

Equilibrium Properties of a Voltage-dependent Junctional Conductance

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ABSTRACT The conductance of junctions between amphibian blastomeres is strongly voltage dependent. Isolated pairs of blastomeres from embryos of *Ambystoma mexicanum*, *Xenopus laevis*, and *Rana pipiens* were voltage clamped, and junctional current was measured during transjunctional voltage steps. The steady-state junctional conductance decreases as a steep function of transjunctional voltage of either polarity. A voltage-insensitive conductance <5% of the maximum remains at large transjunctional voltages. Equal transjunctional voltages of opposite polarities produce equal conductance changes. The conductance is half maximal at a transjunctional voltage of ~15 mV. The junctional conductance is insensitive to the potential between the inside and outside of the cells. The changes in steady-state junctional conductance may be accurately modeled for voltages of each polarity as arising from a reversible two-state system in which voltage linearly affects the energy difference between states. The voltage sensitivity can be accounted for by the movement of about six electron charges through the transjunctional voltage. The changes in junctional conductance are not consistent with a current-controlled or ionic accumulation mechanism. We propose that the intramembrane particles that comprise gap junctions in early amphibian embryos are voltage-sensitive channels.

INTRODUCTION

Cells in many embryonic and adult tissues are coupled by intercellular pathways that are permeable to ions and small molecules. Gap junctions are generally accepted as the structures that mediate the coupling (cf. Bennett and Goodenough [1978]). Recent studies have shown that the permeability of gap junctions to charge carriers and small molecules can be diminished by various experimental treatments. The underlying cellular mechanisms may operate in vivo to determine the distribution of biologically significant molecules (or electrical potential) among a population of cells and, during embryogenesis, for example, might be involved in determination and differentiation.

We have found that the junctional conductance between amphibian blastomeres is voltage dependent (Harris, 1979; Spray et al., 1979). Initially, we observed that junctional conductance decreased over a period of a few hundred milliseconds when sufficient current was applied to one cell of a

coupled pair. Because of difficulties in determining equilibrium and kinetic features of the voltage-dependent conductance when membrane potential was changing in both of the coupled cells, the junctional membrane was voltage clamped and junctional currents were measured directly.

The present paper, the first of a series, describes the conductance of gap junctions at equilibrium as a function of transjunctional voltage. The second paper (Harris et al., 1981) describes the kinetics of the conductance changes and the implications for mechanisms gating the conductance of the junctional membrane. A subsequent paper will interpret the uncoupling seen in current-clamp conditions in terms of the characterization of the junctions developed in the first two papers and will explore the possible biological importance of the voltage dependence. For convenience, and because it is likely to be correct, we will assume in all three papers that the voltage-dependent conductance is mediated by channels in the junctional membrane. The channels are presumed to correspond to the intramembrane particles seen in freeze-fracture replicas of the gap junctions (Hanna et al., 1980). Preliminary communications of some of these data have been published (Harris et al., 1978 and 1979; Spray et al., 1978; Bennett et al., 1978 *b*).

METHODS

After removal of the jelly coat and vitelline membrane with fine forceps, pairs of blastomeres were isolated from axolotl (*Ambystoma mexicanum*) or anuran (*Xenopus laevis* and *Rana pipiens*) mid-cleavage stage embryos (stages 6–8; Harrison, 1969) with glass dissecting needles. Cell pairs were placed in physiological saline (Niu-Twitty solution; Flickinger, 1949) containing 58.2 mM NaCl, 0.7 mM KCl, 0.3 mM $\text{Ca}(\text{NO}_3)_2$, 0.8 mM MgSO_4 , 0.8 mM Na_2HPO_4 , 0.1 mM KH_2PO_4 , and 0.4 mM NaHCO_2 adjusted to pH 7.8 with NaOH, to which was added up to 0.1% colchicine to inhibit mitosis. After isolation cell pairs were allowed to stabilize for at least 0.5 h before experimentation was started. The cells ranged in diameter from 40 to 100 μm . All stages and species showed similar electrical properties; all data shown is from axolotl, unless otherwise noted.

A double voltage-clamp procedure was devised to directly measure junctional currents (Fig. 1). Each cell of a coupled pair was impaled with two microelectrodes (filled with 3 M KCl; resistances, 5–20 $\text{M}\Omega$), one for recording voltage and one for delivering current. Each of the two cells was independently voltage clamped to the potential recorded on penetration.

The current supplied by one of the clamps was measured as the voltage developed across a 1- $\text{M}\Omega$ resistor in the current injection pathway. Total current entering the bath was measured in some experiments by a virtual ground current probe. Usually, voltage steps were delivered to one of the cells, cell B, while the other cell, cell A, was held at a constant potential. In this situation, any current flowing through the junctions from cell B into cell A associated with the voltage step (junctional current, I_j) was exactly matched by current of opposite polarity injected into cell A, supplied by its clamp to keep its membrane potential constant. Thus, this clamp current was equal to $-I_j$, and gave a direct measure of junctional conductance (g_j) when divided by the transjunctional voltage (V_j). The settling time of the clamping system was <3 ms.

The relations between the conductances, voltages, and measured currents are derived below, where V_a and V_b are the respective cell voltages referred to a common

holding potential, I_a and I_b are the currents supplied by the voltage clamps in addition to holding current, and g_a and g_b are the conductances of the nonjunctional membranes. We directly measured I_a as the voltage drop across the resistor in series with the current electrode in cell A. Total current measured by the virtual ground (I_t) is the sum of the two clamping currents: $I_t = I_a + I_b$. Defining current flow from cell A

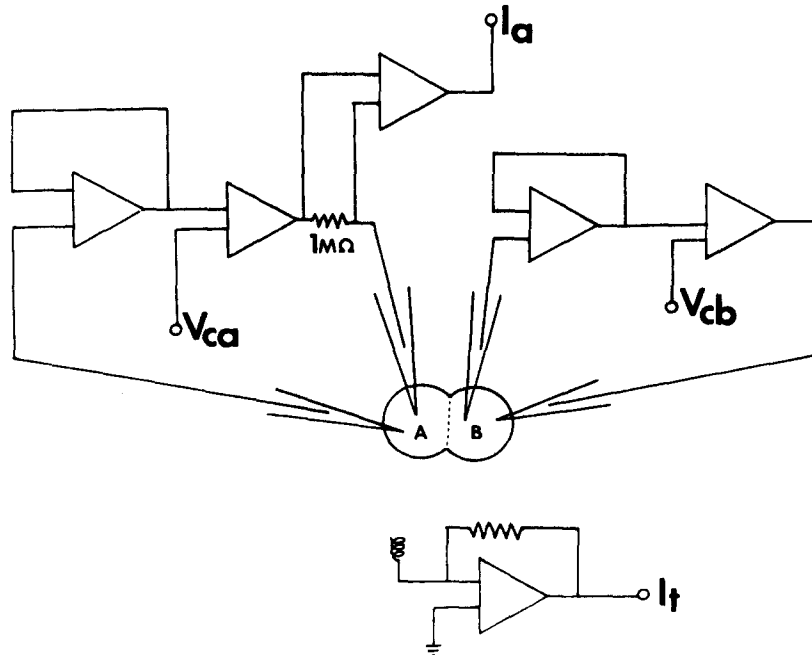


FIGURE 1. Voltage-clamp circuits for measuring junctional current. Each cell of an isolated coupled pair was impaled by two microelectrodes for measurement of voltage and delivery of current. Each cell was clamped to the potential recorded after impalement, and the holding potentials were adjusted to zero transjunctional voltage (see Methods). For most experiments, the voltage in cell B was stepped to desired potentials, and the junctional current was measured as that supplied to cell A by its clamp to maintain its membrane potential constant. This current, I_a , was measured in the current-injection pathway as the voltage across the labeled resistor. The junctional conductance is I_a divided by the magnitude of the transjunctional voltage step. Total current, I_t , entering the bath was monitored in some cases by a virtual ground current probe (lower circuit diagram). Command pulses to cells A and B were delivered at V_{ca} and V_{cb} , respectively.

to cell B as positive: $I_j = V_j g_j$, where $V_j = V_a - V_b$. Currents supplied to cells A and B are the sum of currents through nonjunctional and junctional conductances:

$$I_a = V_a g_a + (V_a - V_b) g_j \quad (1)$$

and

$$I_b = V_b g_b + (V_b - V_a) g_j. \quad (2)$$

When V_a is held at zero and V_b is stepped, $I_a = -V_b g_j$ and $V_j = -V_b$; thus $I_j = I_a$ and

$$g_j = I_a / V_j \quad (3)$$

Although we have not used the measurement in these studies, the current through the nonjunctional membrane of cell B is I_t when V_a is held at zero.

Coupled cells that have different intrinsic resting potentials will have different membrane potentials even when coupled, provided the coupling coefficient is less than unity. If two such cells are clamped to their membrane potentials as described above, the junctional membrane will be clamped to a nonzero potential, and a steady-state junctional current (I_h) will exist. This current is driven by the difference in intrinsic resting potentials of the nonjunctional membranes of the cells. At the termination of a voltage step that makes the junctions low conductance, the holding potentials are offset from one another, but because little current can flow through the junctions, a current of magnitude near I_h is provided to each cell by its voltage clamp. As the junctional conductance recovers, the current provided by the clamps decreases, and the nonjunctional membranes of the two cells provide the current as before. A change in the current being supplied to the cells by the clamps during recovery of junctional conductance, therefore, indicates that the cells are not clamped to the same potential. In principle, to clamp the junctional membrane to zero voltage, the holding potential for cell A could be adjusted so the current supplied to cell A was zero when the junctional conductance was very low. Then the holding potential for cell B could be adjusted so that current did not change during recovery of junctional conductance. Thus, cell B would be held at the potential of cell A, and the transjunctional potential would be zero. In practice, the holding potential of cell A was adjusted so that I_a did not change after termination of a pulse. In this way, both cells were clamped at a potential between their intrinsic resting potentials, and the holding potential across the junction was zero.

Voltages and current were displayed on a Tektronix, Inc. (Beaverton, Oreg.) storage oscilloscope and photographed with a Grass Instrument Co. (Quincy, Mass.) camera. In some cases the traces were recorded on paper by a Gould Inc. (Cleveland, Ohio) Brush recorder (high frequency cutoff, about 55 Hz). Computational analysis was performed on a Hewlett-Packard Co. (Palo Alto, Calif.) model 9830A programmable calculator. Data were digitized by hand with a Hewlett-Packard model 9864A digitizer. In some cases, junctional current records were filtered above 200 Hz by a three-pole active filter (A.P. Circuit Corp., New York).

RESULTS

Linearity of Nonjunctional Membrane

For most experiments, junctional current was measured as the current supplied to cell A while its potential was held constant, and voltage dependence of nonjunctional conductance would have had no effect on measurements of junctional properties. However, for those experiments in which cell A was stepped, and for interpretation of current-clamp experiments (Spray et al., 1979), it was important to establish the current-voltage relation of nonjunctional membrane. The current-voltage relation in voltage-clamped, isolated blastomeres was linear over a range of at least ± 50 mV from the holding potential (Fig. 2). Furthermore, no tail currents were observed when the potential of isolated blastomeres was returned to the holding potential from

voltage steps of either sign. Thus, neither resting potential nor conductance was affected by voltages in this range.

Junctional Currents during Transjunctional Voltage Steps

When both cells of a coupled pair were voltage clamped to a common voltage (near their resting potentials) transjunctional voltage was zero. When small voltage steps were then delivered to one cell, the junctional current remained

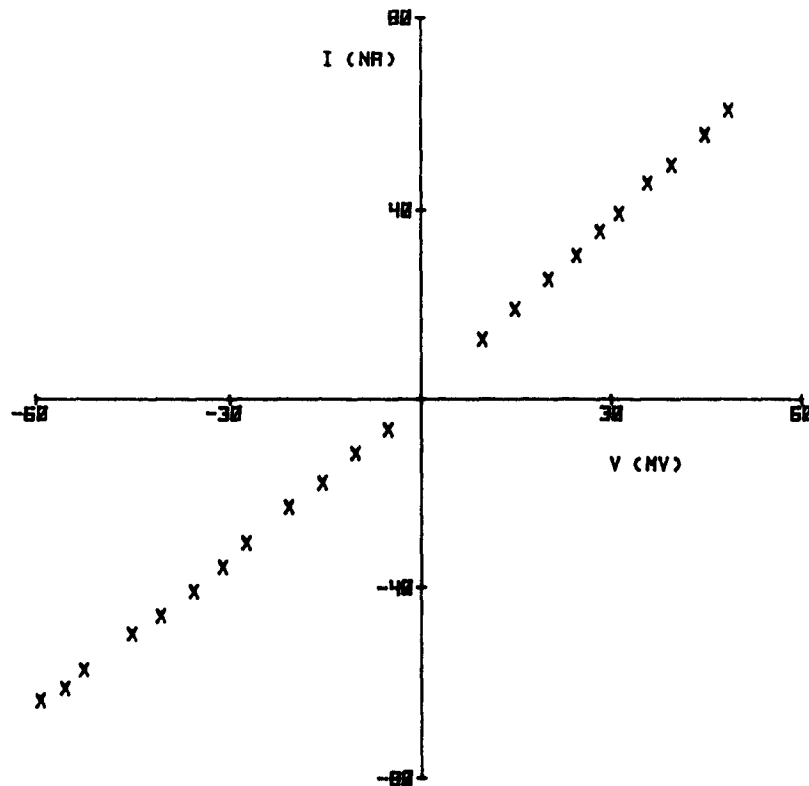


FIGURE 2. Current-voltage relation of a single blastomere. The cell was clamped to its resting potential, and its voltage was stepped from this potential with command pulses of both polarities. Membrane currents were constant after capacitative transients, and their values are plotted as a function of the voltage steps. This relation is essentially linear over a wide range.

nearly constant for the duration of the step (Fig. 3 A_{1-2} B_{1-2}). When larger transjunctional voltage steps were given, the junctional current began at a higher value and decayed over several hundred milliseconds to a lower, steady-state level (Fig. 3 A_{3-5} and B_{3-5}) that was constant for the duration of the voltage steps (tested for as long as 100 s). The decline of junctional current with constant transjunctional voltage reflects the decline of junctional con-

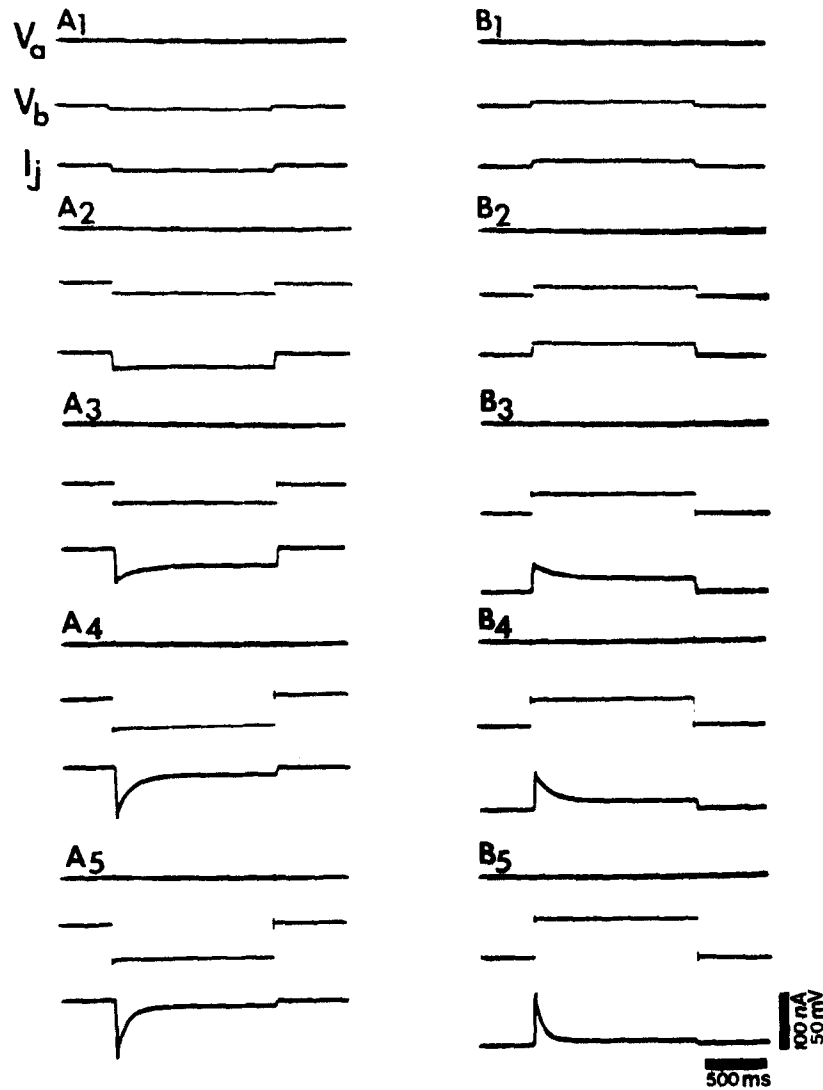


FIGURE 3. Effects of magnitude and polarity of transjunctional voltage steps on junctional conductance. Each cell of a coupled pair was voltage clamped, and cell B was stepped to various potentials (V_b , middle trace). Junctional current (I_j , lower trace) is the current delivered to cell A to maintain its potential constant (V_a , upper trace). Junctional current was constant for small voltages but fell to new steady-state values in response to moderate hyperpolarizing (column A) and depolarizing (column B) voltage steps. The fall in current was more rapid and to a lower level for larger steps (compare, for example, A_3 and A_5). The effects of voltage steps of opposite polarities were symmetrical.

ductance. The junctional conductance changed symmetrically in response to voltage steps of either polarity (Fig. 3 A_{3-5} and B_{3-5}). It is apparent from these records that both the magnitude and time-course of the conductance changes depended upon the size of the transjunctional voltage step. After termination

of a transjunctional voltage step, junctional conductance recovered over several hundred milliseconds (Harris et al., 1981). The time-course of the conductance changes and its voltage dependence are treated in the following paper (Harris et al., 1981).

The steady-state junctional conductance, g_{∞} , showed a pronounced voltage dependence. The steady-state conductances of the experiment in Fig. 3 are plotted as a function of transjunctional voltage in Fig. 4. Beyond a few millivolts, the steady-state conductance fell steeply, and the change was symmetrical around zero transjunctional voltage.

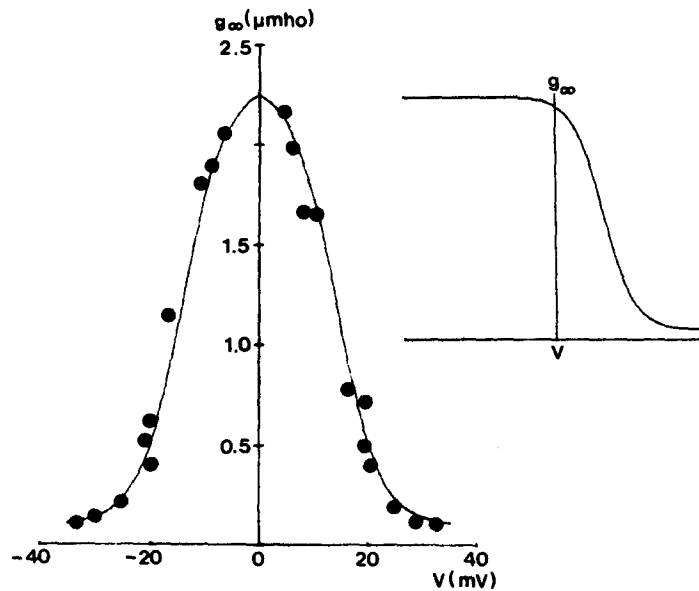


FIGURE 4. The steady-state conductance-voltage relation. The steady-state junctional conductances (g_{∞}) for the data of Fig. 3 are plotted as a function of transjunctional voltage. The junctional conductance decreased as a steep function of transjunctional voltage. The conductance decreased to $\sim 5\%$ of its maximum value with a transjunctional voltage of ± 30 mV. The conductance changes are equal for equal voltages of opposite polarity. The points are well fit by the relation given in Eq. 7 utilizing parameters determined as in Fig. 6 and equal changes for voltages of either sign. The *inset* shows a plot of Eq. 7 for the voltage sensitivity of a single polarity with comparable parameters.

Even with large transjunctional voltage steps, the junctional conductance did not reach zero. A small component a few percent of the original conductance remained when transjunctional voltages > 40 mV were applied (Fig. 5).

Effect of Transjunctional Voltage at Various Displacements from the Resting Potentials

The steep relationship of steady-state junctional conductance to transjunctional voltage is insensitive to the potential between cytoplasm and exterior. In the experiment of Fig. 5, the voltage in cell B was stepped to various levels

(*upper inset*) and steady-state conductance was determined as for Fig. 4. Then the voltage in cell A was stepped over a similar range while the voltage in cell B was stepped to either a large positive or negative value with a longer lasting pulse (Fig. 5, *lower two insets*). The step in cell A was sufficiently long for

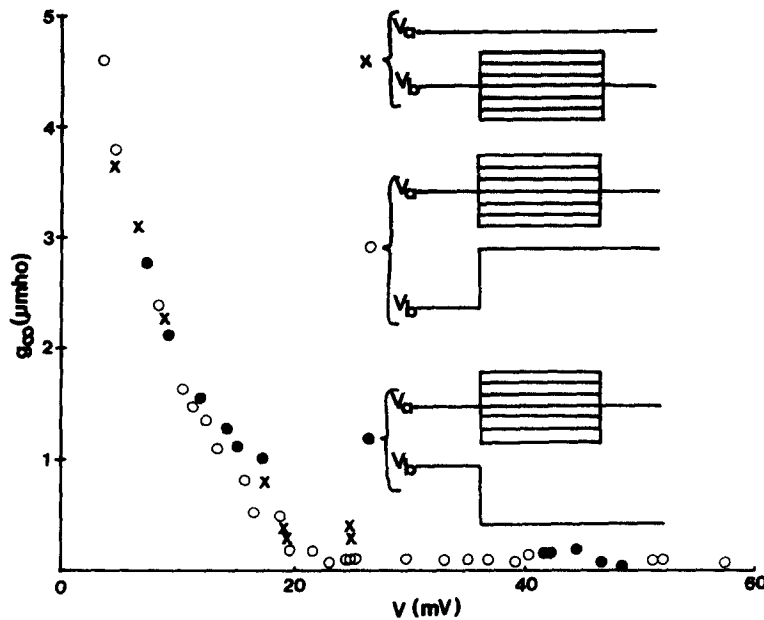


FIGURE 5. Conductance-voltage relation of the junctions at various voltages with respect to the external medium. To determine this relation, voltage steps were delivered to a pair of cells such that the same transjunctional voltage occurred at different potentials with respect to the external medium. Three stimulus patterns were employed: cell B was stepped to various potentials (*upper inset*) in the same way as in previous experiments (Fig. 3), and cell B was given longer lasting, 28-mV steps of positive and negative polarity while cell A was stepped to various potentials (*lower two insets*). While both cells were being stepped, the junctional conductance reached a steady-state level. Just after the termination of the shorter pulse, the instantaneous conductance of the junction was determined from the current provided to cell A to hold its potential constant when the transjunctional potential was ± 28 mV. These conductances are plotted as a function of absolute value of the transjunctional voltage. The \times 's, open circles, and filled circles indicate the different stimulus patterns as indicated in the *insets*. The three conductance-voltage relations superimpose, indicating that junctional conductance is determined solely by the transjunctional voltage over this range. Also, it is evident that the small voltage-insensitive component of the junctional conductance remains constant at large transjunctional voltages.

junctional conductance to reach its steady-state value. The current supplied to cell A, however, included current through its nonjunctional conductance because V_a was also being stepped. When the shorter pulse to cell A was terminated and only the longer pulse to cell B remained, the "instantaneous"

junctional conductance was the steady-state conductance resulting from the preceding voltage steps which was determined as in previous experiments. For a step to cell B of +28 mV and graded steps up to ± 28 mV to cell A, junctional conductance was measured for transjunctional voltages from zero to 56 mV, cell B positive. For a step to cell B of -28 mV and graded steps up to ± 28 mV to cell A, junctional conductance was measured for transjunctional voltages from zero to 56 mV, cell B negative. When plotted as a function of absolute transjunctional voltage, both of these relations are superimposable with each other and with the conductances determined when only cell B was stepped. Given the demonstrated linearity of the nonjunctional membrane, we conclude that over this voltage range, steady-state junctional conductance is determined solely by transjunctional voltage and is unaffected by the potential between the inside and outside of the cells.

Modeling of the Equilibrium Data

The voltage dependence of the steady-state junctional conductance can be modeled if we assume that the conductance is mediated by a population of channels, each of which exists in either a high-conductance or a low-conductance state. We further assume that the energy difference between the states is a linear function of transjunctional voltage of each sign and that transitions between the states are reversible and first order. According to the Boltzmann relation, the equilibrium ratio of channels in high- and low-conductance states is related exponentially to the energy difference between those states. In the system described here, the voltage field across the junctions is presumed to act on charges or dipoles in the channel molecules, and to contribute to the energy difference, thus influencing the equilibrium distribution of the channels between the two states. If this simple construct holds, the fraction of open channels, n , divided by the fraction of closed channels, $1 - n$, should be given by

$$n/(1 - n) = \exp [-A(V - V_0)], \quad (4)$$

where V is the transjunctional voltage, V_0 is the transjunctional voltage at which the conductance is half maximal ($n = 1/2$), and A is a constant expressing the voltage sensitivity (cf. Ehrenstein et al. [1970], Schein et al. [1976], and Harris et al. [1981]).

If one assigns single-channel conductances to the high- and low-conductance states, it follows that

$$n = (g_\infty - g_{\min})/(g_{\max} - g_{\min}) \quad (5a)$$

and

$$1 - n = (g_{\max} - g_\infty)/(g_{\max} - g_{\min}), \quad (5b)$$

where g_∞ is the steady-state conductance and g_{\max} and g_{\min} are the maximum and minimum conductances, respectively (see Appendix of Harris et al. [1981]).

Thus, from Eqs. 4 and 5,

$$(g_\infty - g_{\min})/(g_{\max} - g_\infty) = \exp [-A(V - V_0)] \quad (6)$$

(This equation also holds when there is a fixed conductance in parallel with the voltage-sensitive conductance; see Appendix of Harris et al. [1981]). Therefore, if the model is appropriate, a plot of $\ln [(g_{\infty} - g_{\min}) / (g_{\max} - g_{\infty})]$ vs. voltage should be linear and determine the values of A by its slope and of V_0 by its voltage axis intercept. The data of Fig. 4 are plotted in this manner in Fig. 6, and a good fit to a straight line is obtained. The sensitivity to voltages of each polarity was such that the junctional conductance was significantly less than g_{\max} at $V = 0$. Therefore, g_{\max} was not a measurable parameter and was evaluated by the curve-fitting procedure outlined in the Appendix. In this procedure, the effects of voltage on the conductances were assumed to be symmetrical around zero transjunctional voltage.

The values of A and V_0 determined from Fig. 6 are $A = 0.25$ and $V_0 = 14.1$

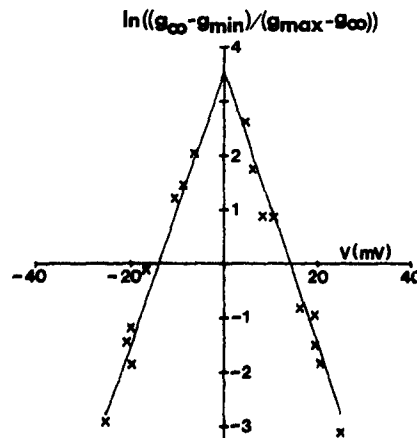


FIGURE 6. Plot of $\ln [(g_{\infty} - g_{\min}) / (g_{\max} - g_{\infty})]$ as a function of transjunctional voltage. This relation should be linear for the proposed model (see text). The data are well fit by straight lines calculated by linear regression. The slope A reflects the voltage sensitivity of the transition between the two conductance states. The voltage-axis intercept (V_0) is a function of the voltage sensitivity and the energy difference between these two states in the absence of a field. In this example, $A = 0.25$ and $V_0 = 14.1$ mV.

mV. The constant A can be expressed as nq/kT (Ehrenstein et al., 1974 and 1977; Harris et al., 1981), where n is the equivalent number of electron charges q that move through the entire membrane voltage in order to effect the state change and kT has its usual significance. For these data, $n = 6.25$.

The model can also be tested by its ability to predict the form of the conductance-voltage relation on linear axes. Solving Eq. 6, for g_{∞} :

$$g_{\infty} = \{(g_{\max} - g_{\min}) / (1 + \exp[A(V - V_0)])\} + g_{\min}. \quad (7)$$

With the values of A and V_0 determined from Fig. 6, g_{∞} is plotted as a function of voltage according to Eq. 7 in Fig. 4. As for Fig. 6, conductance symmetry was assumed for voltages of either sign. (A plot for voltage sensitivity of a

single polarity calculated with comparable parameters is shown in Fig. 4, *inset*.) The fit between the data and Eq. 7 is excellent. Similar fits were obtained for data from at least 10 other experiments, with A ranging from 0.20 to 0.25 and V_0 ranging from 14 to 15 mV.

Junctional Conductance Is Not Current Dependent

An alternative to voltage dependence that should be considered is current dependence, due, for example, to the accumulation of ions or a polarization effect. The improbability of current dependence may be seen from voltage-clamp records which show that, at small transjunctional voltages, larger steady-state junctional currents flow than at much larger voltages after

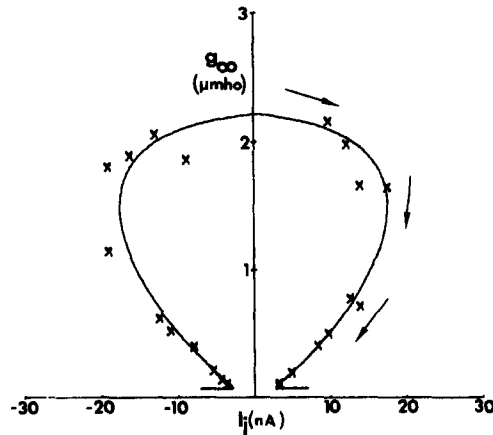


FIGURE 7. Relation of junctional conductance to junctional current. Junctional conductance is plotted as a function of junctional current utilizing the data of Figs. 4 and 6. The smooth curve is according to Eq. 8. Over a significant range of junctional current, the conductance is not single valued. The arrows indicate the direction of increasing voltage.

junctional conductance has decreased (Fig. 3). Furthermore, the conductance-voltage relation of Eq. 6 can be solved for current I_j in terms of conductance to give

$$I_j = (g_\infty/A) \{ \ln [(g_{\max} - g_\infty)/(g_\infty - g_{\min})] + AV_0 \}. \quad (8)$$

When this relation is plotted as conductance vs. junctional current, more than one value of conductance is possible over a significant range of current (Fig. 7). The multiple values of possible conductance as a function of current lead to the characterization of the conductance as voltage rather than current dependent. The lower values of conductance (limited by g_{\min}) were not reached in this experiment because of the larger transjunctional voltages required.

Voltage-dependent Junctions in Other Amphibian Embryos

Similar voltage dependence of junctional conductance was obtained from early embryos of *Rana pipiens* and *Xenopus laevis*. The conductance-voltage relations for these anuran species (Fig. 8 *A* and *B*) and the parameters V_0 and A obtained from fitting to Eq. 6 are similar to those for *Ambystoma*.

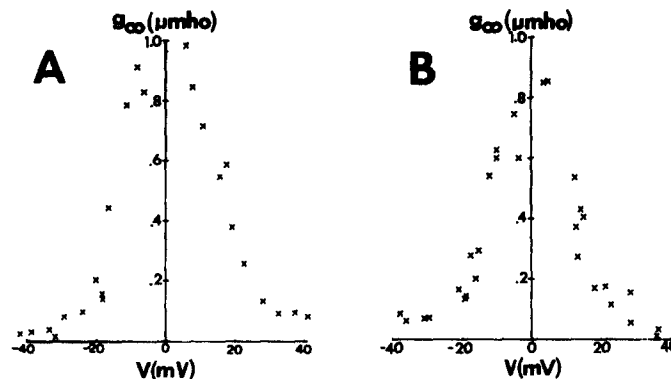


FIGURE 8. Steady-state conductance-voltage relations of junctions between *Rana* and *Xenopus* blastomeres. These relations for *Rana* (*A*) and *Xenopus* (*B*) blastomeres indicate a voltage dependence of junctional conductance similar to that for *Ambystoma*. In the plots shown the constants are $V_0 = 16$ mV and $A = 0.19$ for *Rana*, and $V_0 = 13$ mV and $A = 0.19$ for *Xenopus*.

DISCUSSION

Characterization of the Conductance Elements

Considerable evidence indicates that electrotonic coupling between cells is in most cases mediated by gap junctions. The coupling pathway has been characterized as a hydrophilic channel formed by a dimer of the particles seen in freeze-fracture replicas of gap junctions, one particle embedded in each membrane lipid bilayer (Bennett and Goodenough, 1978). In transmission electron micrographs and freeze-fracture replicas of gap junctions between axolotl blastomeres, the gap junctions and constituent particles are not morphologically distinguishable from those observed in other tissues (Hanna et al., 1980). As is also true of the rectifying electrotonic synapses in the crayfish (Hanna et al., 1978), no morphological correlate of voltage sensitivity has been identified. Furthermore, the *Ambystoma* junctions are permeable to the fluorescent dye Lucifer Yellow (mw443), a molecule that is near the maximum size that permeates other gap junctions (Stewart, 1978; Bennett et al., 1978 *c*; Kater and Galvin, 1979), and the permeability in *Ambystoma* is voltage dependent (Spray et al., 1979). The unremarkable morphology and permeability of gap junctions in *Ambystoma* embryos suggests that the basic structure of the junctional channels is similar to that of these junctions in

other tissues. The structural difference conferring voltage sensitivity on the channel has little effect on permeability or structure as determined by present techniques.

The junctional conductance remaining at large transjunctional voltages is as much as 5% of the maximum junctional conductance. This residual conductance may arise from a small population of voltage-independent junctional channels, a nonnegligible conductance of the channels when in the low-conductance state or coupling across extracellular space between the closely apposed membranes of the cells. Transient exposure to CO₂-equilibrated saline decreases junctional conductance to levels below that remaining at large transjunctional voltages in normal saline. The effect of CO₂ is reversible and occurs with no obvious change in the abutting membranes of the cells (Spray et al., 1981). Therefore, coupling by way of extracellular space, which should not be affected by CO₂, cannot fully account for the voltage-independent component of junctional conductance. Either of the other two explanations remain as possibilities. As shown in the following paper (Harris et al., 1981), the form of the conductance-voltage relation is the same for voltage-dependent channels whose low-conductance state is nonzero as for voltage-independent channels in parallel with voltage-dependent channels that close completely. The two mechanisms presumably would be differentiable by permeability measurements of the low-conductance junctions, but long time constants of equilibration would make such determinations difficult (Bennett et al., 1978 *c*).

The Parameter Acting on the Conductance Elements

Two experimentally produced conditions reported to reduce gap junctional conductance are high concentrations of intracellular ionized calcium (Rose and Loewenstein, 1975; Stern et al., 1980) and intracellular pH a few tenths of a unit below normal (Turin and Warner, 1977; Bennett et al., 1978 *a* and 1978 *b*; Spray et al., 1981). We must consider the possibility that the effect of voltage on junctional conductance is secondary to a voltage-induced increase in intracellular calcium or hydrogen ions at the junctional membrane.

Furthermore, buffering systems of calcium and hydrogen ions are interdependent, and an increase in the concentration of free ion of either species could lead to an increase in the concentration of the other through competition for binding or sequestering sites. For example, injection of calcium into *Helix* neurons has been shown to cause a decrease in intracellular pH (Meech and Thomas, 1977), and cytoplasmic acidification of *Xenopus* blastulae causes a slight increase in intracellular free calcium (Rink et al., 1980).

An effect on the junctional membrane due to voltage-dependent calcium entry through the nonjunctional membrane is ruled out by the observations that voltage steps of both polarities cause identical changes in junctional conductance, and that the junctional conductance is insensitive to nonjunctional voltages. A calcium-releasing structure coupled to the surface membrane similar to sarcoplasmic reticulum can also be rejected on this basis. Also, the modest transjunctional voltages required to make the junctions high resistance

are unlikely to produce voltage gradients sufficient to affect intracellular calcium-releasing organelles. We conclude that junctional voltage does not act on the junctional membrane through calcium entry or release from intracellular stores.

Current flowing through the junctional membrane during a voltage step could cause the iontophoretic accumulation at the junctional membrane of ions that affect its conductance. The accumulation would arise from differences in the ability of ions of differing charge or size to permeate the channel. For example, calcium or hydrogen ions would accumulate near the junctional membrane if they were less permeable than other charge carriers. This is unlikely in view of the permeability of gap junctions to much larger ions. The charged (and presumably impermeant) macromolecules that buffer calcium and hydrogen ions in the cytoplasm might accumulate at the junctional membrane. If the concentration of these molecules were sufficiently great and the ionization constants favorable, the local calcium level or pH would be altered. Furthermore, an accumulation of less permeant ions at the junctions could change the local space charge sufficiently to reduce the concentration of permeant ions near the membrane. Finally, the current flowing through the junctional membrane could produce a local membrane voltage opposite to the imposed voltage by changing the concentrations of ions of differing permeability in the channel.

In an accumulation or polarization mechanism, the differences in ionic permeability will give rise to maintained concentration gradients near the membrane only if there is maintained current through the junction. If the current falls, the voltage gradient near the membrane is reduced, the concentration gradient of ions near the membrane relaxes, and any effect on the junction reverses. The mechanisms just suggested are therefore current-controlled mechanisms and, as such, are not supported by the data. In the high-conductance condition, steady-state junctional currents can be passed that are larger than those required to maintain the low-conductance condition (Figs. 3 and 7). We conclude, therefore, that accumulation mechanisms are not responsible for the changes in conductance of the gap junction channels and, specifically, that hydrogen and calcium ions are not involved. The probable mechanism, further supported by data in the following paper (Harris et al., 1981), is that the conductance of the gap junction channel is controlled by the voltage across it.

APPENDIX

Evaluation of Parameters

The parameters of voltage sensitivity predict that a small proportion of the channels sensitive to voltage remain closed at $V = 0$. For representative values of the parameters ($A = 0.25$; $V_0 = 14$ mV), the fraction of channels open is 0.97 at $V = 0$ determined according to Eq. 6 for voltage sensitivity of each polarity. Therefore, for voltage sensitivity of each polarity, there would be an error of at least 3% if g_{\max} were evaluated as the instantaneous conductance at the onset of a voltage step. For this reason, g_{\max} was not directly measurable, and its value was estimated by a procedure of successive approximation.

In Fig 9 A, for g_{∞} evaluated from Eq. (7) using the values for A and V_0 above, $g_{\max} = 1$ and $g_{\min} = 0.05$, $\ln [(g_{\infty} - g_{\min}) / (kg_{\max} - g_{\infty})]$ is plotted as a function of V for several values of k near one. Over the relevant voltage range, the linearity of the plots is sensitive to small errors in g_{\max} , i.e., to small deviations in k from unity. For this plot, reading from the uppermost curve

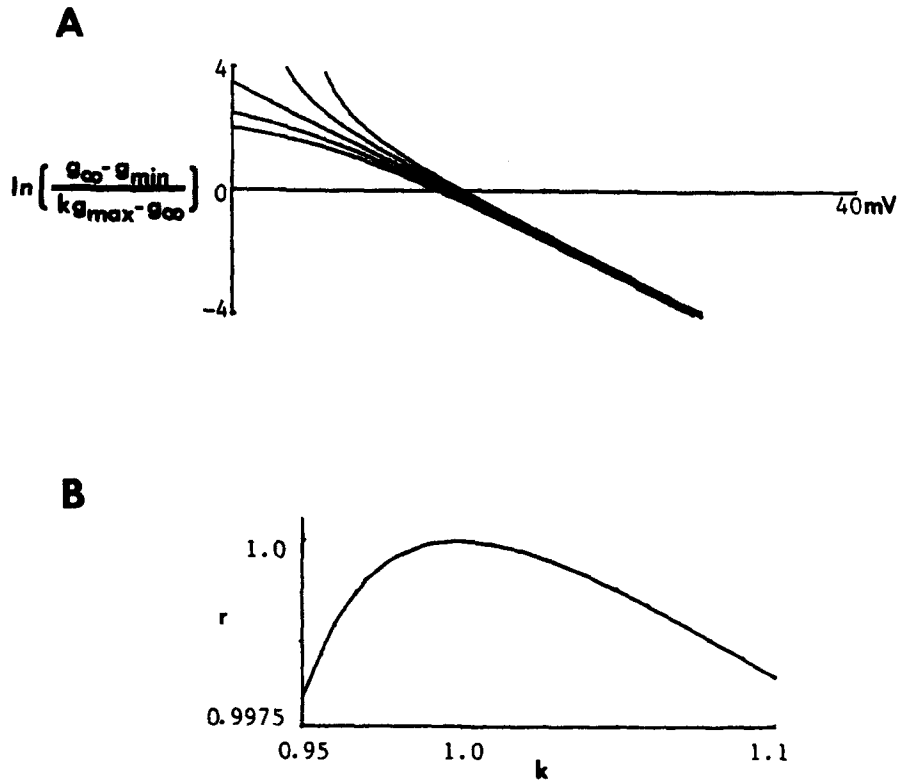


FIGURE 9. The effect of errors in g_{\max} on linearity of plots of Eq. 6. (A) Steady-state conductance g_{∞} was computed according to Eq. 7 for parameters $A = 0.25$, $V_0 = 14$, $g_{\max} = 1$, and $g_{\min} = 0.05$. Then Eq. 6 was plotted semilogarithmically with the g_{\max} term multiplied by an error factor k near 1. Reading from the uppermost plot downward, k was set at 0.90, 0.95, 1.00, 1.05, and 1.10. (B) Relation between the parameter describing goodness of fit to linearity r to the equation used above as a function of the error in g_{\max} . Because r decreases monotonically on either side of $k = 1$, the use of the value of g_{\max} giving the maximal value of r for a plot of Eq. 6 is justified.

downward, k was set at 0.90, 0.95, 1.00, 1.05, and 1.10. Furthermore, Fig. 9 B shows that the goodness of fit parameter r for these curves decreases monotonically as a function of k on either side of a maximum at $k = 1$.

For the evaluation of parameters, symmetry around zero transjunctional voltage was assumed for the conductance changes. The value for g_{\min} was

chosen by inspection. A range of g_{\max} 's was also chosen by inspection, and $\ln [(g_{\infty} - g_{\min}) / (g_{\max} - g_{\infty})]$ was calculated as a function of absolute values of voltage over that range of g_{\max} 's incrementing g_{\max} by small amounts. A linear regression was performed on the data for each g_{\max} . When the parameter r describing goodness of fit to linearity was maximal, that value of g_{\max} and the corresponding values of A and V_0 were taken as the true values. This value of g_{\max} was little affected by the existence of voltage sensitivity to both polarities, because it was evaluated over a transjunctional voltage range where $<1\%$ of the channels are closed by sensitivity to the opposite polarity ($|V| > 5$ mV; see Harris et al. [1981]).

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