but the outer one is quite different in character and not sensitive to \( \text{C}_9^+ \) (11).

### SELECTIVITY AND CONDUCTION

With a mental picture of the channel’s architecture, could we imagine how the channel distinguishes between Na\(^+\) and K\(^+\) ions? According to the gross architectural picture, K\(^+\) apparently dehydrates at least partially while going through the filter. Both Na\(^+\) and K\(^+\) have a charge of 1 e (electronic charge) and are simple hard-sphere cations (12), so why cannot the smaller Na\(^+\) (which has a Pauling radius of 0.95 Å) pass through a filter that accepts K\(^+\) (1.33 Å)? This problem became acute when Pancho and I discovered that Na\(^+\) in the internal medium partially blocked K channels at high membrane voltage (13). Aided in our thinking by the close-fit hypothesis used by Lorin Mullins (14), we proposed the scheme given in Figure 2a. The top row shows K\(^+\) and Na\(^+\) ions in water, with the oxygens of water molecules packed closely around both, binding more tightly to the smaller Na\(^+\) than to K\(^+\) (which has a lower hydration energy). The filter is lined with hydrophilic groups, e.g., carbonyl oxygens, and its diameter makes it a good fit for K\(^+\) (Figure 2a, lower left). Thus, K\(^+\) is bound about equally well in water or in the channel’s filter. For Na\(^+\), however, binding in the filter is not favorable because the filter’s walls are rigidly fixed and prevent close approach of the carbonyl oxygens to the ion (Figure 2a, lower right). The result is that Na\(^+\) has much higher energy in the filter than in water; it is therefore unlikely to leave water and enter the filter. This is precisely what is needed for selective transport through a channel: Selectivity is conferred by having a low entry...
Figure 2

(a) A theory for K⁺ selectivity. K⁺ and Na⁺ ions are shown in water and in a carbonyl-lined filter that is a good fit for K⁺. The energy of K⁺ in water and in the filter is approximately the same. The energy of Na⁺ in the filter is much higher than in water because the filter walls are too rigid to allow the carbonyls to close around this small ion. Through the use of an elementary calculation, the situation drawn would provide a selectivity that is orders of magnitude greater than observed, implying that the walls are not completely rigid. Modified from Reference 13. (b) K⁺ is hydrated in the vestibule, dehydrates to enter the filter, and rehydrates at the outer end. Modified from Reference 16. The energy diagram (lower diagram) shows that K⁺ faces no large barriers in transit through the channel. Na⁺ is excluded by a high barrier (cf. the appendix in Reference 13).

It is useful to think of the energy of a K⁺ ion in five positions as it passes through the filter (Figure 2b, right to left): (1) hydrated in the water of the vestibule, (2) in the transition state as it dehydrates to enter the filter, (3) in the filter, (4) as it leaves the filter and rehydrates, and (5) rehydrated in the external solution. Because the transport rate of K⁺ through the channel is fast, K⁺ must encounter no large energy barriers as it passes through. Dehydration at both ends of the filter must be performed quite efficiently to keep the barrier low. Additionally, the energy of K⁺ in the filter must be similar to the energy of K⁺ in H₂O, where the ion is bound tightly (hydration energy 79.3 kCal mol⁻¹; see Reference 15). If so, K⁺ binding in the selectivity filter must be approximately as tight as in H₂O. A clever experiment verifies this: K⁺ remains bound in the filter for minutes when K⁺ is removed from internal and external solutions while the channel gate is closed (17). Ion replacement, on the other hand, is rapid because the energy barrier for an entering, replacement ion is low (Figure 2b).

GATING

To me the most fascinating aspect of channels has always been gating: how the channels respond to voltage and how their gates open and close in fractions of milliseconds. Any student of gating willing to study the papers of Hodgkin & Huxley (1) has a big head start. Here I greatly simplify their work. They showed that axons conduct action potentials in much the same way that submarine telephone cables (the first trans-Atlantic telephone cable was laid in 1956) conduct telephone messages. The objective is to get high-frequency transmission along an axon or cable that consists of a conductor
surrounded by an insulator, both immersed in salt water. The signal current flowing along an axon/telephone cable continually weakens as current leaks out of the conductor (axonplasm/copper) through the resistance and capacitance of the insulator (membrane/insulator). These losses are particularly severe in axons, in which the signal attenuates in millimeters, versus miles for a telephone cable. In both cases the solution is to put in boosters, which detect the weakened signal coming in from upstream and amplify it back to full strength. The boosters in axons are Na channels, and in telephone cables they are electronic amplifiers. Axons also have K channels to get them repolarized and ready for the next action potential.

Hodgkin & Huxley (1) put all this together in a masterful summary that deserves to live forever. They quantitatively described separate Na and K conductances (now known to be mediated by channels). Both conductances can be activated over approximately the same voltage range; activation begins near $-50$ mV and is complete near 0 mV. The activation process for both is similar, but Na conductance is faster, and the action potential is well established by Na influx before the K conductance turns on. Activation of the K conductance allows an efflux of K ions that pulls membrane voltage back toward the resting level. This is made easier by a second gate on the Na channel, termed the inactivation gate, which shuts down the Na conductance, facilitating repolarization. Hodgkin & Huxley did not and could not know the mechanisms involved in selectivity, activation, and inactivation. They seemed to favor the idea that Na and K each had a selective carrier that ferried (conducted) ions across the membrane when the carrier was activated by control particles sensitive to membrane voltage. K carriers, e.g., were switched on or off by the movement of four hypothetical particles termed $n$, each with a charge of 1–2 e. The particles could be either positive and move outward to activate the carrier, or negative and move inward; Hodgkin & Huxley had no way to know. All four particles had to be activated to switch on the carrier/conductance. The Na carrier was similar but with three control particles ($m$) rather than four. Movement of a single control particle ($h$) with a charge of $\sim 2$ e “inactivated” the Na carrier.

The Hodgkin & Huxley scheme accounted nicely for the kinetics of activation and deactivation of the carriers/conductances. At rest all the control particles were deactivated on one side of the membrane, and they activated one by one following depolarization, moving to the other side. Because all had to be activated to switch on the carrier, there was a pronounced lag in activation, making both Na and K conductance increase sigmoidally. In contrast, on repolarization a conducting carrier was switched off by deactivation of a single one of its control particles; the time course was exponential (for sufficiently negative voltage) and had no initial lag. The requirement for multiple control particles to explain the kinetics gave a premonition of the multisubunit (or multidomain) nature of the molecules underlying the conductances.

DESTROYING Na INACTIVATION

The most accessible of the gating functions turned out to be Na inactivation. The finding that K channels could be given a semblance of inactivation by C9 ions raised the question of whether inactivation of Na channels had a similar inactivation mechanism, a terrifying departure from the formulation of Hodgkin & Huxley. The question received additional impetus from the finding that Na inactivation could be removed without much effect on activation by perfusing an axon internally with pronase, a proteolytic enzyme mix first used in axons to facilitate internal perfusion. An overdose of pronase caused changes in the voltage clamp currents that were first interpreted as an effect on K channels (18). I thought the effect looked more like removal of Na inactivation, so Pancho, Eduardo Rojas, and I set out to see, and got, the result shown in Figure 3 (19).
Figure 3

Pronase destroys inactivation when applied internally. Families of superimposed $I_{\text{Na}}$ traces are shown before and after pronase application, in an axon with $I_K$ blocked by TEA$^+$. (Left) Before pronase application $I_{\text{Na}}$ increases after depolarization as the channels activate, then decreases as inactivation occurs. On repolarization there is a small inward tail of $I_{\text{Na}}$, through the small fraction of channels that did not inactivate. (Right) After pronase application, inactivation does not occur. On repolarization there is a large inward current through the still-active channels. This current decays rapidly as the channels deactivate. Each trace shown represents a depolarization from $-70$ mV to the indicated voltage, with a 2-s interval between each depolarization. $I_{\text{Na}}$ is outward at 60 and 80 mV where the voltage drives Na$^+$ outward through the channels. Modified from Reference 19.

After blocking of the K channels of an axon with TEA$^+$, the Na channels fail to inactivate after pronase treatment, even though activation and deactivation occur normally. Pronase removes inactivation from a channel in a single clip: The inactivation rate of those channels not yet touched by pronase is completely normal (not shown in Figure 3). The picture of a ball-and-chain mechanism for Na inactivation was almost irresistible: A single internal C$_9^+$-like particle (ball) diffuses in and blocks a Na channel after its internally located activation gate is partially or fully activated. Pronase cuts a peptide chain that links the ball to the inner end of a channel, leaving the activation gate untouched. This seemed nice but was distinctly unlike the model of Hodgkin & Huxley, who had never been wrong! Further elucidation had to wait on other evidence.

**Activation**

The experiments described above provided evidence that ions go through channels that have gates at their cytoplasmic end. It was a necessity from the physicist’s point of view that the gate be controlled by something analogous to the charged particles (m, n) postulated by Hodgkin & Huxley (see above section, “Gating”). A magnetic sensor, for example, would be sensitive only to current or to $dV/dt$ and could be excluded. Whatever the identity of the charges, they inevitably produce a current as they move through the membrane. The control, or gating, current would presumably be quite small and of short duration because the charges would at most move from one side of the membrane to the other. It would be outward as the gate opens and inward as it closes. But could it be detected? With stimulus from Rojas, Pancho and I set out to find it. We suppressed all the ionic current, subtracted out the linear portion of the capacitive current, and turned up the amplification.

After intense effort and much sorting through artifacts, we were rewarded by the recordings in Figure 4 (20). Trace i was in very low external Na$^+$ and shows inward $I_{\text{Na}}$ preceded by a transient outward current. After adding tetrodotoxin (TTX) to block ion movement through Na channels, we got trace ii. The peak of the current is the same as in the absence of TTX, and the current’s decay toward zero is visible thanks to the absence of $I_{\text{Na}}$. Trace ii has the time course...
expected for gating current: It starts early and dies away as the channels open. The current is outward on depolarization and inward on repolarization (not shown in Figure 4), as expected for the passive movement of charge in the electric field of the membrane. There is no way to tell from the current itself whether the gating charge is positive and moving outward on depolarization, or whether it is negative and moving inward.

From trace ii (Figure 4) it seems that the gate opens normally in the presence of TTX. Hille (21) had postulated that TTX binds to a receptor near the outside end of the Na channel. The absence of an effect of external TTX on gating current is consistent with an internal activation gate that is unaffected by TTX on the other side of the membrane. This further supports the idea that both the activation and the inactivation gates of Na channels are inside. Above all, we were happy that gating current, which had to exist, not only existed but was detectable.

Gating current is small and sometimes frustrating to work with. One must continually remember that it is obtained by subtracting out the best estimate of the linear capacitive current and that this estimate can never be perfect. Furthermore, there were two immediate lessons, one about activation and one about inactivation (the latter is described below). Before inactivation occurs, the (inward) gating current on repolarization decays at approximately the same rate as INa as the activation gate closes. The Hodgkin & Huxley model of three independent gating particles predicted that it should be three times faster. This made it clear that information on activation/deactivation would have to be assembled empirically and that no preexisting theory could help much (22, 23). If the channel were made of protein, as seemed likely from the pronase results, one could think of the gating transitions not in terms of gating particles but as transitions among conformational states of a channel protein.

A lover of speculation, I proposed the model channel shown in Figure 5 (24). The hypothetical protein had (a) four subunits surrounding a pore and (b) a gate that could open only when all four subunits were activated. Activation and deactivation for each subunit were governed by a zipper-like arrangement of charges (Figure 5b). Making the inside of the membrane positive caused relative motion between the two halves of the zipper, driving a conformational change that, by an unknown mechanism, activated the subunit. This made it possible to activate the next subunit and so on until all subunits were activated and the gate could open. When Na (25) and K channels (26, 27) were cloned some years later, definitively proving at last that the channels were proteins, I was pleased to see some echoes of these speculations evident in the amino-acid sequence.
**Figure 5**

An early speculation about channel gating. The model channel had four subunits and a gate. In each subunit, or in the junction between, was a zipper-like arrangement of charge (b). The small relative motion shown (deactivated ↔ activated) transferred one full electronic charge across the membrane because all the charges moved in the membrane field. This motion drove a conformational change (details unknown but easy to imagine) (a) that activated the zipper's subunit, with activation pictured diagrammatically as outward rotation. This made it possible for the next subunit to be activated by its zipper and so on, until the gate was free to open. Modified from Reference 24.

**KIR channel:** a K channel that acts like a one-way valve, blocking outward current. KIR channels are opened by K⁺ influx, caused by negative V_m and/or high extracellular K⁺.

**S4:** the fourth transmembrane segment, containing four to eight positively charged amino acids

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**THE SEQUENCING OF Na AND K CHANNELS**

Once channels were sequenced they became much more tangible. Abandoning chronology for simplicity, let us begin with the two families of K channels, Kᵥ and Kᵢᵣ. Members of both families are made of four identical subunits (peptides); Kᵢᵣ peptides are approximately one-fourth as large as those of the Kᵥ family. The N and C termini are inside for both. For each Kᵢᵣ subunit there are 2 transmembrane crossings (T₁ and T₂), yielding a total of 8 crossings for a Kᵢᵣ channel, whereas a Kᵥ subunit has 6 (S₁–S₆), and a Kᵥ channel, thus, 24. T₁ and T₂ are analogous to S₅ and S₆ and are now known to form the ion conduit of the channel. Segments S₁–S₄ are found in voltage-sensitive channels. The very obvious voltage sensor is the S₄ segment, which contains seven positively charged amino acids in some Kᵥ peptides, much like the positive side of the zipper. The complementary negative charges, which are an energetic necessity, are still something of a mystery, as discussed below.

Na channels seem to have come later in evolution. They involve mutations that stitched the subunit peptides together to form a very large peptide containing four domains. As in the Kᵥ family, the Na channel apparently...
has an ion conduit made by apposition of S5 and S6 from the four domains, forming a central core and four subdomains (S1–S4) surrounding the core (details are still uncertain). After the stitching, the domains differentiated, presumably to confer Na selectivity, faster gating, and inactivation.

**THE DISCOVERY OF THE PORE REGION**

A prescient Na channel model from Guy & Seetharamulu (28) aided the effort to associate regions of the sequence with function. These researchers identified the region between S5 and S6 (in present terminology) as the likely pore and selectivity filter. A search for the binding site of the potent K channel blocker charybdotoxin (29) led within a few years to the identification of the pore region in K channels (30, 31) and of a signature sequence (32) that made it possible to identify potential K channels in the protein database. Among these was a bacterial Kir family channel, KcsA (33).

**PORE, FILTER, AND GATE: THE KcsA CRYSTAL STRUCTURE**

MacKinnon and colleagues (34) then began the arduous task of crystallizing the KcsA channel and subjecting it to X-ray analysis. The following is a brief summary of their work, but the original papers are a pleasure that should not be missed. In each of the four subunits that comprise the channel, there are two membrane-crossing helices, T1 and T2, that correspond to S5 and S6 in the Kv family and are so labeled here. S5 and S6 are joined by a long linker that dips into the membrane to make a pore helix that supports the selectivity filter. Figure 6 shows the KcsA structure with S5 omitted. The S6 helices form a cone that contains the pore helix and the filter, form the walls of a cavity, and line the inner part of the pore. They converge toward the inner side of the membrane; the gate region is at the convergence. The gate is closed in KcsA: There are three points in the convergence zone at which methylene groups crowd together too closely for even a dehydrated K⁺ ion to pass. Because these groups are hydrophobic, each of the three seals makes a very high energy barrier to ion movement, a very effective gate. The Kv family also has a gate in this part of the S6, although the structure of this family’s S6, based on the sequence, is probably quite different, as Yellen (35) has pointed out. The Kv gate closes tightly enough to exclude small cations (36). The open state of KcsA has not been crystallized, but a reasonable model is provided by another bacterial channel, MthK (37), in which the convergence zone is widely spread apart and more than wide enough to admit a hydrated K⁺ ion.

![Figure 6](https://www.annualreviews.org)
A K⁺ ion going from axoplasm to external fluid passes through the gate region, enters the water-filled cavity, and approaches the inner end of the selectivity filter. To enter the filter, the ion must dehydrate and the carbonyl groups at both ends of the filter must be carefully arranged to lower the energy barrier for dehydration/rehydration. Once in the filter, the dehydrated ion is surrounded by carboxyls of the filter wall. Starting inside, there are four binding sites—4, 3, 2, and 1—and probably only two are simultaneously occupied, 4 and 2, or 3 and 1. An ion approaching from the cavity would first repel the occupying ions from positions 4 and 2 to positions 3 and 1. When the entering ion occupies site 4, the ion in 3 would move to 2, and the ion in 1 would rehydrate as it left the filter. The occupying ions at this point are again in 4 and 2, and the cycle can repeat. The figures of Reference 39 show dehydration in progress.

**INACTIVATION OF Na CHANNELS**

After the gating current measurements (22, 23), it was reasonable to believe that Na inactivation resembled Cs⁺ block in that the voltage dependence of block/inactivation arose from coupling to activation: Both Cs⁺ and the inactivation particle simply diffused into their blocking site when the activation process made the site available. However, there were clear differences. The Na channel can close readily when inactivated, useful functionally in preventing leak during recovery from inactivation. Related to this, approximately one-third of gating charge remains mobile after Na inactivation. This is unlike in TEA⁺ block, which immobilizes all gating charge in K channels. The not-immobilized charge moves on repolarization to close the channel promptly (no foot in the door), averting leak during recovery. Also, Na channels need not open fully before inactivating (40), although most inactivation in an action potential occurs from the open state. There is much recent evidence on Na inactivation, which will be the subject of a future communication.

**INACTIVATION OF K CHANNELS**

Some K channels, notably those termed Shaker B, inactivate rapidly. Yellen and colleagues (40a) found that K inactivation resembles Cs⁺ block in that it occurs only when the channel is open. Furthermore, like Cs⁺, the inactivation particle is subject to knock off by K⁺ ions moving inward through the filter after repolarization, and it acts like a foot in the door, preventing closing of the activation gate. Hoshi et al. (41) identified the inactivation particle as a "ball" at the N terminus of the Shaker B peptide. Four balls are attached by flexible chains to the inner surface of the channel and diffuse into the channel mouth when the gate is open. Mutations that shorten the chain reduce the number of possible conformations of the chain and thus increase the inactivation rate. Decreasing the number of balls by mutation (42) or enzymatically (43) reduces the rate of inactivation, proving that there are four inactivation particles rather than one, as in the Na channel (19). MacKinnon and colleagues (44) have X-ray pictures of the ball within the cavity.

Aldrich and colleagues (45) described a second type of inactivation, referring to it as C inactivation to distinguish it from N-type (or ball-and-chain) inactivation. The mechanism for inactivation is not completely clear, but the experiments of Yellen and colleagues (46) strongly suggest that it involves a constriction near the outer mouth of the selectivity filter.

**S4 MOTION DURING ACTIVATION**

As a voltage-dependent channel activates, gating charge must move; as noted above, there is no physical alternative. Common sense and some evidence tie this to motion of the S4 segments (47, 48). The original estimate of Hodgkin and Huxley was that approximately...
six electronic charges move to activate a channel, but current estimates for the Shaker K channel are more than twice that (49, 50). Precisely how the motion occurs is not yet clear. Simplest conceptually are the zipper model (Figure 5), its helical screw variant (51), and the paddle model (52). All three models have problems. The negative counterions for the first two models have never been identified in sufficient number. The absence of counterions in the paddle model raises, in my mind, the insurmountable energetic problem of putting multiple arginines (charged and hydrophilic) into lipid, at a cost of approximately 15 kCal mol$^{-1}$ for each arginine (53).

A more complex model postulates crevices that penetrate around the S4 segments and go part way through the membrane from both sides (54, 55). These crevices admit anions from the internal and external solution to serve as counterions. A new crystal structure of Kv1.2 is helpful but not definitive because it shows the S4 only in one state, probably open (56). There are no obvious crevices. How the S4 moves during gating remains a challenging question.

**HOLDING THE ACTIVATION GATE OPEN**

Yellen and colleagues (57) found a thought-provoking way of holding open the gate of a Kv channel even when $V_m$ dictates it should be closed. They introduced a cysteine residue (replacing a valine) in the gating region and added cadmium. The cadmium binds to the introduced cysteine and forms a bridge to a native histidine 10 residues further down and in a different subunit. Remarkably, the gate is tied open, unable to close on repolarization.

**HOLDING THE GATE CLOSED WITH 4-AMINO PYRIDINE**

4AP$^+$ is a K channel blocker with an interesting mode of action (58, 59). It enters and leaves the vestibule from inside when the activation gate is open, as do TEA$^+$ and Cs$^+$. Its blocking affinity is very low when the gate is open ($K_D \sim 55$ mM) but high when it is closed ($K_D \sim 30 \mu$M). Presumably, 4AP$^+$ fits loosely in the vestibule in the open state but the vestibule contracts around it in the closed state. 4AP$^+$ then serves as an adhesive to keep the vestibule contracted. The gate can open, but slowly and with low probability. In short, 4AP$^+$ enters through the open gate and pulls the gate closed behind it. This is the opposite of TEA$^+$, which tends to hold the gate open, like a foot in the door. Interestingly, when 4AP$^+$ is trapped in the vestibule by the closed gate, the S4 segments of all subunits can move in and out almost normally in response to voltage changes (58, 60). Total charge movement, however, is reduced by 5% or 10% because the final opening step and its associated charge movement are unlikely to occur. Also, the Q-V curve, which relates gating charge movement and $V_m$, is shifted to the right by 5–10 mV, reflecting the small amount of extra work that is required to move the S4s outward with 4AP$^+$ trapped in the vestibule. This fact tells a good deal about the coupling of S4 to the gate.

**COUPLING OF S4 TO THE GATE**

Open probability $P_o$ can vary from $\sim 10^{-9}$ at $-80$ mV (49, 50) to $\sim 0.7$ at $+20$ mV. Do the S4s pull the gate open when they are activated? Or do they lock the gate shut when they are deactivated? Or both? TEA$^+$ and 4AP$^+$ interact with the gate, and studying these interactions is quite helpful in deciding such questions. First, consider deactivation of the channel. TEA$^+$ in the vestibule holds the gate open and freezes S4 motion (61). Figure 7a shows a mechanical explanation: Flanges on the S6 segments prevent the S4s from moving downward as long as the gate is held open by TEA$^+$. (K$^+$ and Rb$^+$ in the vestibule also impede closing, suggesting that the vestibule contains only water when the gate closes normally.) Apparently there is rigid coupling between S4 deactivation and gate closing: The S4 cannot move inward while the gate is open.
Thinking about the coupling of S4 and the activation gate. (a) When TEA\(^+\) is in the vestibule, the channel gate is held open, and the S4s are immobilized in activated position. Projections (flanges) on the S6 prevent the S4s from moving inward as long as the gate is open. When TEA\(^+\) leaves the vestibule, the gate can close, and negative internal voltage drives the S4s inward to the deactivated position. The deactivated S4s lock the gate shut. (b) 4AP\(^+\) in the vestibule stabilizes the closed state by an energy roughly equivalent to that of a hydrogen bond, thus making the gate unlikely to open. Experimentally, with 4AP\(^+\) in the vestibule the S4s can move almost normally, from deactivated (left) to activated (right).

Thus, an elastic connection such as the gating spring diagrammed must be very compliant. Arrows show the forces exerted on the gate by the gating springs (via imaginary pulleys) when they have been tensed by outward movement of the S4s. The forces are quite small. Modified from Reference 63.

Does S4 motion pull the S6 open by means of some so-far-unknown gating spring (62)? 4AP\(^+\) holds the gate closed but impedes S4 motion only slightly (see above). This suggests that any link between the S4 and the gate is very weak and easy to stretch (63). In Figure 7b, a hypothetical S4-S6 connection is drawn as an S4-linked gating spring attached via a pulley (to get the force in the right direction) and an inelastic cord to S6. If the spring is very stiff, the S4s cannot move when 4AP\(^+\) holds the gate shut. At the other extreme, if the spring is completely compliant, no extra work will be required to move the S4s, and the center point of the Q-V curve will be unaffected. As noted above, the experimental curve shows only a small displacement, signifying enough energy to change P\(_n\) by a factor of less than 10, many orders of magnitude less than the experimental range of P\(_n\). This strongly suggests that the main function of the S4 is to lock the gate closed and that S4 makes only a minor contribution to pulling it open. This idea gains some support from the crystal structure of Kv1.2 (64), which shows that the S4-S5 linker is in a position that would appear to lock the gate shut when S4 is deactivated. Mutational alterations in this region of the S6 and the facing region of the S4-S5 linker profoundly alter gating (65, 66).

**WHY IS THE ACTIVATION GATE ALL OR NONE (USUALLY)?**

The gate-opening step is all or none and must involve coordinated movement in the S6 segment of all four subunits. This is clear from single-channel records, in which the opening is a single jump that cannot be resolved into smaller jumps. One factor almost surely is that the gate region is either large enough to admit a hydrated K\(^+\) ion, or it is not: Even partially dehydrating a K\(^+\) in a hydrophobic environment like the gate region would create a large energy barrier and make ion flux too small to detect. A related but more difficult question is why all four S4s must be activated to open the gate. A possible answer is that the gate region’s hydrophobic lining must be pulled in four directions to effectively break the hydrophobic bonds that almost certainly help to hold it closed. An analogy would be...
the need to pull in at least two directions to open a plastic sack whose sides are clinging together electrostatically (63).

**SUMMARY AND CONCLUSIONS**

Ion channels have gone from concept to reality in the past 50 years. They have been probed, sequenced, and crystallized, and much is now understood about their general properties, selectivity, and mechanisms of gating. They are essential in virtually all physiological regulatory mechanisms, perception, memory, and thought. Their genetic defects are involved in an ever-growing number of diseases, and they offer enormous possibilities for therapeutic intervention. Ah, to be able to start over again!

**LITERATURE CITED**


1. The foundation of ion conduction. Difficult to read because of older conventions but rewarding.
2. Elucidates the gross architecture of K channels. Among my papers, this is my favorite.
4. An awful title, but the selectivity theory is good.


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Errata
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