Voltage Clamp Limitations of Dual Whole-Cell Gap Junction Current and Voltage Recordings. I. Conductance Measurements

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ABSTRACT Previous correction methods for series access resistance errors in the dual whole-cell configuration did not take into account the effect of nonzero resting potentials (E_rest) and junctional reversal potentials (E_junc). Dual whole-cell currents were modeled according to resistor-circuit analysis and two correction formulas for the measurement of junctional currents (I_j) were assessed. The equations for I_j, derived from Kirchoff’s law before and after baseline subtraction of the nonjunctional current, were assessed for accuracy under a variety of whole-cell patch-clamp recording conditions. Both equations accurately correct for dual whole-cell voltage-clamp errors provided that the cellular parameters are included in the nonbaseline subtracted I_j derivations. Junctional conductance (g_j) estimates are most reliable at high junctional resistance (R_j) values and minimize the need for corrective methods based on electrode series and cellular input resistances (R_elec and R_in). In the “open-cell” configuration, low R_j values relative to R_in are required for accurate g_j estimates. These methods provide the basis for accurate quantitative measurements of junctional resistance (or conductance) of gap junction channels or connexin hemichannels in the dual whole-cell or open-cell configurations. Reevaluation of V_j-dependent gating of rat connexin40 g_j produced nearly identical Boltzmann fits to previously published data. Continuous g_j-V_j curves generated by variable slope V_j ramps provide for more accurate fits and assessment of the time-dependence of the half-inactivation voltage and net gating charge movement.

INTRODUCTION

The dual whole-cell recording configuration, where two cells are independently voltage clamped by their own whole-cell patch electrode, is routinely applied to the measurement of junctional conductance (Veenstra, 1996). Most junctional current recordings are obtained for the primary purpose of measuring the macroscopic junctional current (I_j) or resistance (R_j) in response to experimental variables that modify channel gating (Kolb and Somogyi, 1991; Veenstra, 1991a). Although the development of the patch-clamp technique made it possible to voltage clamp individual ion channels with a single electrode (Hamill et al., 1981), the nature and location of the gap junction channel precludes the direct patch approach to this plasmalemmal intercellular channel. Occasionally, small cells with high input resistances (R_in) expressing relatively few gap junction channels when paired permit the resolution of single-gap junction channel currents (i_j) (Neyton and Trautmann, 1985; Veenstra and DeHaan, 1986; Rook et al., 1988). Corrective measures for patch electrode series access resistance (R_elec) errors are rarely required for i_j recordings, but become increasingly important as junctional conductance (g_j = 1/R_j) increases (Weingart, 1986). Two correction methods, one derived from voltage clamp analysis of a dual whole-cell resistor circuit (Veenstra and Brink, 1992), and another that modeled whole-cell currents using Kirchoff’s law (Van Rijen et al., 1998), published slightly different equations that permit off-line analysis of I_j and R_j. Both methods require knowledge of R_elec and R_in for each cell and subtraction of nonjunctional membrane currents (I_in) from the whole-cell current to obtain the value of I_j. This is best accomplished in a nonvoltage pulsed cell, because I_in will remain relatively constant provided that R_in remains stable. However, these previous derivations always assumed the voltage of the nonpulsed cell (cell 2 or the post-junctional cell) was 0 mV. When the dual whole-cell patch clamp technique is applied to living cells in primary or established cell cultures, the cellular resting potential (E_rest) should be considered, because setting the holding potential equal to E_rest will minimize unwanted nonjunctional membrane currents and improve the resolution of I_j. Furthermore, any asymmetry in E_rest or the whole-cell recording conditions of both cells produces small discrepancies in the initial recording conditions that must be corrected for in the experimental I_j and R_j measurements. In this manuscript, correction methods for I_j and R_j measurements are developed that more accurately reflect actual recording conditions and the effects of intrinsic cellular properties (e.g., E_rest or cellular membrane resistance, R_in) modified by the establishment of the dual whole-cell patch electrode configuration. Asymmetric junctional properties such as heterotypic gap junction channels, bi-ionic potentials, or unequal whole-cell voltage clamp conditions are also considered in the derivations.

The transjunctional voltage (V_j) gating of the rat connexin40 (rCx40) gap junction was reevaluated using continuous I_j-V_j relations in conjunction with I_j correction procedures, and the results are presented. Slow V_j ramps (200 ms/mV) produced half-inactivation voltage (V_1/2) and
gating charge valence \((z)\) values that agreed closely with previous results using conventional voltage pulse protocols. The time-dependence of \(V_{1/2}\) and \(z\) were ascertained using a family of \(V_j\) ramps with different ramp speeds (ms/mV). Correction for reductions in \(V_j\) and \(I_j\) due to ionic resistance errors produced a slight reduction in the measured \(V_j\)-insensitive normalized \(g_j\) \((G_{\text{norm}})\) from 0.30 to 0.23. Using a continuous \(g_j-V_j\) curve from fewer experiments than required using voltage pulse protocols reduced the variability of the fitted Boltzmann parameters.

**METHODS**

Figure 1 is a resistor circuit \((A)\) and current vector diagram \((B)\) for the dual whole-cell configuration. Each cell has its own resting potential \((E_{\text{rest}})\) determined by \(I_{\text{rest}} \cdot R_m\) prior to establishment of the whole-cell patch electrode configuration. After \(G_1\) seal \((R_s)\) formation and membrane patch disruption, \(E_{\text{rest}}\) equals \(I_{\text{rest}} \cdot R_m\) where \(R_m = (R_m \cdot R_j)/(R_m + R_j)\). A defined current value \((I_{\text{total}})\) was used instead of a defined \(E_{\text{rest}}\) to model the shunting of the resting membrane potential by a whole-cell patch electrode. It is true that \(I_m = 0\) at \(E_{\text{rest}}\) but this is because the inward and outward membrane ionic currents exactly balance each other. Because the Nernst potentials for \(K^+\), \(Na^+\), etc. are not changed and there is a finite resting \(g_{\text{Na}}, g_{\text{K}}, \text{etc.}\), the current values at which this dynamic balance is achieved is fixed for a resting membrane.

Each patch electrode has a resistance \((R_j)\) in series with \(R_m\) and \(R_t\) that will affect those measured values. The command potentials of each patch electrode \((V_1\) and \(V_2)\) initially begin with \(V_1 = V_2\) followed by alteration of one command potential \((\Delta V)\) to impose a voltage gradient across \(R_s\) and determine the value of \(I_j\) from the resultant whole-cell currents \((I_1\) and \(I_2)\). To evaluate the model circuit, a command \(V_{j,\text{comm}} = V_1 - V_2\) was varied by \(\pm 100\) mV in 10-mV increments. Initial values of \(R_{\text{el1}}\) and \(R_{\text{el2}}, R_{\text{el}}, R_{\text{m1}}\) and \(R_{\text{m2}}, R_j\) and \(I_{\text{rest1}}\) and \(I_{\text{rest2}}\) were assigned, and the whole-cell voltages \((V_{\text{m1}}\) and \(V_{\text{m2}})\) and currents \((I_1\) and \(I_2)\) were calculated for each \(V_{j,\text{comm}}\). Optimal recording conditions initially assigned to the dual whole-cell circuit were \(R_{\text{el}} = 100\) M\(\Omega\) \((\leq 100 \cdot R_m)\), \(R_j = 10\) G\(\Omega\) \((\geq 10 \cdot R_m)\), \(R_s = 1\) G\(\Omega\) \((\leq R_m)\), and \(R_m = 1\) G\(\Omega\). These values approximate dual whole-cell conditions from connexin-transfected mammalian cell lines or primary cell cultures with cell diameters of \(\leq 20\) \(\mu m\). The following set of equations defines the whole-cell current and voltage values of the dual-cell circuit before and after establishment of the dual whole-cell patch electrode configuration.

**Dual whole-cell voltage clamp equations**

\[
I_{\text{total}} = I_1 + I_2 
\]

\[
I_1 = I_{\text{in1}} + I_j 
\]

\[
I_2 = I_{\text{in2}} - I_j 
\]

\[
V_{\text{m1}} = V_1 - (I_1 \cdot R_{\text{el}}) 
\]

\[
V_{\text{m2}} = V_2 - (I_2 \cdot R_{\text{el}}) 
\]

\[
E_{\text{rest1}} = I_{\text{rest1}} \cdot R_{\text{in1}} 
\]

\[
E_{\text{rest2}} = I_{\text{rest2}} \cdot R_{\text{in2}} 
\]

To model the whole-cell currents, the following expressions were derived from the resistor circuit diagram (Fig. 1):

\[
I_1 = \frac{V_{\text{m1}} - E_{\text{rest1}}}{R_{\text{m1}}} + \frac{V_{\text{m1}} - V_{\text{m2}} - E_{\text{rev}}}{R_j} 
\]

\[
\frac{[V_1 - (I_1 \cdot R_{\text{el}})] - E_{\text{rest1}}}{R_{\text{m1}}} + \frac{[V_1 - (I_1 \cdot R_{\text{el}})] - [V_2 - (I_2 \cdot R_{\text{el}})] - E_{\text{rev}}}{R_j} \] (4a)

\[
I_2 = \frac{V_{\text{m2}} - E_{\text{rest2}}}{R_{\text{m2}}} - \frac{V_{\text{m1}} - V_{\text{m2}} - E_{\text{rev}}}{R_j} 
\]

\[
\frac{[V_2 - (I_2 \cdot R_{\text{el}})] - E_{\text{rest2}}}{R_{\text{m1}}} - \frac{[V_1 - (I_1 \cdot R_{\text{el}})] - [V_2 - (I_2 \cdot R_{\text{el}})] - E_{\text{rev}}}{R_j} \] (4b)

FIGURE 1 Equivalent resistive circuit for the dual whole-cell configuration. \((A)\) Equivalent resistive circuit for a dual whole-cell voltage clamp using patch electrodes where the seal resistance, \(R_s\), is in parallel with the membrane resistance, \(R_m\), of each cell. A command voltage, \(V_j\), is applied to cells 1 and 2 independently via patch clamp amplifiers and the membrane potential, \(V_{\text{m1}}\) of each cell is equal to \((V - I \cdot R_{\text{el}})\). The resting potential, \(E_{\text{rest}}\), of each cell, determined initially by \(I_m \cdot R_m\) becomes \(I_m \cdot R_m\) in the whole-cell configuration. Any source of asymmetry (connexin or ionic composition) between the two cells can produce a voltage difference, \(E_{\text{rev}}\), across the junctional resistance, \(R_j\). When \(V_1 = V_2\) and \(R_{\text{el1}}/R_{\text{m1}} \approx R_{\text{el2}}/R_{\text{m2}}, V_{\text{m1}} = V_{\text{m2}}\) and the net transjunctional potential, \(V_j\), and current, \(I_j\), equal zero, provided that \(E_{\text{rev}} = 0\). \((B)\) The resulting current vectors in response to a \(V_j + \Delta V_j, V_2, E_{\text{rest}}, I_{\text{rest}}\), and \(E_{\text{rest}}\) applied to the dual whole cell resistor circuit diagrammed in panel \(A\). A net \(V_j \approx \Delta V_j\) and \(I_j\) proportional to \(-\Delta I_j\) (see Eqs. 1g and 1i) are produced, and the exact value of \(R_j = V_j/I\) can be determined by the net \(\Delta V_j/I\). \(I_{\text{rest}}\) is a DC component of \(I_j\) and \(I_{\text{rest}}\) is a DC component of \(I_m\) for each cell.
These expressions for the ideal whole-cell currents cannot be solved for $I_1$ and $I_2$ because it is not possible to solve for $V_{m1}$ and $V_{m2}$ without knowing the values of $I_1$ and $I_2$. To solve for $I_1$ and $I_2$ independently knowing only the initial $V_{m1}$, $I_{res1}$, and $R$ values of the model circuit, we again solve for Eqs. 1b and 1c knowing that $I = V/R_{total}$ for each current path. For each cell

$$I_{in} = \frac{V \cdot R_{in} - E_{rest} \cdot (R_{el1} + R_{in})}{(R_{el1} + R_{in}) \cdot R_{in}},$$  

and

$$I_j = \frac{[(V_1 - V_2) \cdot R_j] - [E_{rev} \cdot (R_{el1} + R_j + R_{el2})]}{(R_{el1} + R_j + R_{el2}) \cdot R_j}. \quad (6)$$

The appropriate expressions for $I_1$ and $I_2$ become

$$I_1 = V_1 \cdot R_{m1} - E_{rest1} \cdot (R_{el1} + R_{m1}) \frac{(R_{el1} + R_{m1}) \cdot R_{m1}} \right.$$  

$$+ \left[ \frac{[(V_1 - V_2) \cdot R_j] - [E_{rev} \cdot (R_{el1} + R_j + R_{el2})]}{(R_{el1} + R_j + R_{el2}) \cdot R_j} \right] \quad (4c)$$

and

$$I_2 = V_2 \cdot R_{m2} - E_{rest2} \cdot (R_{el2} + R_{m2}) \frac{(R_{el2} + R_{m2}) \cdot R_{m2}} \right.$$  

$$- \left[ \frac{[(V_1 - V_2) \cdot R_j] - [E_{rev} \cdot (R_{el1} + R_j + R_{el2})]}{(R_{el1} + R_j + R_{el2}) \cdot R_j} \right] \quad (4d)$$

Whole-cell junctional current and resistance equations

Experimental determination of $R_j$ from dual whole-cell currents requires estimation of $I_j$ and calculation of $R_j$ according to Ohm’s Law, $R_j = V/I_j$. $R_j$ is experimentally determined by measuring $I_j$ in response to an applied $V_j$. $V_j$ is defined as the difference in the two command potentials $V_1$ and $V_2$. Initially $V_j = V_2$ and a $\Delta V$ pulse is applied to one cell conventionally defined as cell 1 (prejunctional cell). One cell must be chosen as the reference for calculating $V_j$ if $V_j$ is defined as $V_j = V_1 - V_2$; then $V_j = \Delta V$. However, $R_{el1}$ is in series with $R_{m1}$ of each cell so the actual value of $V_j = V_{m1} - V_{m2}$

$$V_j = [(V_1 + \Delta V) - V_2] - [(R_{el1} \cdot I_1) - (R_{el2} \cdot I_2)] \quad (2c)$$

(Rook et al., 1988; Veenstra and Brink, 1992). $I_j$ appears in both whole-cell current signals but with opposite sign (Eqs. 1b and 1c) (Veenstra and DeHaan, 1986). Expressions for $I_j$ using either whole-cell signal were derived from Kirchoff’s law where

$$I_j = I_1 \cdot \left(1 + \frac{R_{el1}}{R_{m1}}\right) - \frac{V_1}{R_{m1}}, \quad (1e)$$

or

$$I_j = -I_2 \cdot \left(1 + \frac{R_{el2}}{R_{m2}}\right) + \frac{V_2}{R_{m2}} \quad (1f)$$

(Weingart, 1986; Rook et al., 1988; Eq. A8 and A9 of Van Rijen et al., 1998) because $R_{m1}$ measured in the whole-cell configuration is $R_{m2}$. Because $V_j$ is altered by the $\Delta V$ step, $I_{m1} = [V_j (+ \Delta V)]/R_{m1}$ does not remain constant. Therefore, $-I_2$ is conventionally used to measure $I_j$ (Veenstra and DeHaan, 1986; Weingart, 1986). Veenstra and Brink (1992) derived a related expression for $I_j$ based on resistive circuit analysis where

$$I_j = -\Delta I_2 \cdot \left(1 + \frac{R_{el2}}{R_{m2}}\right) \quad (1g)$$

(Eq. 6 in Veenstra and Brink, 1992). If one subtracts Eq. 1f when $V_j = V_1 + \Delta V$ from the initial condition when $V_j = V_2$, one obtains Eq. 1g because $V_2$ and $R_{m2}$ (ideally) remain constant and the $R_{m2}$ terms cancel out. This is the original definition for $I_j = -\Delta I_2$ (see Veenstra, 1991b). The condition that $V_{m2}$ when $V_j = V_2$ equals $V_{m2}$ when $V_j = V_2$ does not actually hold true because a small fraction of $I_j$ must flow across $R_{m2}$ in the whole-cell configuration, resulting in a small change in $V_{m2}$

$$\Delta V_{m2} = I_j \left[\frac{R_{el2} \cdot R_{m2}}{(R_{el2} + R_{m2})}\right] = I_j \cdot R_{el2} \quad (2d)$$

during the $\Delta V_j$ step. This equation first appeared as Eq. 9 in Veenstra and Brink (1992). The cellular resistance ($R_j$) was defined as the input resistance of the whole-cell relative to the junction. The Kirchoff’s law expressions for $I_j$, when the “real” cell parameters of $E_{rest1}$ and $E_{rest2}$ (Fig. 1) are considered become

$$I_j = I_1 \cdot \left(1 + \frac{R_{el1}}{R_{m1}}\right) - \frac{(V_1 - E_{rest1})}{R_{m1}}, \quad (1h)$$

and

$$I_j = -I_2 \cdot \left(1 + \frac{R_{el2}}{R_{m2}}\right) + \frac{(V_2 - E_{rest2})}{R_{m2}}. \quad (1i)$$

Because $R_j = V_j/I_j$, the expressions for estimating $R_j$ from dual whole-cell voltage clamp currents are

$$R_j = \frac{[(V_1 + \Delta V_j) - V_2] - [(R_{el1} \cdot I_1) - (R_{el2} \cdot I_2)] - E_{rest}}{-I_2 \cdot (1 + \frac{R_{el2}}{R_{m2}}) + \frac{(V_j - E_{rest2})}{R_{m2}}} \quad (5a)$$

or

$$R_j = \frac{[(V_1 + \Delta V_j) - V_2] - [(R_{el1} \cdot I_1) - (R_{el2} \cdot I_2)] - E_{rest}}{-\Delta I_2 \cdot (1 + \frac{R_{el2}}{R_{m2}})} \quad (5b)$$

The major difference between these two approaches is in the estimation of $I_{m2}$ in Eq. 5a and the subtraction of $I_j$ ($\approx I_{m2}$) when $V_j = V_2$ in Eq. 5b. The uncompensated $R_j$ estimate for the purpose of evaluating the correction methods was defined as

$$R_j = \frac{V_1 - V_2}{-\Delta I_2}, \quad (5c)$$

where

$$V_j = V_1 - V_2 \quad (2e)$$

and

$$I_j = -\Delta I_2 \quad (1j)$$
RESULTS

Experimental determination of $R_{el}$ and $R_{in}$

The whole-cell capacitive transient decay time constant in response to a small voltage step ($\Delta V_1 = \Delta V_2$) is used to determine the value of $R_{el}$ according to

$$\tau_{cap} = R_{el} \cdot C_{in}$$

(Hamill et al., 1981; Sakmann and Neher, 1995). This measurement also requires integration of the cellular input capacitance ($C_{in}$) for each cell from the total charge ($Q_{in}$) of the whole-cell capacitive transient according to Faraday’s law ($C_{in} = Q_{in}/\Delta V$). $R_{in1}$ and $R_{in2}$ are assessed experimentally by varying $V_1 = V_2$ simultaneously to minimize $I_j$ ($V_j \approx 0 \text{ mV}$). $\Delta V$ can be a single step or a voltage ramp or staircase that determines $R_{in}$ over a range of voltages (e.g., -100 to +60 mV, physiological voltage range). This experimental determination of $R_{in}$ ignores any preexisting $V_j$ due to $E_{rest1} \neq E_{rest2}$, $V_{in1} \neq V_{in2}$, or $E_{rev} \neq 0 \text{ mV}$. For example, if $I_1 \cdot R_{el1} \neq I_2 \cdot R_{el2}$ even when $V_1 = V_2$, $E_{rest1} = E_{rest2}$, and $E_{rev} = 0$, then $V_{in1} \neq V_{in2}$ (Eqs. 2a and 2b) and $I_j \neq 0$ (Eqs. 1g and 1l). In actual terms, $V_{in1} = V_{in2}$ only when $V_1 = V_2$, ($R_{el1}/R_{in1}$) = ($R_{el2}/R_{in2}$), and ($V_1 - E_{rest1}$) = ($V_2 - E_{rest2}$). So minor asymmetries in the dual whole-cell circuit will result in $V_j \neq 0$ when $V_1 = V_2$. These minor differences are often negligible (<1 mV).

Experimental determination of $E_{rest}$

By definition, $E_{rest1} = V_1$ when $I_1 = 0$ and $V_1 = V_2$ (and $E_{rest2} = V_2$ when $I_2 = 0$). However, Eq. 1b infers that $I_1 = 0$ only when $I_{in2} + I_j = 0$ (or $I_2 = 0$ when $I_{in2} - I_j = 0$, Eq. 1c). Therefore, any asymmetries in $R_{el}/R_{in}$ and $V - E_{rest}$ will produce errors in the actual $E_{rest1}$ and $E_{rest2}$ measurements. These errors are typically small unless $R_{in}$ is low or $R_{el}$ is high and can be essentially ignored. Precise determination of $E_{rest}$ requires determining the applied $V$ where $I = 0$ and solving Eqs. 4c and 4d for $E_{rest}$,

$$E_{rest1} = \frac{V_1}{R_{el1} + R_{in1}} - \frac{(V_{in1} - V_{in2} - E_{rev})}{R_j} \cdot R_{in1},$$

and

$$E_{rest2} = \frac{V_2}{R_{el2} + R_{in2}} + \frac{(V_{in1} - V_{in2} - E_{rev})}{R_j} \cdot R_{in2}.$$  

A nonzero $E_{rev}$ can develop from an asymmetric (heterotypic) gap junction channel or asymmetric ionic conditions in addition to unequal dual whole-cell parameters. The condition of $E_{rev} \neq 0$ will be considered in another manuscript on the subject of experimental $E_{rev}$ measurements. Eqs. 7a and 7b are not practical when $R_{el}$ is unknown, as in a biological dual whole-cell experiment when $V_{in1} \neq V_{in2}$.

Because [(($V_{in1} - V_{in2})/R_j$)] equals $I_j$, the $E_{rest}$ measurements when $I_1 = 0$ and $I_2 = 0$ will be in error. However, when $I_1 + I_2 = 0$ the unknown $I_j$ term cancels out and the solution to Eqs. 4c + 4d becomes

$$E_{rest1} + E_{rest2} = \frac{V}{R_{el1} + R_{in1}} + \frac{1}{R_{el2} + R_{in2}}.$$  

Eq. 8 can be solved from the whole-cell input resistance and capacitance current signals required to make any series resistance corrections of dual whole-cell $I_j$ or $R_j$ measurements. It is apparent that

$$E_{rest} = \frac{V \cdot R_{in}}{R_{el} + R_{in}}$$

for each cell. In general terms, $E_{rest} = V$ provided that $E_{rev} = 0$ and $V_{in1} = V_{in2}$.

Dual whole-cell recording conditions

To evaluate the accuracy of the three experimental $R_j$ estimates (Eqs. 5a–c) under a variety of dual whole-cell voltage clamp conditions, $R_{el}$, $R_{in}$, and $E_{rest}$ were altered from initial optimal dual whole-cell conditions. Whole-cell currents were modeled using Eqs. 4c and 4d and the $I_j$ and $R_j$ estimates using Eqs. 1g and 1l, and Eqs. 5a–c were calculated for each set of experimental conditions. The whole-cell parameters that were altered for each set of experimental conditions are listed in Table 1. Cellular parameters that remained constant were $R_{in1} = R_{in2} = 1 \text{ G}\Omega$ and $E_{rev} = 0$.

### Table 1: Resistor and voltage values for dual whole-cell experiments

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>$R_{el1}$ (MΩ)</th>
<th>$R_{el2}$ (MΩ)</th>
<th>$R_{in}$ (GΩ)</th>
<th>$R_{in1}$ (GΩ)</th>
<th>$R_{in2}$ (GΩ)</th>
<th>$E_{rest1}$ (mV)</th>
<th>$E_{rest2}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.91</td>
<td>0.91</td>
<td>-36.4</td>
<td>-36.4</td>
</tr>
<tr>
<td>High $R_j$</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>0.91</td>
<td>0.91</td>
<td>-36.4</td>
<td>-36.4</td>
</tr>
<tr>
<td>Low $R_j$</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.50</td>
<td>0.50</td>
<td>-20</td>
<td>-20</td>
</tr>
<tr>
<td>$V \neq E_{rest}$</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.91</td>
<td>0.91</td>
<td>-72.7</td>
<td>-72.7</td>
</tr>
<tr>
<td>Realistic</td>
<td>25</td>
<td>20</td>
<td>10</td>
<td>1.30†</td>
<td>1.67‡</td>
<td>-34.8†</td>
<td>-33.3³</td>
</tr>
</tbody>
</table>

*R$_{in1} = (R_{el} \cdot R_{in})/(R_{el} + R_{in}).

+E$_{rest1} = I_{rest1} \cdot R_{in}$, $I_{rest2}$ was -40 or -80 pA.

3$E_{rest1} = 1.30$ GΩ because $R_{in1}$ was increased to 1.5 GΩ. $I_{rest1}$ was reduced to -26.7 pA.

4$R_{in2} = 1.67$ GΩ because $R_{in2}$ was increased to 2.0 GΩ. $I_{rest2}$ was reduced to -20 pA.

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The estimated $R_j$ values from Eqs. 5a–c were determined at the specified $R_j$ values of 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, and 5.0 GΩ, and the percent error of the estimated value was plotted accordingly. Eqs. 5a, b, and c provide only slightly different $R_j$ estimates due to different definitions of $I_j = -\Delta I_2 \cdot [1 + (R_{el1}/R_{m1})]$ or $-\Delta I_2 \cdot [1 + (R_{el2}/R_{m2})] + I_{m2}$, respectively. Eq. 5c represents an uncompensated $R_j$ estimate based only on the values of $(V_{el1} - V_{el2})$ and $-\Delta I_2$. Optimal dual whole-cell recording (DWCR) conditions of $R_{el1} = R_{el2} = 1\%$ of $R_{m1} = R_{m2}$ and $R_j = R_{el1} = 10 \cdot R_{m1}$, where $R_{m1} = 1$ GΩ were used for these calculations (see Table 1). The common holding potential ($V_{el1} = V_{el2}$) was $-40$ mV, $E_{rev} = 0$ mV, and $E_{rest1} = E_{rest2} = -36.4$ mV in the whole-cell configuration because $R_{m1} = R_{m2} = 0.91$ GΩ and $I_{rest1} = I_{rest2} = -40$ pA. (B) The same as in part A except that $R_{el1} = R_{el2} = 2\%$ of $R_{m1} = R_{m2}$. The percent error increases for all three $R_j$ estimates, but the percent error remains $<5\%$ for all values of $R_j$ according to Eq. 5a and 5b. The percent error in the uncompensated $R_j$ estimate rises rapidly and exceeds $10\%$ for $R_j > 1$ GΩ. (C) The percent error in the $V_j$ and $I_j$ estimates according to Eq. 2c and 1g or 1i. The $I_j$ estimates using Eq. 1g and 1i were identical for $R_j < 1$ GΩ and varied slightly from each other at higher $R_j$ values.

**Optimal dual whole-cell conditions**

Bilateral symmetry and a common holding potential near the intrinsic resting potential ($V \equiv E_{rest}$) characterize the optimal dual whole-cell experiment. The actual $E_{rest1} = E_{rest2} = -36.4$ mV instead of the $-40$-mV value of the intact cell because an $R_{m1} = 10 \cdot R_{m}$ yields a $R_{in1} = 0.91 \cdot R_{m}$. This produces a small holding current ($I_{in}$) of $-3.5$ pA at the common holding potential of $-40$ mV. Figure 2 A illustrates the percent error in the $R_j$ estimate obtained with Eqs. 5a, b, and c. Because the modeled whole-cell currents already account for series resistance errors due to $R_{el1}/R_{m1}$, the $[1 + (R_{el1}/R_{m1})]$ term was omitted from the $I_j$ estimates for Eqs. 5a and 5b. To model the attenuation expected from the whole-cell circuit, the $-\Delta I_2$ term was divided by the $[1 + (R_{el2}/R_{m2})]$ term for Eq. 5c. The results of Eqs. 5a and 5b vary only slightly with $R_j$ increasing to a maximum error of $-1.5\%$ at $R_j = 50$ MΩ ($g_j = 20$ nS) while an uncompensated $R_j$ estimate (Eq. 5c) rises to an error of $+41\%$ under the identical conditions. The major source of error is the drop in the actual $V_j$ of up to $-30\%$ due to the increasing $I_j$, $R_j$ as $R_j$ decreased. The uncompensated value of $-\Delta I_2$ closely matched (within $1\%$) the percent error in the applied $V_j$ at all $R_j$ values (Fig. 2 C). The small difference in the percent error between $-\Delta I_2$ and $V_j$ equals the $R_{el1}/R_{in1}$ attenuation factor of $1.1\%$.

**Series resistance factors**

Because the $R_{el1}/R_{in1}$ ratio only increases the error in the current signal by $1\%$ for every $10$ MΩ/GΩ, doubling $R_{el1}$ and $R_{el2}$ has only modest effects on the compensated $R_j$ estimates, whereas the percent error in the uncompensated...
$R_j$ estimate doubles (Fig. 2 B). Again, the major source of error is the decrease in actual $V_j$ due to the voltage drops across each electrode (Fig. 2 C). As $I_j$ increased, the percent error in $R_j$ using either Eqs. 5a or 5b increased to only $-3.8\%$ at $g_j = 20$ nS compared to $+83\%$ using Eq. 5c. Hence, calculating $V_{m1}$ and $V_{m2}$ using Eqs. 2a and 2b can significantly enhance the accuracy of $R_j$ estimates. The simple correction of using $V_j = V_{m1} - V_{m2}$ instead of $V_j = V_1 - V_2$ will account for most ($>90\%$) of the error in the $R_j$ estimate except under the worst circumstances ($R_{el} > 10\%$ of $R_m$).

Nonjunctural current considerations

Lowering $R_{in}$ There are two ways that $R_{in}$ can be affected. Larger cells have lower $R_{in}$ values due to the increased membrane surface area times the specific membrane resistivity, such as adult versus neonatal cardiac myocytes. Alternatively, a lower $R_j$ value in parallel with $R_m$ will reduce $R_{in}$ by the relative proportion of $R_{in}/(R_j + R_{in})$. Alterations in $R_{in}$ are the most likely experimental variable during dual whole-cell recordings using the same cell preparation. To model the effect of a reduced $R_{in}$ on $I_j$ and $R_j$ estimates, $R_{in1}$ and $R_{in2}$ were lowered to 1 GΩ each. Because $R_{in1}$ and $R_{in2}$ also equal 1 GΩ, $R_{in1}$ and $R_{in2}$ drop to 0.50 GΩ. The effects are threefold. $I_{in}$ will approximately double for the same command potential $V$, the series resistance factor will be approximately doubled, and any intrinsic $E_{rest}$ will be further shunted, causing an additional increase in $I_{in}$. Given that $I_{in}$ is still a relatively high 500 MΩ, $I_{in}$ will increase by only 2 pA/mV difference in $V - E_{rest}$. For $V_1 = V_2$ at $-40$ mV, this results in an increase in $I_{in}$ from $-3.5$ to $-40$ pA, or only an additional 400 μV initial voltage drop across each electrode. These modest alterations in the dual whole-cell circuit increases the percent error in the $R_j$ estimates using Eqs. 5a and 5b only slightly to $-2.5\%$ compared to $+43\%$ for Eq. 5c. However, large errors in the estimation of $I_j$ and $R_j$ occur if $E_{rest2}$ is considered to be 0 mV as in Eq. 1f (Eq. A9 in Van Rijen et al., 1998). These large errors in the estimation of $I_j$ cause the $R_j$ estimate to fluctuate from large negative to large positive values of percent error eventually stabilizing within $\pm 2\%$ error when $R_j \leq 100$ MΩ (data not shown, see Fig. 3).

Holding and resting potential discrepancies. Errors in the estimation of $I_j$ and $I_{in}$ from whole-cell currents can also arise from differences in the common holding potential of the dual-cell voltage clamp and the intrinsic resting potential of a myocyte or neuron. To model an $E_{rest}$ of $-80$ mV, $I_{rest}$ was increased to $-80$ pA. In the whole-cell configuration with $R_m = 10$ GΩ, $E_{rest}$ decreased to $-72.7$ mV. Maintaining a $V_j = V_2$ value of $-40$ mV again imposes an $I_{in}$ value that will often exceed the value of $I_j$ and produce significant errors in the $R_j$ estimate unless $E_{rest}$ is appropriately considered as in Eq. 5a. Given the optimal $R_{el}/R_{in}$ of 1.1% and $R_{in} \approx 1$ GΩ, the effects of the additional $+36$ pA of $I_{in}$ are negligible and the $R_j$ estimates with Eqs. 5a and 5b remain within $-1.5\%$ error as the uncompensated $R_j$ estimate rises to $+42\%$ at $R_j = 50$ MΩ.

Whatever the cause of an increase in $I_{in}$, $\Delta V_{in}$ will increase and accurate estimates of $I_{in}$ become more critical when $I_j$ is low if Eq. 5a is to be used to estimate $R_j$. Baseline subtraction of all whole-cell currents when $V_j = V_2$ offers the advantage of being insensitive to the initial value of $I_{in}$ and any changes in $I_{in}$ during a dual whole-cell recording can be accurately monitored by tracking the baseline value of $I_j$ during an experiment. Another feature of Eq. 5b that is not apparent from the average percent error values illustrated in Fig. 2 A is the behavior as a function of $V_j$. To illustrate the differences between Eqs. 5a and 5b under more realistic dual whole-cell conditions, slight asymmetries were assigned to the circuit and the $R_j$ estimates over a $\pm 100$ mV were evaluated.

Realistic experimental conditions

On average, the 4–5 MΩ patch electrode acquires an $R_{el}$ of 20 MΩ after patch break (Wang et al., 1992), $R_m = 200–500$ MΩ (Veenstra et al., 1992; Beblo et al., 1995), and the $R_{in}$ of connexin-transfected N2A cells $\approx 1$ GΩ. Small asymmetries were assigned to $R_{el}$ and $R_{in}$ to mimic realistic experimental conditions as listed in Table 1. The common holding potential of $-40$ mV also differs slightly from $E_{rest}$ because the value of $E_{rest}$ is typically not evaluated before beginning an experiment. The results are illustrated in Fig. 3 where Eqs. 5a and 5b again provide similar $R_j$ estimates of 490 MΩ, or $-2.0\%$ error. The uncompensated $R_j$ estimate was 555 MΩ or $+11.1\%$ error. Eqs. 1g and 1i also produced similar $I_j$ estimates that differed by a maximum of 1.0 pA with an average percent error from ideal $I_j$ values of $-8.3\%$. What was not readily apparent from Fig. 3 B was that the standard deviation of the percent error in $I_j$ was $0.5\%$ with Eq. 1i and $<0.2 \times 10^{-6}\%$ with Eq. 1g. This again illustrates the better stability of Eqs. 1g and 5b in estimating $I_j$ and $R_j$. If $E_{rest2}$ is omitted from the $I_j$ estimation (Eq. 1f), $I_j$ is shifted by $+20$ pA and the percent error rises sharply at low $V_j$ values where $I_j$ is small (Fig. 3 A). This results in $R_j$ estimates that also fluctuate toward negative and positive extremes as $I_j$ approaches 0 pA (Fig. 3 A). This is similar to the variations in $R_j$ obtained when $E_{rest2}$ is omitted from the $I_j$ estimates for the low $R_m$ and high $E_{rest2}$ examples. The asymmetry in the actual $V_j$ was only 200 μV and the percent error was $10.1\%$ (Fig. 3 C). Eq. 5b produces stable $R_j$ estimates, provided that $R_{in2}$ remains stable, whereas Eq. 5a is very sensitive to the $I_{in2}$ estimate at each $V_j$ analyzed. The accuracy of both correction methods is comparable when performed correctly to within $5\%$ error for $R_j \leq 50$ MΩ although Eq. 5b has the advantages of requiring a simpler calculation of $I_j$ and better stability over a range of $V_j$ values.

In Figs. 2 and 3, the compensated $R_j$ estimates of Eqs. 5a and 5b underestimated $R_j$ by 1 to 4%, depending on the dual
whole-cell recording conditions. Figure 3 B illustrates the point that the recorded $-\Delta I_2$ current (Eq. 1j) closely matched the drop in $V_j$ across the dual patch clamp circuit (Eq. 2c), whereas Eqs. 1g and 1i compensated for the fraction of $I_j$ that flowed across $R_{in2}$ instead of $R_{el2}$. However, it follows that the alteration in $V_j$ produced by $\Delta I_2 \cdot R_{el2}$ must equal $\Delta V_{m2} \cdot R_{in2}$ because, by definition, both must equal $\Delta V_{m2}$ (see Eq. 2d). This means that the previously published Eqs. 2c/1f and 5b for corrected $R_j$ estimates contain a small error (Veenstra and Brink, 1992; Van Rijen et al., 1998). If Eq. 2c is to be used to calculate the actual applied $V_j$, then the whole-cell current attenuation factor $[1 + (R_{el2}/R_{in2})]$ must not reappear in the denominator of Eqs. 5a and 5b. To test this derivation, Eq. 1i was modified to

$$I_j = -I_2 + \frac{(V_j - E_{rest2})}{R_{in2}}$$  

(1k)
and Eqs. 2c/1j to

\[
R_j = \frac{[V_1 + \Delta V_1 - V_2] - [(R_{el1} \cdot I_1) - (R_{el2} \cdot I_2)] - E_{rev}}{-\Delta I_2}. \tag{5d}
\]

The results are shown in Fig. 3D where the percent error in the compensated \(R_j\) estimates for the realistic dual whole-cell circuit is illustrated. The percent error was reduced from \(-1.1\) to \(-2.3\), and \(-2.0\%\) to \(-0.04\), \(-0.18\), and \(-1.25\%\) for the \(R_{el1} = 10 \text{ M}\Omega\), \(20 \text{ M}\Omega\), and realistic circuits when Eq. 5d was used to estimate \(R_j\) (\(= 500 \text{ M}\Omega\)) instead of Eq. 5b. The difference in the percent error between Eq. 5a and 2c/1k or Eq. 5c and 5d, equal to \(R_{el}/R_{in}\), was typically \(<2\%\) for all dual whole-cell conditions examined. Eq. 5d was most accurate (\(<0.5\%)\) when symmetry was maintained for the dual whole-cell circuit. Even though Eqs. 5a and 5b remain accurate to within \(-5\%\) over a 100-fold range of \(R_1\) values, Eq. 5d remains more accurate than Eq. 5b by the percentage equivalent of \(R_{el}/R_{in}\) under actual dual whole-cell experimental conditions where \(R_j\) is unknown.

**Alternative recording configurations**

The limitations and possible errors of estimating \(I_1\) and \(R_j\) in the dual whole-cell configuration are evident from Eqs. 5a and 5b. An alternative method for studying the regulation of \(R_j\) was developed using adult cardiac myocytes (Noma and Tsuboi, 1987; Sugiuara et al., 1990). The “open-cell” configuration relies on \(R_{in1}\) measurements obtained after \(R_{in2}\) was shunted to 0 \(\Omega\). This configuration was modeled using the circuit diagram in Fig. 1 by setting \(R_{el2}\) and \(R_{in2} = 0 \Omega\). Because \(V_2 = 0 \text{ mV}\), the common holding potential for minimizing \(I_1\) becomes \(V_1 = 0 \text{ mV}\). Another worthy consideration of these initial open-cell conditions is the effect on \(E_{rest1}\) now that \(R_j\) and \(R_{in1}\) are in parallel. The net resistance of the open-cell (\(R_{oc}\)) is

\[
R_{oc} = \frac{R_{in1} \cdot R_j}{R_{in1} + R_j}, \tag{9}
\]

the open-cell current (\(I_{oc}\)) when \(V_1 = 0 \text{ mV}\) is

\[
I_{oc} = I_{rest1} + \frac{E_{rev}}{R_j}, \tag{10}
\]

and the open cell voltage (\(E_{oc}\)) is

\[
E_{oc} = \left[I_{rest1} + \frac{E_{rev}}{R_j}\right] \cdot R_{oc}. \tag{11}
\]

This equation applies to any whole-cell macroscopic current in the open-cell configuration. The open-cell preparation can result from a coupled cell pair, a freshly isolated cell containing functional gap junctions, or a single cell with functional connexin hemichannels (Mazet et al., 1985; Noma and Tsuboi, 1987; Sugiuara et al., 1990; Zhou et al., 1997; Zhang et al., 1998). These conditions do not apply to direct patch recordings of hemichannels or gap junction channels in the cell-attached patch or excised patch configurations originally described during the development of the patch clamp technique (Hamill et al., 1981; Sakmann and Neher, 1995).

**Open-cell \(R_j\) measurements**

The whole-cell currents were modeled using Eq. 4c for a range of \(R_j\) and \(R_{in1}\) values. The \(I–V\) curves obtained in the open-cell configuration with \(R_j = \infty, 1.0 \text{ G}\Omega\), or \(0.5 \text{ G}\Omega\) are illustrated in Fig. 4A. \(R_{oc}\) was estimated from 1/slope of the linear \(I–V\) plot and the actual and measured values are listed in Table 2. The corresponding \(R_j\) value was calculated using Eq. 9.

Because \(R_{el}/R_{oc}\) was only 1.1%, the \(I–V\) plots overestimated \(R_{oc}\) by 1–4.3%, whereas the \(I–V_{m}\) plots were accurate to within \(-1.5\%\) at all values tested. The \(R_{el}/R_{oc}\) ratio increased to 2.0% for \(R_j = 1 \text{ G}\Omega\) and decreased to 0.8% for \(R_m = 1.5 \text{ G}\Omega\). The \(R_j\) estimates in Table 2 account for the changes in \(R_j, R_{oc}\), and \(R_m\) of the model circuit. However, in an open-cell experiment, where the change in \(R_m\) was not accounted for, the \(R_j\) estimates would be 354.4 and 616.6 \(\text{M}\Omega\), respectively. Using the slope of the \(I–V_{m}\) plot does not improve these estimates (335.4 and 589.9 \(\text{M}\Omega\)) because the \(\approx 30\%\) and 20% errors are due to the estimate of \(R_m\). The only
A possible way to account for a change in $R_e$ is to return to a control condition where $R_{sec}$ was known initially, otherwise changes in $R_e$ will dramatically affect the $R_j$ estimate in the open-cell configuration.

### Experimental application of $I_j$ and $R_j$ correction methods

#### Assessment of cellular and junctional parameters

To assess the feasibility of these two correction methods relative to an uncompensated $R_j$ estimate, Eqs. 5a–c were applied to dual whole-cell current recordings from rCx40-transfected N2A cells. $R_{cl}$ was determined from Eq. 6 in response to a 10-ms, −40 to −35-mV voltage step applied simultaneously to both cells. To obtain all of the necessary cellular parameters, both cells were simultaneously ramped from −140 to +60 mV from a common holding potential of −40 mV. The whole-cell current traces from one experiment are illustrated in Fig. 5. The $R_{mi}$ and $R_{ci}$ values were 3.6 and 1.5 GΩ as determined from trace 1 in the −140 to −20-mV range. The command potential ($V_{comm}$) was varied by 1 mV per 20 ms. The slope of the $I–V$ curve was determined for each cell by linear regression analysis over the linear range of the $I–V$ curve and $E_{rest}$ was determined for each cell by solving for the condition of $I_1 + I_2 = 0$ to obtain the value of $V_{comm}$ necessary for Eq. 8b. Alternatively, the value of $E_{rest}$ can be determined directly from the common value of $V_m$ where $I_1 + I_2 = 0$ in the two $I–V_m$ plots. $V_m$ was calculated from the whole-cell currents using Eqs. 2a and 2b. The value of $g_j$ was subsequently determined from the same voltage ramp applied alternately to cell 1 and cell 2 (Fig. 5, traces 2 and 3) to generate the corresponding $I_j–V_j$ plots. The $R_j$ measurements of 169 and 200 MΩ from traces 2 and 3 with Eqs. 5a, 5b, or 5c/1f were calculated from the reciprocal of the linear slope in the −20 to +20-mV range. Eqs. 2a/1k and 5d produced identical slopes with slightly higher $R_j$ values (173 and 202 MΩ, Fig. 6, A and B). The respective uncompensated $R_j$ measurements were 232 and 262 MΩ. These ≥30% higher $R_j$ measurements occurred even though the $R_{j}/R_{ci}$ ratios were only 0.75% and 1.95% for cells 1 and 2 because the $(R_{cell} + R_{el})/R_{j}$ ratio was ≥29%. The significance of including $E_{rest}$ in the Kirchoff’s law solution to $I_1$ was also examined by omitting this term from Eq. 5a (Fig. 6, A and B, data not shown).

The results of four rCx40 cell pairs revealed only two differences between the four $R_j$ estimates. Eq. 5c, the uncompensated $R_j$ estimate, overestimated $R_j$ in every experiment, whereas Eqs. 2c/1k and 5d always produced identical slopes of the $I_j–V_j$ curves. The effect of omitting the $E_{rest}$ term from the $I_j$ estimate was a parallel shift in the $I_j–V_j$ curve along the $V_j$ axis. The $x$-intercept was ±1 mV in three of four experiments using Eqs. 5c and 5d. The value of the $x$-intercept was higher in three of four experiments using Eq. 2c/1k and exceeded 1 mV in 50% of the $I_j–V_j$ curves. Omitting the $E_{rest}$ term from Eq. 1k produced higher voltage offsets in 50% of the cases.

One advantage of this approach is that an estimate of $R_j$ can be obtained in both directions from the slope of the linear region of the $I_j–V_j$ plots. Asymmetries in the dual whole-cell circuit can be accurately assessed from the data acquired in <1 min. Another advantage is that $g_j$ (or $R_j$) can be calculated in a continuous manner over a ±100-mV range. The primary disadvantage is that $I_j$ is obtained in an asymmetric manner relative to $+V_j$ and $−V_j$ values because each $V_j$ ramp commenced with a large $V_j$ value and ap-
proached the opposite $V_j$ polarity from an initial value of 0 mV. This is best illustrated in Fig. 6, where the $I_j-V_j$ and $g_j-V_j$ plots for the experiment shown in Fig. 5 are presented. When $V_j$ is used as calculated in Eq. 2c, the large transient in $I_j$ in response to an instantaneous −100-mV $V_{\text{comm}}$ step occurs on opposite ends of the $I_j-V_j$ curve (Fig. 6, A and B). The apparent asymmetry in the applied $V_j$ prevents valid comparisons of $V_j$-dependent changes in $g_j$ at opposite $V_j$ values. The $I_j$ transient also illustrates the point that $V_j$ is changing during the initial voltage step because the value of $I_j \cdot R_{\text{el}}$ is changing in time. This could explain the variability observed in the decay time constants of $I_j$ in previous reports of $V_j$-dependent gating (Veenstra, 1991b; Wilders and Jongsmma, 1992; Wang et al., 1992; Chanson et al., 1993).

The $g_j-V_j$ curves calculated with Eqs. 5d or 2c/1k also illustrate the differences in the $-\Delta I_j$ (Eq. 1g) and $-I_j + I_{m2}$ (Eq. 1k) approaches to estimating $I_j$. Anytime $I_j$ or $V_j$ approaches 0, the $R_j$ and $g_j$ estimates become unstable and approach $\pm \infty$. These fluctuations in the $g_j$ estimates are generally larger with Eq. 2c/1k than with Eq. 5d. The $g_j$ fluctuations were closer to 0 mV with Eq. 5d because the $x$-intercept is also smaller relative to Eq. 2c/1k (Fig. 6, C and D).

$V_j$-dependent regulation of $g_j$

Because the steady-state $G_j-V_j$ curve for rCx40 derived from a conventional voltage pulse protocol was already known (Beblo et al., 1995), we chose to reexamine the intrinsic $V_j$-gating of rCx40. The primary disadvantage of the $V_j$ ramp was alleviated by gradually increasing $V_j$ from 0 mV in both directions and the ramp speed was varied to determine what ramp duration was required to continuously approximate steady-state $g_j$ values. $V_j$ was varied from −40 to −140 mV and from −40 to +60 mV in 1-mV increments with rest intervals of varying duration between each voltage ramp to allow for recovery from any $V_j$-gating that might have occurred. Each $V_j$ ramp was repeated five times and the $I_j$ and $I_{m2}$ traces were ensemble averaged prior to calculation of $I_j$, $V_j$, and $g_j$. Ramp durations of 5, 10, 15, 20, 25, 30, 40, 50, 100, and 200 ms/mV were repeated five times on each of five cell pairs. The results from one experiment are shown in Fig. 7 A that demonstrate the progression toward steady-state values with increasing ramp duration. Only Eq. 1j was used to estimate $I_j$ because Eq. 5d produced accurate and more stable $R_j$ estimates than did Eq. 2c/1k without requiring determination of $E_{\text{rest2}}$ values. All continuous $I_j-V_j$ relationships were linear in the $V_j$ range of ±25 mV. The slope of the $I_j-V_j$ relationship in the 0 to ±25-mV $V_j$ range was used to normalize $g_j$ ($G_j$) of each experiment. The $G_j-V_j$ curve was fitted by the equation

$$G_j = \frac{G_{\text{max}} - G_{\text{min}}}{1 + \exp((zF/RT)(V_j - V_{1/2}))},$$

with pClamp8.0 software (Axon Instruments, Inc., Foster City, CA).

The $G_{\text{max}}$ and $G_{\text{min}}$ values were constant for all voltage ramp speeds (5, 10, 15, 20, 25, 30, 40, 50, 100, and 200 ms/mV). The average $G_{\text{max}} = 1.01 \pm 0.01$ and the average $G_{\text{min}} = 0.24 \pm 0.04$ for all ten voltage ramp durations. The half-inactivation voltage ($V_{1/2}$) and gating charge valence ($z$) values varied reciprocally with the voltage ramp speed as illustrated in Fig. 7, B and C. The $V_{1/2}$ and $z$ values for $-V_j$ and $+V_j$ were fitted with a single exponential function to determine the time constant ($\tau$) for each process. The standard deviations were $V_{1/2} < 0.8$ mV and $z < 0.10$ for each data point. The $\tau_{V_j}$ was $36 \pm 8$ and $23 \pm 6$ ms/mV for $-V_j$ and $+V_j$ and $\tau_j$ was 81 and 149 ms/mV. Because $V_j$ was not constant, $\tau$ possesses the units of ms/mV. The time-dependence of these $G_j$-gating parameters has not been previously described. The shortest ramp duration of 5 ms/mV ensured that the voltage clamp had achieved steady state prior to the next 1 mV step for all experiments. Figure 7 C indicates that, as soon as $V_j$ was stable, a net valence of approximately two gating charges had already moved (i.e., instantaneous) with another 1.5–2.0 charges to follow with a $\tau$ of approximately 80–150 ms. This suggests that part of the rCx40 $V_j$-gate is located within the $V_j$ field while another

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<th>Resistance Value (GΩ)</th>
<th>Actual $R_{\text{oc}}$</th>
<th>$R_{\text{oc}}$</th>
<th>$I-V$</th>
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<td>512.1</td>
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*Actual $R_{\text{oc}}$ was calculated using Eq. 9 with $R_{\text{el}} = 10$ GΩ, $R_{\text{oc}} = 1.0$ GΩ, and $R_{\text{el}} = 10$ MΩ unless otherwise indicated.

$^\dagger R_j$ was calculated from Eq. 9.

$V_{\text{oc}}$ was calculated with Eq. 2a and the $I_j-V_{\text{oc}}$ plot was fitted by linear regression.

$^\dagger R_j = 0.5$ GΩ for all calculations.
FIGURE 5 Experimental determination of \( R_{\text{g}} \). A voltage ramp from \(-140 \) to \(+60 \) mV with a slope of \( 0.05 \) V/s (i.e., \( 20 \)-mS/mV increment) was applied sequentially to both cells simultaneously (trace 1), cell 1 alone (trace 2), and cell 2 alone (trace 3) from a common holding potential of \(-40 \) mV. The corresponding whole-cell current traces are shown for (A) cell 1 and (B) cell 2. \( R_{\text{m}} \) was calculated from the slope of trace 1 after calculating \( V_{\text{m}} \) for each cell. Only \( V_{\text{m}} < -20 \) mV was used to calculate \( R_{\text{m}} \) for N2A cells. \( R_{\text{m1}} \) and \( R_{\text{m2}} \), previously determined from whole-cell capacitive transient decay constants, were 28 and 30 M\( \Omega \). \( R_{\text{m1}} \) and \( R_{\text{m2}} \), were 3.6 and 1.5 G\( \Omega \), and the resulting series resistance errors were 0.8% and 2.0%, respectively. \( E_{\text{rev}} \) was \(-24.5 \pm 0.2 \) mV for both cells. \( I_{\text{g}} \) was calculated from traces 2 in panel B and 3 in panel A for the purpose of estimating \( R_{\text{g}} \).

component of approximately equal charge moves into the \( V_{j} \) field with a finite reaction rate that can be determined using a variable duration voltage ramp method. The final values for \( V_{1/2} \) and \( z \) also closely approximate the previously determined values for rCx40 using a conventional voltage step protocol.

Because the \( V_{1/2} \) converged to a constant value for the 100 and 200-ms/mV duration \( V_{j} \) ramps, the 200-ms/mV \( G_{j-V_{j}} \) curves were compared to the steady-state \( G_{j} \) curve for rCx40 obtained using a 6-s duration pulse protocol (Beblo et al., 1995). The results from six rCx40 cell pairs are summarized in Fig. 8 A and Table 3. The slope of the instantaneous \( I_{j-V_{j}} \) relationship was used to normalize \( g_{j} \) for each experiment from Beblo et al. (1995). Because there was no instantaneous \( I_{j-V_{j}} \) relationship for the ramp protocols, the linear slope of the 0 to \( \pm 25 \)-mV \( I_{j-V_{j}} \) relationships were used to normalize the \( g_{j} \) of each experiment. The mean \( g_{j} \) was \( 2.74 \pm 2.58 \) and \( 2.49 \pm 2.05 \) nS for the six \( -V_{j} \) and \( +V_{j} \) ramps compared to \( 2.26 \pm 1.50 \) nS from previous results (\( N = 28 \), only 10 were used for the Boltzmann fit, Beblo et al., 1995). The slight reduction in the mean slope \( g_{j} \) between \( -V_{j} \) and \( +V_{j} \) ramps indicates that the 15-s rest interval needs to be extended to permit full recovery from \( V_{j} \)-dependent inactivation.

A pulse protocol similar to the one used in Beblo et al. (1995) was also applied to the same six rCx40 cell pairs (Fig. 8 B). The mean \( g_{j} \) was \( 2.79 \pm 2.11 \) nS for the six instantaneous \( I_{j-V_{j}} \) relationships (\( r \approx 0.97 \) except for the lowest \( g_{j} \) experiment, \( r = 0.93 \)). The best fit with Eq. 12 to this data set yielded \( G_{\text{max}} = 1.13 \pm 0.23 \) or \( 0.81 \pm 0.06 \), \( G_{\text{min}} = 0.20 \pm 0.07 \) or \( 0.23 \pm 0.05 \), \( V_{1/2} = -34.9 \pm 8.2 \) or \(+44.2 \pm 3.8 \), and \( z = -1.7 \pm 0.7 \) or \(+4.0 \pm 2.1 \) for \( -V_{j} \) and \(+V_{j} \) values. The mean instantaneous and steady state \( g_{j} \) values were \( 2.81 \pm 2.17 \) and \( 2.45 \pm 2.06 \) nS. These results further demonstrate the advantage of the continuous \( V_{j} \) ramp over the pulse protocol in producing steady state \( G_{j-V_{j}} \) results with reduced variability from the same experimental population. Each ensemble-averaged \( V_{j} \) ramp was acquired in the same amount of time as a single \( V_{j} \) pulse protocol.

DISCUSSION

The most sensitive assay for gap junction communication is electrical current because it can be resolved to the level of a single channel. The regulation of gap junction communication typically requires only the assessment of the value of \( R_{j} \) or \( g_{j} \) in response to the modulating treatment. There are limitations to the measurement of \( R_{j} \) from dual whole-cell patch clamp recordings that are often not appreciated. Two related methods of correcting for series resistance errors in the estimation of \( I_{j} \), \( V_{j} \), and \( R_{j} \) have been published (Veenstra and Brink, 1992; Van Rijen et al., 1998). Neither of these methods considered the possible effects of physiologic cellular resting potentials or gap junction diffusion potentials on these electrical measurements. The derivations presented in this manuscript provide the mathematical solutions to these “real” cell conditions and assess the performance of the Kirchoff’s law (Eqs. 1h and 1i) and baseline subtraction expressions (Eqs. 1g and 1j) for \( I_{j} \) under a variety of experimental conditions that mimic actual dual whole-cell experimental conditions. The experimental determination of nonzero \( E_{\text{rev}} \) values will be considered in another manuscript on relative ionic permeability measurements of gap junction channels.
The results presented in Figs. 2 and 3 demonstrate that both methods are accurate to within 5% error even with 20-nS \( g_j \) cell pairs and 50 M\( \Omega \) whole-cell patch electrodes. The differences in the two correction methods are most prevalent when \( I_j \) is small and variations in the estimate of \( I_{in} \) from the whole-cell current dramatically influence the relative value of \( I_j \). Because Eq. 1g results from the baseline subtraction of Eq. 1i when \( V_1 = V + \Delta V \) and \( V = V_1 = V_2 \), the only expected difference between these two expressions will result from different assessments as to the value of \( I_{in2} = V_2/R_{in2} \) (Eq. 1f; Eq. A9 in Van Rijen et al., 1998). The effect of this one difference is illustrated in Figs. 5 and 6 where the variations in the \( g_j \) estimate increase as \( V_j \) and necessarily \( I_j \) nears zero. The model and experimental results of dual whole-cell recordings demonstrate that the \( -\Delta I_2 \) expression is inherently more stable than the \( -I_2 + I_{in2} \) expressions derived from Kirchoff’s law. As expected from the initial conditions, the stability of the \( -\Delta I_2 \) method depends on the stability of the \( I_2 \) \((V_1 = V_2) \) baseline, not the value of \( V_2/R_{in2} \) as is true for the Kirchoff’s law \( I_1 \) equation. This is true even when the value of \( E_{rest} \) is included in the \( I_{in2} \) calculation (Eq. 1i). Eq. 1g also outperformed Eqs. 1f and 1i in 75% of the experiments in estimating the \( x \)-intercept of the \( I_j-V_j \) relationship to be nearest to 0 mV (<1 mV error). Because it is not necessary to calculate \( E_{rest} \) to estimate \( I_j \) when using the \( \Delta \) approach, this method is also easier to implement experimentally. Except when \( I_j \) is small, the two approaches agree very closely. So either method is acceptable although the ease of use and stability of the \( I_j \) measurements favor the use of the \( -\Delta I_2 \) method. Another
occur with different ramp durations (5, 20, 50, and 200 ms/mV). The ms/mV ramps, respectively. (5.1 and 5.2, 4.6 and 5.4, and 3.4 and 4.3 nS for the 5, 20, 50, and 200 FIGURE 7 The time-dependence of Vj from one rCx40 experiment illustrate the time-dependent changes in 5–6 rCx40 experiments were fitted with Eq. 12 and the value of the Junctional Current and Conductance Measurements 2243

\[
\frac{g_j}{V_j} = \frac{V_{m1} - E_{rest1}}{R_{m1}}
\]

(11)

and

\[
I_j = -I_2 + \frac{V_{m2} - E_{rest2}}{R_{m2}}.
\]

(1m)

These equations still depend on the precise calculation of \(E_{rest2}\) and \(V_{m2}\) to accurately determine the value of \(I_{m2}\). Because \(\Delta V_{m2}\) equals \(I_2 \cdot R_{m2}\) (\(\approx I_1 \cdot R_{m2}\), Eq. 2d), the difference between \(V_2\) and \(V_{m2}\) is typically small. Hence, the \(I_j\)–\(V_j\) curve will be shifted only by an amount equal to \(\Delta V_{m2}\). half-inactivation voltage, \(V_{i2}\), for 10 different \(V_i\) ramps were fitted with a single exponential function to determine the time constant. The solid points represent the value of \(V_{i2}\) obtained from a conventional long-pulse voltage protocol (Beblo et al., 1995). The \(V_{i2}\) converged toward a final value of \(-49\) or \(+53\) mV with a time constant of 36 or 23 ms/mV. \(G_{m1} = 1.03\) and \(G_{m2} = 0.23\) were constant for all slope voltages. \(N = 5\) for all ramp durations except the 200-ms/mV ramp (\(N = 6\)). (C) The net valence \(z\) of the gating charge movements for the same data shown in panel B. The solid points again represent the value of \(z\) obtained from a conventional long-pulse voltage protocol (Beblo et al., 1995). The initial value of \(z\) was \(\pm 2.3\) (\(\pm V_i\)) and it converged to final values of \(-3.8\) and \(+4.6\) with time constants of 81 and 149 ms/mV.

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The normalized $g_j$ was calculated for six different rCx40 cell pairs from the $I_j-V_j$ plot divided by the linear slope conductance $\pm 25$ mV, $V_j$ ($\Delta V_j$) was varied by 200 ms/mV from a common holding potential of −40 mV to −140 and +60 mV with a 15-s rest interval between the $-V_j$ and $+V_j$ voltage ramps. Each ramp was repeated five times and the $G_j-V_j$ curve was calculated from the ensemble-averaged $I_j-V_j$ trace. Every data point represents the 20-ms average $G_j$ at a constant $V_j$ (10 points/mV) from each experiment. The solid line is the best fit to the cumulative data points using Eq. 12: $G_j = (G_{\text{max}} - G_{\text{min}}) / [1 + \exp(\pm 2\Delta V_j / V_{1/2})]$. Each half of the $G_j-V_j$ plot was fitted independently, and the Boltzmann parameters are listed in Table 3. (B) The same steady-state $G_j-V_j$ curve acquired using a pulse protocol where $V_j$ was varied in 10-mV increments over a ±100-mV range. The duration of each $\Delta V_j$ pulse and rest interval was 7.5 sec. The values of the fitted parameters for Eq. 12 are listed in the text. The instantaneous $g_j$ was used to normalize the steady-state $g_j$ value of each experiment.

The percentage improvement in the $R_j$ estimates for Eq. 2c/1m over Eq. 2c/1k or Eq. 5a is also only 1 or 2 times the percentage value of $R_{el2}$ relative to $R_{in2}$. As was shown for Eqs. 1f and 1i, the behavior of the two expressions will be similar because the only difference is in the initial estimate of $I_{in2}$. The data in Figs. 2, 3, and 6 indicate that the $-\Delta I_j$ method (Eq. 5d) is inherently more stable, easier to apply, and frequently more accurate than the Kirchoff’s law expression.

Both Eqs. 5a and 5b require knowing the value of $R_{el}$ to make any corrections. The occasional experimenter can accurately account for the error in the $R_j$ estimate by remembering that the actual resistance being measured in the dual whole-cell voltage clamp experiment is $R_{el1} + R_j + R_{el2}$. It follows that the proportion of the command $V_j$ actually applied to the junction is

$$V_j = [(V_1 - V_2) \cdot R_j] / (R_{el1} + R_j + R_{el2}).$$

The direct measurement of $I_j$ by one whole-cell electrode combined with this corrected $V_j$ value will accurately estimate $R_j$. It is most convenient to use the cell where $V_j$ is held constant because $I_{in}$ will not change dramatically, provided that $R_{in}$ remains stable. Contrary to Eq. 1 and 2 from Van Rijen et al. (1998), it is important to keep the same point of reference when alternatively varying $V_1$ and $V_2$ to produce a defined $V_j$. A $-\Delta V_j$ pulse produces a negative $V_j$ relative to $V_2$ when applied to cell 1 but a positive $V_j$ relative to $V_2$ when $-\Delta V_j$ is applied to cell 2. Hence, the net voltage gradient across the gap junction will be oppositely directed whenever the same $\Delta V$ is applied alternately to cells 1 and 2. This is especially important when bilateral symmetry is not maintained across the junction, because the resulting $E_{rec}$ or rectifying $I_j$ (and $g_j$) must maintain the same polarity whether $V_1$ or $V_2$ is varied to produce $V_j$ (Barrio et al., 1991; Bukauskas et al., 1995; Suchyna et al., 1999). Figures 5 and 6 illustrate this point in relation to the $V_j$-gating of rCx40 gap junctions. The −100-mV $\Delta V_j$ step was applied alternately to cells 1 and 2 (traces 2 and 3) and resulted in $I_j$ transients of opposite $V_j$ values.

Because $V_j = [V_1 - (I_j \cdot R_{el1}) - V_2 + (I_2 \cdot R_{el2})]$ (Eq. 2c), whenever time-dependent changes in $I_j$ occur, the applied $V_j$ will not remain constant. Any asymmetry in $I \cdot R_{el}$ will produce differences in the applied $V_j$ during a $\Delta V_j$ pulse that result in an asymmetric $G_j-V_j$ curve if the command $V_j$ value is used in the final analysis. Because $R_j$ increases as $I_j$ decreases during a constant $\Delta V$, a time-dependent increase in the actual applied $V_j$ also develops. Hence, $V_j$ is not constant during an instantaneous $\Delta V_j$ step and exponential

### Table 3: Boltzmann Parameters for Steady-state rCx40 $G_j-V_j$ Plots

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beblo et al., 1995</th>
<th>Ramps</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_{\text{max}}$</td>
<td>1*</td>
<td>1*</td>
</tr>
<tr>
<td>$G_{\text{min}}$</td>
<td>0.33</td>
<td>0.28</td>
</tr>
<tr>
<td>$V_{1/2}$</td>
<td>−54 mV</td>
<td>+47 mV</td>
</tr>
<tr>
<td>$z$</td>
<td>3.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

$^*G_{\text{max}}$ was fixed to a value of 1.0 for the Boltzmann fits in Beblo et al., 1995.
fits of the decay phase of $I_j$ can result in variable kinetic time constants ($\tau$). The best correction for this variability is to calculate $V_j$ and $I_j$ using correction formulas $2c$ and $1j$ (or $1m$) for every digitized point. However, variations in $V_j$ still depend on the proportion of the initial voltage drop (i.e., $R_{gj}/R_j$) across the electrode, and kinetic variability will still exist even with the corrections. The operative factor here is $\partial V_j/\partial t$ that may account for some of the kinetic variations in $\tau_{V_j}$ (Veenstra, 1991b; Wilders and Jongsmma, 1992; Wang et al., 1992; Chanson et al., 1993). A method for determining the equilibrium properties of the steady state $G_j-V_j$ curve devoid of instantaneous fluctuations in $V_j$ was developed. The results of the continuous $V_j$ ramps are reported herein.

Any time there is an asymmetry across the gap junction, a finite voltage will exist across $R_j$. This will produce a small $I_j$ even when $V_1 = V_2$. It does not matter if the source of the asymmetry is a heterotypic gap junction, asymmetric bilateral ionic salt gradients, or asymmetries in the two whole-cell circuits, $V_j \neq 0$ mV. According to the $\Delta I_j$ method, this residual $I_j$ will be subtracted out with the $-I_2$ ($V_1 = V_2$) baseline. Hence, small errors can occur using the $\Delta I_j$ method anytime there is an initial $I_j$ component when $V_1 = V_2$ (Eq. 1g and 5b). The alternative expressions derived from Kirchoff’s law offer an improvement over this condition, provided that $I_{rest2}$ is included in the calculation of $I_{in2}$ as presented in Eq. 1i and 5a. For the sake of accuracy, the correct expressions are Eq. 1m and 1c/1m for the $I_j$ and $R_j$ calculations using the Kirchoff’s law expressions. This was demonstrated in Fig. 2, A and B where the percent error in the $R_j$ estimate was slightly lower at high $R_j$ values ($R_j \geq 2$ G$\Omega$). However, most often when $R_j \geq 2$ G$\Omega$, single-gap junction channel currents are visible in the $I_j$ recording and single-channel analysis methods are used. When single-channel current amplitudes ($i_j$) are measured from macroscopic $I_j$ traces, this difference between Eqs. 1g and 1i is alleviated. The merits of all-points (“real-time”) current histograms also ensure that $i_j$ and $I_j$ are represented as they appeared in the originating whole-cell current recording (Veenstra and Brink, 1992). Under single-channel recording conditions, $R_{gj}$ rarely exceeds 1% of $R_j$ or $R_{in}$ and the series resistance errors are minimized. Furthermore, the discrepancies between $V_{in1}$ and $V_{in2}$ when $V_1 = V_2$ rarely exceed 1 mV under adequate whole-cell voltage clamp conditions. This fact is demonstrated in Figs. 5 and 6, where actual $I_j$ recordings from homotypic rCx40 gap junctions under symmetrical ionic conditions produced lower x-intercept (<1 mV) values with Eqs. 1g and 1j than with Eqs. 1f, 1i, or 1m in 75% of the experiments. The one exception was an experiment where $R_{in1} < 1$ G$\Omega$ and the x-intercept was 2–3 mV from the origin using Eq. 1g compared to 0.9–1.4 mV with Eq. 1i for the corresponding $I_j-V_j$ curves. In actual application, Eq. 1g (the $\Delta I_j$ method, Veenstra and Brink, 1992) is more accurate than Eq. 1i (derived from Eq. 1f(A9) in Van Rijen et al., 1998) in estimating $I_j$ from $-I_2$.

Another approach to study the regulation of $g_j$ was to expose the gap junction by shunting $R_{in}$ and then perfuse with intracellular ions that may modulate cellular function such as H$^+$, Ca$^{2+}$, Mg$^{2+}$, and ATP$^4-$ (Noma and Tsuboi, 1987; Sugiuura et al., 1990). The derivations for the “open-cell” configuration (Eqs. 9–11) reveal that it is necessary to determine the value of $R_j$ and $R_{in1}$ prior to establishing this configuration ($R_{oc} = R_j || R_{in}$) from the dual whole-cell configuration to be quantitatively accurate. Because the open-cell $R_j$ estimate is equally sensitive to $R_{in1}$, the accuracy of all open-cell $R_j$ measurements is favored by a low $R_j/R_{in}$ ratio. Therefore, this approach is amenable to $R_j$ measurements only when the experimental variable to be tested does not equally affect $R_{in}$. The primary advantage of this approach is the ability to internally perfuse a gap junction with a variety of ionic solutions in a reversible manner.

The ability to obtain accurate $R_j$ measurements were advanced by the use of $V_j$ ramps to the measure of steady-state $V_j$-dependent gating (Figs. 7 and 8). $V_j$ was symmetrically increased from 0 to $\pm 100$ mV in 1-mV increments of varying duration. Ensemble averages of five $V_j$ ramps of equal duration were normalized to the slope $g_j$ of the $I_j-V_j$ curve from 5–6 different rCx40 cell pairs. The $G_j-V_j$ curves of all experiments were pooled together and fitted with a Boltzmann function (Eq. 12) to estimate the half-inactivation voltage ($V_{1/2}$) and gating charge valence ($z$) for the $V_j$-gating of rCx40. $G_{max}$ was within 1% of the normalized value of 1.00, and $G_{min}$ was 0.24 $\pm$ 0.04 for all ramp durations tested. These values were in close agreement with previous results using 10-mV, 6-s duration $V_j$ steps (Table 3; Beblo et al., 1995). The slightly lower $G_{min}$ may result from the calculation of actual $V_j$ using Eq. 2c in the $g_j$ calculations or from the 200-ms/mV continuous variation of $V_j$ (20 sec per 100 mV ramp). The new data indicate that the $V_{1/2}$ and $z$ values are similar for $g_j = 2–7$ nS cell pairs when corrected. The largest variations in the actual $G_j-V_j$ curves result from the two experiments where $g_j < 1$ nS because individual channel openings and closings accounted for a larger percent of $I_j$. The previous $G_{max}$, $G_{min}$, and $V_{1/2}$ values were within 2–5% of the values obtained here using $V_j$ ramps and all-points $I_j$ and $V_j$ correction methods. The net gating charge valence ($z$) increased from 3 to 4 with the continuous steady-state $G_j-V_j$ curve. The continuous $I_j$, $V_j$, and $g_j$ analysis provided by the $V_j$ ramps allowed for more accurate fitted curves from fewer experiments as evidenced by the lower standard deviation of the fitted parameters relative to the same results obtained with a pulse protocol. Each experiment also required less time to acquire a single steady-state $I_j-V_j$ curve. This method should be useful to all experimental applications where equilibrium constants are to be determined for an $I_j$ blocking reaction.

In addition to the confirmation of the $V_j$-gating parameters of rCx40, the rate at which $V_{1/2}$ and $z$ varied with $V_j$ were obtained for the first time. The $V_{1/2}$ decreased from approximately $\pm 70$ mV for the 5-ms/mV ramp to a final
value of approximately \( \pm 50 \text{ mV} \) with a \( \tau \) of 20–40 ms (Fig. 7). According to the Boltzmann model, \( \tau = 1/(\alpha + \beta) \), the opening, \( \alpha \), and closing, \( \beta \), rates for the gap junction channels (assuming only two states) and \( \alpha = \beta \) at \( V_{1/2} \) (Harris et al., 1981; Spray et al., 1981). This means that the equilibrium between \( \alpha \) and \( \beta \) has a time constant of \( \approx 20 \text{ ms} \), and the opening and closing reaction rates at this \( V_j \) are \( \approx 0.1 \text{ per ms} \). The gating charge increased from an initial valence of approximately 2 to a final value of approximately 4 with a \( \tau \) of \( \approx 100 \text{ ms} \). Most importantly, these data indicate that a gating charge movement with a net valence of 2 occurred as quickly as the settling time of the dual whole-cell voltage clamp circuit. Two additional charges moved in a time- and voltage-dependent manner. This result favors a two-domain \( V_j \)-gating mechanism, where one half of the charge lies within the \( V_j \) field (membrane) and one-half moves in and out of the \( V_j \) field in a time-dependent manner. This is consistent with a proposed mechanism for Cx26 and Cx32, where amino acid residues near the cytoplasmic amino-terminus and the first extracellular loop of the connexins control the polarity of the \( V_j \)-gating mechanism (Verselis et al., 1994). Recent evidence further indicates that only a single subunit is required to inactivate the connexin hemichannel, and that the NH2 terminus lies within the \( V_j \) field and undergoes local conformational changes (Oh et al., 2000). The new ramp \( V_j \)-gating data provides additional kinetic information that may further identify the mechanism for \( V_j \)-dependent gating of connexin channels in a manner analogous to N-type (“ball-and-chain”) inactivation of delayed rectifier \( K^+ \) channels (Hoshi et al., 1990).

In summary, corrections for \( I_j \) and \( V_j \) can accurately account for series resistance errors in \( R_j \) and \( g_j \) estimates in the dual whole-cell patch clamp configuration. Nonzero values for cellular resting potentials \( (E_{\text{rest}}) \) and junctional potentials \( (E_{\text{rev}}) \) are also considered and found to be especially important at high \( R_j \) values. The quality of the voltage clamp improves during \( V_j \)-gating in a time-dependent manner. The alternative open-cell recording configuration requires quantitative assessment of \( R_{\text{in1}} \) prior to shunting \( R_{\text{in2}} \) and accuracy is improved if \( R_j \ll R_{\text{in1}} \). \( V_j \) ramps accurately reproduce steady-state \( g_j \) properties and provide the advantage of producing a continuous \( I_j-V_j \) curve in equal or less time than a conventional pulse protocol. Varying the speed of the \( V_j \) ramp can also assess the time-dependence of the net gating charge movement \( (z) \) and equilibrium voltage \( (V_{1/2}) \) at the expense of determining the voltage- and time-dependence of the individual rate constants \( (\alpha \) and \( \beta )\).

REFERENCES


