Molecular Basis of Gating Charge Immobilization in Shaker Potassium Channels

FRANCISCO BEZANILLA,* EDUARDO PEROZO,†
DIANE M. PAPAZIAN, ENRICO STEFANI

Voltage-dependent ion channels respond to changes in the membrane potential by means of charged voltage sensors intrinsic to the channel protein. Changes in transmembrane potential cause movement of these charged residues, which results in conformational changes in the channel. Movements of the charged sensors can be detected as currents known as gating currents. Measurement of the gating currents of the *Drosophila* Shaker potassium channel indicates that the charge on the voltage sensor of the channels is progressively immobilized by prolonged depolarizations. The charge is not immobilized in a mutant of the channel that lacks inactivation. These results show that the region of the molecule responsible for inactivation interacts, directly or indirectly, with the voltage sensor to prevent the return of the charge to its original position. The gating transitions between closed states of the channel appear not to be independent, suggesting that the channel subunits interact during activation.

SEVERAL TYPES OF VOLTAGE-DEPENDENT ION CHANNELS open upon depolarization (activation) and they spontaneously close, even when the depolarization is maintained (inactivation). This dual effect of membrane depolarization was first described for the sodium conductance of the squid axon (*1*). On the basis of gating current measurements, it was proposed that inactivation is the result of channel blockage by an inactivating particle that can swing into position after part or all of the conformational change required for activation has taken place (*2*).

Inactivation of Shaker potassium (K) channels is similar to that of sodium channels. The primary structures of Shaker channel proteins have been elucidated by molecular cloning of the *Drosophila* Shaker gene (*3*). Each channel is thought to be made up of four such proteins (*4*). Deletion analysis indicates that a region near the NH_2-terminus of the monomeric channel protein is necessary for inactivation of the channel (*5*). This region corresponds to the "ball and chain" device that blocks the channel in the inactivated state; inactivation can be restored in channels with NH_2-terminal deletions if the deleted peptide fragment is added to the cytoplasmic side of the channels (*6*). Although the channel is multimeric, one inactivating particle is probably sufficient to block the channel (*7*).

Gating currents represent the electrical expression of the conformational changes that lead to channel opening. Data from whole-cell and single-channel electrophysiological recordings and gating-current experiments have made it possible to infer a kinetic model for voltage-dependent gating and inactivation. One prediction of the ball and chain model is that the charge that moves during activation becomes immobilized during inactivation. Return of the voltage sensor to its original position is hindered when the channel is blocked by the inactivating particle. This proposal has been tested by measuring gating currents of normal Shaker channels and mutant channels with an NH_2-terminal deletion after expressing both channels in *Xenopus* oocytes (*8*). The data presented support the hypothesis that the inactivating particle interacts with regions of the channel that take part in the charge-moving conformational change required for activation.

A kinetic model for activation of the Shaker channel has been proposed in which four identical and independent subunits undergo voltage-dependent conformational changes between closed states. Subsequently, a voltage-independent transition opens the channel (*9*). This model supports the idea that each subunit of the channel has a voltage sensor, which may include the positively charged S4 segment (*10*), and that gating charge must move in each subunit in order for the channel complex to open. The experiments described here indicate that the subunits of the Shaker K channel interact during the activation process, as previously suggested by mutagenesis of the S4 segment in Shaker channels (*10*).

Charge movement associated with activation and inactivation of the Shaker K channel. Gating currents were recorded from an electrically isolated patch of oocyte membrane by a technique that allows both fast control of the membrane potential and control of the composition of the intracellular fluid by internal perfusion (*11*). The gating currents from normal Shaker channels increased in amplitude and decayed faster with increased depolarizations (Fig. 1A). For small depolarizations, the current rose abruptly (at the speed of the voltage clamp) and at the end of the pulse, the current decayed as a single exponential process. With larger depolarizations, a slower rising phase of charge movement was evident at both the beginning and end of the pulse. The amplitude of the current at the end of the depolarizing pulse was smaller than at the onset of the pulse and the charge recovery was slow, taking several tens of milliseconds to return fully. This indicates that the charge was immobilized during the depolarizing pulse. Gating currents from a mutant that lacks inactivation because of a deletion in the NH_2-terminal region of the channel monomer differed from those of the complete channel in that the charge recovered completely and relatively quickly at the end of the pulse (Fig. 1C). Therefore,

F. Bezanilla, E. Perozo, and D. M. Papazian are at the Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90024. E. Stefani is at the Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

*To whom correspondence should be addressed.
†Present address: Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024.

removal of inactivation also removes gating charge immobilization.

The integrals of the gating current records were plotted as normalized gating charge (Fig. 1, B and D) along with the normalized peak conductance. Some of the charge moved at potentials at which no ionic currents were detected, an indication that charge moves during conformational changes between closed states. The charge distributions (Fig. 1, B and D) can be fit by a simple Boltzmann function (continuous curves), from which an effective valence of 1.8 electronic charges per elementary gating event was calculated. This value may correspond to the amount of charge moved by the voltage sensor in one subunit. This valence is similar to the value of 2.3 electronic charges proposed in a kinetic model for activation (*9*). The limiting slope of the logarithm of the conductance plotted as a function of voltage (*12*) shows 4.1 electronic charges, which may correspond to the amount of charge moved by all the voltage sensors in the channel before it opens. Therefore, a minimum of two to three subunits of the channel must move before the channel opens.

The gating currents (Fig. 1) are not compatible with models of activation that include identical subunits responding to voltage independently. If the subunits were identical and independent, the gating current would be expected to rise instantly and then decay monotonically (*13*). Instead, the gating current begins with a slower (non-instantaneous) rising phase. The kinetics of this rising phase and the amount of gating charge moved were altered by conditioning potentials (Fig. 2A) (*14*). After 20-ms conditioning pulses to various potentials, gating currents were elicited by a pulse to 0 mV. The gating current rose much more slowly after a conditioning pulse to −110 mV than after a pulse to −40 mV, and more charge was moved, as indicated by the area under the trace. This observation is consistent with the existence of several closed states through which the channel transits before it finally opens. At hyperpolarized potentials, closed states further away from the open state may be more populated. Even though the channel subunits may be identical, transitions between closed states are apparently not equivalent. That the gating current turns on faster after being pulsed to −40 mV than after a pulse to −110 mV indicates that the transition between the initial states is slower or less dependent on voltage than the subsequent transitions. These properties are similar to those of gating currents of K channels in squid axon and of DRK1 K channels expressed in oocytes (*15*).

If the subunits functioned independently, the gating current would turn off four times more slowly than the ionic current because a closing transition in one subunit would be sufficient to close the channel despite the fact that only one quarter of the charge had returned. For a comparison of the closing kinetics, gating currents and ionic currents were elicited with pulses to 0 mV, and then currents were recorded for each by returning the potential to −80 mV (Fig. 2B). The off-gating current had an initial slow phase which may have been caused by the delay in the return of the charge that results from the less voltage-dependent (or voltage-independent) transition from the open state to the nearest closed state (*9*).

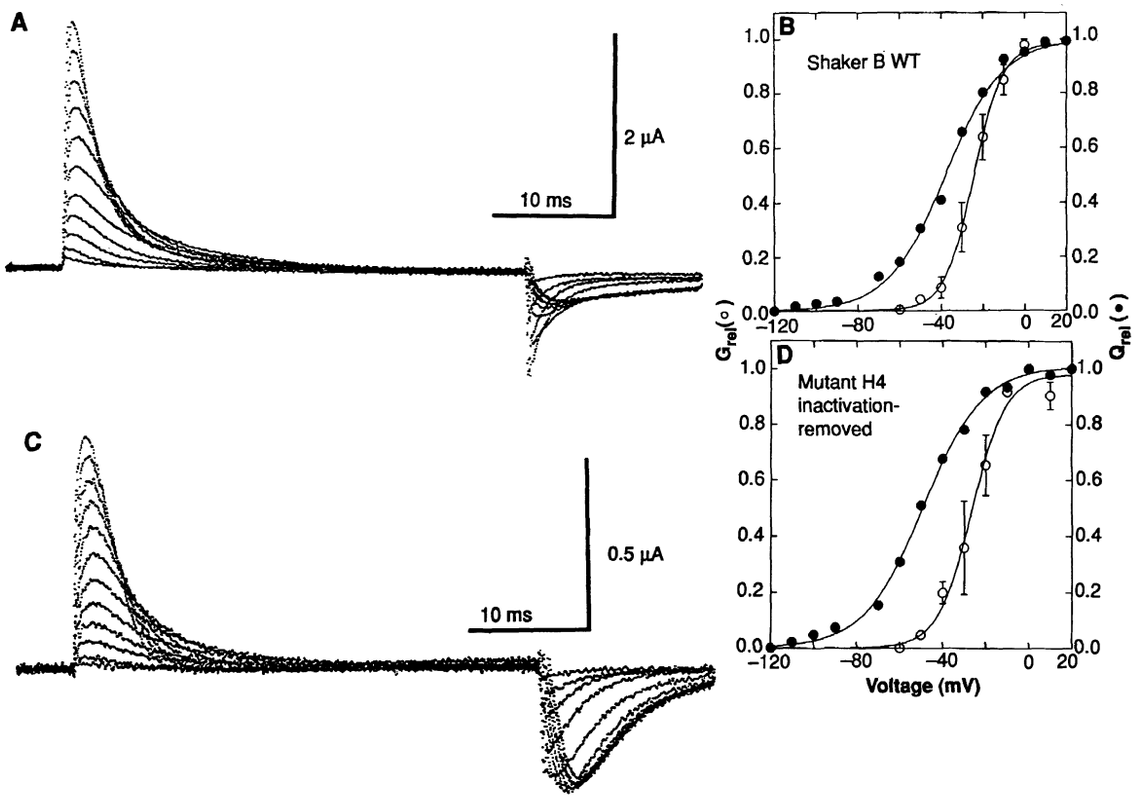

Fig. 1. Gating currents recorded in normal Shaker potassium channels and a mutant of the channel expressed in *Xenopus* oocytes. Recordings were made two to four days after RNA injection. Gating currents recorded from Shaker B channels (**A**) and the non-inactivating mutant channels (**C**) were obtained by subjecting the channels to depolarizing pulses ranging from −70 to 30 mV in 10 mV steps. The holding potential was −80 mV. The potential at which the subtracting pulses were started (subtracting holding potential) was −120 mV; subtraction of nonspecific linear currents (leakage currents) and linear capacitance was done with a divided pulse procedure (p/−4 procedure) (*20*). The subtracting holding potential could be varied between −120 and −150 mV without changing the gating currents recorded. Data were acquired with a computer controlled acquisition system (*21*), filtered at 10 kHz and digitized at 50 μs per point. Records shown are averages of signals for ten cycles for the Shaker channel and four cycles for the non-inactivating mutant. Data were subsequently digitally filtered at 2 kHz. Temperature was 18°C. The internal solution contained 120 mM *N*-methylglucamine-glutamate, 1 mM EGTA, and 10 mM Hepes (pH 7.3), and the external solution was composed of 120 mM of tetraethylammonium-methylsulfonate (TEA-MES), 1.8 mM $CaCl_2$, and 10 mM Hepes (pH 7.3). Relative conductance (open circles) and on-gating charge (closed circles) were plotted as a function of voltage for Shaker B (**B**) and the non-inactivating mutant (**D**). Results are representative of eight experiments. The relative conductance is the value of the conductance divided by the maximal conductance. Conductance values were calculated at each potential by the ratio of the current jump (including the tail) to the voltage difference (*22*), and the values are the means of three experiments; bars represent SEM. The on-gating charge was obtained as the integral of the on-gating current for the duration of the depolarizing pulse. Each data set was fitted by a Boltzmann relation:

$$Q = 1/(1 + \exp(ze(V - V_{1/2})/kT)).$$

The valence z was 1.8 for the wild-type and mutant channels. The $V_{1/2}$ values were −37.9 mV and −49 mV for the gating charge and −24.4 mV and −26.6 mV for the conductance of the normal Shaker and mutant channels, respectively. The limiting slopes of the conductances as a function of voltage gave a limiting effective valence of 4.14 for the normal Shaker channel and 3.62 for the mutant channel.

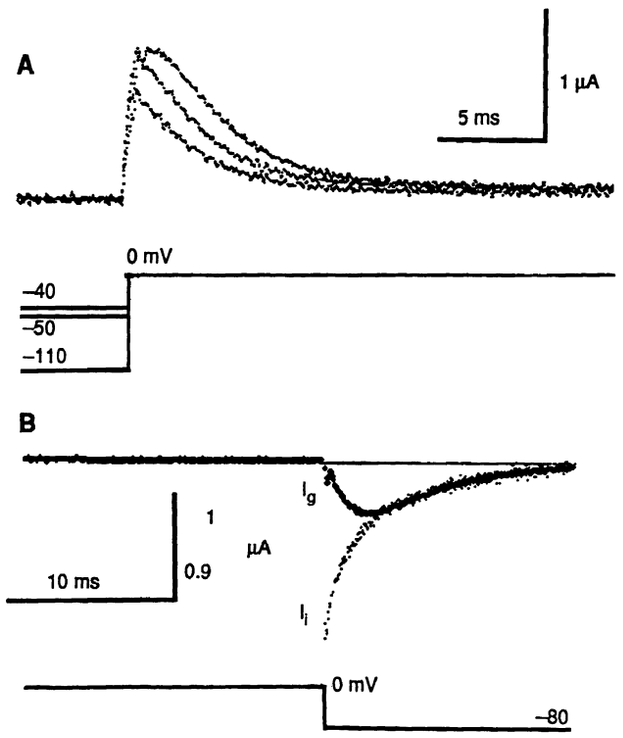

Fig. 2. Time course of the on- and off-gating currents. (**A**) Cole-Moore shift of gating currents. The 20-ms pulses to −110, −50 and −40 mV as indicated were followed by a depolarizing pulse to 0 mV. The signal is an average of four cycles. (**B**) Comparison of the gating current and ionic current at the end of a depolarizing pulse. The gating current (I_g, signal average of 5 cycles, scale bar = 1 μA) and ionic current (I_i, 1 cycle, scale bar = 0.9 μA) records were obtained from different oocytes injected with RNA encoding the non-inactivating mutant channel.

Subsequently, time courses of ionic and gating currents were superimposable, indicating that they have the same kinetics. Therefore, the subunits appear not to respond to voltage independently.

In the mutant, the onset of the off-gating current was slower at greater depolarizations (Fig. 1C). This is more easily seen in the mutant channel than in the normal Shaker channel because of the lack of charge immobilization. These slower kinetics may be explained, as in the case of the Shaker channel, by an initial step that is slower or that carries less charge in the series of events associated with the return of the gating current. The gating currents of the mutant at the beginning of the pulse were similar to those of the normal Shaker channel. However, the initial phases of both the on- and the off-gating currents were slower in the mutant than in the original channels.

If the subunits are indeed identical and each moves the same amount of charge, we can conclude that the gating subunits of the Shaker channel interact during activation. The kinetics of the on-gating currents (Fig. 2A) indicate that during activation, transitions that occur later (closer to the open state) are faster than those that occurred earlier. The kinetics of the off-gating currents (Figs. 1C and 2B) indicate that during deactivation (that is, the return from the open to closed states) the transitions become faster as they progress further away from the open state.

Induction of charge immobilization by the inactivating particle. To determine the time course of charge immobilization, gating currents from normal Shaker (Fig. 3A) and mutant (Fig. 3B) channels were measured at the end of depolarization pulses that were applied for various periods of time. The charge was not immobilized in the mutant even by long depolarizations or very positive potentials (Fig. 1C). The ratio of the off-gating charge (Q_{off}) to the on-gating charge (Q_{on}) was determined as a function of pulse duration (Fig. 3C). To calculate Q_{on}, gating currents obtained with depolarizations of different durations were integrated over the entire pulse. To calculate Q_{off}, the gating current tails were integrated for 20 ms after the end of the pulse (*16*). The ratio Q_{off}/Q_{on} for Shaker channels indicates the time course of charge immobilization, which resembles the time course of inactivation measured in whole cells.

For a short depolarization (about 1 ms), the initial phase of the normal Shaker K channel off-gating current was too fast to record. Subsequently, all the charge returned in one exponential process (Fig. 3A). After longer depolarizations, the off-gating currents decayed more slowly and the decay consisted of at least two components. Long integration periods were required to recover the full charge. After these long depolarizations, the fast component of the off-gating current may correspond to the charge that returns quickly in transitions between the *n* inactivated states of the channel

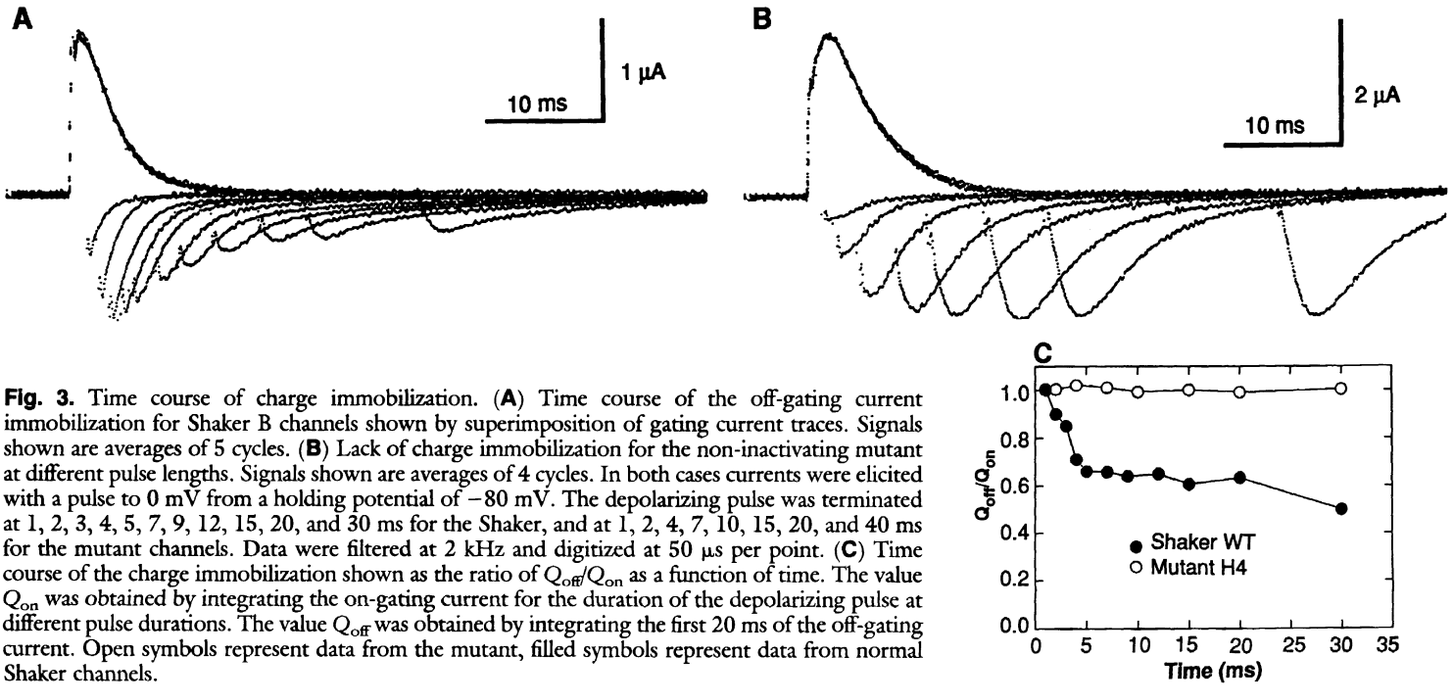

Fig. 3. Time course of charge immobilization. (**A**) Time course of the off-gating current immobilization for Shaker B channels shown by superimposition of gating current traces. Signals shown are averages of 5 cycles. (**B**) Lack of charge immobilization for the non-inactivating mutant at different pulse lengths. Signals shown are averages of 4 cycles. In both cases currents were elicited with a pulse to 0 mV from a holding potential of −80 mV. The depolarizing pulse was terminated at 1, 2, 3, 4, 5, 7, 9, 12, 15, 20, and 30 ms for the Shaker, and at 1, 2, 4, 7, 10, 15, 20, and 40 ms for the mutant channels. Data were filtered at 2 kHz and digitized at 50 μs per point. (**C**) Time course of the charge immobilization shown as the ratio of Q_{off}/Q_{on} as a function of time. The value Q_{on} was obtained by integrating the on-gating current for the duration of the depolarizing pulse at different pulse durations. The value Q_{off} was obtained by integrating the first 20 ms of the off-gating current. Open symbols represent data from the mutant, filled symbols represent data from normal Shaker channels.

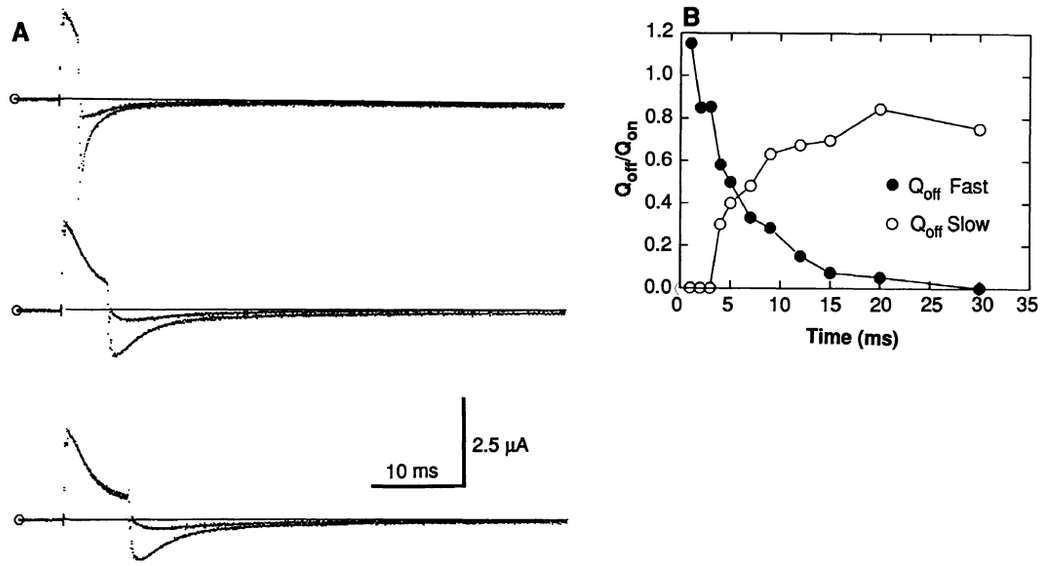

Fig. 4. Kinetics and voltage dependence of the off-charge remobilization. (**A**) Gating current traces on an extended time scale. Traces were obtained with pulses of three different durations to 0 mV from a holding potential of −80 mV followed by a return to either −60 mV or to −110 mV. The subtracting holding potential was −120 mV. Data were filtered at 2 kHz and digitized at 50 μs per point. The signals shown are averages of 5 cycles. (**B**) Time course of the gating charge return to −110 mV was separated into a two-component process. The filled symbols represent the fast-gating remobilization, the open symbols represent the slow-gating remobilization as a function of pulse duration. The fast and slow components were separated by fitting a single exponential to the slow component at long times and extrapolating to the beginning of the off pulse. The slow component was then subtracted from the total off-gating current to obtain the fast component. This fast component or the fitted slow exponential was integrated and divided by the on-gating charge to give the fraction of charge returning via the slow or fast components of the gating current, respectively.

($I_n \leftrightarrow I_{(n+1)}$ transitions), and the slow component may correspond to the charge that leaks back from the inactivated states toward the closed states ($I_n \leftrightarrow C_n$ transitions), that is, charge remobilization.

To obtain information on the voltage dependence of charge remobilization, we measured off-gating currents from Shaker K channels subjected to depolarizing pulses of different durations, that were followed by a return to either −60 or −110 mV at the end of the pulse (Fig. 4A). When the membrane potential was returned to −110 mV, more of the charge was recovered, indicating that the rate of recovery is dependent on membrane voltage. The fast and slow components of the off-gating current were more visible at −110 mV than at −60 mV. To separate the components of the tail, the slow component of the gating tail was fit to a single exponential that was subtracted from the total gating tail to give the fast-gating component. Once separated, each component was individually integrated to obtain the rate of fast and slow charge return after the pulses of various durations (*17*). As the fast component of the charge disappeared, the slow component appeared, so that the total charge remained approximately constant (Fig. 4B). The time constant of the slow component of the tail (approx 15 ms) was not voltage-dependent in the voltage range studied, an indication that recovery from inactivation via $I_n \leftrightarrow C_n$ transitions is probably voltage-independent (*9*). In the case of the non-inactivating mutant, the charge that moved at the end of a depolarizing pulse was recovered in only one fast kinetic component (Fig. 2B). This recovery presumably reflects transitions between closed states because the inactivated states were eliminated by the mutation. In this case, the development of an initial non-instantaneous phase in the off-gating current follows approximately the time course of the gating current decay during depolarization, suggesting that the initial slow phase is correlated with the population of channels in the open state.

A simple interpretation of the above results is that the inactivation particle not only blocks the conduction pathway but also prevents the return of the charged segments to their resting, closed positions. The amount of charge immobilized is about half of the total charge, indicating, for a tetrameric channel, that at least two of the segments can move back (or all four can move half-way back) while the inactivating particle is still in its blocking position; the rest of the charge can only move back after the ball has left its binding site.

Induction of charge immobilization by internal TEA. Other molecules that interact with the internal mouth of the channel may behave like the inactivating particle (*18*). Perfusion of the internal side of the channel with the tetraethylammonium ion (TEA), a blocking agent, not only blocked the ionic current but also induced

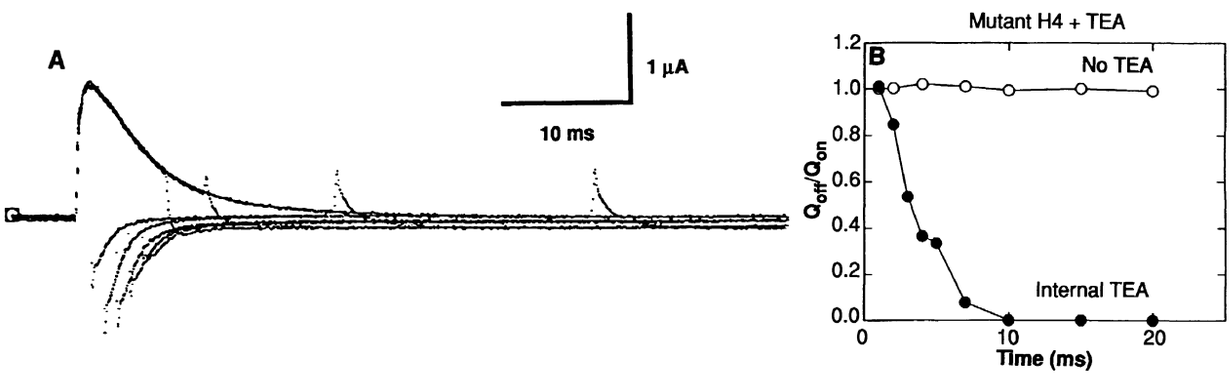

Fig. 5. Effect of internal TEA on the gating currents of the non-inactivating mutant. (**A**) Superimposed records of gating currents for a pulse to 0 mV for different durations. Signals shown are averages of 5 cycles. (**B**) Time course of the charge immobilization shown as the ratio of Q_{off}/Q_{on} as a function of time in the presence of TEA for the mutant channel. The values Q_{on} and Q_{off} were obtained as in Fig. 2. Open symbols represent data in the absence of TEA, filled symbols represent data in the presence of internal TEA. The holding potential was −80 mV and the subtracting holding potential was −120 mV. The internal solution contained 120 mM TEA-glutamate, 1 mM EGTA, and 10 mM Hepes (pH 7.3), and the external solution contained 120 TEA-MES, 1.8 mM $CaCl_2$, and 10 mM Hepes (pH 7.3).

charge immobilization. Gating currents from the non-inactivating mutant channel were recorded in the presence of internal TEA for pulses of different durations (Fig. 5A), and the Q_{off}/Q_{on} ratios were plotted as a function of pulse duration (Fig. 5B). The immobilization in this case was complete, and it occurred faster than immobilization of the normal Shaker K channel, being closer to the time course of channel opening rather than inactivation. This implies that the channel must open to be blocked by TEA and for immobilization of the total charge to occur. In the presence of TEA, the remobilization was slower than the normal recovery from inactivation and induced a new, anomalous charge movement in the negative voltage region seen as an upward deflection in the gating current tail (Fig. 5A). This anomalous deflection could be eliminated if a positive holding potential (40 mV) was used for subtraction (*19*). The immobilization produced by internal TEA in the non-inactivating mutant channel was also observed in the normal Shaker channel as a complete charge immobilization. The interaction of TEA with the activating segments of the channel is such that none of them can move back until the TEA exits the channel. This difference in action with the inactivating particle may help in understanding the interaction that produces inactivation in the Shaker K channel. The polypeptide comprising the ball and chain may not be able to interact simultaneously with all four putative activating segments to immobilize them. In contrast, TEA may be able to do so because of its four ethyl groups.

Note added in proof: Gating currents have been measured from mutant chimeric potassium channel (*27*). Those authors used patch-clamp techniques and TEA in the internal solution. They found that the charge at the end of the pulse was immobilized regardless of the ability of the mutant to inactivate, and the immobilization occurred more quickly than the inactivation of the ionic currents. Their results can be explained on the basis of our observation that TEA induced charge immobilization and their finding of slow deactivation in the chimeras.

REFERENCES AND NOTES

1. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **117**, 500 (1952).
2. C. M. Armstrong and F. Bezanilla, *J. Gen. Physiol.* **70**, 567 (1977).
3. D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *Science* **237**, 749 (1987); A. Baumann *et al.*, *EMBO J.* **6**, 3419 (1987); B. L. Tempel, D. M. Papazian, T. L. Schwarz, Y. N. Jan, L. Y. Jan, *Science* **237**, 770 (1987); A. Kamb, L. E. Iversen, M. A. Tanouye, *Cell* **50**, 405 (1987).
4. R. MacKinnon, *Nature* **350**, 232 (1991).
5. T. Hoshi, W. N. Zagotta, R. W. Aldrich, *Science* **250**, 533 (1990).
6. W. N. Zagotta, T. Hoshi, R. W. Aldrich, *ibid.*, p. 568.
7. R. MacKinnon, W. M. Zagotta, R. W. Aldrich, *Biophys. J.* **59**, 404a (1991).
8. The wild-type Shaker construct consisted of the Sac II–Eco RI fragment of the ShB cDNA clone (*23*) subcloned into Bluescript (Stratagene). The mutant construct ShH4-IR, obtained from R. MacKinnon (*24*) was similar, but contained a deletion of amino acids 6 through 46 to remove fast inactivation (*5*). A portion of the mutant construct was derived from ShH4 (*25*), a clone with two amino acid differences with ShB (ShH4 has a Val inserted after Leu^{512} and contains one less Gln than ShB between positions 558 to 564). Although the initially published sequences also differed at amino acids 584 and 585, ShB was found to be identical to ShH4 at these positions on resequencing. For RNA transcription, the Bluescript plasmids were linearized with Eco RI and transcribed with T7 RNA polymerase (Promega) as described (*26*). RNA was injected into Xenopus oocytes after minimal dilution to give high levels of expression. Injected oocytes were bathed in modified Barth's saline at 18°C and were defolliculated in collagenase (4 mg/ml) (Sigma) for 2 hours at room temperature.
9. W. N. Zagotta and R. W. Aldrich, *J. Gen. Physiol.* **95**, 29 (1990).
10. M. Noda *et al.*, *Nature* **312**, 121 (1984); W. Stuhmer *et al.*, *ibid.* **339**, 597 (1989); D. M. Papazian, L. C. Timpe, Y. N. Jan, L. Y. Jan, *ibid.* **349**, 305 (1991).
11. The open oocyte vaseline gap technique was used as described [M. Taglialatela, L. Toro, E. Stefani, *Biophys. J.*, in press]. In brief, defolliculated oocytes were placed in a perspex chamber that established three compartments: top pool P, for recording; middle pool, GS, for guard; and bottom pool, I, for current injection. Seal resistances between compartments were one to five Mohms. There were three voltage clamps: the first clamp controlled the P pool to the command potential; the second clamp maintained the potential (monitored with a microelectrode) under the membrane in the P pool to ground by injecting current into the I pool; the third clamp maintained the potential of the GS pool to the same potential as the P pool to isolate electrically the recording of the current from the current injection pool. Membrane currents were recorded from the P pool, which had a diameter of 750 μm, by means of a current-to-voltage converter with feedback resistors ranging from 100 kohms to 1 Mohm. The capacity transient time constant of decay was 20 to 50 μs. The bottom of the oocyte was cut to allow injection of current intracellularly and was cannulated with a plastic tube connected to a syringe pump to exchange the intracellular milieu. Oocytes mounted in this chamber could typically be used to record currents for about two hours.
12. W. Almers, *Rev. Physiol. Biochem. Pharmacol.* **82**, 96 (1978).
13. J. Stimers, F. Bezanilla, R. E. Taylor, *J. Gen. Physiol.* **89**, 521 (1987).
14. K. S. Cole and J. W. Moore, *Biophys. J.* **1**, 161 (1960); R. E. Taylor and F. Bezanilla, *J. Gen. Physiol.* **81**, 773 (1983).
15. M. M. White and F. Bezanilla, *J. Gen. Physiol.* **85**, 539 (1985); M. Taglialatela and E. Stefani, in preparation.
16. In principle, all charge moved after the onset of the pulse should be recovered after the pulse ends. However, if some charge returns very slowly, the measured value, which must be obtained by a finite integration period, could be an underestimate because of baseline noise and offset. Therefore, we defined an operational integration period of 20 ms after the pulse ended. This was long enough to recover all the off-charge in the non-inactivating mutant but it did not include much of the slow component of the charge return of the normal Shaker channel.
17. The slow component of the charge return was obtained as the analytical integral of the fitted exponential (when $t \rightarrow \infty$) and the fast charge return was estimated as the area of the remaining gating current tail after subtraction.
18. K. L. Choi, R. W. Aldrich, G. Yellen, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 5092 (1991). L. Toro, R. Latorre, E. Stefani, *Neurosci. Soc. Abs.*, in press.
19. F. Bezanilla, R. E. Taylor, J. M. Fernandez, *J. Gen. Physiol.* **79**, 21 (1982).
20. F. Bezanilla and C. M. Armstrong, *ibid.* **70**, 549 (1977).
21. J. Stimers, F. Bezanilla, R. E. Taylor, *ibid.* **85**, 65 (1985).
22. W. F. Gilly and C. M. Armstrong, *ibid.* **79**, 935 (1982).
23. T. L. Schwarz, B. L. Tempel, D. M. Papazian, Y. N. Jan, L. Y. Jan, *Nature* **331**, 137 (1988).
24. G. Yellen, M. E. Jurman, T. Abramson, R. MacKinnon, *Science* **251**, 939 (1991).
25. A. Kamb, J. Tseng-Crank, M. A. Tanouye, *Neuron* **1**, 421 (1988).
26. L. C. Timpe *et al.*, *Nature* **331**, 143 (1988); L. C. Timpe, Y. N. Jan, L. Y. Jan, *Neuron* **1**, 659 (1988).
27. W. Stuhmer, F. Conti, M. Stocker, O. Pongs, S. H. Heinemann, *Pluegers Arch.* **418**, 423 (1991).

28. We thank R. MacKinnon for ShH4IR clone, M. Pillos for DNA sequencing and RNA production, E. de Robertis for the *Xenopus* oocytes, A. M. Correa for participation in some of the experiments, and G. Cantrell for building the voltage clamp apparatus. Supported by USPHS grants GM30376, GM43459, HL37044, and HD25616.

14 June 1991; accepted 11 September 1991