

Kinetic Properties of a Voltage-dependent Junctional Conductance

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ABSTRACT We have proposed that the gap junctions between amphibian blastomeres are comprised of voltage-sensitive channels. The kinetic properties of the junctional conductance are here studied under voltage clamp. When the transjunctional voltage is stepped to a new voltage of the same polarity, the junctional conductance changes as a single exponential to a steady-state level. The time constant of the conductance change is determined by the existing transjunctional voltage and is independent of the previous voltage. For each voltage polarity, the relations between voltage, time constant, and steady-state conductance are well modeled by a reversible two-state reaction scheme in which the calculated rate constants for the transitions between the states are exponential functions of voltage. The calculated rate constant for the transition to the low-conductance state is approximately twice as voltage dependent as that for the transition to the high-conductance state. When the transjunctional voltage polarity is reversed, the junctional conductance undergoes a transient recovery. The polarity reversal data are well modeled by a reaction scheme in which the junctional channel has two gates, each with opposite voltage sensitivity, and in which an open gate may close only if the gate in series with it is open. A simple explanation for this contingent gating is a mechanism in which each gate senses only the local voltage drop within the channel.

INTRODUCTION

As described previously, the conductance of the junctions between blastomeres of early amphibian embryos is strongly dependent on transjunctional voltage (Harris, 1979; Spray et al., 1979 and 1981). The steady-state conductance for each polarity of transjunctional voltage is well fit by equations describing equilibrium behavior of a two-state system in which the energy difference between the states is a linear function of transjunctional voltage and transitions between states occur by a reversible first-order process. We therefore proposed that there are channels through the junctional membrane that can exist in either a high- or a low-conductance state, depending on the transjunctional voltage (Spray et al., 1979 and 1981).

The present paper examines the junctional conductance during transitions between equilibrium states at different voltages. For transjunctional voltages

of one sign, the conductance changes are well described by first-order kinetics. The time-course of transitions when transjunctional potentials are reversed in sign suggests that there are two mirror symmetrical gates in series in the junctional channels, each of which is affected by the transjunctional voltage only when the apposed gate is open.

METHODS

Blastomeres from early axolotl (*Ambystoma mexicanum*) embryos were mechanically dissociated as pairs, impaled with microelectrodes, and voltage clamped as described in the preceding paper (Spray et al., 1981). Each cell of a pair was voltage clamped to a common holding potential, and the voltage of one cell of the pair (V_b) was stepped to various levels. Junctional current (I_j) was measured as the current delivered to the other cell to hold its potential (V_a) constant.

RESULTS

Time-Course of the Junctional Conductance Changes

During a small transjunctional voltage step of either polarity, the junctional current was constant (Fig. 1 *A*), but, for larger voltage steps, junctional currents declined (Fig. 1 *B*; see also Fig. 3 of Spray et al. [1981]). The relaxation of the junctional conductance g from its initial level g_0 to its steady-state level g_∞ was well fit by an exponential of the form (see Appendix)

$$g = g_\infty + (g_0 - g_\infty) \exp(-t/\tau). \quad (1)$$

For Fig 1 *C* and *D*, the junctional conductances during three transjunctional voltage steps were calculated from digitized junctional currents and transjunctional voltages, and the changes were plotted semilogarithmically with respect to time (Fig. 1 *C*). The relations are linear, with a negative slope that is steeper for larger transjunctional voltages. These data support the assertion that the conductance changes for voltages of a single polarity are produced by a simple first-order process.

There was no detectable delay in the onset of the decline of junctional conductance within 3 ms after a voltage step, the settling time of the current record. Furthermore, the conductances calculated from extrapolation to time zero are nearly identical. Identity would obtain if there were no delay in the effect of transjunctional voltage on the conductance or if the delay were proportional to the time constant of decline of the conductance at all voltages, which would suggest that it was part of the same kinetic process.

The recovery of the junctional conductance when the transjunctional voltage was returned to zero after a large voltage step also followed an exponential time-course (Fig. 2). The increase in conductance was monitored by following the large step with shorter test pulses at various delays (Fig. 2, *inset*). The junctional current flowing at the onset of the test pulse reflected the instantaneous junctional conductance. These data are plotted in Fig. 2 as $\ln [(g_\infty - g)/g_\infty]$ vs. time after return to zero transjunctional voltage and are well fit by a straight line. The exponential time-course of conductance recovery is also consistent with a first-order process.

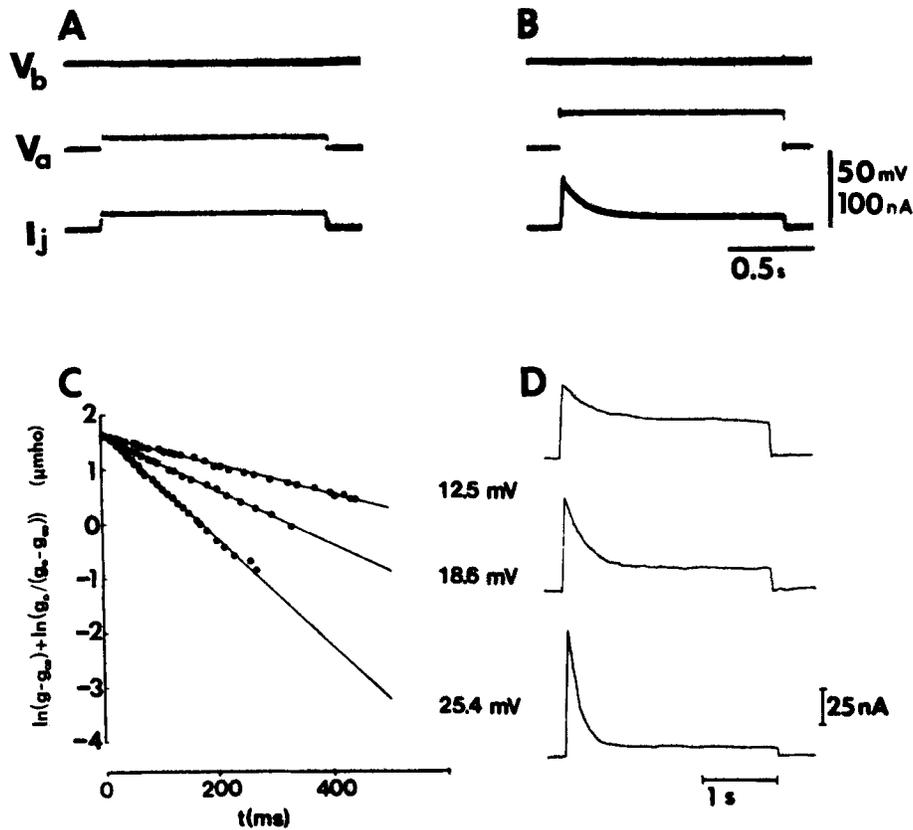


FIGURE 1. Exponential decay of junctional conductance. A pair of cells were voltage clamped and stepped to various voltages, and junctional currents were measured as described in the preceding paper (Spray et al., 1981). The voltages of the two cells are V_a and V_b , and the junctional current I_j is that supplied to one to keep its voltage (V_a) constant. (A) During a small transjunctional voltage step, the junctional current remained constant. (B) During a larger transjunctional voltage step, the junctional current fell to a low steady-state level over several hundred milliseconds (from Fig. 4 of Spray et al. [1981]). (C) Semilog plot of junctional conductance for various transjunctional voltages. Junctional currents during transjunctional voltage steps were digitized, and plots of these values are shown in D. Current, divided by the transjunctional voltage, yielded junctional conductance. For each voltage, the steady-state conductance g_∞ was subtracted from the changing conductance g and $\ln(g - g_\infty)$ was plotted against time. The slope and y intercept, $\ln(g_0 - g_\infty)$, where g_0 is conductance at time zero, was evaluated by linear regression. The value of g_0 was determined from $\ln(g_0 - g_\infty)$ and g_∞ . The same data and regression lines were then plotted in C as $\ln(g - g_\infty) + \ln[g_0 / (g_0 - g_\infty)]$ vs. time, which gives $\ln(g_0)$ as the y intercept. The points are well fit by straight lines, indicating that junctional conductance declined as simple exponential function of time. The rate of decay of the junctional conductance increased with larger transjunctional voltages, and the computed values of g_0 are nearly identical.

Effect of Previous History on the Relaxation Time Constant

If the conductance changes were due to a reversible first-order process in which the forward and reverse rates were solely a function of voltage, the relaxation time constant at a given voltage would be independent of the previous voltage history of the junction. Thus, the relaxation of the current to its steady-state level should occur with the same rate, whether the transjunctional voltage is stepped to a given level from larger or smaller potentials. Experiments of the type shown in Fig. 3 showed that the time constant of the relaxation at a given voltage after a step from higher or lower voltages was

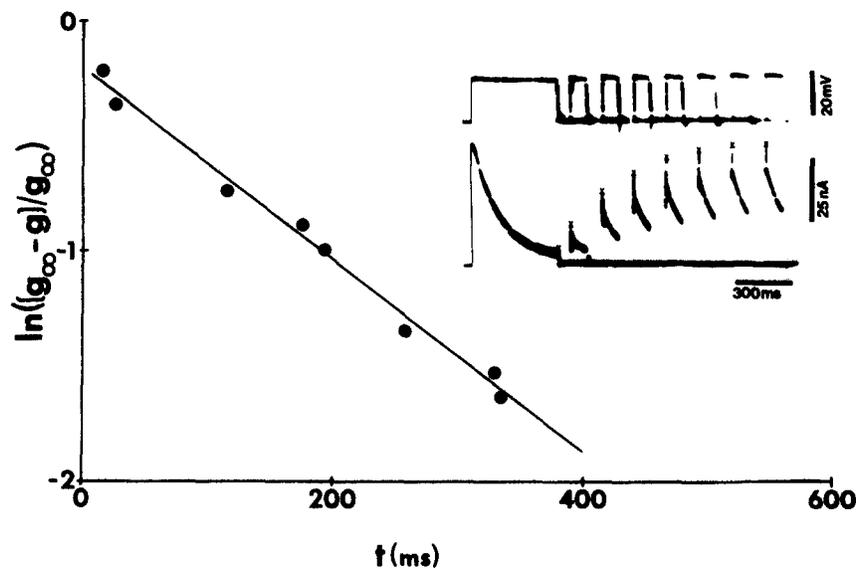


FIGURE 2. Exponential recovery of conductance after termination of a transjunctional voltage step. After identical long lasting voltage steps that lowered the junctional conductance, shorter voltage steps were given in successive trials at various times thereafter to assess the recovery of junctional conductance (*inset*). The difference between the instantaneous conductance g at the beginning of each of the shorter steps and its (steady-state) value for long intervals between conditioning and test pulses g_{∞} was determined. Then $\ln [(g_{\infty} - g)/g_{\infty}]$ was plotted against time. A fit determined by linear regression fits the points well, which indicates that junctional conductance recovers in an exponential fashion.

the same, whether the conductance increased or decreased (all voltages being of the same sign; the transitions when polarity was reversed are considered below). In the plots of $\ln |g - g_{\infty}|$ against time, the slopes are equal, indicating that a single time constant characterized the relaxation at each transjunctional voltage.

Voltage Dependence of the Relaxation Time Constant

The ability to measure the time constant independent of previous history facilitated measurement of the time constant over a wide range of transjunc-

tional voltages. For large steps from zero voltage, the conductance decrease was large, and its time-course readily measured. For small voltage steps, the conductance change was small, and its time-course more difficult to measure. In stepping to a small voltage from a large one, large increases in conductance were obtained whose time-courses were more readily measured. Using such a stimulation paradigm (Fig. 3, *inset*), the relaxation time constants were determined over a wide range of transjunctional voltages. The time constants are plotted as a function of voltage in Fig. 4 A (X's and filled circles are the experimentally determined values for decreasing and increasing conductances, respectively).

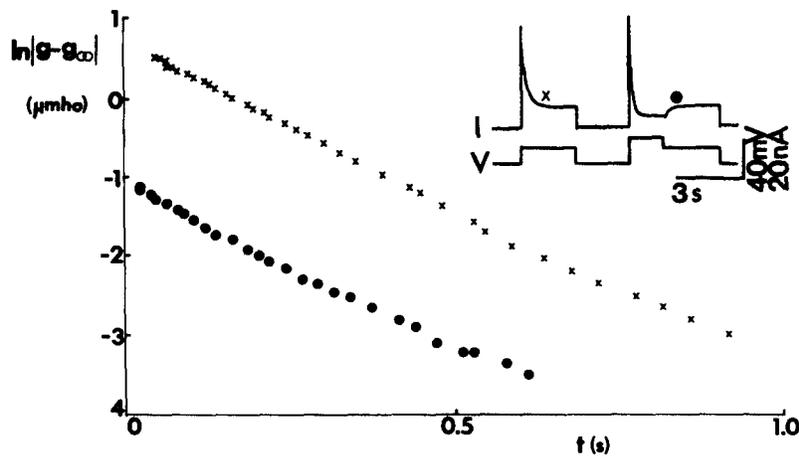


FIGURE 3. Lack of effects of previous history on relaxation time constant. The *inset* shows the stimulation paradigm used to step to a given voltage from zero voltage and from a large prepulse voltage. The relaxations of the conductance to its steady state value $|g - g_{\infty}|$ were plotted semilogarithmically as a function of time for pulses with (●) and without (×) a prepulse. The slopes of these lines are equal, indicating that the rate of relaxation of the currents is not influenced by the previous voltage history of the junction.

Calculation of Rate Constants

The kinetic data presented in Figs. 1–4 are consistent with the conductance changes mediated by a first-order process dependent on voltage. Such a process may be modeled assuming that the conductance changes are due to the distribution of a population of channels between high- and low-conductance states according to the free energy difference between the states, where the energy difference is a function of transjunctional voltage. The rate of the transition of a population of channels from one conductance state to the other is determined by the difference between the free energy of the initial state and the free energy of the transition state through which the channel must pass to reach the other state (Glasstone et al., 1941). These energy differences are the activation energies and, for a voltage-dependent system such as this, are

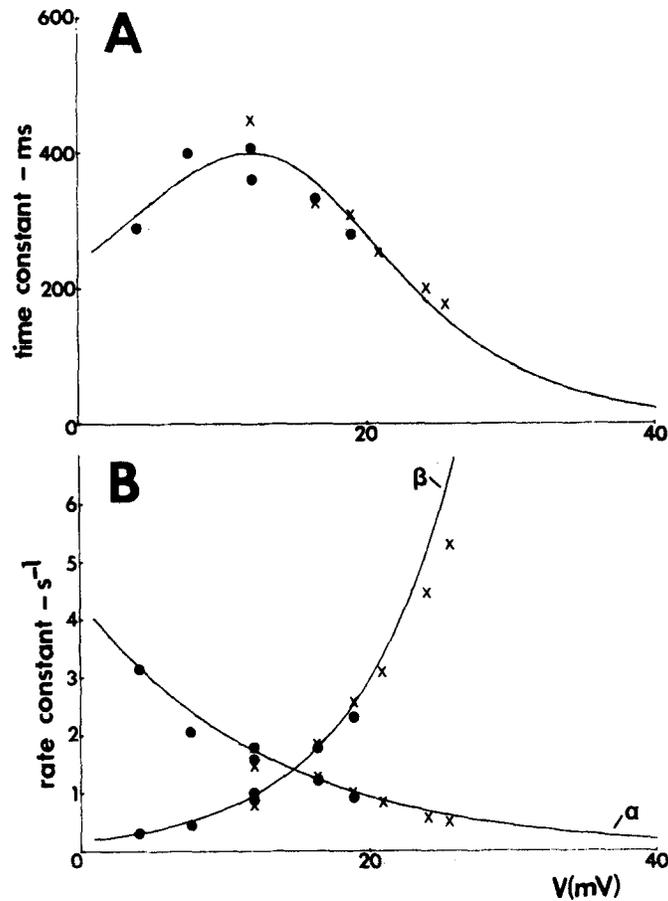
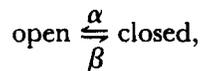


FIGURE 4. Experimental and calculated time and rate constants. (A) Dependence of the time constant on voltage. The time constants of conductance changes were measured with the stimulation paradigms shown in Fig. 3, and plotted as a function of voltage. The X's represent values derived from relaxations during decreasing conductance (no prepulse), and the filled circles values from relaxations during increasing conductance (larger prepulse). The continuous curve was calculated according to $\tau = 1/(\alpha + \beta)$, where α and β were determined by Eqs. 6 and 7. (B) Dependence of rate constants on voltage. The values for opening and closing rate constants were calculated from Eqs. 2 and 3. The continuous curves are exponentials determined by linear regression fits to semilog plots (Eqs. 6 and 7). The calculated curves for time and rate constants closely approximate the data, which indicates that the kinetics of this system are consistent with the model proposed.

changed by voltage due to the interaction of charges in the channel molecule with the electric field (see Discussion). The reaction may be represented by



where α and β are voltage dependent. For such a system, at a given voltage, α and β are calculable from the time constant τ and the normalized steady-state conductance G_∞ (see Eqs. A3 and A4 in Appendix):

$$\alpha = G_\infty/\tau \quad (2)$$

and

$$\beta = (1 - G_\infty)/\tau. \quad (3)$$

In these equations, G_∞ can be considered an activation parameter representing the fraction of the voltage-sensitive component of the conductance reached at steady state for a given voltage. According to a model in which the activation energies are linear functions of transjunctional voltage (see Discussion), the relation between the rate constants and voltage should be described by

$$\alpha = \lambda \exp [-A_\alpha(V - V_0)] \quad (4)$$

and

$$\beta = \lambda \exp [A_\beta(V - V_0)], \quad (5)$$

where A_α and A_β are the respective voltage sensitivities of the rates, V_0 is the voltage at which the steady-state conductance is half maximal, and λ is a constant, the rate for which $\alpha = \beta$, reached at $V = V_0$.

For the experiment whose time constant data are shown in Fig. 4 A, α and β were calculated from Eqs. 2 and 3 using the values of τ shown and values of G_∞ determined by the equilibrium modeling procedure described in the preceding paper (Spray et al., 1981). The calculated rates are plotted as a function of voltage in Fig. 4 B and are well fit by exponentials determined from linear regression of semilogarithmic plots of α and β (not illustrated). The slopes of the semilogarithmic relations give the voltage sensitivities, and the coordinates of their point of intersection are V_0 and λ . From these data

$$\alpha = 0.0013 \exp [-0.077(V - 14.7)] \quad (6)$$

and

$$\beta = 0.0013 \exp [0.14(V - 14.7)]. \quad (7)$$

The voltage dependence of the closing rate is approximately twice that of the opening rate.

From Eqs. 6 and 7, τ was calculated as $1/(\alpha + \beta)$ (Eq. A4) and plotted as a continuous line in Fig. 4 A. The adequacy of the fit of the calculated curves to the experimentally derived values for time constants and rate constants indicates that for either polarity of applied voltage the kinetics are consistent with a two-state model in which the activation energies for the transitions depend linearly on voltage. As discussed in the next section, transitions between voltages of opposite sign require a more complicated reaction scheme.

Voltage Polarity Reversal

Because voltages of either polarity affected the junctional conductance, it was of interest to investigate the conductance changes when the transjunctional

voltage was switched from one polarity to the other. Junctional currents obtained during a voltage step of constant magnitude when given alone and when preceded by voltage steps of various magnitudes of the opposite polarity are shown in Fig. 5. When the test pulse was preceded by a prepulse of opposite polarity that reduced the junctional conductance (Fig. 5, *middle*

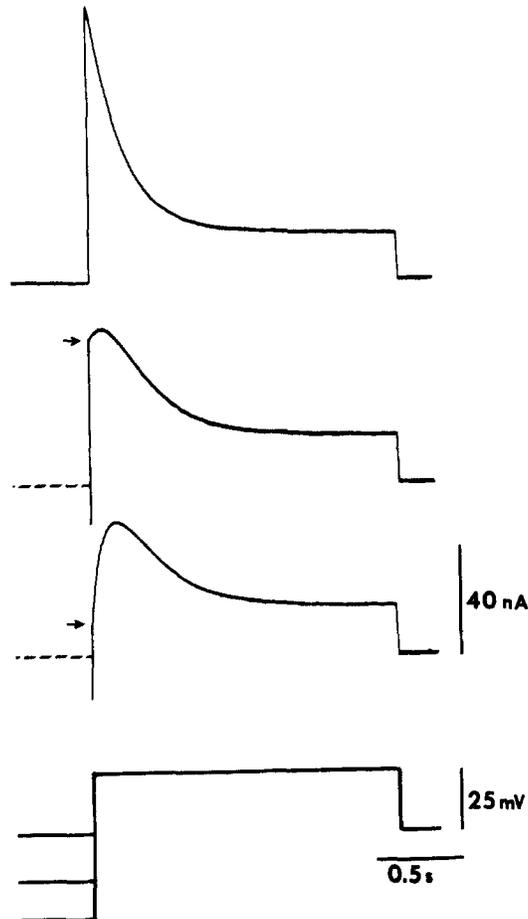


FIGURE 5. Transient recovery of junctional conductance with voltage polarity reversal. For the upper current trace, a transjunctional voltage step was given during which junctional current declined exponentially to a steady-state value. For the second and third current traces, the same voltage step was given, but was preceded by voltage steps of opposite polarity. In both traces, junctional current during the test pulse showed a transient recovery from the initial values (arrows) before declining to its steady-state level. The transient increase indicates that in moving between two low-conductance states induced by voltages of opposite polarity, the junctional membrane passed through a state of higher conductance. The patterns of voltage application are superimposed in the bottom trace.

traces), the current during the test pulse underwent a transient increase and then decreased to the value appropriate for the test pulse. This finding indicates that at least some of the conductance elements pass through a high-conductance state when transiting between low-conductance states caused by opposite voltage polarities. In superimposed current records (Fig. 6 *A*), the current flowing after a prepulse significantly "overshoots" that in response to a test pulse alone. That is, for a major portion of the relaxation of the currents,

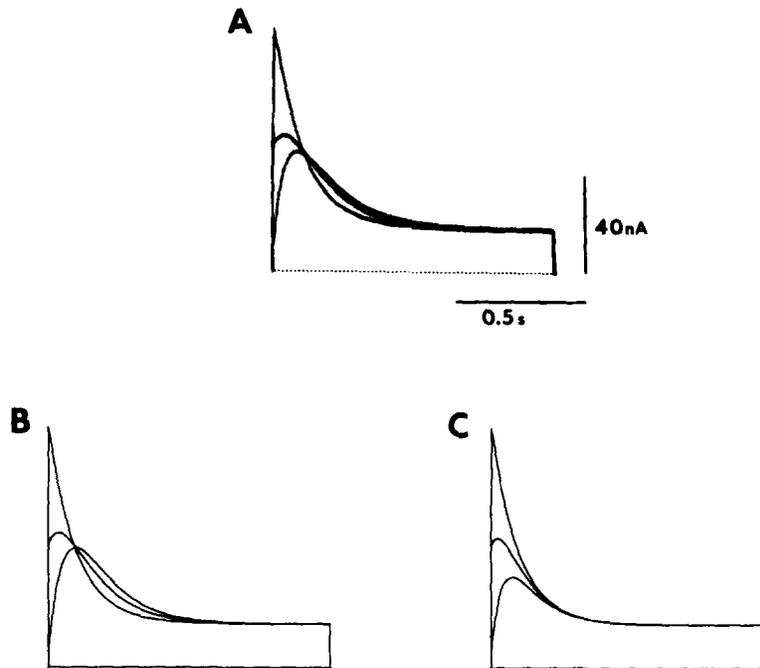


FIGURE 6. Comparison of polarity reversal data with calculated conductances for contingent and independent gating. (*A*) Polarity reversal data (from Fig. 5). The junctional currents during the test pulse are photographically superimposed to allow comparison of the currents flowing when stepped to the test voltage from zero voltage and from prepulses of the opposite voltage polarity. (*B*) Time-courses calculated from the contingent gating model (Eqs. 8–11). The contingent gating model requires that with polarity reversal the gate closed by the prepulse must open before the gate in series with it senses the transjunctional voltage. (*C*) Time-courses calculated from the independent gating model (Eqs. 12–13). The independent gating model requires that each gate respond to the transjunctional voltage independently of the other. The conductance time-courses in *B* and *C* were calculated for the test voltage and prepulse voltages that produced the junctional currents shown in *A*. For both *B* and *C*, in addition to the limitation of α_1 described in the text, the voltage dependence of the rates defined by Eqs. 6 and 7 was scaled by factor of 0.925 to achieve closer fit to the equilibrium conductances. The form of the data is best reproduced by the contingent gating model.

the current during the test pulse after polarity reversal exceeds that during the test pulse given alone. Furthermore, the overshoot is greater for larger prepulse voltages.

To account for the conductance changes after polarity reversal, the system was modeled as if there were two mirror symmetrical gates in the junctional channel, each responding to voltage with the first-order relation described above. Mirror symmetry allows for the two gates on a channel to close in response to voltages of opposite sign. The operation of the two gates may be independent of or contingent upon one another, possibilities considered below.

Contingent Gating

Assume that each gate senses local voltage within the channel and that the gates have zero conductance when closed. Then in the presence of a voltage that closes one gate, the entire transjunctional voltage is developed across that gate, and the voltage across the open gate is zero. When the voltage polarity is reversed, the closed gate must open before the voltage acts on the other gate. The operation of one gate is, therefore, contingent on the state of the other. This hypothesis can be closely modeled by a three-state reaction scheme:



where the rates are given by Eqs. 6 and 7, and the subscripts refer to the operation of gates 1 and 2, which have voltage sensitivities of opposite polarities. The general solution for this system is given by Arndt and Roper (1975).

We will consider the case in which the voltage is stepped to a test value V_2 of the polarity that closes gate 2 from zero voltage and from various voltages V_1 of the polarity that closes gate 1. The contribution of the rate constant governing the closing of gate 1 (β_1) may be reasonably neglected in calculating the conductance during V_2 , because β_1 is already small at zero voltage (see Fig. 4 B).

The solution for the normalized conductance during the test pulse V_2 is the sum of three normalized components, s_0 for channels initially open, s_1 for channels initially closed by gate 1 and s_2 for channels initially closed by gate 2, the contribution of each component being weighted by its fraction of the total number of channels. The solutions for the normalized components are:

$$s_0 = \frac{\alpha_2}{\alpha_2 + \beta_2} + \left(1 - \frac{\alpha_2}{\alpha_2 + \beta_2}\right) \exp [-(\alpha_2 + \beta_2)t], \quad (8)$$

$$s_1 = \frac{\alpha_2}{\alpha_2 + \beta_2} - \frac{\alpha_1\beta_2}{(\alpha_2 + \beta_2 - \alpha_1)(\alpha_2 + \beta_2)} \exp [-(\alpha_2 + \beta_2)t] + \frac{\alpha_1 - \alpha_2}{\alpha_2 + \beta_2 - \alpha_1} \exp (-\alpha_1 t), \quad (9)$$

and

$$s_2 = \frac{\alpha_2}{\alpha_2 + \beta_2} (1 - \exp [-(\alpha_2 + \beta_2)t]), \quad (10)$$

where α_2 and β_2 are evaluated at $V = V_2$, and α_1 at $V = -V_2$ (because its voltage sensitivity is of the opposite polarity). The component s_0 is a simple exponential decay reflecting relaxation to the new equilibrium condition where a fraction of channels are closed by gate 2; s_1 is the sum of two exponentials according to the opening of gate 1 and subsequent closing of a fraction of gate 2; and s_2 is the exponential relaxation of channels initially closed by gate 2 to the new equilibrium where more of these channels are open. The normalized conductance is

$$G = p_0 s_0 + p_1 s_1 + p_2 s_2, \quad (11)$$

where p_i is the fraction of channels in each state at the potential before the test pulse according to $p_0 = 1/(1 + \beta_1/\alpha_1 + \beta_2/\alpha_2)$, $p_1 = (\beta_1/\alpha_1)/(1 + \beta_1/\alpha_1 + \beta_2/\alpha_2)$, and $p_2 = (\beta_2/\alpha_2)/(1 + \beta_1/\alpha_1 + \beta_2/\alpha_2)$, where the rates are evaluated at $V = V_1$, and $p_0 + p_1 + p_2 = 1$.

In the preceding sections, the voltage dependence of the rate constants for voltages of a single polarity was characterized. In the contingent gating model, the rates calculated according to Eqs. 6 and 7 were applied to each gate of a channel, where positive voltages increased the closing rate and decreased the opening rate of one gate, and negative voltages had the same effects on the other gate. In the polarity reversal experiments, the field across gates that closed in response to a voltage of polarity V_1 was reversed during the test pulse, and V_2 was of opposite sign to the voltages used to determine Eqs. 6 and 7. The method of determining the rate constants did not allow investigation in this voltage range because of the closing of the series gate, and the value used in the calculations was an extrapolation of the computed line in Fig. 4 *B* to the other sign of voltage. For the rates determined according to Eqs. 6 and 7, the calculated current fit the data for the test pulse alone, but the calculated currents after a prepulse rose and fell too rapidly and reached too large a peak value. The fit was greatly improved by requiring α_1 to saturate at the value predicted by Eq. 6 at $V = -5$ mV rather than at the full magnitude of V_2 . Thus, polarity reversal allows investigation of voltage sensitivity of the gates in a region otherwise inaccessible.

The solutions incorporating the limiting value of α_1 are graphed in Fig. 6 *B* and agree well with the time-courses of Fig. 5 shown superimposed in Fig. 6 *A*. In both experimental and calculated curves, the later portion of the current during a test pulse after a prepulse significantly exceeds that when no prepulse is given, and the magnitude of this difference is greater for larger prepulses. For the voltage steps shown, the overshoots are much larger than those predicted by independent gating (Fig. 6 *C*; see below).

It can be shown analytically for the contingent gating model that the overshoot of current after polarity reversal occurs for any values of the rate constants and increases with magnitude of the prepulse. As an example,

consider the case of large prepulses of polarity V_1 and test pulses of polarity V_2 in which essentially all the gates are initially open without a prepulse ($p_0 \approx 1$), all the gates on one side are closed at the end of the prepulse ($p_1 = 1$), and all the gates on the opposite side are closed at the end of the test pulse ($p_2 = 1$). Since the integrals of s_1 and s_0 from $t = 0$ to $t = \infty$ are equal, the current during the test pulse after the prepulse, which initially is zero, must later become larger and exceed s_0 , which decreases exponentially. The equality of the current integrals follows simply from consideration of the original model. If the open gates on one side do not detect transjunctional potential until the closed gates in series are open, then the channels will remain open for the same length of time and pass the same amount of current, whether the series gates start open or closed.

The voltage sensitivity of the gates is such that near $V = 0$, some of the conductance elements are closed by gate 1 (due to voltage sensitivity of polarity V_1) and some are closed by gate 2 (due to voltage sensitivity of polarity V_2). For $V = 0$, these two populations are equal and are each $\sim 3\%$ of the total number of channels. During a pulse of polarity V_2 the channels closed by gate 1 will open according to Eq. 9, causing the relaxation of the current to deviate from exponentiality. The deviation is too small to be resolved by present techniques. The channels closed by gate 2 also reduce the initial number of open channels, but relax exponentially with the same time constant as open channels and, therefore, do not alter the time-course of the conductance. In addition to these deviations predicted by the model, one would expect that near $V = 0$ some channels would be closed by both gates 1 and 2. This fraction would presumably be no greater than the product of p_1 and p_2 near $V = 0$, and therefore would be quite small.

Independent Gating

An alternative model for voltage reversal that should be considered is that each gate sees the entire transjunctional voltage independent of whether the other gate is open or closed. This model may be represented by the following reactions for each gate occurring simultaneously and independently:



where the rate constants are voltage dependent as described above.

Each gate will exhibit the first-order kinetics described by Eq. A9:

$$G_i = G_{i\infty} + (G_{i0} - G_{i\infty}) \exp(-t/\tau_i), \quad (12)$$

where the subscript i refers to gate 1 or 2, and the other symbols have their previous meanings. Because each gate is independent, the fraction of channels open is the product of the fraction of channels with gate 1 open (G_1) and the fraction of channels with gate 2 open (G_2). For a step of polarity V_2 preceded by a prepulse of polarity V_1 , the conductance is described by (neglecting α_1 as

before):

$$G = \{1 + [G_{10} - 1] \exp [-(\alpha_1 t)]\} \{G_{2\infty} + [G_{20} - G_{2\infty}] \exp [-(\alpha_2 + \beta_2)t]\}, \quad (13)$$

where $G_{2\infty} = \alpha_2/(\alpha_2 + \beta_2)$, the rates are evaluated at V_2 , and the fractions of each gate initially open, $G_{10} = \alpha_1/(\alpha_1 + \beta_1)$ and $G_{20} = \alpha_2/(\alpha_2 + \beta_2)$, are evaluated at V_1 .

This solution is graphed in Fig. 6 C, with the same limit on α_1 as for the contingent gating. It was necessary to limit α_1 to obtain approximately the same peak current following a prepulse. The calculations do not fit the experimental data as well as those from the contingent gating model. In particular, the current after a prepulse does not noticeably exceed that without a prepulse. We conclude that independent gating as modeled does not account for the data.

DISCUSSION

Phenomenology of the Conductance Changes

The form of the junctional currents during transjunctional voltage steps of a single polarity allows certain conclusions regarding the underlying processes. The currents change with an essentially exponential time-course during a transjunctional voltage step, but deviation from exponentiality soon after onset of the voltage step is not ruled out. The absence of S-shaped kinetics or delay seen, for example, in sodium and potassium conductances in squid axons (Hodgkin and Huxley, 1952 *a* and 1952 *b*) implies the absence of cooperative interaction between the elements controlling the conductance changes, and suggests a first-order reaction mechanism for voltages of each polarity.

The conclusion that a first-order process underlies the conductance changes could be drawn from modeling of the steady-state conductances without reference to kinetic data (Spray et al., 1981). This simple conductance-voltage relation is seen in a number of natural and artificial conductance systems. Potassium channel inactivation in skate electroplaques (Grundfest, 1973) and slow sodium inactivation in squid axons (Chandler and Meves, 1970; Rudy, 1978) show similar simple dependence on voltage, as does excitability inducing material (EIM) in oxidized cholesterol bilayers (Ehrenstein et al., 1974) and voltage-dependent anion channel (VDAC) inserted into phospholipid bilayers (Schein et al., 1976). Several elements, each of which exhibits reversible first-order kinetics, could interact cooperatively to give the more complex activation kinetics of sodium and potassium channels (cf. Hodgkin and Huxley [1952 *c*]). This possibility is supported by the finding that the charge movement associated with the activation of sodium gates ("gating currents") in the squid axon shows a first-order voltage dependence (Keynes and Rojas, 1974).

The conductance changes of junctions between amphibian blastomeres are relatively slow (hundreds of milliseconds) compared with many other voltage-

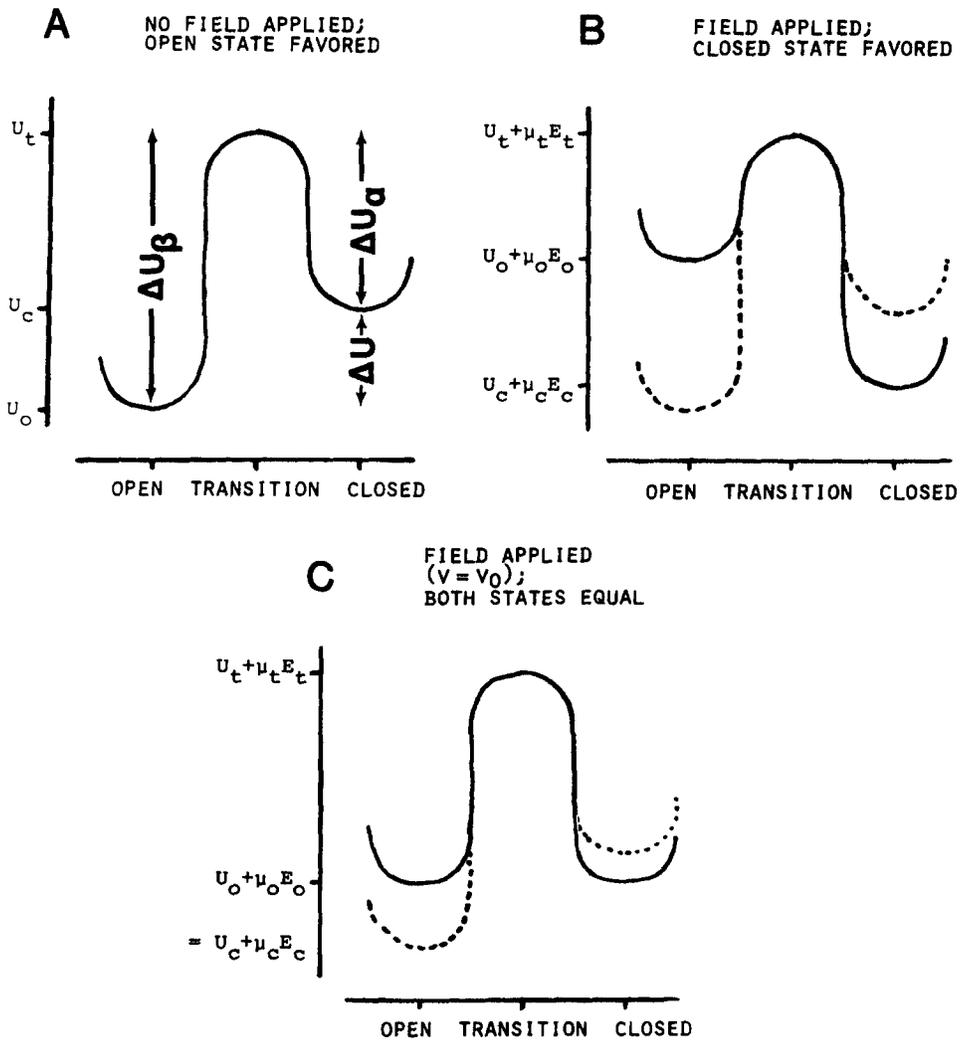
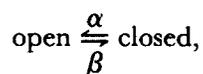


FIGURE 7. Free energy diagrams for voltage-dependent transitions in gap junction channels. *A* shows the energy barrier profile for a gate in the absence of a field, where the relationships between the energies of the open, closed, and transition states are due to the conformational energies of the molecule alone. The activation energies for channel closing and opening are ΔU_β and ΔU_α , respectively. Because $\Delta U_\beta > \Delta U_\alpha$, the opening rate is faster than the closing rate, and the open state is favored. *B* shows the energy profile in the presence of an applied voltage field ($V > V_0$). The activation energies have been changed due to the contributions of the voltage-dependent energy term to each state (see text). Because the activation energy for closing is less than that for opening, the closing rate is faster than the opening rate, and the closed state is favored. At $V = V_0$ (*C*), the change in energy of each state is such that the activation energies are equal. Therefore, the rate constants are equal, and the channels have equal probabilities of being in either state. The energy diagrams are drawn for a gate

dependent conductances. There are, however, similar or slower conductance changes reported from biological membranes (slow inactivation of sodium conductance [Rudy, 1978]) and from biological material studied in artificial bilayers (matrix protein [Schindler and Rosenbusch, 1978], VDAC [Schein et al., 1976], Colicin K [Schein et al., 1978], EIM [Ehrenstein et al., 1974] and hemocyanin [Lattore et al., 1975]).

Significance of the Kinetic Modeling

In the first-order reaction scheme that we propose, channels can exist in high- and low-conductance states:



where α and β are functions of voltage. Conventionally, one designates the free energies W_o and W_c for open and closed states and W_t for the free energy at the peak of the barrier (or transition state) between them. From reaction rate theory (Glasstone et al., 1941):

$$\alpha = \nu_\alpha \exp [-(W_t - W_c)/kT] \quad (14)$$

and

$$\beta = \nu_\beta \exp [-(W_t - W_o)/kT], \quad (15)$$

where ν_α and ν_β are frequency factors, k is Boltzmann's constant and T is absolute temperature. The expressions $W_t - W_c$ and $W_t - W_o$ represent the activation energies for opening and closing, respectively. For each state, the free energy W of a voltage-sensitive molecule can be divided into two components, the conformational free energy in the absence of an applied field, U , and a field-dependent component μE , where E is the effective field strength and μ is the equivalent dipole moment of the molecule normal to the field. The μE term is the energy supplied by the applied field to the channel molecule.

The energy relations may be clarified by reference to the diagram of Fig. 7. In the absence of the field, the energy of the open state is lower than that of the closed state (Fig. 7 A). Since $W_t - W_c < W_t - W_o$, the opening rate α is faster than the closing rate β , and most of the channels are open. When a large field is applied, the relative energy of the closed state becomes lower (Fig. 7 B). Now, since $W_t - W_o < W_t - W_c$, β is faster than α , and most of the channels assume the closed configuration. At an intermediate field where $V = V_0$, the energies of the two states are equal (Fig. 7 C). At this voltage, W_t

closed by a positive voltage, and are aligned arbitrarily at the energy of the transition state, which is also likely to be voltage dependent. The dotted lines in the diagrams where a field is applied show the relative energy relations in the absence of a field. Due to the differing voltage sensitivities of the rates, for a given voltage the activation energy of the closed state is changed less than that of the open state.

– $W_c = W_t - W_o$, α equals β , and the channels are equally distributed between the two states. Because $W_o = W_c$ at V_0 , the sum of the values of the voltage-dependent energy terms added to each state is equal to the difference in energy between the states in the absence of a field.

Magleby and Stevens (1972) have argued that μ is likely to be field independent for a protein molecule embedded in a membrane, and, therefore, may be considered a purely state-dependent parameter. We will propose below that only a fraction of the total field may be developed across a particular part of the channel molecule and that this fraction need not be the same in each state. We therefore allow E to be a state-dependent parameter as well. For each state i , then

$$W_i = U_i + \mu_i E_i. \quad (16)$$

The field strength may be defined by $E_i = V/M_i$ (assuming constant field for the portions of the voltage drop under consideration), where V is the trans-junctional voltage and M_i is the effective membrane thickness over which V is developed. Therefore, at a given V , we may write

$$W_i = U_i + V(\mu_i/M_i). \quad (17)$$

The units of μ_i/M_i are of charge, and this term represents the equivalent amount of charge acted on by the entire membrane voltage in state i . Substituting into Eqs. 14 and 15:

$$\alpha = \nu_\alpha \exp \{-[\Delta U_\alpha + V\Delta(\mu/M)_\alpha]/kT\} \quad (18)$$

and

$$\beta = \nu_\beta \exp \{-[\Delta U_\beta + V\Delta(\mu/M)_\beta]/kT\}, \quad (19)$$

where

$$\Delta U_\alpha = U_t - U_c,$$

$$\Delta U_\beta = U_t - U_o,$$

$$\Delta(\mu/M)_\alpha = \mu_t/M_t - \mu_c/M_c,$$

and

$$\Delta(\mu/M)_\beta = \mu_t/M_t - \mu_o/M_o,$$

where the dipole moments of open and closed states are of opposite sign. Since at V_0 the rate constants are equal, we may define a new constant, λ , as their value at V_0 :

$$\lambda = \nu_\alpha \exp \{-[\Delta U_\alpha + V_0\Delta(\mu/M)_\alpha]/kT\}$$

and

$$= \nu_\beta \exp \{-[\Delta U_\beta + V_0\Delta(\mu/M)_\beta]/kT\},$$

from which we obtain

$$\alpha = \lambda \exp [-\Delta(\mu/M)_\alpha(V - V_0)/kT]$$

and

$$\beta = \lambda \exp [-\Delta(\mu/M)_\beta(V - V_0)/kT],$$

or, as used above,

$$\alpha = \lambda \exp [-A_\alpha(V - V_0)] \quad (4)$$

and

$$\beta = \lambda \exp [A_\beta(V - V_0)], \quad (5)$$

where

$$A_\alpha = \Delta(\mu/M)_\alpha/kT \quad (20)$$

and

$$A_\beta = -\Delta(\mu/M)_\beta/kT. \quad (21)$$

Eqs. 4 and 5 are exponentials that accurately describe the voltage dependence of α and β in Fig. 4 *B*. The accuracy of this description requires that A_α and A_β be field independent over the range of interest, which would be true if each of the state-dependent variables, μ_i and M_i , were field independent. However, because A_α and A_β are proportional to the difference between two μ_i/M_i terms, equal field dependence of these terms would still result in field independence of A_α and A_β . Thus, an exponential relation between voltage and rate constant does not require that dipole moments be independent of field.

The contingent gating modeling of polarity reversal (Fig. 6*B*) requires that the opening rate α_1 be limited. Evidently, its value saturates for moderate negative voltages, thus deviating from exponential dependence on voltage. Moreover, it is possible that the closing rate β begins to saturate within the voltage range shown in Fig. 4 *B*; the experimental points lie below the exponential relation at the higher voltages. These possible deviations from exponential of the voltage dependence of the rate constants could be accounted for by a field dependence of the $\Delta(\mu/M)$ term. That is, outside the relatively narrow range of voltages for which rates were determined (5–25 mV for each polarity of voltage sensitivity), the dipoles may orient within the imposed voltage field such that $\Delta(\mu/M)$ changes. A recent report states that for high voltages applied to the ACh channel in one species of *Rana* (but not another), the logarithm of the closing rate is not linearly related to voltage, which can be interpreted as arising from the voltage dependence of the relevant $\Delta(\mu/M)$ term (Neher and Stevens, 1979).

The adequacy of the equations of the two-state kinetic model in describing the relation of time constant, steady-state conductance, and rate constants as a function of voltage of a single polarity shows that this system behaves kinetically as if it were first order. The different voltage dependencies of the two rate constants indicates that an applied voltage affects the energy of the closed state more than that of the open state. This finding is not unique (cf. Hodgkin and Huxley [1952 *c*] and Rudy [1978]).

Significance of the Equilibrium Modeling

The preceding development allows the determination of a number of parameters from the equilibrium data (Spray et al., 1981) that have physical meaning in the context of the two-state model. Since $G_\infty = \alpha/(\alpha + \beta)$, as noted in the Appendix, we can substitute the expressions for α and β of Eqs. 4 and 5 to obtain

$$G_\infty = 1/\{1 + \exp [A(V - V_0)]\}, \quad (22)$$

where

$$A = A_\alpha + A_\beta. \quad (23)$$

This equation is one form of the Boltzmann relation (Eq. 6 of the preceding paper [Spray et al., 1981]). Since the units of μ/M are units of charge, we can set $A = nq/kT$, where n is the equivalent number of electron charges q that move through the entire transjunctional field in the transition between open and closed states.

From the equilibrium data of the preceding paper, $A = 0.20$ to 0.25 , and it follows that $n = 5.0$ to 6.3 (Spray et al., 1981). This amount of charge movement corresponds to an e -fold change in the ratio of open to closed channels for every 4–5 mV. For the sodium channel, voltage sensitivity due to the movement of a similar amount of charge was modeled as the result of three separate but identical processes (Hodgkin and Huxley, 1952 *c*).

The difference in energy of the open and closed states ΔU may be readily calculated from V_0 and A . Since at V_0 the energies of open and closed states are equal, it follows from Eq. 17 that

$$\Delta U = U_o - U_c = (\mu_o/M_o + \mu_c/M_c)V_0,$$

and, substituting from Eqs. 20, 21, and 23

$$\Delta U = AV_0kT. \quad (24)$$

Since V_0 is 14–15 mV (Spray et al., 1981), ΔU is ~ 2 kcal/mol.

An Explicit Model

The combination of morphological and physiological data allows the proposal of a more detailed model. From the structure of the gap junction (cf. Bennett and Goodenough [1978]) and the symmetry of the voltage dependence of the conductance, it is plausible that there are two gates per channel, one in each membrane but oppositely oriented. The two-gate hypothesis is consistent with the effects of voltage reversal, where each channel appears to pass through the open state before it closes. A simple mechanism that provides for contingent gating as indicated by Fig. 6 would be one in which each gate senses local voltage drop along its portion of the channel. When both channels were open, the voltage across each gate would be something less than half the total voltage; when one gate was closed, the entire transjunctional voltage would be developed across that gate (for the closed gate, $E_c/E_o \geq 2$), and no voltage would be seen by the open gate. The greater voltage sensitivity of the closing

rate indicates that $\mu_o E_o$ is about two times greater than $\mu_c E_c$ (see Eqs. 6 and 7). Because for each gate E_c is likely to be greater than E_o by a factor of at least two, μ_o should be more than four times as great as μ_c . In other words, the difference in dipole moments between open and transition states should be more than four times as great as that between closed and transition states. In the diagram of Fig. 8 we have indicated this difference by a greater redistribution of charge in moving from transition to open state than in moving from transition to closed state. The diagram arbitrarily shows zero dipole moment for the gating charges in the transition state. Because differences in charge

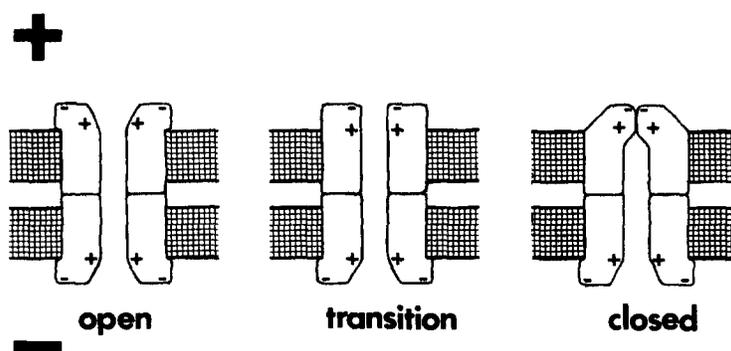


FIGURE 8. Proposed model for gating of the gap junction channel. Each channel is composed of two oppositely oriented hemichannels, each containing a gating structure sensitive to local voltage within the channel. The action of a large voltage of appropriate polarity on dipoles of the hemichannel affects the energy of the molecule such that it is more stable in the conformation that closes the channel. Furthermore, when in the closed conformation, the component of the dipole normal to the field is reduced, so that the energy contributed to a closed hemichannel by a field of a given strength is less than that contributed to an open channel. This accounts for the greater voltage sensitivity of the closing rate. The orientation of the dipole in the transition state is arbitrarily drawn parallel to the field.

distribution are the relevant point, this assumption causes no loss of generality. Also, for the figure, we have assumed that the gate that closes is in the membrane on the side of positive voltage. We have no evidence as to which gate is affected by a given polarity of voltage.

In the modeling of contingent gating that follows from the diagram of Fig. 8, we have implicitly assumed that the channels closed completely. If conductance in the closed state had a nonzero value (which could be no greater than the "voltage-insensitive" component G_{\min} , i.e., <5%), a small fraction of the transjunctional voltage would be developed across the open gate when the gate in series was closed. The field seen by the open gate would not be zero, and the open gates would therefore close more rapidly than predicted by contingent gating. Similarly, the field at the closed gate would be less than if

it closed completely, but this difference would already have been included in rates calculated from voltage sensitivity of one polarity. We have yet to calculate how large the effect of incomplete closing would be, but we doubt that we could detect it with our present measurements given the small value of the residual conductance.

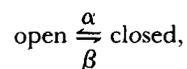
One might ask whether both gating mechanisms of the channel could sense the transjunctional voltage drop through the walls of the channel molecule, even when one gate was closed. This possibility seems unreasonable from the geometric relations of the hemichannels in each membrane; the wall is narrow compared with the length of the molecule (Makowski et al., 1977; Unwin and Zampighi, 1980). Furthermore, as shown in the preceding paper (Spray et al., 1981), there is no effect on the steady-state conductances of voltages between the inside and outside of the cells. Such voltages would be likely to produce a field component within the walls of the channels that would act on voltage sensors within them. Calculations indicate that the access resistance to the portion of the channel molecule exposed to the extracellular space between the coupled cells is at least two orders of magnitude less than the resistance of an area of cell membrane of the same size as the junction. Therefore, the potential between the extracellular space within the junctions and the interior of the cells would have been changed during this set of experiments. We conclude that the gates respond only to voltages within the channels.

The two-gate model is not the only possibility for a three-state system that accounts for the effects of voltage reversal. We can conceive of a turnstile or toggle model in which a single gating entity has a central open position and oppositely oriented closed positions. Such a structure might be more plausible for voltage-dependent channels in single membranes that exhibit contingent gating. VDAC (Schein et al., 1976) and EIM (Ehrenstein et al., 1978) may show such behavior, but detailed studies of the kinetics of conductance change with voltage reversal for these channels have not been reported. The model we propose is attractive because of membrane symmetry, although there could be a single gating structure formed at the junction of the hemichannels. Unwin and Zampighi (1980) have proposed a model for closing of gap junction channels by a torsion that shuts the cytoplasmic end of the channel. Such a torsion could be produced by a transjunctional voltage provided the dipole changes were appropriate. For convenience, the charge movements diagrammed in Fig. 8 are in the radial rather than in the circumferential direction suggested by the Unwin and Zampighi model. Details of the mechanism may come with greater knowledge of the molecular structure.

APPENDIX

Equations Derived from First-Order Kinetics

Consider a population of channels that can exist in either of two states, an open state of high conductance and a closed state of low conductance:



where α and β are rate constants that are functions of voltage independent of prior conditions.

Let n be the fraction of open channels and $1 - n$ the fraction of closed channels. If kinetics are first order, the rate of change of n at a constant voltage is given by

$$dn/dt = -\beta n + \alpha(1 - n). \quad (\text{A1})$$

The solution of this equation is

$$n = n_{\infty} + (n_0 - n_{\infty}) \exp(-t/\tau), \quad (\text{A2})$$

where n_0 and n_{∞} are the initial and final values of n , respectively, and τ is the time constant. The parameters n_{∞} and τ are related to α and β as follows:

$$n_{\infty} = \alpha/(\alpha + \beta) \quad (\text{A3})$$

and

$$\tau = 1/(\alpha + \beta). \quad (\text{A4})$$

If γ_o and γ_c are conductances of single open and closed channels, respectively, and N is the total number of channels, the conductance g is given by

$$g = \gamma_o n N + \gamma_c (1 - n) N. \quad (\text{A5})$$

The maximum conductance g_{\max} and the minimum conductance g_{\min} will be $\gamma_o N$ and $\gamma_c N$, respectively. From Eqs. A2 and A5

$$g = g_{\infty} + (g_0 - g_{\infty}) \exp(-t/\tau), \quad (\text{A6})$$

where g_0 and g_{∞} are initial and final values of g .

We may define G as the voltage-sensitive component of the conductance normalized to its maximum value:

$$G = (g - g_{\min})/(g_{\max} - g_{\min}). \quad (\text{A7})$$

And, substituting in terms of single channel conductances,

$$G = (\gamma_o n N + \gamma_c (1 - n) N - \gamma_c N)/(\gamma_o N - \gamma_c N) = n. \quad (\text{A8})$$

Thus, the normalized value of conductance G can be substituted for the fraction of channels open. In particular,

$$G = G_{\infty} + (G_0 - G_{\infty}) \exp(-t/\tau) \quad (\text{A9})$$

If there is a voltage-insensitive leakage component g_1 in parallel with the voltage-sensitive component, this value must be added to each term in Eq. A6. Thus, $g + g_1 = (g_{\infty} + g_1) + [(g_0 + g_1) - (g_{\infty} + g_1)] \exp(-t/\tau)$, which can be written $g^* = g_0^* + (g_0^* - g_{\infty}^*) \exp(-t/\tau)$, where asterisks represent observed values, e.g., $g^* = g + g_1$. The equation has the same form as Eq. A6, and, therefore, we cannot distinguish from conductance measurements alone one set of voltage-sensitive channels that close incompletely from two sets of parallel channels, one of which is voltage sensitive and one of which closes completely.

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