Voltage-Dependent Gating and Single-Channel Conductance of Adult Mammalian Atrial Gap Junctions

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In the heart, the rapid propagation and synchronization of action potentials necessary for a normal heart rhythm and an effective cardiac output are mediated by specialized ionic channels that link adjacent cells and are known collectively as gap junctions. Cardiac gap junctions are gated by various physiological and pharmacological agents, but the role of voltage in their gating is unclear. Whereas embryonic or neonatal ventricular cells have voltage-gated gap junctions, adult cells are reported to have only voltage-independent gap junctions. We studied the voltage dependence of adult rat atrial gap junctions by individually voltage clamping each cell of a connected cell pair and controlling the transjunctional voltage (Vj), measuring transjunctional current (Ij), and calculating junctional conductance (gj). Two distinct populations of cell pairs were observed: highly coupled pairs with the peak gj ranging from 3.4 to 4.0 nS and weakly coupled pairs with the peak gj ranging from 0.3 to 2.0 nS. gj was dependent on Vj, and Ij decayed exponentially, with the time constants being voltage dependent. Voltage dependence was most apparent when cells were poorly coupled. The gj did not decrease to zero. The normalized conductance–Vj plot was fit with a two-state Boltzmann model as a first approximation, resulting in a half-inactivation potential and gating charge of 42.5 mV and 1.14 eV, respectively, for the weakly coupled cell pairs. For highly coupled cell pairs, the half-inactivation potential shifted to 53.3 mV. Single gap junctional channels had a gj of 36.2 ± 7.1 pS (range, 27–49 pS), which was Vj independent. The junctional current as obtained by ensemble average of single-channel recordings has a time constant of current decay comparable to that observed for Ij at similar Vj. These findings suggest that the voltage-dependent kinetics of Ij result from the all-or-none gating of a population of 36-pS conductance channels. It is interesting to speculate that voltage gating of gap junction channels provides rapid fine control of cell-to-cell interaction, particularly in poorly coupled cells, and importantly determines both cardiac excitability and impulse propagation.

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KEY WORDS • cardiac electrophysiology • gap junctions • connexons • voltage dependence • single-channel current • cardiac myocytes

Cardiac cells are interconnected by low-resistance hydrophilic channels, which constitute a specialized plasma membrane known as the gap junction.1,2 These channels mediate the rapid transfer of electrical signals responsible for the synchronized activity necessary for an effective cardiac output.3,4 Each channel consists of two hemichannels or connexons. The connexon extends from the myoplasm of a cell through its lipid bilayer into a 2–3-nm gap between the cells, where it meets the connexon extending from the neighboring cell to form the communicating channels.2,5 Each connexon is believed to be a hexamer of identical or homologous protein subunits.6,7 The major protein components, connexins, from several tissues have been isolated and shown to be a member of a gene family,8 and the topography of connexins has been examined using proteases9,10 and antibodies against specific peptides.10–13 The activity of cardiac gap junction channels is reported to be gated by changes in [Ca2+]i, pH, second messengers, lipophilic substances, and other factors.4,14–16 Membrane potential importantly gates nonjunctional transmembrane channels, such as the Na+, Ca2+, and K+ channels, but the role of voltage in gating cardiac junctional channels is unclear. Whereas embryonic or neonatal mammalian ventricular myocytes have voltage-gated gap junctions, adult ventricular and sinoatrial cells are reported to lack such voltage-dependent gap junctions.17–21 Little is known about the properties of atrial gap junctions and their gating. We report, for the first time, voltage dependence of adult mammalian atrial gap junctions as assessed by voltage-clamping each cell of connected pairs of adult atrial myocytes.

Materials and Methods

Dissociated adult rat atrial myocytes were cultured for 18 hours at a low concentration, resulting in many doublets.22 The majority of such cells have normal resting and action potentials.23 Both cells of a coupled
cell pair were clamped at a common holding potential of 0 mV (the resting potentials for the given bath and pipette solutions) with independent patch-clamp amplifiers (Axopatch ID, Axon Instruments). Experiments were performed at room temperature. The patch electrodes contained (mM) CsCl 120, CaCl₂ 1, MgCl₂ 2, EGTA 10, MgATP 5.0, and HEPES 5 (pH 7.2). The bath contained (mM) NaCl 133, KCl 3.6, MgCl₂ 0.3, glucose 16, and HEPES 3 (pH 7.3–7.4). The resistance of the micropipettes measured in the bath solution ranged from 3 to 5 MΩ. Hyperpolarizing voltage steps (10 mV) over a range of ±120 mV and of varying durations (usually 4 seconds) were applied to one of the cell pair (Vj) while the follower cell was held at 0 mV (Vs) so that transjunctional voltage (Vj) equaled Vj−Vs or Vj. The conductance values were normalized to the steady-state state and the charge transference (Gj) was measured at 42.5 mV and a gating charge of 1.14 eV for the Gj−Vj relation. The Vj and gating charge were 39.5 mV and 1.61 eV, respectively, for the steady-state g−Vj relation when not normalized. In the 17 highly coupled cell pairs (gj>$2 nS), voltage- and time-dependent decay of Vj was not observed for Vj≥30 mV. As Vj was increased further (±40 mV), the voltage dependence became apparent. Peak Ij was not voltage dependent. The Boltzmann fit of the Gj−Vj relation (Figure 2) showed that Vj = 53.5 mV, approximately a 10-mV shift on the voltage axis as compared with the weakly coupled cell pairs. The corresponding gating charge was 0.98 eV. In both the weakly and highly coupled cells, Gj was never reduced to zero; the mean residual Gj was approximately 22% of the peak Gj for the weakly coupled cells and was higher (=50%) for the highly coupled cells.

Voltage dependence was studied for both steady-state and instantaneous (peak) Ij. The steady-state conductance and the normalized conductance (Gj−steady-state/ instantaneous peak) versus Vj plots were fit with a two-state Boltzmann distribution24 as a first approximation to allow comparison of our results with those published by others. The time course of current decay was best fit with a single- or double-exponential function as deemed appropriate. The resulting time constants of decay (τs) were plotted against Vj to study their voltage dependence. Single-channel conductance (γj) was obtained from all-point amplitude histograms at each Vj as used by Veenstra,21 and individual conductances were plotted versus Vj to study the voltage dependence, reversal potential, and multiplicity of channels.

**Results**

Recordings sufficient for analysis were obtained in 32 cell pairs, and gj ranged from 0.03 to 40 nS with a mean peak gj of 24±10.7 nS. Two distinct populations of cell pairs were observed: highly coupled pairs (n=17) with the peak gj ranging from 3.4 to 40 nS and the steady-state gj ranging from 1.9 to 37 nS, and weakly coupled pairs (n=15) with the peak gj ranging from 0.3 to 2.0 nS and steady-state gj ranging from 0.03 to 1.4 nS. In weakly coupled cells (gj≤2 nS), gj was steeply time and voltage dependent. An example of such voltage dependence is shown in Figure 1A. Steady-state and peak current–voltage relations were determined as in Figure 1B, and the corresponding gj values for each Vj are plotted in Figure 1C. Peak gj occasionally showed voltage-dependent decay as shown in Figure 1C. For the majority of cell pairs studied, however, the peak current (and hence conductance) was Vj insensitive. Instantaneous peak conductance, steady-state conductance, and Gj were plotted for each Vj (Figure 1C).

The voltage dependence was measured in eight of 15 weakly coupled cell pairs; in the other seven, conductance was very small, and unitary channel openings and closings were measured as discussed below. The steady-state conductance and mean Gj were determined for various Vj's. In the eight weakly coupled cell pairs (gj≤2 nS), the two-state Boltzmann fit was used as a first approximation (Figure 2), resulting in a half-inactivation potential (Vj) of 42.5 mV and a gating charge of 1.14 eV for the Gj−Vj relation. The Vj and gating charge were 39.5 mV and 1.61 eV, respectively, for the steady-state g−Vj relation when not normalized. In the 17 highly coupled cell pairs (gj>$2 nS), voltage- and time-dependant decay of Vj was not observed for Vj≤30 mV. As Vj was increased further (±40 mV), the voltage dependence became apparent. Peak Ij was not voltage dependent. The Boltzmann fit of the Gj−Vj relation (Figure 2) showed that Vj = 53.5 mV, approximately a 10-mV shift on the voltage axis as compared with the weakly coupled cell pairs. The corresponding gating charge was 0.98 eV. In both the weakly and highly coupled cells, Gj was never reduced to zero; the mean residual Gj was approximately 22% of the peak Gj for the weakly coupled cells and was higher (=50%) for the highly coupled cells.
FIGURE 1. Voltage dependence of adult atrial gap junctions. Panel A: Recordings of voltage-dependent decay of junctional currents (IJs). IJ decays in time at a transjunctional voltage (Vj) of \( \geq 20 \) mV. For this cell, Vj ranged from 120 to 0 mV. For clarity, IJ values for Vj of 120, 100, 30, 10, and 0 mV only are shown. Low-pass filter frequency was 50 Hz, and the digital sampling rate was 500 Hz. Panel B: IJ versus Vj plots for both peak (closed circles) and steady-state (open circles) IJs using the complete set of experimental data shown in panel A. Panel C: Voltage dependence of junctional conductance (gj) and normalized junctional conductance (Gj). The instantaneous conductance, steady-state conductance, and normalized steady-state conductance (ss/inst) are plotted as a function of Vj.

Discussion

The major findings of this study are that adult atrial gj is dependent on Vj, voltage dependence is most apparent in weakly coupled cells, the time course of inactivation has two components with both the faster (\( \tau_1 \)) and slower (\( \tau_2 \)) components being voltage dependent, gj never reduced to zero, \( \tau \) of the microscopic current is similar to \( \tau \) of the macroscopic current, and gj is \( \approx 36 \) pS and is voltage independent.

The observation of voltage-dependent gating of adult rat atrial cells was unexpected, because, as mentioned, adult ventricular and adult sinoatrial gap junctions have been reported as being independent of Vj.\textsuperscript{18-20} Voltage-dependent gap junctions have been found in amphibian blastomeres, salivary glands from Chironomus and Drosophila melanogaster, rat hepatocytes, and Xenopus oocytes expressed from connexin32 or connexin26 cRNA and in electrical synapses of crayfish and hatchet fish and other invertebrates and embryonic systems (see reviews in References 4, 14, and 16).

We found that the behavior of coupled cell pairs with a low conductance differed from that of more highly coupled cell pairs. In weakly coupled cells (gj \( \leq 2 \) nS), a Vj \( \geq 20 \) mV was associated with a time-dependent decay of IJ. Vj and gating charge were similar to that reported for embryonic chick ventricular cells by Veenstra.\textsuperscript{21} The voltage dependence of highly coupled cells (at Vj \( \geq 50 \) mV) is similar to that observed for neonatal ventricular cells with unitary channel conductances of \( \approx 50 \) pS\textsuperscript{20} and to that observed when a cardiac junctional protein, connexin43, was expressed in a hepatoma cell line, although endogenous channels did exist in this “communication-deficient” cell line, producing two distinct unitary conductances of \( \approx 60 \) and 100 pS.\textsuperscript{25-27} The implications of these correspondences are yet to be determined, although the developmental regulation of expression of junctional proteins and tissue and species differences might be important determinants.

For highly coupled cell pairs, the voltage dependence was not apparent unless Vj was \( \approx 40 \) mV. This observed shift of Vj for affecting voltage dependence in highly coupled cell pairs could be attributed, in part, to uncompensated series resistance when the junctional and series resistance are comparable or to a channel density–dependent effect, as previously described.\textsuperscript{19-21} Recent modeling studies suggest that in highly coupled cells (high density of open channels) there is a substan-
FIGURE 2. Voltage dependence of normalized junctional conductance ($G_j$). Voltage-dependent decay of $G_j$ is shown for both highly coupled (diamonds) and weakly coupled (solid circles) cells. The normalized steady-state (steady-state/instantaneous peak) $G_j$ is plotted as a function of junctional voltage ($V_j$). Each point represents the mean±SEM. The solid lines are the theoretical fit of the data by the Boltzmann relation as a first approximation. The half-inactivation potential, effective gating charge, and residual conductance for the weakly coupled and highly coupled cells are 42.5 and 53.3 mV, 1.14 and 0.98 eV, and 0.22% and 0.48% of peak, respectively. The steady-state conductance for weakly coupled cells when not normalized with respect to the instantaneous peak showed the half-inactivation potential and gating charge of 39.5 mV and 1.61 eV, respectively.

The characteristics of the decay of $I_j$ with two $\tau$s suggest that the kinetics of voltage-dependent inactivation is complex. A similar biexponential decay of $I_j$, especially at larger $V_j$s, has been reported in embryonic chick ventricular myocytes. Alternatively, two $\tau$s may reflect two different populations of channels. We also

fore, is shifted appropriately toward the right on the voltage axis, a higher voltage difference would be required to show a voltage dependence similar to that in weakly coupled cells.

A $V_j$ of ≥20 mV between interiors of the opposing cells occurs in experimental preparations (References 29–33). Similarly, such voltage gradients exist in the normal and abnormal heart. We would expect the highest $V_j$ to occur in the sick heart, with abnormal action potential morphology and duration and with marked temporal dispersion of refractoriness. It is interesting to speculate that voltage gating of gap junction channels provides rapid fine control of cell-to-cell interaction, particularly in poorly coupled cells, and might regulate both cardiac excitability and impulse propagation. As can be seen in Figure 3B, the time course of decay of $I_j$ is fast and is comparable to or even shorter than the time course of action potential duration. Alternatively, perhaps voltage gating helps maintain the closed state of hemichannels that may be present in the nonjunctional region of the cell membrane, thereby assuring cellular integrity. A transmembrane potential of >50 mV is commonly observed in cardiac myocytes. Recently, hemichannel-like activity has been observed in intact cells as well as in reconstituted membranes,34–39 and the anatomic substrates for such channels have been localized in several recent studies40,41 including direct visualization with atomic force microscopy41 and immunolocalization of antibodies against the normally inaccessible extracellular domains in the intercalated disc of rat heart. With injury, prolonged cellular depolarization could keep voltage-dependent hemichannels open, resulting in further injury and perhaps cell death.

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FIGURE 3. Time course of decay of junctional current. Panel A: Decay of junctional current ($I_j$) from a representative experiment. Junctional voltage ($V_j$) is 100 mV. The decay phase is fit with a double-exponential function (solid line, $r=0.987$) with a fast time constant of decay of 62 msec and a slow time constant of decay of 1,847 msec. Panel B: Voltage dependence of time constants. Time constants for decay were obtained for six cell pairs at each of several $V_j$s. The average time constants (mean±SEM) are plotted for each $V_j$. The faster time constant ($\tau_1$) is indicated by the inverted closed triangles; the slower time constant ($\tau_2$) is indicated by the closed circles. The digital filter frequency was 250 Hz.
found a residual $I_j$ in both poorly coupled and well-coupled cell pairs. A residual $I_j$ has been found in voltage-dependent gap junctions from all tissues and species and has frequently been suggested to represent a different channel population or alternately may be a reflection of the kinetic property of the junction channels that have a non-zero open probability even at a large $V_j$.  

Single-channel currents, such as plotted in Figure 4B, were well fit with a first-order regression line suggesting that $\gamma_i$ was independent of $V_j$. The $\gamma_i$ values that we obtained ranged from 27 to 49 pS (mean, $36.2\pm7.6$ pS) and were comparable to those reported for ventricular gap junction channels. Previous reports of unitary conductance $<60$ pS in cardiac cells have used patch solutions with large organic anions rather than chloride, suggesting that perhaps the atrial channels are smaller, but this must be considered speculation. The time course of the decay of microscopic current suggests that the decay has a single $\tau$ that is comparable to the $\tau_i$ of the macroscopic $I_j$. This could suggest that the voltage-dependent kinetics of the macroscopic $I_j$ in adult atrial myocytes result from the all-or-none gating of a population of single channels with a conductance of $\approx 36$ pS. The residual current could perhaps be represented by yet another voltage-independent single channel or the non-zero open probability of the channels at a large $V_j$. Multiple junctional channel proteins have been found to coexist in the same junctional membranes.

Gap junctions from atrial cells have not been well characterized, but there is some evidence that atrial gap junctions may differ from ventricular gap junctions structurally and/or biochemically. Our findings of voltage-dependent gating in atrial cells would be consistent with such a possibility, and our finding of inactivation of current with multiple $\tau$s would suggest that perhaps complex channel kinetics have evolved for the specific requirements of atrial cells. Alternately, possibly reexamination of sinoatrial nodal and ventricular tissues under conditions similar to those used in this study would reveal voltage dependence of $g_\phi$ as well.

**Figure 4.** Single-channel conductance. Panel A: Selected tracings of currents showing single-channel activity. Junctional currents ($I_j$) for junctional voltages ($V_j$) of 80, 60, 40, and 10 mV are shown. For the sake of clarity, only the $I_j$s are shown, although comparable $I_{j2}$ and $I_{j3}$, and has been obtained from the complete data of the experiment shown in panel A. Each point represents single-channel current determined from all-point amplitude histograms (inset), which compensates for noise. For example, the illusion of apparently different current sizes in the 80- and 60-mV tracings arises from the fact that the noise level in the 80-mV tracing is at least twice as large as that in the 60-mV tracing, and this is accounted for using the "all-point fit." The average currents as determined by all-point fit are 4.04, 3.91, and 3.65 pA for the 80-mV tracings and 3.12 and 2.76 pA for the 60-mV tracings. The solid line is the best-fit linear regression line ($r=0.974$) with a slope of 0.0466, giving a single-channel conductance of 46.6 pS, and suggests that single-channel conductance is voltage independent.

**Figure 5.** Time course of decay of microscopic junctional current, $I_j$. The data are obtained from two identical sets of experiments at a junctional voltage of 90 mV. The decay phase is fit with a single-exponential function (solid line, $r=0.948$) with a time constant of decay of 240 msec.
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