Action potential modulation of connexin40 gap junctional conductance

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Lin, Xianming, and Richard D. Veenstra. Action potential modulation of connexin40 gap junctional conductance. Am J Physiol Heart Circ Physiol 286: H1726–H1735, 2004.—Connexin40 (Cx40) is abundantly expressed in the atrial myocardium, ventricular conduction system, and vascular endothelium and smooth muscle cells of the mammalian cardiovascular system. Rapid conduction through cardiac tissues depends on electrotonic transfer of the action potential between neighboring cells. To determine whether transjunctional voltages (Vj) elicited by an action potential can modulate conductance of Cx40 gap junctions, simulated myocardial action potentials were applied as voltage-clamp waveforms to Cx40 gap junctions expressed in mouse neuro2A (N2A) cells. Junctional currents resembled the cell-to-cell voltage for each CL (Vj), returned toward 0 mV. Time-dependent conductance curves for Cx40 were modeled with one inactivation and two recovery voltage-dependent components. There was a temporal correlation between development of conduction delay or block and the inactivation phase of junctional conductance. Likewise, recovery of junctional conductance was coincident with recovery from refractoriness, suggesting that gap junctions may play a role in the genesis and propagation of cardiac arrhythmias.

Previously, we reported that the transjunctional voltage (Vj) gating properties of Cx43 are sufficient to produce phasic changes in junctional resistance (Rj) between two cells experiencing voltage gradients equivalent to the amplitude of the ventricular cardiac action potential (21). Because increasing Rj between cardiac myocytes can support slower conduction velocities (0) than reduced excitability, we wanted to determine whether the junctional conductance (gj) of Cx40 was similarly modulated by time-dependent Vj gradients (15, 27, 28). Intercellular conduction times can increase from 200 μs to >20 ms before complete conduction block develops (18, 36, 37, 41). Under these conditions, the safety factor for propagation depends increasingly on the L-type calcium current (25, 28, 31). Cx40 gap junctions have a half-inactivation voltage (V1/2) of −50 mV, 10 mV less than values reported for Cx43 (1, 33, 38, 39). Therefore, Cx40 gap junctions should experience similar, if not greater, changes in Rj during the depolarization and repolarization phases of the cardiac action potential relative to Cx43. In this study, we report our findings on the modulation of Cx40 gj by action potential-generated Vj gradients and develop a model for calculating the time-dependent gj curves at six different steady-state cycle lengths (CL) of stimulation.

MATERIALS AND METHODS

Stable rat Cx40 transfectants of mouse neuro2A neuroblastoma (N2A) cells were cloned as previously described (1). Double whole cell patch-clamp recordings were achieved using the procedures defined for correcting for junctional series resistance errors (35). Electrode resistance was 6–31 MΩ (16.0 ± 6.4 MΩ) after membrane patch disruption, with whole cell input resistances of 1–5 GΩ and cell input capacitances of 1.5–3.0 pF. All experimental results included in the final analysis were limited to <5% error in the applied Vj for the entire duration of the protocol. All reported gj measurements represent corrected values, and junctional current (Ij) was defined as −ΔIj (35). All whole cell current recordings were obtained using previously described methods with dual Axopatch 200B (Axon Instruments, Union City, CA) or BioLogic RK-400 (Molecular Kinetics/Bio-Logic, Pullman, WA) patch-clamp amplifiers (21, 35). All records were low-pass filtered at 500 Hz (LPF-202A 4-pole Bessel filter, Warner Instruments, Sarasota, FL) and digitized at 4 kHz using a Digidata 1320 analog-to-digital board and pCLAMP 8.2 software (Axon Instruments). Data analysis was performed using the CLAMPfit program, and Ij and Vj calculations were performed offline. Graphs were constructed using Origin version 6.1 or 7.0 software (OriginLab, Northampton, MA).

The Luo-Rudy cardiac action potential voltage-clamp waveforms were the same as those previously described (21, 23), and stimulus CL of 250, 500, 750, 1,000, 1,500, and 2,000 ms were applied until a steady state was achieved (≤200 simulated beats). The output voltage action potential waveform was applied to cell 1 (V1), whereas the command voltage for cell 2 (V2) was set constant to the simulated diastolic resting potential for each CL (−88.1 mV ≤ V2 ≤ −90.2 mV).

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RESULTS

Time-dependent changes in \( I_j \) and \( g_j \) during an action potential. Cx40 cell pairs were stimulated at a constant CL of 1,000 ms for 200 s as a control. The initial \( I_j \) response to the first applied action potential and the steady-state average of the last 100 action potentials are shown in Fig. 1A. There was a 12-ms delay before the onset of the action potential, and the action potential delay at 95% repolarization (APD\(_{95} \)) was 153 ms at CL = 1,000 ms. The peak \( g_j \) of this cell pair was 1.56 nS. The initial and average steady-state \( I_j \) traces qualitatively resemble the shape of the action potential but decline from the action potential peak to an almost constant steady-state value achieved near the midpoint of the action potential plateau. To determine the decline in \( g_j \) during constant pacing at CL = 1,000 ms, \( I_j \) was divided by the corrected \( V_j \) value for the initial and average steady-state action potentials, and the time-dependent changes in \( g_j \) during the train of 200 action potentials were plotted. Figure 1B illustrates that, for this example, \( g_j \) declined by >50% from the peak of the action potential to a minimum quasi-steady-state plateau value. From this minimum plateau value, \( g_j \) gradually began to recover during the final phase of repolarization. To average the results from all eight experiments at CL = 1,000 ms, \( g_j \) was normalized \((G_j)\) to the peak \( g_j \) of the first action potential in each experiment, and the initial and average steady-state \( G_j \) curves were plotted as described above (Fig. 1C).

At a frequency of 1 Hz, the average behavior of a Cx40 gap junction in response to constant pacing at 1 beat/s closely resembled the behavior of the single experiment shown in Fig. 1, A and B. During the first 25 ms of the action potential, \( G_j \) declined by 37% from the normalized peak \( G_j \). \( G_j \) declined by another 18% during the plateau phase of the action potential to a nearly constant minimum of 0.45. From this minimum of 0.45, \( G_j \) rose gradually to 0.65 at APD\(_{95} \) (153 ms; Fig. 1C). \( G_j \) recovered to its full initial value over the next 30 ms, 182 ms after the onset of the action potential.

The behavior of individual Cx40 gap junction channels was assessed from low-\( G_j \) experiments, which permitted the resolution of quantal channel current fluctuations. The \( I_j \) recordings from one low-\( G_j \) experiment (<0.6 nS) are illustrated in Fig. 2. Ten individual \( I_j \) traces, the ensemble average of those 10 traces, and the ensemble average of all 200 traces are displayed in Fig. 2A. The ensemble average of as few as 10 \( I_j \) traces, inclusive of all channel conductance states, reproduced the behavior of the higher-\( G_j \) macroscopic recordings, where distinct channel current fluctuations were not observed because of the activity of \( \geq 10 \) gap junction channels. A maximum of four 150-pS Cx40 gap junction channels were estimated to be present in this experiment (Fig. 2B). On average, approximately two 150-pS channels were open initially, with only one equivalent channel remaining open during the plateau phase of the action potential in this experiment. The activity of the inactivated channel began to recover during phase 3 repolarization. These data provide direct evidence for the \( V_j \)-dependent gating of individual Cx40 gap junction channels during the action potential and indicate that this is the primary mechanism for the modulation of \( G_j \).

The same procedures were followed for the five other CL, and the results are summarized in Fig. 3A. APD\(_{95} \) for each CL was used to plot the steady-state \( G_j \) curve, because the \( G_j \) calculations became more variable as \( V_j \) \( \rightarrow \) 0, thus excluding the last 6–7 mV of repolarization from the final \( G_j \) analysis. \( G_j \) declined to a minimum of 0.45–0.50 relative to the peak \( G_j \), except at CL = 250 ms, at which a minimum time-dependent \( G_j \) of only 0.60 was achieved, owing to the shorter action potential duration. In all cases, \( G_j \) increased toward initial peak values as \( V_j \) decreased from 85 mV toward 0 mV. \( G_j \) returned
to the peak value at $V_j \leq 10$ mV during the return phase of the CL-dependent $G_j$ curve. The mean $g_j$ was $1.75 \pm 1.33$ (SD) for all experiments ($n = 28$).

To determine the time-independent relation between $G_j$ and $V_j$ for Cx40, a steady-state $G_j$-$V_j$ curve was obtained by pooling the ensemble average of five 200 ms/mV $V_j$ ramps from 0 to $\pm 100$ mV from each of four different experiments (Fig. 3B). The average $G_j$-$V_j$ curve was fitted with a Boltzmann equation as follows

$$G_j^{\text{ss}} = \frac{G_{\text{ss}}^{\text{max}} \cdot \exp\left[A \cdot (V_j - V_{1/2})\right]}{1 + \exp\left[A \cdot (V_j - V_{1/2})\right]}$$

(1)

where $G_{\text{ss}}^{\text{max}}$ is $1(G_j = g_j/g_{j,max})$, i.e., the resting normalized slope conductance for each experiment; $G_{\text{ss}}^{\text{min}}$ is the minimum value of $g_j/g_{j,max}$; $A$ is the slope factor for the Boltzmann curve ($zF/RT$ where $F$ is Faraday’s constant, $R$ is the gas constant, and $T$ is temperature); and $V_{1/2}$ is the half-inactivation voltage. The slope factor is proportional to the gating charge movement ($z$) of the state transition (14, 30). For the curve shown in Fig. 3B that best describes the averaged data, $G_{\text{ss}}^{\text{max}} = 0.24$ and 0.22, $V_{1/2} = -52$ and +48 mV, and $z = -2.9$ and +3.7 elementary charges for negative and positive $V_j$ values, respectively. These results are in close agreement with previous findings using this $V_j$ protocol (1, 35).

The minimum $G_j$ achieved during the action potential plateau does not achieve the $G_{\text{ss}}^{\text{min}}$ of the Cx40 steady-state $G_j$-$V_j$ curve. The recovery phase of the CL-dependent $G_j$ curves coincided with the increase in steady-state $G_j$ as $V_j$ decreased from +85 mV.

Voltage-dependent changes in $I_j$ decay constants. The basis for the decline in $g_j$ during the early phases of the cardiac action potential to the quasi-steady-state minimum $G_j$ was examined with $V_j$ pulses between $-40$ and $-140$ mV to determine the decay constants at fixed $V_j$ values. $V_j$ was made negative, because there is a slow time- and voltage-dependent membrane conductance that activates above $-20$ mV in N2A cell membranes that can become quite large by the end of a 2.5-s $V_j$ pulse (7.5 s for $V_j = 60$ mV). A train of 5 or 10 $V_j$ pulses of equal amplitude was applied at a rate of 1 pulse every 30 s, and the ensemble-averaged current was fitted with an exponentially decaying function. The decay time constants of selected $V_j$ pulses from a single experiment are shown in Fig. 4A. Only the initial 400 ms of the 2.5-s pulses are displayed to better illustrate the rapid decay phase of the $I_j$ signal recorded from cell 2. At some $V_j$ values, there was a second slower decay phase with time constants on the order of 1 s that were not analyzed, because they amounted to <20% of the total
The novel observation that the CL-dependent $G_I$ recovers during the late phases of the cardiac action potential was examined further by applying full-amplitude premature action potentials with incrementally increasing 10-ms delays to Cx40 gap junctions at CL = 1,000 ms. Figure 5A demonstrates that the $I_I$ amplitude clearly increases with each additional delay. Each $I_I$ trace from this single experiment represents the ensemble average of 20 sweeps for each stimulus delay. The premature stimulus delay was increased from 120 to 190 ms from the onset of the normal CL = 1,000 ms action potential. The average $G_I$ from 5 or 10 experiments is indicative of a consistent increase in $G_I$ from the minimum $G_I$ of 0.45–0.50 obtained during the action potential to 85% of the initial $G_I$ value during the first 30 ms of the diastolic interval (Fig. 5B). These observations confirm the increase in $G_I$ observed in Figs. 1C and 3A.

To assess the time and $V_J$ dependence of the $G_I$ recovery phase, a pulse protocol that stepped from the plateau action potential $V_J$ of approximately +80 to +70 mV ≤ $V_J$ ≤ +10 mV was utilized. Results from this protocol revealed that $I_I$ increased in a time-dependent manner with time constants of 10–40 ms for $V_J$ = +60 mV (Fig. 5C, Table 1). Small

$$1/\tau = A^0 \cdot \exp(V_J/V_c)$$

The decay constants decline $e$-fold per 17.6 mV ($=V_c$, the voltage constant) from an initial amplitude ($A^0$) of 0.0004 ms$^{-1}$ [time constant ($\tau$) = 2,500 ms] at $V_J = 40$ mV. This $V_J$-dependent decline in the $I_J$ decay constants was determined only by examining $V_J$ > ±100 mV. The increasingly rapid first-order decay kinetics indicate that the $V_J$-gating properties of Cx40 are fast enough to modulate $g_J$ during the time course of a cardiac action potential.

**Time-dependent recovery of $I_I$ and $G_I$**

**Fig. 3.** Time-dependent and steady-state $G_I$ curves for Cx40. A: ensemble-averaged $G_I$ over the last 100 action potentials from 5–8 experiments calculated relative to ADP$A_{50}$ for each CL. $G_I$ declined to a minimum plateau value of 0.45–0.60 and recovered to 0.70–1.00 of its initial value at ADP$A_{50}$ for each CL. B: steady-state $G_J$-junctional voltage ($V_J$) curve for Cx40 obtained from continuous $V_J$ ramps from 0 to ±100 mV in 200 ms/mV increments. Solid curved line is best fit of a Boltzmann distribution (Eq. 1) to data from 4 experiments. Ensemble-averaged $G_J$ curves from 5 $V_J$ ramps per experiment were pooled to calculate mean $G_I$ data points (symbols).

**Fig. 4.** Voltage-dependent decay of Cx40 $I_J$. A: whole cell 2 currents ($I_J$) in response to a $V_J$ pulse ($\Delta V_J$) applied to cell 1. Common holding potential ($V_J = 0$ mV) was –40 mV. $I_J$ traces represent unsubtracted whole cell current ($I_J$) values. $I_J = -\Delta I_J$, where $\Delta I_J$ is baseline ($V_J = V_J = -40$ mV) subtracted value. $V_J$-dependent decay time constants were obtained from exponential fit (solid curved lines) of ensemble-averaged $I_J$ trace from 5–10 $V_J$ pulses. B: reciprocal of average (mean ± SD) $V_J$-dependent decay time constants from 4–11 experiments well described by an exponential function with a voltage constant of 17.6 mV. $1/\tau$ was assumed to decline to zero with decreasing $V_J$ from an estimated rate of 0.0004 ms$^{-1}$ at 40 mV (Eq. 2).
time-independent increases in $G_j$ were also observed at $V_j = +40$ mV. There was no increase in $G_j$ at $+70$ mV $V_j$. The time constants were poorly correlated with the $V_j$ of the recovery pulse ($r = 0.60$ for linear or exponential fits). The time-dependent increase in $G_j$ approached the values of the steady-state Cx40 $G_j$-$V_j$ curve for each $V_j$ (Fig. 5D). These data were consistent with the hypothesis that the rise in $G_j$ resulted from a proportional $V_j$-dependent increase in steady-state open probability as observed in the steady state $G_j$-$V_j$ curve (Eq. 1 and Fig. 3B). This occurred despite the convergence of the minimum $G_j$ attained during the action potential to twice the $G_{min}$ of the steady-state $G_j$-$V_j$ curve. The recovery time constants were $\pm 40$ ms in the $V_j \leq 60$-mV range, at least two orders of magnitude faster than the decay time constants at these same voltages. The rapid inactivation and recovery time constants at high and low $V_j$ values reinforce the concept that the gating kinetics of gap junctions can achieve values in the range of 10 ms.

**Modeling phasic $G_j$ alterations.** Figure 6 illustrates the temporal correlations between the inactivation of $G_j$ during the large $V_j$ gradients associated with the onset of the action potential and the recovery phases of $G_j$ with the two phases of action potential repolarization. Projection of the average $G_j$

curve at $CL = 1,000$ ms into the $V_j$ plane clearly demonstrates that inactivation predominantly occurred at $>100$ mV and that $G_j$ rose at $V_j < 90$ mV. If the changes in $G_j$ during the cardiac action potential at each CL are due to the time-dependent kinetics and steady-state $G_j$-$V_j$ relation of Cx40 gap junctions (Figs. 3B and 4B), it should be possible to model these phasic alterations in $G_j$ under the same conditions. The time integral of $G_j$ was calculated using the two-component model developed for Cx43 (21). The inactivation components for Cx40 were defined as follows

$$G_j^{1+} = G_j^{2+} = (G^{0} - G_{min1&2}) \cdot \left[ 1 - dt \cdot A^0 \cdot \exp((V_j^{+1} - 40)/17.6) \right] + G_{min1&2} \quad (3)$$

where the two components described for Cx43 were set equal to each other for Cx40, because the decay of $I_j$ was adequately described by a single-exponential decay (Fig. 4A). The initial conditions for each inactivation component were defined as follows

$$G_j^{1+0} = G_j^{2+0} = (1 - G_{min})/2 + G_{min}/2 \quad (4)$$

so that

$$G_{max1} + G_{max2} + G_{min1} + G_{min2} = 1 \quad (5)$$

The equivalent time-dependent inactivation components ($G_j^1$ and $G_j^2$) were each given a $G_{min}$ equal to one-half the $G_{min}$ of the steady-state $G_j$ Boltzmann curve (Eq. 1 and Fig. 3B), and the $V_j$-dependent time constants were computed on the basis of the exponential decay time constants described in Fig. 4B (Eq. 2). Equation 2 provides an accurate description of the decline in $G_j$ from initial resting values at each CL, as illustrated in Fig.
Fig. 6. Functional correlation of $G_j$ in time and voltage. A: temporal correlation between $G_j$ and $V_j$ for action potential at $CL = 1,000$ ms. Vertically hatched bar indicates the first 25 ms of an action potential during which conduction block develops; Cx40 $G_j$ inactivates by 37% during this critical time period. Horizontally hatched bar indicates initial phase of $G_j$ recovery during phase 3 repolarization, which produces a 15% increase in $G_j$ from a minimum of 0.45. The diagonally hatched bar indicates the final phase of $G_j$ recovery during final repolarization. Phases of $G_j$ recovery also coincide with relative refractory and supernormal excitability periods of the cardiac action potential. B: autocorrelation plot of $G_j$ and $V_j$ best indicates voltages where inactivation, initial recovery, and final recovery phases of $G_j$ occur. Inactivation is driven by high $V_j$ values above $+100$ mV, and complete recovery requires repolarization to $V_j < 10$ mV.

7. The dashed line in Fig. 7 represents the numerical solution to Eq. 3 for each CL. The exact $G_{\text{max}}$, $G_{\text{min}}$, and $A^0$ values used to fit the decay phases of the $G_j$ curve at each CL are listed in Table 2. The value of $A^0$ was decreased from the estimated value of 0.0004 from Eq. 2 (Fig. 4B) for most CL owing to the different response times of the voltage-clamp recordings. The time lag between the model and $G_j$ curves in Fig. 7, A–F, reflects the difference between the model calculated from the real-time $V_j$ values and the finite response time of the recorded $I_j$ values.

Figure 6A further indicates that the two phases of $G_j$ recovery coincided with the two rates of repolarization of the action potential during phase 3 and early diastole. The rates of repolarization for these two temporal phases are approximately $-2.0$ and $-0.2$ mV/ms, yet the majority of the increase in $G_j$ occurs at $V_j \approx 10$ mV. There is a time dependence to the increase in $G_j$ at $V_j \approx +60$ mV, but the time constants varied only between 10 and 40 ms (Table 1 and Fig. 5C). $G_j$ exceeded the steady-state $G_j$ values at $V_j \approx 50$ mV, because the minimum $G_j$ achieved during the action potential was approxi-

mately twice the steady-state $G_{\text{min}}$, and the recovery is due to the increasing open probability of only the inactivated channels. As shown previously for Cx43 (21), a dual-exponential function of voltage, related to the two phases of repolarization, adequately described the increase in $G_j$ relative to $V_j$ (Fig. 6B). The two components of the recovery phases of $G_j$ were defined as follows

$$R_1^i = AR_1 \cdot (G_{\text{max}1} + G_{\text{min}1} - G_j^i) \cdot \exp(V_j^i/24.5)$$

(6)

and

$$R_2^i = AR_2 \cdot (G_{\text{max}2} + G_{\text{min}2} - G_j^i) \cdot \exp(V_j^i/5.5)$$

(7)

where

$$R_1^i = R_{\text{max}1}$$

(8)

and

$$R_2^i = R_{\text{max}2}$$

(9)

are the final values of $R_1^i$ and $R_2^i$. The actual values for $R_{\text{max}1}$, $R_{\text{max}2}$, $AR_1$, and $AR_2$ used to provide the fitted recovery curves in Fig. 7 are provided in Table 3. At $CL = 250$ ms, the contribution of $R_j^1$ was minimal, whereas the amplitudes of $R_j^1$ and $R_j^2$ were relatively constant at the other CL. The voltage constant in Eq. 6 was changed from 24.5 to 32.0 mV for $CL = 250$ and 1,000 ms to provide a better fit of the data in Fig. 7, A and D. The numerical solutions to Eqs. 6 and 7 are illustrated by the short- and long-dotted lines in Fig. 7.

The $V_j$-dependent solutions to Eqs. 3, 4, 6, and 7 provide an accurate description of the phasic changes in Cx40 $G_j$ observed during the cardiac action potential over frequencies of 30–240 beats/min (Fig. 7). The final expression for $G_j$ is

$$G_j^i = G_j^{i-1} + G_j^{i+1} + R_1^i + R_2^i$$

(10)

which can be solved for continuously in time and readily lends itself to incorporation into existing models of cardiac action potential propagation. These results demonstrate the flexibility of the dual-component model to fit continuous time-dependent $G_j$ curves as a function of $V_j$ for a variety of CL and cardiac gap junctions comprising different homotypic connexin combinations.

**DISCUSSION**

Our data demonstrate that $G_j$ is in a non-steady state during the first 20–40 ms of a cardiac action potential and declines with a time constant beginning in the 10-ms range until a quasi-steady-state $G_j$ is achieved during the slowly changing plateau voltages. At this juncture, $G_j$ increases in proportion to the steady-state $G_{1-V_j}$ curve for Cx40 gap junctions. This results in a slow and steady increase in $G_j$ as the $V_j$ gradient decreases from 85 to 10 mV. At $V_j < 10$ mV, $G_j$ returned to peak values. According to the original model for voltage gating of $G_j$, each gap junction channel has two gates in series that close with one specific voltage polarity (14, 30). As $V_j$ increases from 0 mV with one polarity, the open probability will decrease toward a minimum residual conductance state ($G_{\text{min}}$) on one side while remaining fully open on the other (Fig. 3B). As $V_j$ declines, the closed gates reopen with a $V_j$-dependent rate constant ($\alpha$) that is different from that for inactivation ($\beta$) (14, 30, 39) (Figs. 4 and 5 and Table 1). The modulation of $G_j$ by the cardiac action potential occurs by similar mechanisms in
Fig. 7. Model CL-dependent $G_j$ curves. Average $G_j$ curve (thin solid line) and calculated time-dependent changes in $G_j$ according to Eq. 10 (model, thick solid line) for each CL are illustrated. Integration time step ($\Delta t$) was 250 $\mu$s. Computed output of the 2 identical $V_j$-dependent inactivation components (Eq. 3; $G_1$ & $G_2$, thick dashed line), initial recovery (Eq. 6; $R_1$, long-dotted line), and final recovery (Eq. 7, $R_2$, short-dotted line) phases of $G_j$ are also indicated. Mathematical model provides an accurate description of Cx40 data averaged from 5–8 experiments at each CL. Parameter values for fitted curves are provided in Tables 2 and 3.

Table 2. Cx40 $G_j$ inactivation parameter values

<table>
<thead>
<tr>
<th>$G_j$</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1,000</th>
<th>1,500</th>
<th>2,000</th>
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<td>$G_{max1}$</td>
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<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
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<tr>
<td>$G_{max2}$</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
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<td>0.39</td>
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<td>$A_1$</td>
<td>0.00055*</td>
<td>0.00036</td>
<td>0.00025</td>
<td>0.00027</td>
<td>0.00023</td>
<td>0.0002</td>
</tr>
<tr>
<td>$A_2$</td>
<td>0.00055*</td>
<td>0.00036</td>
<td>0.00025</td>
<td>0.00027</td>
<td>0.00023</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

$G_j$, junctional conductance. $G_{max1}$ always equals $G_{max2} = (1 - G_{max})/2$ from Eq. 1. $G_{max}$ always equals $G_{max2} = G_{max}/2$ from Eq. 1. *A1 always equals A2. A2* from Eq. 2. $A^0$ was adjusted to provide the best fit at each cycle length (CL).

Table 3. Cx40 $G_j$ recovery parameter values

<table>
<thead>
<tr>
<th>$G_j$</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1,000</th>
<th>1,500</th>
<th>2,000</th>
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<tr>
<td>$R_{max1}$</td>
<td>0.40</td>
<td>0.30</td>
<td>0.30</td>
<td>0.32</td>
<td>0.30</td>
<td>0.33</td>
</tr>
<tr>
<td>$R_{max2}$</td>
<td>0.05</td>
<td>0.26</td>
<td>0.23</td>
<td>0.25</td>
<td>0.25</td>
<td>0.22</td>
</tr>
<tr>
<td>$AR_1^*$</td>
<td>1.92</td>
<td>0.91</td>
<td>1.18</td>
<td>1.15</td>
<td>1.15</td>
<td>1.35</td>
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<tr>
<td>$AR_2^*$</td>
<td>0.29</td>
<td>1.78</td>
<td>1.13</td>
<td>1.15</td>
<td>1.15</td>
<td>1.14</td>
</tr>
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</table>

*For $R_1^*$ and $R_2^*$ to achieve the final value of $R_{max1}$ and $R_{max2}$. Voltage constant for $R_1^*$ was increased from 24.5 to 32.0 mV for CL = 250 and 1,000 ms.

Cx40- and Cx43-containing gap junctions (21). Although they are qualitatively similar, there are some quantitative differences in the rates of inactivation and recovery reported for these two cardiovascular connexins. The most significant difference is the monoexponential inactivation of Cx40 gap junctions relative to the biexponential decay observed for Cx43. There was also a difference in $G_j$ recovery at $+70$ mV, where Cx40 exhibited no increase in $G_j$ while Cx43 did. These differences are likely due to the modestly different $V_j$ dependencies of Cx40 and Cx43 on $V_j$. 

H1732 ACTION POTENTIAL MODULATION OF Cx40 CONDUCTANCE
In the atrium, depolarization of an isolated atrial myocyte from ~90 mV to the threshold for the sodium current of ~60 mV will require ~1.4 pC of charge. This calculation is based on the average atrial myocyte diameter of 15 μm, length of 100 μm, membrane surface area of 4,700 μm², membrane resistivity of 2.6–3.0 kΩcm², and specific membrane capacitance of 1 μF/cm², all of which yield an estimated input resistance of 55 MΩ and membrane capacitance of 47 pF (2, 3, 16). With the assumption of a peak \( V_j \) of 130 mV, an \( I_f \) of 14 nA would deliver enough charge (\( Q_j \)) to activate the cell in 100 μs. This requires a \( g_j \) of 108 nS or an \( R_j \) of <9.3 MΩ. From \( R_j \) measurements between isolated pairs of adult ventricular myocytes, an \( R_j \) of 1.7 MΩ (\( g_j = 590 \) nS) can be estimated (40, 41). There are no similar measurements for adult atrial myocyte pairs, but assuming a 2-fold reduction in cell surface area and a proportional increase in atrial myocyte pairs, but assuming a 2-fold reduction in cell surface area and a proportional increase in \( R_j \) provides a reasonable estimate. Hence, \( R_j = 4 \) MΩ or \( g_j = 250 \) nS, only a 2.5-fold excess of gap junction channels.

Values of \( \theta \) of 0.5 ms in the atrial myocardium and 1.0 ms in a Purkinje fiber translate into a conduction time of ~100–200 μs/cell. At these \( \theta \) values, up to 50% of the 200-μs conduction time per cell can be attributed to the junctional delay in activation (10). An action potential amplitude of 130 mV and maximum upstroke velocity (\( dV_{up}/dt \)) of 100 V/s in the atrium indicates that the action potential upstroke has a duration of 1.3 ms, or approximately six cell lengths. Hence, the maximum \( V_j \) under normal conditions is only ~20 mV, so the intercellular conduction delay and \( V_j \) gradient are not sufficient to induce \( V_j \)-dependent inactivation in well-coupled myocardium (4). However, this changes when \( \theta \) is reduced to ~0.1 ms. At \( \theta < 0.1 \) ms, the intercellular conduction delay approaches the full duration of the action potential upstroke. This indicates that the slow conduction often associated with reentrant arrhythmias is associated with \( V_j \) gradients >100 mV and intercellular conduction delays >1 ms. It is under these conditions of decremental conduction that this newly described \( V_j \)-dependent gating mechanism can influence action potential propagation on a beat-to-beat basis. Furthermore, if, under normal conditions, each cell experiences only a fraction of the action potential amplitude during propagation (4), then the number of open gap junction channels required to deliver the 14 nA of excitatory current within 100 μs increases reciprocally. Hence, the number of open gap junction channels plays a more important role than previously suggested under normal conditions as well. Furthermore, recent evidence suggests that cardiac sodium channels are locally distributed near the Z lines and intercalated disks of the sarcolemma (43). This would serve to maximize the depolarization of the t tubule and intercalated disk membranes necessary to sustain excitation-contraction coupling and intercellular propagation.

Models of linear longitudinal propagation demonstrate that \( dV_{up}/dt \) increases and then decreases as \( g_j \) is reduced and that \( \theta \) varies linearly with the number of gap junction channels from >10,000 to <1,000 channels (5, 28). These models vary quantitatively in terms of the value of \( g_j \) that must be achieved before \( \theta = 0.1 \) mm/ms, but there is general agreement that this occurs when <1,000 channels are present. This represents a >95% reduction in the estimated number of open gap junctions between ventricular myocytes (21) but only an estimated 40% reduction in the number of open gap junctions between atrial myocytes. Below \( \theta = 0.1 \) mm/ms, \( \theta \) begins to vary nonlinearly with \( g_j \) (5, 28). Given that \( dV_{up}/dt \) is not yet decreasing at the corresponding value of \( g_j \), this implies that the junctional delay has increased >10-fold, to >90% of the observed conduction time, for this 5-fold reduction in \( \theta \). It is estimated that reductions in \( g_j \) can support 200-fold reductions in \( \theta \), whereas decreased excitability can produce only a 3-fold reduction in \( \theta \) before complete block develops (28). Hence, in regions of slowed conduction between 0.1 and 0.5 m/s, conduction delays of ≤1 ms occur between myocytes, \( V_j \)-dependent inactivation would be minimal, and the reduction in \( g_j \) associated with the 10-fold increase in junctional conduction delay must occur by other regulatory mechanisms (13). However, further reductions in \( g_j \) support extremely slow \( \theta \) and allow sufficient time for development of \( V_j \)-dependent inactivation. A doubling of the conduction time from this condition leads to a decreased safety factor for propagation and eventual conduction block (28).

Conduction delays of a few milliseconds normally occur in nodal tissues and between the Purkinje and ventricular cell layers of the ventricular subendocardial surface. These conduction delays were postulated to occur as a result of higher \( R_j \) values in these tissues (8, 17, 32). Conduction delays between ventricular cardiomyocytes can also increase to >20 ms before conduction fails completely (18, 36, 37, 41). Hence, conditions can exist where \( V_j \)-dependent changes in \( g_j \) become prominent within regions of the mammalian heart, particularly in transitional border zones between tissues where physiological or pathophysiological differences in \( g_j \) and membrane excitability occur. It is the purpose of this model to become incorporated into models of action potential propagation, where the effects of \( V_j \)-dependent activation on \( \theta \) can be examined at different levels of resting \( g_j \) and various cellular properties.

The rapid decline in \( g_j \) during early repolarization is especially relevant to slowed conduction, because it was demonstrated that phase 1 repolarization and the slow inward calcium current play critical roles in the propagation of the cardiac action potential under conditions resulting from high \( R_j \) values (25, 28, 31). The transient increase in \( g_j \) that occurs during phase 3 repolarization is also significant to cardiac electrophysiology, because this phenomenon coincides with the vulnerable period of cardiac excitability when premature extrasystoles can arise (26, 27, 32). During this window in time and voltage, a single extrasystole can induce unidirectional conduction block, which elicits electrical disorganization and fibrillation. Therefore, in poorly coupled tissues with heterogeneous depolarization and repolarization patterns, changes in \( R_j \) may facilitate the occurrence and spread of a premature impulse in the time and space domains. This increases the probability of unidirectional conduction block and fibrillation in the ventricular conduction system and working myocardium.

Another possible explanation for the differences in the dynamic model for the gating of Cx40 and Cx43 gap junctions is the slightly higher whole cell access resistances of the Cx40 \( I_f \) recordings. The channel access resistance determines how the current is divided among the individual gap junction channels in a plaque. One account for the observed loss of \( V_j \) sensitivity with increasing \( g_j \) was the postulated increase in access resistance as gap junction plaques become larger (42). This means that gap junction channels in the center of a gap junction plaque would contribute less current to the overall \( I_j \). It is estimated that the normal-sized myocardial gap junction plaque would possess three times more access resistance if it existed as a uniformly packed 220-μm circular patch than if it took the form of a 20 nm × 2 μm strip (12). Nonuniform packing of gap junction channels within large-diameter macular gap junction plaques and reduction of plaque.

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width to <0.6 μm serve to minimize the effect of access resistance and maximize conductance efficiency (12). These models indicate that the spatial distribution of gap junction channels within a plaque will influence a channel’s access resistance (12, 42).

The loss of Vj-dependent gating in the dual whole cell configuration is unavoidably influenced by patch electrode resistance (35). To illustrate how patch electrode series resistance (Rseries) affects the kinetics and steady-state Vj gating parameters, whole cell currents (I) were modeled as follows

\[ I_1 = V_j(R_{el1} + R_{in}) - (E_{rest}/R_{in}) \]

\[ + (V_j - V_i)(R_{el1} + R_i + R_{el2}) \]  (11)

\[ I_2 = V_j(R_{el2} + R_{in}) - (E_{rest}/R_{in}) \]

\[ - (V_j - V_i)(R_{el2} + R_i + R_{el1}) \]  (12)

where the input resistance (Rin) of each cell is equal to the parallel combination of the membrane (Rm) and gigaseal (Rs) resistances: Rin = (RmRs)/(Rm + Rs). For this demonstration, Rin was kept constant at 1 GΩ by making Rm = 2 GΩ each. Actual experimentally obtained Rin values were slightly higher: 1–5 GΩ. The resting potential (Erest) of each cell was defined as the resting diastolic potential (−89.77 mV) during the action potential at CL = 1,000 ms. Figure 8 illustrates the results obtained for I1 = −ΔI2 under various experimental dual whole cell recording conditions during application of the action potential at CL = 1,000 ms. The dynamic gap junction model for Cx40 was added to the whole cell current model according to the parameters listed in Tables 2 and 3 for Eqs. 3, 6, and 7. Slowing of the inactivation kinetics is evident at gj = 1 nS as the resistance of each whole cell patch electrode increases from 0 (ideal) to 25 MΩ (upper limit of experimental recording conditions). Diminished inactivation is more pronounced under normal recording conditions (Rel1 = Rel2 = 10 MΩ) as gj increases from 1 to 40 nS (Rj = 1 GΩ–25 MΩ). The operative factor for the reduced inactivation is the diminution of the applied Vj gradient in proportion to \([R_j/R_i + R_{el1} + R_{el2}]/(V_j - V_i)\), ignoring the contribution of the nonjunctional membrane currents to the total whole cell current (35). The decay time constants for Cx40 gap junctions will decrease e-fold for every 17.6-mV drop in applied Vj (Eq. 2). Our experimental results include data from low-conductance pairs consisting of a few Cx40 gap junction channels to cell pairs with gj = 8 nS with minimal quantitative differences in the relative steady state to peak gj values (Figs. 2 and 3) (21, 35). The results with Cx40 also demonstrate the flexibility of the basic four-component Gj model to fit continuous time-dependent Gj curves as a function of Vj for a variety of CL and distinct connexin gap junctions by simple modification of the component amplitudes and voltage constants.

The importance of Cx40 to myocardial conduction is manifested in the conduction disturbances observed in the targeted Cx40 gene knockout studies in transgenic mice and in the recently reported incidence of familial atrial standstill (12, 19, 29). Additionally, the absence of Cx40 and/or Cx43 from vascular endothelium and smooth muscle impairs the regulation and conduction of vasomotor tone via coupled electrical and chemical signaling pathways (9, 20). Although tonic contractions are more prevalent in the vasculature than phasic contractions associated with action potentials, the possibility exists that long-duration intercellular gradients affect junctional communication by the same Vj-dependent mechanisms described here. It is also probable that long-duration Vj gradients >20 mV will produce significant reductions of gj by spermine block of Cx40 gap junction channels (24). In conclusion, these data and the dynamic model for the regulation of Cx40 gj by the ventricular cardiac action potential suggest that phasic changes in gj may contribute to the development of conduction block and fibrillation between poorly coupled ex-
citable and inexcitable cells in a tissue. Implementation of these mathematical models for homotypic Cx40 and Cx43 gap junctions will allow for the future development of models related to various cardiac action potential waveforms, connexin composition of cardiac gap junctions, and propagation through cardiac tissues possessing different geometries and ionic currents.

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