Fast and Slow Steps in the
Activation of Sodium Channels

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ABSTRACT Kinetic features of sodium conductance (gNa) and associated gating current (Ig) were studied in voltage-clamped, internally perfused squid axons. Following a step depolarization Ig ON has several kinetic components: (a) a rapid, early phase largely preceding gNa turn-on; (b) a delayed intermediate component developing as gNa increases; and (c) a slow component continuing after gNa is fully activated. With small depolarizations the early phase shows a quick rise (<40 μs) and smooth decay; the slow component is not detectable. During large pulses all three components are present, and the earliest shows a rising phase or initial plateau lasting ~80 μs. Steady-state and kinetic features of Ig are minimally influenced by control pulse currents, provided controls are restricted to a sufficiently negative voltage range. Ig OFF following a strong brief pulse also shows a rising phase. A depolarizing prepulse producing gNa inactivation and Ig immobilization eliminates the rising phase of Ig OFF. gNa, the immobilized portion of Ig ON, and the rising phase reappear with similar time-courses when tested with a second depolarizing pulse after varying periods of repolarization. 30 mM external ZnCl2 delays and slows gNa activation, prolongs the rising phase, and slows the subsequent decay of Ig ON. Zn does not affect the kinetics of gNa tails or Ig OFF as channels close, however. We present a sequential kinetic model of Na channel activation, which adequately describes the observations. The rapid early phase of Ig ON is generated by a series of several fast steps, while the intermediate component reflects a subsequent step. The slow component is too slow to be clearly associated with gNa activation.

INTRODUCTION
The molecular events that regulate opening and closing of Na channels in axon membrane are driven by the rearrangement of charged gating structures in response to a change of membrane potential. Movement of these charged structures generates a detectable “gating current” (Armstrong and Bezanilla, 1973, 1974; Keynes and Rojas, 1973, 1974; Meves, 1974). Thus far, gating current associated with Na activation has been observed, but nothing unequivocally related to the other major gating processes in axons, Na inactivation or K activation, has been found.

Previous studies concentrating on the relation between activation and...
inactivation have led to the conclusion that the two processes are not independent. Inactivation is not associated with detectable gating current and is thought to derive its voltage sensitivity from coupling to activation; i.e., inactivation can occur only after activation of a channel is nearly complete (Armstrong and Bezanilla, 1977; Bezanilla and Armstrong, 1977; Meves and Vogel, 1977a,b). A similar conclusion had been reached from studies of the kinetics of the g_{Na} (Hoyt, 1968; Goldman and Schauf, 1972).

We turn here to a study of the kinetic features of gating current with the aim of learning more about the activation process. Activation involves a number of steps and gating current reflects all of them, giving information that cannot be obtained from studying conductance changes alone. Important information about the steps that lead to opening of the channel is contained in the first 100 μs of the gating current transient. This interval is close to the limits of time resolution and has proved difficult to study. The Hodgkin and Huxley equations (1952) predict an instantaneous rise and exponential decay for gating currents. Most published records, on the other hand, show an instantaneous jump in the current, followed by either a plateau or a rise to a peak.

Is this “rising phase” a machine artifact or the result of nonlinear charge movement during the control pulses? We have examined these questions experimentally and conclude that the rising phase probably originates from neither of these sources but is instead a genuine feature of gating current and gives information about the relative transition rates among closed states of the channel. Perhaps, more importantly, we also demonstrate a relatively slow component of gating current that had previously escaped detection. This component can be seen clearly for steps positive to about -20 mV, and probably arises from the last and slowest step in activation, the actual opening of the channel.

**METHODS**

Experiments were performed on isolated giant axons of *Loligo pealii*, obtained at the Marine Biological Laboratory, Woods Hole, Mass. Cleaned axons were internally perfused and voltage clamped. Except for minor changes, the techniques were the same as those described in Bezanilla and Armstrong (1977). The changes were as follows: (a) The internal electrode was not a “piggy-back.” Instead, an axial wire was inserted by one manipulator, and a voltage pipette (containing an electrically floating Pt wire) was inserted from the same cut end of the axon by another manipulator. This had the minor advantage that none of the surface of the axial wire was covered with glue. (b) Pulses were generated by a 12-bit D/A converter interfaced to the computer. (c) The current measuring region of the chamber in most experiments was 6.2 mm rather than 3.1 mm. This seemed to improve the signal-to-noise ratio appreciably.

In order to prevent saturation of the amplifiers and A/D converter by the very large surge of capacity current following a voltage step, a linear transient proportional in amplitude to the voltage step applied was subtracted from the membrane capacity current transient. The combined signal was then fed to an integrating input stage (Bezanilla and Armstrong, 1977), digitized by a 12-bit A/D converter with a sample interval of 10 μs, and then fed to a sample and hold amplifier. 720 samples were taken
per sweep; the last 600 points were compressed 5 to 1, giving one point per 50 μs.
Most of the records shown were obtained with the P/4 procedure (Armstrong and Bezanilla, 1974). Some traces were digitally filtered by replacing the $n^{th}$ sample point, $I_n$, by the sum $(I_n/2) + (I_{n-1} + I_{n+1})/4$.

Performance of the clamp and the recording apparatus for a step input of current is shown in Fig. 1a. The step shown was generated by a voltage clamp pulse applied to a resistor. The rise time of the recorded signal was ~20 μs.

Tests were performed on model membranes of two types in checking the linearity of the system: (a) a purely resistive “membrane” made simply from a resistor or (b) a capacitor. Cancellation of the signal from either resistor or capacitor (assuming ideality) should have been perfect using the P/4 procedure. In fact, cancellation with the capacitor was usually not perfect, and a small signal lasting about 30 μs usually remained. This is illustrated in Fig. 1b, which is the result from 10 cycles of the P/4 procedure for a 150-mV pulse. The residual signal is small in comparison to gating current, but it is large enough to cast doubt on the first 30 μs of the recordings.

Names and compositions of all solutions are given in Table I. In most experiments $I_{Na} + I_g$ was recorded in 1/4 Na seawater, and $I_g$ was subsequently determined after total removal of Na, addition of TTX, or both. $I_g$ was then subtracted from $I_{Na} + I_g$ to yield the $I_{Na}$ traces illustrated.

**RESULTS**

**Temporal Relation between $I_g$ and $I_{Na}$**

Fig. 2 shows $I_{Na}$ and $I_g$ (after Na removal and TTX addition) recorded at several voltages from a fiber perfused with 200 TMA to eliminate potassium current. At ~20 mV or ~10 mV, $I_g$ has a quick rise, complete in 40 μs, and then decays in two distinct phases. The faster phase is almost finished before $I_{Na}$ becomes detectable. The second, slower phase continues until shortly after $I_{Na}$ reaches its peak. This component of current, which was not well resolved in earlier records, is most clearly visible in the range between ~20 mV and 0 mV and will be called the intermediate component. At more negative voltages, e.g., ~40 mV, the intermediate component is too small to resolve. For large
depolarizations, positive to +20 mV, it is obscured by additional charge movement that is too slow to be associated with channel opening.

The trace at -20 mV suggests that activation is the result of one or more fast steps, giving rise to the fast component of \( I_p \), followed by a slower one that generates the intermediate component. The additional slow charge movement seen at very positive voltages may arise from a transition between two open states (Armstrong and Bezanilla, 1977), but there is no firm evidence.

**Isovecolarity along the Axon**

Much of this paper is concerned with the rapid kinetics of gating current, and a first question is whether the voltage is sufficiently uniform along the axon to allow confidence in the current measurements. The amount of longitudinal nonuniformity is strongly dependent on the membrane conductance, and a large inward \( \text{Na}^+ \) current when conductance is high should present the most severe test of uniformity. Fig. 3 shows an experiment testing for voltage nonuniformities under conditions of both high and low membrane conductance.

Internal voltage was measured simultaneously at two points separated by 5.6 mm, using the combination electrode shown in Fig. 3 a (see legend for details). The external voltage \( (V_o) \) was measured at only one point, in the center of the current measuring region (Fig. 3 b), and was subtracted from each of the two internal voltage signals to yield \( V_1 \) and \( V_2 \). The controlled voltage was \( V_1 \).

In Fig. 3 c and d the electrode was positioned with the voltage control point 5.6 mm to the right of the chamber's center. When \( V_1 \) (not illustrated) was

| TABLE I |
| SOLUTIONS* |
| Internal solution | TMA-glutamate | TMA-fluoride | Trizma§ 7.0 | Sucrose |
|-------------------|----------------|--------------|-------------|---------|
| mM                | mM             | mM           | mM          | mM      |
| 200 TMA           | 150            | 50           | 10          | 560     |

| External solutions | Trizma§ 7.0 | Tris base | NaCl | CaCl₂ | ZnCl₂ |
|--------------------|-------------|-----------|------|-------|-------|
| mM                 | mM          | mM        | mM   | mM    | mM    |
| Tris TTX†          | 480         | —         | —    | 50    | —     |
| ASW                | —           | —         | 450  | 50    | —     |
| ½ Na               | 360         | —         | 113  | 50    | —     |
| Tris 30 Zn         | 471         | 24        | —    | 10    | 30    |
| Tris 10 Ca         | 540         | —         | 113  | 10    | 30    |
| 1:4 Na 30 Zn       | 353         | 18        | 116  | 10    | 30    |
| 1:4 Na 10 Ca       | 424         | —         | 116  | 10    | 30    |

* Osmolarity of all solutions was between 950-1,000 mosM; pH was between 6.9 and 7.1.
† Tetramethylammonium ion.
§ Tris (hydroxymethyl) aminomethane, Sigma Chemical Co., St. Louis, Mo.
† Tetradotoxin, 200 nM.
stepped from −70 to −20 mV, \( V_2 \) (at the center of the chamber) did not follow exactly but was always within a few millivolts of the command value. \( V_2 \) and the current generated in the central region are shown in Fig. 3 c.

Fig. 3 e shows the same procedure, after withdrawing the electrode so that the control point was at the center of the current measuring region, and \( V_2 \) was measured 5.6 mm to the left. In this case \( V_2 \) followed the command voltage rather accurately.

In Fig. 3 c and e there is a strong inward current and a high expectation of nonuniformity in voltage. Fig. 3 d and f, on the other hand, show \( V_1 \) and \( V_2 \) during a pulse when membrane resistance is high. \( V_1 \) was stepped from −70 to −120 mV, with the control pipette at either +5.6 mm (d) or 0 mm (f). In both cases, membrane current is very small (not illustrated). The voltage at both the control point and 5.6 mm away is a rapid step (rise time about 2 μs), regardless of the location of the control point. The conclusion is that the
membrane is isopotential during voltage steps when membrane resistance is high, as it is during gating current measurements.

**I₉ Has a Rising Phase for Large Depolarizations**

Gating currents from two axons depolarized to 0 mV and to +50 or +60 mV are shown in Fig. 4 on an expanded time scale. In both cases the current transient for the smaller pulse shows a practically instantaneous rise and smooth decay. At +50 or +60 mV, however, there is a distinct rising phase lasting about 80 μs.
Voltage traces are also shown, for both steps in Fig. 4 a, and for the larger one in Fig. 4 b. It is clear that the membrane potential has settled to its final level before the rising phase is completed. Moreover, the shapes of the rise in gating current at these two voltages are qualitatively different, even though the voltage steps at 0 and +50 mV (Fig. 4 a) are very similar in shape. The lowest trace ($V_m$, P/4) in Fig. 4 b is the difference between one test (to +60 mV) and four control voltage steps for 10 cycles with the P/4 procedure.

**Figure 4.** ON gating current for small and large steps of membrane potential. (a) Upper traces: $I_g$ ON at 0 mV and +50 mV. Lower traces: corresponding voltage steps. Note the sharp rise in $I_g$ ON at 0 mV and the rising phase at +50 mV that continues after the membrane potential has stabilized. Same axon as Fig. 2. (b) Similar records of $I_g$ ON at 0 mV and +60 mV in another axon. Voltage trace is shown only for +60 mV. The lowest trace ($V_m$, P/4) shows $V_m$ subjected to 10 cycles of the P/4 procedure (see text). Matching of test and control pulses is essentially perfect. Axon SE018G. Tris TTX/200 TMA. Holding potential -70 mV. Control pulses: P/4 from -180 mV. 8°C.

**Figure 3 (Opposite).** Isopotentiality along the axon under conditions of high and low membrane conductance. (a) Combination electrode for measuring intracellular voltages at two points spaced 5.6 mm apart. Two enameled Pt wires, bare only at the tips, were attached to the outside of a pipette, which in turn was attached to a platinized axial wire. A Ag-AgCl junction in the pipette was connected to the input of a voltage follower. The Pt wire ending at the pipette mouth was connected to the same input through 0.01 μF. The output of this follower less the external voltage was the feedback signal to the clamp regardless of electrode position (see below). The other Pt wire, ending 5.6 mm away, was capacitively coupled to another voltage follower. The external voltage at the center of the chamber was subtracted from both follower outputs to yield $V_1$ (pipette tip) and $V_2$ (wire). (b) Scale diagram of the chamber showing relative spacings of the center of the current measuring region (0 mm) and the points ±5.6 mm away in the guard regions. The electrode assembly in a was advanced or withdrawn so that voltage at either 0 or +5.6 mm could be controlled. (c) Voltage ($V_1$) was controlled at +5.6 mm and stepped from −70 to −20 mV with a rise time of −2 μs (not illustrated). Top trace shows $V_2$ at 0 mm. Bottom trace shows $I_m$ in artificial seawater (ASW). $V_2$ is not perfectly controlled when $g_m$ is large. (d) $V_1$ was controlled at +5.6 mm and stepped from −70 to −120 mV. $V_2$ (at 0 mm) is satisfactorily controlled when $g_m$ is low. (e) As in c, but with voltage ($V_1$) controlled at 0 mm after moving the electrode 5.6 mm to the left. (f) As in d with $V_1$ controlled at 0 mm.
sampled, subtracted, and signal-averaged in exactly the same manner as were the corresponding currents. The subtraction is good, and the appearance of a rising phase with large depolarizations is therefore not due to poor matching between test and control pulses.

Tests for Distortion of \( I_g \) by Control Pulse Current

In an earlier study Armstrong and Bezanilla (1974) pointed out that with the \( \pm P \) method a prominent rising phase (lasting up to 250 \( \mu s \)) resulted from nonlinear charge movement during the control pulse. We used several tests to assess whether control pulse current is contributing to the rising phase seen, e.g., in Fig. 4. The first was a measure of the total nonlinear charge movement in the control pulse range.

The \( Q-V \) curve for a typical fiber (Fig. 5 \( \text{top} \)) saturates at both ends, near \(-140\) and \(+40\) mV. (For convenience the zero current line is taken as the asymptote near \(-140\) or \(-150\) mV.) Some of the currents from which this curve was obtained are shown in Fig. 5 \( \text{bottom} \). The fiber was held at \(-70\) mV, and the inward currents are for negative steps from this voltage (see diagrams). The curve shows that capacitance behaves linearly from \(-140\) to \(-170\) mV, but is nonlinear between \(-140\) and \(-70\) mV (c.f. Bezanilla and Armstrong, 1975). The control pulses for large depolarizations overlap part of this range, and the resulting nonlinear charge movement subtracts from that during the test pulse, causing an underestimate of the latter. With the \( P/4 \) procedure, the underestimate is four times the charge moved during a single control pulse.

For example, the point at \(+90\) mV in Fig. 5 \( \text{top} \) should have added to it four times the charge at \(-130\) mV, which was the voltage during the control pulse. The correction at several voltages (estimated from the smooth curve) is shown by the open circles in the figure. It slightly changes the shape of the \( Q-V \) curve, but has a negligible effect below \(+30\) mV.

In terms of total charge movement, the contamination by control pulse current is small using the \( P/4 \) procedure. Distortion of the early time-course of \( I_g \) could nonetheless be appreciable. We used three further tests to assure ourselves that the distortion is probably not serious.

(a) The top trace in Fig. 6 \( a \) shows gating current recorded at \(+50\) mV with control pulses from \(-170\) to \(-140\) mV (same axon as Fig. 5). The middle trace gives the estimated contaminating current during the control pulses obtained by taking the difference between records at \(-170\) mV and \(-140\) mV (from the experiment in Fig. 5 \( \text{top} \)) and multiplying by \(-4\). Adding the resultant back to \( I_g \), and thus removing the contamination, gives the noisy trace on the bottom of Fig. 6 \( a \). For comparison, the uncorrected \( I_g \) trace has been superimposed. Although the rising phase is somewhat faster after correction, there is still a plateau-like initial phase. The same result was obtained in four other fibers.

(b) Similar information was derived in another axon by a different procedure. Fig. 6 \( b \) shows the effect of varying the voltage from which the control pulses originate (as indicated in the figure), again using the \( P/4 \) procedure. Gating
Figure 5. Nonlinear charge movement over the voltage range $-170 \text{ mV}$ to $+90 \text{ mV}$. (Top) Steady-state voltage distribution of nonlinear charge movement. Solid points were obtained by integrating records below for 7 ms following the step. Smooth curve has been drawn by eye. Very little nonlinear charge movement can be detected negative to $-130 \text{ mV}$. Open circles are data points corrected for the estimated nonlinear charge movement in the control pulses (see text for details). (Bottom) Nonlinear capacity currents (P/4 procedure) with control pulses originating from $-170 \text{ mV}$. Membrane potential during the pulse is indicated for each trace. Axon SE058D. Tris TTX//200 TMA. Holding potential $-70 \text{ mV}$. $8^\circ \text{C}$.
currents for all three cases are similar, but there is a tendency for the rising phase to become slightly faster and more plateau-like as the control pulse level is made more negative.

(c) Fig. 6c shows gating currents following a depolarization to +50 mV with control pulses of either 30 or 7.5 mV amplitude, starting from −170 mV. The upper trace, with 7.5 mV control pulse, is noisy, but the rising phase is prominent as in the lower trace.

![Figure 6](image)

**Figure 6.** Tests for distortion by control pulse current. (a) Ig ON at +50 mV before correction. Control pulses were +30 mV starting from −170 mV. II: Contaminating current during the control pulses (see text). I and II were added together to give III, the corrected trace. The rising phase is not drastically altered by the correction. Same axon as Fig. 5. (b) Ig ON at +50 mV with +30 mV control pulses originating from −150, −170, or −190 mV as indicated. The rising phase is not much affected. Same axon as Fig. 4 b. (c) The effect of varying control pulse amplitude. Upper trace: Ig ON at +50 mV using +7.5 mV control pulses starting from −170 mV. The record is noisy because of the low signal-to-noise ratio with this small control pulse. Lower trace: Ig ON for same amplitude test pulse but with control pulses of +30 mV from −170 mV (P/4). There is no detectable change in the shape of Ig ON with this reduction in control pulse amplitude. Axon SE088A. Tris TTX//200 TMA. Holding potential −70 mV. 3.5°C.

**Ig OFF Has a Rising Phase after a Short Pulse**

Fig. 7a shows INa and Ig OFF as the Na channels close at −70 mV following a 0.5-ms pulse to +50 mV. INa reaches its maximum amplitude by 40 μs and then decays abruptly. Ig, in contrast, increases in amplitude for about 80 μs before beginning to decay with a rounded time-course. As has been reported (Armstrong and Bezanilla, 1974; Meves, 1974; Neumcke et al., 1976), the decay of Ig is only a bit slower than that of INa, the respective time constants here being 321 and 239 μs.

Ig OFF traces from another experiment are shown in Fig. 7b after a short pulse and after one long enough to cause Na inactivation and charge immo-
bilization. After the long pulse \( I_g \) OFF has a much sharper early time-course, and the rising phase is less pronounced. In addition there is a prominent slow phase after the long pulse, which represents the delayed return of gating charge that was immobilized by inactivation (c.f. Armstrong and Bezanilla, 1977).

\( I_g \) ON is also affected in amplitude and time-course by inactivation, as described in a subsequent section.

**Zn Slows Na Activation and the Rising Phase of \( I_g \) ON, but Closing of the Channels Is Unaffected**

External Zn is one of the few agents that slows Na activation kinetics, as shown by Hille et al. (1975). It also slows gating current (Armstrong and Bezanilla, 1975). Fig. 8 a shows that 30 mM external \( \text{ZnCl}_2 \) significantly slows the rising phase of \( I_g \), extending its duration even further beyond the settling time of the clamp (c.f. Fig. 4). Zn also depresses the peak amplitude of \( I_g \), slows its decay (Fig. 8 a), and slows the activation of \( I_{Na} \) (Fig. 8 b). For large depolarizations, such as that shown in Fig. 8, Zn produces no change in total gating charge moved, but does cause a steady-state \( g_{Na} \) block of 20–35%. To facilitate comparison, the Zn trace was scaled up by a factor of 1.25 in Fig. 8 b.

Unexpectedly we found that Zn has little or no effect on channel closing. Fig. 8 c and d show \( I_g \) and \( I_{Na} \), respectively, at -100 mV following a strong, brief depolarization. Traces in Zn have been superimposed on the zincless traces. To facilitate comparison, \( I_{Na} \) in Zn (Fig. 8 d) was scaled by a factor of 1.5 to account for the \( g_{Na} \) blocking action of Zn during the pulse.

The definite slowing of activation by Zn and its negligible effect on channel closing seems incompatible with the hypothesis that Zn simply shifts the \( g_{Na} \)
activation curve along the voltage axis by altering surface charge density. This was examined further by attempting to superimpose $I_g$ ON records with and without Zn at different voltages. For example, in Fig. 8 $I_g$ ON at +60 mV in Zn (same trace as in Fig. 8 a) superimposes fairly well on that at +40 mV in the absence of Zn. Thus, addition of 30 mM ZnCl$_2$ is roughly equivalent to making the voltage during this step more negative by about 20 mV.

A similar procedure applied to $I_g$ OFF in a number of axons showed that Zn has no consistent effect on either $I_{Na}$ or $I_g$ as channels are closing. The action of Zn is therefore more complex than simply a shift in the steady-state voltage dependence of activation by alteration of surface charge. An alternate explanation is suggested in the discussion, and a detailed analysis of zinc's effects on activation is in preparation.
Prepulses That Produce Na Inactivation Abolish the Rising Phase of \( I_g \)

Armstrong and Bezanilla (1974, 1975, 1977) and Meves and Vogel (1977 a, b) have reported that inactivation of sodium conductance during a long depolarization "immobilizes" a large part of gating charge. Another effect of depolarizing prepulses not specifically discussed by these authors is exerted on the shape of the rising phase. The effect, illustrated in Fig. 9, is like that on \( I_g \) OFF after a long pulse described above, but is rather more pronounced.

Trace \( a \) in Fig. 9 shows \( I_g \) ON recorded with the usual procedure (pulse sequence \( a \)), whereas trace \( b \) is the inactivation or immobilization resistant charge after a prepulse (sequence \( b \); see legend for details). The prepulse reduces total charge movement, abolishes the rising phase, and selectively reduces the slow component. Kinetics of the inactivation resistant charge are thus greatly simplified. For convenience this experiment was done in the presence of 30 mM external Zn to make the rising phase more prominent. Similar results have been obtained without Zn.

For trace \( c \) in Fig. 9 the prepulse was omitted before the control pulses (sequence \( c \)). Records \( b \) and \( c \) are indistinguishable, showing that the prepulse effect on the rising phase is not due to an effect on control pulse currents.

As noted in a previous section, \( I_g \) OFF has a rising phase similar to that of \( I_g \) ON after a short pulse, but not after a long one. This effect occurs with a time-course very similar to the development of charge immobilization and Na inactivation (Armstrong and Bezanilla, 1977; see their Fig. 2).

Reappearance of the rising phase of \( I_g \) ON following a depolarizing prepulse occurs roughly in parallel with the removal of \( I_g \) immobilization and Na
inactivation. Fig. 10a shows gating current (lefthand trace) and \( I_{Na} \) at +60 mV with no prepulse, and the initial plateau phase of \( I_g \) ON is apparent. \( I_{Na} \) was obtained with the same pulse protocol before adding TTX. \( I_g \) and \( I_{Na} \) records following a 15-ms prepulse to +60 mV and recovery at -70 mV for the durations indicated are shown in Fig. 10b-e. As the recovery period lengthens, (a) the initial phase of \( I_g \) ON becomes more plateau-like (i.e., the rising phase develops); (b) the intermediate component of \( I_g \) (see above) reappears; and (c) \( I_{Na} \) reappears.

The rising phase thus disappears and reappears with about the same time-course as development and removal of both \( I_g \) immobilization and Na inactivation, and the effect is not due to changes in nonlinear currents during the control pulses. These observations are explored more fully in the next section.

**DISCUSSION**

Recorded gating current is the difference of currents during test and control pulses, and it is logically impossible to resolve this combination into its...
components with absolute certainty (c.f. Almers, 1978). With this preface in mind, we feel the following points about the time-course of \( I_\text{g} \) are reasonably well established by this paper.

1. \( I_\text{g} \) has an initial plateau or rising phase that is not a machine artifact and is probably not due to nonlinear charge movement during the control pulses. Briefly, the evidence is: (a) A rising phase is seen only with large voltage steps, even though the voltage settles equally rapidly for small and large steps. (b) \( I_{\text{Na}} \) tails have a sharper rise than does \( I_\text{g} \) OFF. (c) External Zn ions prolong the rising phase, and (d) an inactivating prepulse eliminates it. (e) The rising phase is not much altered (if at all) by using control steps of different amplitude, or by varying the voltage from which the control pulses originate.

Nonner et al. (1978) observed a fast rising phase (<30 μs) for \( I_\text{g} \) ON (but not for \( I_\text{g} \) OFF) in frog node of Ranvier and concluded that it probably resulted from contamination by inward current during the control pulses. Their records were obtained with symmetrical pulses that originated from -97 mV. In other experiments, they detected nonlinear current negative to -97 mV and concluded this current was responsible for the rising phase. We agree fully that there is nonlinear capacity current between -97 and roughly -120 mV, but our method avoids this region, and our records nonetheless have a rising phase. Further, their analysis does not provide an explanation for the fact that they (and we) saw a rising phase only for large pulses.

2. The second major point is that over the voltage range from about -30 to 0 mV, \( I_\text{g} \) ON has a fast phase that precedes the conductance change and a less rapid one (the intermediate component) that is roughly proportional to the time derivative of \( g_{\text{Na}} \). It seems inescapable that activation involves one or, more likely, several fast steps, which generate the fast phase of \( I_\text{g} \), followed by a slower step which generates the intermediate component.

3. For steps to +10 mV or more positive, there is additional charge movement (the slow component) that seems too slow to be associated with activation of Na channels.

4. \( I_\text{g} \) OFF has a rounded time-course and a decay phase at -70 mV that is almost as rapid as the fall of \( g_{\text{Na}} \).

5. Finally, when the channels are inactivated, \( I_\text{g} \) ON approximates a single exponential with an almost instantaneous rise (i.e., faster than 30–40 μs).

These results can be thought of in terms of the kinetic diagram in Fig. 11, which is a simplification of one given earlier by Armstrong and Bezanilla (1977). The steps along the horizontal axis represent changes in the activation gate from "fully closed" at the left (\( x_0 \)) to the conducting state (\( x^+ \)) at the extreme right. The horizontal steps are voltage dependent and are associated with gating charge movement, as indicated by the symbols \( Q_1 \) and \( Q_2 \). The vertical steps represent movement of an inactivating particle into (up) or out of (down) its receptor. The inactivation steps are not significantly voltage dependent and generate no detectable gating current. The activation gate is open to the right of the vertical dashed line; the inactivation gate is open below the horizontal dashed line. Both gates must be open for the channel to
conduct, and this is the case only for \( x_f \), the single conducting state. This scheme is adequate for present purposes, but fails to reproduce the voltage dependence of recovery from inactivation (c.f. Armstrong and Bezanilla, 1977).

As noted, all steps along the horizontal axis generate gating current. Necessary conditions for a rising phase are that some of the steps to the right have faster rate constants than do the ones at the left, and/or they have more gating charge movement per step. The intermediate component of \( I_g \) seen near \(-20\) mV suggests that one of the later steps must be relatively slow. Our results can be explained by postulating that the last step in opening a channel, \( x_2 \rightarrow x_f \), is substantially slower than the preceding steps, has more charge movement associated with it, and is therefore more voltage dependent with regard to kinetics and equilibrium constant. This step generates the intermediate component of \( I_g \), which would have the time-course of \( dg_{Na}/dt \) if there were no inactivation.

**Figure 11.** State diagram of a sodium channel. See text for details.

Calculations of \( g_{Na} \) and \( I_g \) were performed using this model, with the initial condition that all of the channels are in state \( x_6 \) at rest (\(-70\) mV). (This is an approximation, for a few of the channels would be in the other states at \(-70\) mV.) \( x_f(t) \), which is directly proportional to conductance, was calculated by numerically integrating the eight first-order differential equations that describe the scheme drawn above. Gating current for each step was calculated from an equation of the type:

\[
I_{g_{x_{6}}} = Q_1 \left( \frac{dx_1^*}{dt} \right) = Q_1(\alpha_{1}x_2 - \beta_{1}x_1^*),
\]

and total \( I_g \) was the sum of that for all steps. For simplicity in the initial stages of fitting, it was assumed that charge movement and rate constants for all of the fast steps (all those to the left of \( x_2 \)) were the same \((Q_s, \alpha_s, \beta_s)\). Ultimately, the \( x_6 \rightarrow x_5 \) step was made slightly slower, but the original charge, \( Q_s \), was retained.

We assumed the following relations for calculating the equilibrium constants \( K_1 \) and \( K_s \) as functions of voltage:
\[ K_1 = \frac{\alpha_1}{\beta_1} = \exp\left(\frac{Q_1(V_m - V_1)}{25 \text{ meV}}\right) \]  
\[ K_x = \frac{\alpha_x}{\beta_x} = \exp\left(\frac{Q_x(V_m - V_x)}{25 \text{ meV}}\right). \]

\( V_1 \) and \( V_x \) are the voltages at which \( K_1 \) and \( K_x \), respectively, are equal to one. Both \( V_1 \) and \( V_x \) are about \(-30 \text{ mV}\), slightly negative to the midpoint of the \( Q-V \) distribution. \( Q_1 \) and \( Q_x \) were selected so that \( g_{Na} \) amplitude was correctly predicted at all voltages. It turned out that the total potential energy change for all steps taken together was 6 meV per millivolt change of membrane potential, precisely the original estimate of Hodgkin and Huxley (1952).

Using these relations and assumptions, the rate constants and \( Q_1 \) and \( Q_x \) were determined by an informal method of successive approximations. Representative calculations using the parameters in Table II are shown in Fig. 12. The model correctly predicts most kinetic features of \( g_{Na} \) and \( I_g \) including the fast and intermediate components of \( I_g \), the rising phase of \( I_g \), and the effects of prepulses and of Zn on \( g_{Na} \) and \( I_g \).

**Table II**

| \( V_m \) | \( \alpha_1 \) | \( \alpha_{2-4} \) | \( \alpha_5 \) | \( \beta_1 \) | \( \beta_{2-4} \) | \( \beta_5 \) | \( \kappa \) | \( \lambda \) | Fig. ref. |
|----------|----------------|-----------------|-------------|-------------|-------------|-------------|---------|---------|-----------|
| \( I_g \) ON | | | | | | | | | |
| -20 | 0.6 | 10 | 7.7 | 0.257 | 7.14 | 5.5 | 0.1 | 0.01 | 12 a |
| +10 | 2.3 | 14 | 10.8 | 0.089 | 3.01 | 2.33 | 0.12 | 0.012 | 12 b |
| +50 | 4.5 | 22 | 17 | 0.007 | 0.953 | 0.735 | 0.15 | 0.015 | 12 c |
| \( I_g \) OFF | | | | | | | | | |
| -70* | 0.137 | 7.6 | 5.87 | 3.2 | 40 | 30.9 | 0.1 | 0.01 | 12 d, e |
| Inactivation effect | | | | | | | | | |
| +60 | 6 | 30 | 23 | 0.004 | 0.87 | 0.62 | 0.15 | 0.02 | 13 a |
| +60‡ | — | — | — | — | — | — | — | — | 13 b |
| Zn effect | | | | | | | | | |
| +60 | 7 | 30 | 23 | 0 | 1 | 0.77 | 0.25 | 0.02 | 13 c |
| +60§ | — | — | — | — | — | — | — | — | 13 d |

Initial condition for all calculations (except *): \( Q_{1-5} = 1 \). \( V_1 \) and \( V_x \) in Eqs. 2 and 3 are \(-30.6 \text{ and} \ -28.4 \text{ mV} \), respectively. \( Q_1 = 2 \) and \( Q_x = e \).

* Initial conditions calculated from \( I_g \) ON at +50 mV out to 1 ms. \( x_{1-5} = 0.107, x_{2-4} = 0.0032, x_1 = 0.859, x_2 = 0.029, x_3 = 0.0016, x_4 = x_5 = g = 0.0016 \).

‡ After prepulse. Initial conditions changed to \( x_5 = 0.35, x_{2-4} = 0.65 \), as judged from \( I_{Na} \) traces in Fig. 10 a, b.

§ Zn-induced state \( x_{2-4} \) added to the left of \( x_4 \). \( \alpha_9 = 40, \beta_9 = 26.7 \) (see text).

_Time-Course of \( g_{Na} \) and \( I_g \)_

Fig. 12 a–c shows experimental traces of \( g_{Na} \) and \( I_g \) ON together with the calculated conductance \( (x^*_5) \) and gating current at three widely spaced voltages. The fits to the conductance traces are reasonably good, except that the calculated rise is slightly too quick. Another fast step would probably be helpful in remedying this.
Fits to the early part of $I_g$ ON are very good at all voltages. In particular, calculated $I_g$ has a rising phase at $+50$ mV but not at $-20$ mV, in agreement with experimental observation. At $+10$ and $+50$ mV the model does not fit the slow component of $I_g$, another suggestion that this component is too slow to be associated with activation.

The fit to $g_{Na}$ and $I_g$ as the channels close (Fig. 12 d and e) is qualitatively correct in that $I_g$ has a more rounded time-course than does $g_{Na}$, and the final rate of decay of $g_{Na}$ and $I_g$ is about the same.

Table II shows that the rate constants behave in a reasonable way as $V$ increases: $\alpha_1$ and $\alpha_x$ increase, and $\beta_1$ and $\beta_x$ decrease smoothly. As noted, the ratios $\alpha_1/\beta_1$ and $\alpha_x/\beta_x$ were calculated from Eqs. 2 and 3, a restriction that made it somewhat surprising that the conductances fit as well as they do. Still better fits could probably be obtained by small adjustments of $Q_1$, $Q_x$, or other parameters; we exhausted our patience but not the possibilities of the model. The rate constants $\kappa$ and $\lambda$ change very little with voltage, e-fold for roughly 175 mV (c.f. Bezanilla and Armstrong, 1977), compatible with the hypothesis that the inactivation step involves little if any measurable charge movement.

**Effect of Inactivation**

At the end of a long depolarizing step most of the channels are inactivated and in state $x_1z$. On repolarization the likely step is $x_1z \rightarrow x_2z$, which is much more rapid than $x_1z \rightarrow x_1^*$. The transition from $x_1z$ to $x_2z$ generates the
inactivation resistant component of gating current, which is exponential since only one step is involved. Thus $I_g$ tails seen after a long pulse do not have the hook seen after shorter steps. Experimentally it is impossible to observe this component without some contamination from charge movement associated with other steps.

In Figs. 9 and 13 $b$ the membrane was repolarized for 0.8 or 1 ms following a long depolarizing prepulse, and a second depolarizing step was then applied.

![Figure 13](image)

**Figure 13.** Experimental and calculated $I_g$ ON showing the effects of a depolarizing prepulse ($a$, $b$) or external Zn ($c$, $d$). Parameters initial conditions are given in Table II. ($a$) $I_g$ ON at +60 mV. ($b$) $I_g$ ON at +60 mV following a 15-ms prepulse to +60 mV and 1-ms recovery at −70 mV. Same experiment as in Fig. 10 $a$, $b$. ($c$) $I_g$ ON at +60 mV. ($d$) $I_g$ ON at +60 mV in the presence of 30 mM ZnCl$_2$. Same experiment as in Fig. 8 $a$.

At the beginning of the second step, most of the channels (about two thirds) are in state $x_2z$, and most of the remainder are in state $x_b$. During the second step the channels in $x_2z$ move to $x_1z$, generating an exponential current, which is added to the more complicated current generated by the channels moving from $x_b$ to $x^+$.* Simulations of the experiment in Fig. 13 $a$ and $b$ are given by the smooth curves. In both experiment and simulation the rising phase of $I_g$ is almost eliminated following a prepulse.

**Zn Effect**

A possible explanation for the action of Zn is that it binds to a negatively charged group that is part of the gating apparatus. The negative group is at
the outer surface of the membrane and accessible to external Zn only when the channel is fully closed, in state $x_6$. On depolarization the extra step required as Zn dissociates, $x_{2z} \rightarrow x_6$, slows activation. Once the channel has progressed to $x_6$, the Zn-binding group has migrated inward and become inaccessible to Zn, which has no further effect on kinetics. Specifically, there is no effect on channel closing (see Fig. 8 c and d). Calculations of $I_{ON}$ based on this model are shown in Fig. 13 c and d, together with experimental traces which are well fitted except for the slow component. Parameters of the fit are given in Table II. It is clear without calculation that $I_{OFF}$ will be unaffected.

$\textbf{InNa during Recovery from Inactivation}$

Experimentally there is no detectable sodium current as channels recover from inactivation (Armstrong, 1978). The model accounts for this because the recovery path, $x_1z \rightarrow x_2z \rightarrow x_6$, bypasses the conducting state.

The model thus has qualitatively correct behavior in all instances tested. Its most important feature is the postulate that the last step is the slowest and most voltage dependent: we regard this as established and inescapable. There are two other significant conclusions. First, all of the early part of gating current is accounted for as being essential to activation of Na channels. Second, there is a slow component that clearly is not explained by the model. Conceivably this component is associated with a transition to a second open state (Armstrong and Bezanilla, 1977). Another possibility is that it is the early phase of gating current from K channels, which may, like Na channels, activate in several fast steps followed by a slow one. This seems somewhat unlikely, since the slow component seems not greatly altered in circumstances where the K channels have ceased to function but it remains a possibility.

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REFERENCES

ALMERS, W. 1978. Gating currents and charge movements in excitable membranes. Rev. Physiol. Biochem. Pharmacol. 82:96-190.

ARMSTRONG, C. M. 1978. Intramembranous charge movement and control of cellular functions by membrane voltage. In Biophysical Aspects of Cardiac Muscle. M. Morad, editor. Academic Press, Inc. New York. 27-29.

ARMSTRONG, C. M., and F. BEZANILLA. 1973. Currents related to movement of the gating particles of the sodium channels. Nature (Lond.). 242:459-461.

ARMSTRONG, C. M., and F. BEZANILLA. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. J. Gen. Physiol. 63:533-552.

1 Almers, W., and C. M. Armstrong. 1980. Survival of K$^+$ permeability and gating currents in squid axons perfused with K$^+$-free media. J. Gen. Physiol. In press.

2 Gilly, W. F., and C. M. Armstrong. Gating current and K channels in the giant axon of the squid. Manuscript submitted for publication.
ARMSTRONG, C. M., and F. BEZANILLA. 1975. Currents associated with the ionic gating structures in nerve membrane. *Ann. N.Y. Acad. Sci.* 264:265–277.

ARMSTRONG, C. M., and F. BEZANILLA. 1977. Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* 70:567–590.

BEZANILLA, F., and C. M. ARMSTRONG. 1975. Properties of the sodium channel gating current. *Cold Spring Harbor Symp. Quant. Biol.* 40:297–304.

BEZANILLA, F., and C. M. ARMSTRONG. 1977. Inactivation of the sodium channel. I. Sodium current experiments. *J. Gen. Physiol.* 70:549–566.

GOLDMAN, L., and C. L. SCHAUF. 1972. Inactivation of the sodium current in Myxicola giant axons. Evidence of coupling to the activation process. *J. Gen. Physiol.* 59:659–675.

HILLE, B. A., A. WOODHULL, and B. I. SHAPIRO. 1975. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions and pH. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* 270:301–318.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* 117:500–544.

HOYT, R. C. 1965. The squid giant axon: mathematical models. *Biophys. J.* 3:399–431.

KEYNES, R. D., and E. ROJAS. 1973. Characteristics of the sodium gating current in the squid giant axon. *J. Physiol. (Lond.)* 233:28–30P.

KEYNES, R. D., and E. ROJAS. 1974. Kinetics and steady state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.)* 239:393–434.

MEVES, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol. (Lond.)* 243:847–867.

MEVES, H., and W. VOGEL. 1977a. Inactivation of the asymmetrical displacement current in giant axons of Loligo forbesi. *J. Physiol. (Lond.)* 267:377–393.

MEVES, H., and W. VOGEL. 1977b. Slow recovery of sodium current and gating current from inactivation. *J. Physiol. (Lond.)* 267:395–410.

NEUMCKE, B., W. NONNER, and R. STÄMPFLI. 1976. Asymmetrical displacement current and its relation with the activation of sodium current in the membrane of frog myelinated nerve. *Pflügers Arch. Eur. J. Physiol.* 363:193–203.

NONNER, W., E. ROJAS, and R. STÄMPFLI. 1978. Asymmetrical displacement currents in the membrane of frog myelinated nerve: Early time course and effects of membrane potential. *Pflügers Arch. Eur. J. Physiol.* 375:75–85.