Sequential gating in the human heart $K^+$ channel Kv1.5 incorporates $Q_1$ and $Q_2$ charge components

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Hesketh, J. Christian, and David Fedida. Sequential gating in the human heart $K^+$ channel Kv1.5 incorporates $Q_1$ and $Q_2$ charge components. Am. J. Physiol. 274 (Heart Circ. Physiol. 46): H1956–H1966, 1999.—On-gating current from the Kv1.5 cardiac delayed rectifier $K^+$ channel expressed in HEK-293 cells was separated into two distinct charge systems, $Q_1$ and $Q_2$, obtained from double Boltzmann fits to the charge-voltage relationship. $Q_1$ and $Q_2$ had characteristic voltage dependence and sensitivity with half-activation potentials of $-29.6 \pm 1.6$ and $-21.9 \pm 2.09$ mV and effective valences of $1.87 \pm 0.15$ and $5.53 \pm 0.27$ e, respectively. The contribution to total gating charge was $0.20 \pm 0.04$ for $Q_1$ and $0.80 \pm 0.04$ (n = 5) for $Q_2$. At intermediate depolarizations, heteromorphing gating current waveforms resulted from relatively equal contributions from $Q_1$ and $Q_2$, with widely different kinetics. Prepulses to $-20$ mV moved only $Q_1$, simplified on-gating currents, and allowed rapid $Q_2$ movement. Voltage-dependent on-gating current recovery in the presence of 4-aminopyridine (1 mM) suggested a sequentially coupled movement of the two charge systems during channel activation. This allowed the construction of a linear five-state model of $Q_1$ and $Q_2$ gating charge movement, which predicted experimental on-gating currents over a wide potential range. Such models are useful in determining state-dependent mechanisms of open and closed channel block of cardiac $K^+$ channels.

Voltage-dependent $K^+$ (Kv) channels have an important role during the repolarization of neuronal and cardiac action potentials. Voltage sensitivity is determined within the S4 transmembrane helices, which contain four to seven positively charged amino acids at every third position, with intervening hydrophobic residues (20, 22, 24, 27). Movement of the S4 helix on depolarization controls channel opening by inducing a cooperative, conformational change in the channel protein. Distinct topological states of the S4 helix have been associated with the resting and open conformations of Shaker $K^+$ channels using cysteine-scanning mutagenesis or fluorescence (17, 18). On depolarization, the S4 helix moves upward, causing specific residues to be exposed to the extracellular space, thereby producing outward on-gating currents ($I_{gon}$). Recent studies using voltage-clamp fluorometry have attempted to define intermediate topological states of the channel protein (1, 6) and have been guided by the idea of two distinct charge systems with different voltage-dependent parameters, dubbed $Q_1$ and $Q_2$ (3). When $Q_1$ is maximally moved, the S4 helix is thought to exist in an intermediate state and only reaches its final destination on movement of $Q_2$.

The existence of two charge systems is of interest because it can allow the separation of gating charge into two distinct components biophysically and structurally. In addition, it is possible that the different charge systems may provide separate and distinct targets for drug interaction with Kv channels. In Shaker $K^+$ channels, $Q_1$ carries somewhat less charge than $Q_2$ does (3), and it has been suggested that $Q_1$ is associated with movement of an auxiliary gating particle that is not part of S4 (6). Recent fluorometric studies, however, suggest that both $Q_1$ and $Q_2$ are caused by movement of the S4 transmembrane helix, although the importance of an auxiliary particle influencing gating is not excluded (1). The two charge components move in a sequential manner such that $Q_1$ must move before $Q_2$ on depolarization (1, 3), and this has several practical and kinetic implications. $Q_1$ movement must impart a delay on $Q_2$ movement that will slow the apparent $Q_2$ kinetics as well as slowing channel activation. $Q_1$ movement may in part be responsible for the Cole-Moore shift of ionic currents in which channel activation is faster after more depolarizing prepulses (3, 9). In addition, the relative stability of the intermediate state at various holding potentials in different channels may help explain widely different voltage-dependent gating in channels with a similar S4 charge valence (1).

The voltage-dependent properties of $Q_1$ and $Q_2$ have been characterized in Drosophila Shaker channels expressed in Xenopus oocytes (3), but studies in mammalian Kv2.1 and Kv1.5 channels have usually limited Boltzmann analysis of charge-voltage (Q-V) relationships to a single charge system (8, 29). In the present study, we have questioned whether the $Q_2/Q_1$ two-charge system model can adequately describe the on-gating currents in the cardiac Kv channel, Kv1.5, and whether sequential coupling of the two systems is required. We have taken advantage of the high expression of Kv channels in small HEK-293 cells and measured $I_{gon}$ to characterize and model the $Q_1$ and $Q_2$ gating charge systems of stably expressed Kv1.5. Two charge systems were immediately suggested by the strikingly biphasic $I_{gon}$ waveforms at intermediate potentials, not apparent in data from other channels. In addition, voltage-dependent parameters of these two charge systems in Kv1.5 have allowed separation of the two components in a manner not possible for the analogous systems in Shaker $K^+$ (3) or cardiac L-type $Ca^{2+}$ channels (16). The sequential nature of the two charge systems was studied in more detail than in...
previous studies (3) by using 4-aminopyridine (4-AP) to prevent late steps in channel opening, and the data strongly argue for a sequential mechanism of both activation and deactivation gating. Finally, it was found that both isolated $Q_1$ and $Q_2$ charge systems showed features suggestive of multiple gating steps for each charge system. On the basis of these data, we constructed a simple linear sequential model made up of four transitions, with two discrete transitions making up each of the $Q_2$ and $Q_2$ gating charge systems.

**MATERIALS AND METHODS**

Cells and solutions. To record gating currents, a stable cell line expressing Kv1.5 with the point mutation W472F was created using the Stratagene Chameleon Kit (Stratagene, La Jolla, CA). The mutation is analogous to the ShH-I4R W434F mutation, which abolishes K+ conduction (23). HEK-293 cells were transfected with a linearized plasmid expression vector, pRC/CMV, containing Kv1.5-W472F, using LipoFectACE reagent (Canadian Life Technologies, Bramalea, Canada) in a 1:10 (wt/vol) ratio. Linearization of the plasmid facilitated recombination of the plasmid DNA with the HEK chromosomal DNA and resulted in the production of clones with expression levels high enough to record gating current. Patch pipettes contained 140 mM N-methyl-d-glucamine (NMG), 1 mM MgCl2, 10 mM HEPES, and 10 mM EGTA, adjusted to pH 7.2 with HCl. The bath solution contained 140 mM NMG, 1 mM MgCl2, 10 mM HEPES, 1 mM CaCl2, and 10 mM dextrose, adjusted to pH 7.4 with HCl. All chemicals were from Sigma Chemical (St. Louis, MO). In experiments utilizing 4-AP, 1 mM 4-AP, adjusted to pH 7.4, was perfused into the bath via a gravity-fed mechanism.

Electrophysiology. Current recording and data analysis were done using an Axopatch 200A amplifier and pCLAMP6 software (Axon Instruments, Foster City, CA). Patch electrodes were fabricated using thin-walled borosilicate glass (World Precision Instruments, Sarasota, FL). After they were fire polished, pipettes used to measure current had resistances of 1–3.5 MΩ when filled with control filling solution. For the nine cells from which complete Q-V relationships were obtained, mean whole cell series resistance was $3.01 \pm 0.52$ (SD) MΩ and mean cell capacitance was $16.8 \pm 4.5$ (SD) pF. It was often possible to measure capacity transients from the cells that decayed with a simple exponential time constant of $<50 \mu$s, as illustrated in Fig. 1C. As we have stated previously (8), the HEK cell membrane was extremely linear at negative potentials, and leakage and capacitive currents were routinely subtracted on-line using a differential amplifier (34) from a holding potential of $-120 \text{mV}$ to $-80$ or $-100 \text{mV}$. No nonlinear charge movement was observed at potentials between $-80$ and $-120 \text{mV}$ that would be able to distort waveforms during leak subtraction. This is illustrated in Fig. 1A, where gating charge is shown at $+60 \text{mV}$ with or without a prepulse to $-120 \text{mV}$.

In almost all the records obtained, a very rapid gating current rise in the first 200 µs after the pulse was applied was limited by the clamp of the membrane, with time constants around 50 µs (see above), as shown in Fig. 1B at $-20 \text{mV}$. This resulted in a fast but sloping rise at almost all potentials (Fig. 2). This was followed by a short plateau phase (Fig. 1B) that became more prominent at increasing depolarizations (Fig. 2A) and could be accompanied by a rising phase (e.g., at $+28 \text{mV}$ in Fig. 2). The current plateau usually took 1.5 ms to reach completion, before current decays (Figs. 1B and 2A) and was not limited by the clamp speed, which allowed detection of a rapid off-gating current as well as the slow on-gating current evident at $-20 \text{mV}$ (Fig. 1B).

Series resistance compensation was not used because of the relatively small size of the gating currents. Data were sampled at 200 kHz for protocols with a short time length (<50 ms) and up to 50 kHz for longer protocols. Data were filtered at 10 kHz for all protocols. All experiments were performed at 22°C. All on-gating charge ($Q_{on}$) measurements were obtained by integrating the on-gating currents until current waveforms decayed to the baseline, which was completed by 20 ms. All results obtained from multiple cells are reported as the means ± SE.

Modeling. A linear sequential model was used to describe on-gating currents from Kv1.5. The model contained five states with forward and backward transitions governing movement between each of these states. $C_r$ represents a resting state of the channel at the most hyperpolarized potentials, whereas $C_a$ represents the state reached at the most positive potentials studied. This model does not contain an open state or an inactivated state and, as such, is not a model of channel activation but, rather, of the transitions preceding activation. Two charge systems, $Q_1$ and $Q_2$ were modeled by transitions between states $C_1$ and $C_2$ and between states $C_2$ and $C_a$. The rationale for this was the minimal model required to simulate the gating current waveforms based on two components of charge movement ($Q_1$ and $Q_2$) obtained from a double Boltzmann function fit to the Q-V curve (Fig. 3). This was of the form

$$Q(V) = Q_{1max}/(1 + \exp [(V_1 - V)/k_1])$$

$$+ Q_{2max}/(1 + \exp [(V_2 - V)/k_2])$$

where $Q_{1max}$ and $Q_{2max}$ are the maximum charges that can be moved by $Q_1$ or $Q_2$ and are proportional to the number (n) of mobile electronic charges ($e^-$) and to the apparent valance of the charge moved ($z'$): $Q_{max} = nz'e^-$, where $z'$ is related to the real charge $z$ by the relation $z' = z\delta$, with $\delta$ representing the fraction of the electric field traversed by the charge. $V_1$ and $V_2$ are the half-activation potentials, and the slope factors $k_1$ and $k_2$ reflect the steepness of the voltage dependence of $Q_1$ and $Q_2$, respectively. $k_1$ and $k_2$ are inversely proportional to $z_1$ and $z_2$ as $k_1 = k_2/z_1z_2$. The rate of the ($z'$) gating charge associated with each state transition was assumed to be symmetrical for the forward and backward transitions. With the use of these simplifying assumptions, the model contained only three free parameters for each transition and only two free parameters at very positive voltages at which the charge of the system was saturated and the backward rates were essentially zero. The gating charge comprising the $Q_1$ and $Q_2$ charge systems was calculated from the steepness of the voltage dependence of the two components of the double Boltzmann function at 1.87 and 5.53 $e^-$, respectively.

The model was constructed using SCoP and SCoPfit (version 3.51, Simulation Resources, Redlands, CA). The number of channels moving between different states was described by a series of first-order differential equations and solved numerically. Rates of these transitions were solved by fitting the model to experimental data comprising isolated $Q_1$ or $Q_2$ traces (Fig. 3) using the three-state model mentioned above for each charge system. The proportion of charge associated with each transition was allowed to float freely during initial fitting and remained relatively constant in data from different cells. For both charge systems best fits were obtained when the first transition carried 33% of the total gating charge ($z'$) associated with $Q_1$ or $Q_2$. The rates of $Q_1$ transi-
tions at voltages greater than $-20$ mV, for which there were no unique $Q_1$ data to fit, were determined by fixing $Q_2$ rates and fitting overall gating current data. The mean values of the rates obtained from data from five different cells were then fit for their relationship to voltage using single or double exponential functions. These functions, describing the voltage dependency of the rates, were then merged into the five-state model described above to generate the model simulations (see Fig. 7). This model is unique because it considers the $Q_1$ and $Q_2$ charge systems to be discrete but to interact solely on the basis of a sequential association.

**RESULTS**

General features of Kv1.5 gating charge movement. Stable expression of Kv1.5-W472F in an HEK-293 cell line allowed the recording of large $I_{\text{gon}}$ with good time resolution. Data illustrated in Fig. 2 were obtained from a single cell, and these waveforms are representative of $I_{\text{gon}}$ and $Q_m$ waveforms from 18 other cells. The data in Fig. 2A show that currents are readily apparent after depolarizing pulses to $-36$ mV and more positive without trace averaging. The kinetics of Kv1.5 $I_{\text{gon}}$ are strongly voltage dependent, because peak current increases and the decay becomes faster at higher depolarizations. A rising phase as well as a complex exponential decay from peak is apparent at most potentials but is most marked at intermediate depolarizations between $-4$ and $+28$ mV (Fig. 2). Decay of $I_{\text{gon}}$ is complete during the 20-ms duration of the depolarizing pulse. Off-gating currents are shown in Fig. 1A, inset. We have described these at some length in prior studies (8, 32). For small depolarizations up to $-10$ mV, these currents have a rapid transient time course, but after more positive depolarizations they begin to develop a slow decay phase (from $-4$ mV to $+12$ mV). At potentials more positive than $+50$ mV, both the rising phase of off-gating current and the decay phase are very slow. The reasons for the slowing are complex and related to changes in channel conformation that occur during opening and slow charge return (2, 8, 25, 34). These likely include a relatively voltage-independent rearrangement that leads to pore opening and, once open, to rapid onset inactivation, which is slow to reverse (2, 8). In this consideration of activation charge systems in Kv1.5 we did not consider these slow rearrangements.
For this reason the model did not contain an “open” state and did not attempt to model charge return.

Time integration of records like those in Fig. 2A allows visualization of $Q_{on}$ as shown in Fig. 2B. Charge moves relatively slowly negative to 0 mV, but after $Q_{on}$ amplitude saturates (+16 mV), the kinetics of $Q_{on}$ continue to get faster as the depolarization increases. The sigmoidal nature of the charge waveforms necessitate the adoption of gating models that comprise at least three states.

Steady-state separation of gating charge into two gating components. The amplitude of $Q_{on}$ at different depolarizing potentials (Q-V curve) reveals a relationship with strong sigmoidicity (Fig. 3). The Q-V curve in Fig. 3 is from the same cell as that from which the $I_{gon}$ waveforms were obtained in Fig. 2, and the general Q-V relationship was consistent across nine complete sets of data obtained. Attempts to fit this relationship with a single Boltzmann function were unsuccessful (data not shown) because of the shallow rise of the curve (the “foot”) and the steep voltage dependence of charge saturation at higher voltages. This suggested a component of the overall gating charge with a more shallow voltage dependence, activated at lower depolarizations, and a second, more voltage-dependent component, activated at more depolarized voltages. The data points were fit with a double Boltzmann function (Fig. 3), and the component single Boltzmann functions were then plotted on the same axes and termed $Q_1$ and $Q_2$. $Q_1$ is a smaller component, is less voltage dependent, and is activated at lower depolarizations than the $Q_2$ component. This two-component system for $Q_{on}$ has been demonstrated previously in two other voltage-gated ion channels, Shaker (3) and human heart L-type Ca$^{2+}$ channels (16). The general voltage-dependent parameters of $Q_1$ and $Q_2$ are conserved in all three channel types, but there are subtle differences that may explain

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**Fig. 2.** Voltage-dependent on-gating current ($I_{gon}$) and on-gating charge ($Q_{on}$) in Kv1.5 in response to depolarizing voltage pulses from −96 mV to +92 mV. A: $I_{gon}$ from a holding potential of −100 mV in 16-mV steps. Inset: off-gating currents at −100 mV from same recordings as in A. Labels refer to potentials during preceding depolarizations. B: $Q_{on}$ measured by integration of $I_{gon}$ transients over time periods sufficient to allow currents to relax to baseline, usually 20 ms. Tracings are shown for depolarizations in 8-mV steps. Data from A and B are from same cell.

**Fig. 3.** Two charge systems can account for steady-state gating charge-voltage (Q-V) relationship. $Q_{on}$ was obtained by integration of $I_{gon}$ over 20 ms (●). Solid line through points was fit to data using a double Boltzmann function (see METHODS, Eq. 1). Mean fit parameters are listed in Table 1. Dotted and dashed lines indicate portion of Q-V relationship attributable to each of the charge systems, $Q_1$ and $Q_2$, respectively. Left inset: $Q_2$ charge isolated after a −20-mV prepulse (see text). Slight decrease of peak $Q_2$ charge levels seen at most positive potentials is an artifact that resulted from a slight nonlinearity during leak subtraction in this example. Right inset: charge movement over voltage range from −49 to −25 mV in which only $Q_1$ moves.
the half-activation potentials for $Q_1$ and $Q_2$ in Kv1.5 are shown in Table 1. The double Boltzmann fit to the charge-voltage curve from five cells displayed the most consistent fits; however, the general properties of the Q-V curves were remarkably similar between the nine cells from which complete Q-V relations were obtained. The 27.5-mV difference between the half-activation potentials $V_1$ and $V_2$ is such that the two charge systems can be separated using relatively simple protocols. Very little $Q_2$ moves at voltages negative to $-20$ mV, so gating current elicited by depolarizing pulses up to $-20$ mV should represent almost exclusively $Q_1$ gating charge (Fig. 3, right inset). In addition, a $-20$-mV prepulse can move most of $Q_1$, so gating current during a test pulse to more depolarized potentials should reflect largely $Q_2$ charge movement (Fig. 3, left inset). $Q_2$ kinetics between $-49$ and $-25$ mV have a shallow voltage dependence and a gradual increase in steady-state charge amplitude with potential. In contrast, positive to $-20$ mV, both the $Q_2$ kinetics and steady-state charge exhibit a strong voltage dependence. Most of $Q_2$ moves between $-10$ and $+10$ mV, and although overall gating charge waveforms exhibit a slightly sigmoidal time course (Fig. 2B), isolated $Q_2$ gating currents show very little sigmoidicity. This results in isolated $Q_2$ gating currents with a briefer peak plateau phase and a less prominent rising phase. Isolated gating charge traces in Fig. 3 are each from different cells and show features (i.e., $Q_2$: faster rise time; $Q_1$: rising phase) similar to those of eight other cells for $Q_1$ charge and 11 other cells for $Q_2$ charge.

Kv1.5 on-gating currents have a unique biphasic nature. Gating currents from Shaker channels (3, 23, 25) and L-type Ca$^{2+}$ channels (16) show monophasic single and double exponential decays with sharp peaks. Kv1.5 gating currents have more rounded peaks and plateaus that, at some potentials, allow visual separation of the two charge systems (Fig. 4A). This biphasic nature of $I_{g1}$ prevented the kinetic separation of $Q_1$ and $Q_2$ obtained from Shaker and L-type Ca$^{2+}$ channel $I_{g1}$ waveforms by dual exponential fits to the current decay (3, 16). The voltage-dependent parameters of $Q_1$ and $Q_2$ are such that the decay phases of $Q_1$ and $Q_2$ do not coincide sufficiently at intermediate potentials to produce smooth double exponential decays. The data in

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<th>$K_1$</th>
<th>$z_1$</th>
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$Q_{1\text{max}}$ and $Q_{2\text{max}}$: maximum on-gating charges that can be moved at extreme depolarizations by charge systems $Q_1$ and $Q_2$, respectively; $V_1$ and $V_2$, half-activation potentials for $Q_1$ and $Q_2$, respectively; $K_1$ and $K_2$, steepness of voltage dependence of $Q_1$ and $Q_2$, respectively; $z_1$ and $z_2$, valences representing multiples of the number of electronic charge units comprising each component ($e^-$) and the proportion of the electric field through which they move ($e^-$).
Fig. 4A show clearly biphasic waveforms at two different voltages. At −20 mV, only Q1 moves and the charge waveform has a clear monophasic decay. At −4 mV, a biphasic trace is evident with an initial rapid decay followed by a slower decay, which produces a “notch” early in the gating current trace (Fig. 4A, arrow). Further depolarization to +12 mV leads to more accelerated Q2 movement and a merging of Q1 and Q2 components that results in an extended plateau phase followed by a monotonic decay (see also Fig. 2A). Depolarizations to +40 mV elicit currents with less rounded peaks followed by clear monophasic decays. The notch and “plateau” evident in gating currents from −4- and +12-mV depolarizing pulses, respectively (Fig. 4), are extremely well conserved across cells. In addition, these features were consistently reduced when preceded by a −20-mV prepulse in four cells, with representative data shown in Fig. 4B.

A test of the interplay between Q1 and Q2 at intermediate potentials was carried out by comparing current waveforms before and after −20-mV prepulses to remove Q1 (Fig. 4B). Gating currents from a −80-mV holding potential show the characteristic biphasic notch and plateau at −4 and +12 mV, respectively. After a −20-mV prepulse, the notch evident at −4 mV is eliminated and the plateau at +12 mV is much reduced. The isolated Q2 also activates more quickly with an absent rising phase, which produces the crossover evident in the pairs of traces. The data in Fig. 4, C and D, show charge integrals of the prepulse and test pulse gating current for the experiments shown in Fig. 4B. The essential feature of these charge integrals is that the charge moved during the −20-mV prepulses (Q1) is not subsequently moved during the test pulses. Thus the different time course and amplitudes of test pulse charges from −80 or −20 mV are caused by the absence of Q1 from −20 mV.

Return of Q1 and Q2 gating charge in presence and absence of 4-AP. An important rationale for the adoption of a sequential model for Q1 and Q2, rather than independent parallel movement of the two charge systems, comes from previous studies of the return of Shaker gating charge. These studies have shown that Q1 and Q2 do not return independently but, rather, interact during deactivation. The result is that the more complete the transfer of Q2, the less mobile is Q1 on repolarization (3). Similar interactions were apparent in Kv1.5 charge return experiments (Fig. 5). To investigate this interaction, a three-pulse protocol was used. First, all charge was moved by fully activating the

Fig. 5. Measurement of recovery time course of Q1 and Q2 in presence or absence of 1 mM 4-aminopyridine (4-AP). In all cases, prepulse was a 20-ms depolarizing pulse to +80 mV and recovery period was a −80-mV hyperpolarizing pulse of variable duration. In all cases, current recovery was assessed with a test pulse to −20 mV (Q1 recovery) or +80 mV (Q2 recovery). In A, B, D, and E, only currents during test pulses are shown. Charge recovery was assessed by integration of test pulse current over a time period of 20 ms. Recovery of Q2 (A) and Q1 (B) in absence of 4-AP is shown following recovery times ranging from 0.5 to 48.5 ms. C: time course of Q1 recovery (○, n = 3, mean ± SE) and Q2 recovery (●, n = 3, mean ± SE). Recovery of Q1 (D) and Q2 (E) in presence of 1 mM 4-AP is shown following recovery times ranging from 0.2 to 5.0 ms. F: time course of Q1 recovery (○, n = 4, mean ± SE) and Q2 recovery (●, n = 4, mean ± SE).
channels with a depolarization to +80 mV. Variable length repolarizations to −80 mV then allowed recovery of the two charge systems. Finally, the time course of Q1 and Q2 recovery was assessed during test pulses to −20 mV (Q1) or +80 mV (Q2). Figure 5A, at +80 mV, and Fig. 5B, at −20 mV, show the recovery of Q2 and Q1 gating currents, respectively (currents during the prepulses have been omitted). The time course of Ig, the recovery of the two systems appears very similar and appears to follow the time course of return of the off-gating current shown in Fig. 5A for Q1. This was confirmed when the Q1 or Q2 charge integrals of the gating currents were plotted against the recovery time (Fig. 5C). Recovery time constants for Q1 and Q2 were 15.2 ± 1.7 ms (n = 3) and 19 ± 1.3 ms (n = 3), respectively. One interpretation of this similarity is that recovery of the two systems is coupled in a sequential manner in which Q2 must recover before Q1 may recover.

The problem is that this interpretation is not exclusive. Q2 charge movement is thought to herald channel opening, which involves a relatively voltage-independent conformational change (3). After channel opening, other processes can also occur, including inactivation (12, 13) and transient sojourns in closed states not in the activation pathway (14). On repolarization, transitions between any of these states and closed states in the deactivation pathway may be rate limiting and, as such, the time course of these transitions would govern recovery of both Q1 and Q2. This would result in a similar time course of recovery of the two systems, and they would also parallel the recovery of off-gating current. In such a way, parallel independent movement of Q1 and Q2 could be concealed and therefore cannot be excluded. 4-AP is thought to prevent opening of Shaker channels by blocking a final allosteric transition in the activation pathway (19). 4-AP and channel inactivation are also thought to be mutually exclusive (5). Similar effects of 4-AP have been shown in Kv1.5 (4, 10), which suggests that it may act in a like manner. We have attempted to use 4-AP to prevent channel opening and entry into any further states that may limit the rate of charge recovery. In this way we have attempted to isolate closed-state transitions when the kinetics of subsequent gating current recovery should more closely represent isolated Q1 and Q2 charge systems.

The same three-pulse experiment as that shown in Fig. 5, A–C was performed in the presence of 1 mM 4-AP, and the results are shown in Fig. 5, D–F. As expected, the time course of the experiment was much faster when rate-limiting transitions were prevented. The time course of the inward off-gating current envelope was very fast and was completed within a few milliseconds. In this situation, recovery of Ig, due to Q2 (Fig. 5D) and Q1 (Fig. 5E) followed a much faster time course than in the absence of 4-AP. However, both Q1 and Q2 recovery still occurred at the same speed. This is clearly seen in the charge return relations in Fig. 5F, plotted on a 10× faster time base than in the absence of 4-AP (Fig. 5C). The recovery time constants for Q1 and Q2 with 4-AP are 1.17 ± 0.1 ms (n = 4) and 0.97 ± 0.06 ms (n = 4), respectively. From these data we gain additional support for a sequential coupled model of Q1 and Q2 charge movement in Kv1.5.

Voltage dependence of Q1 and Q2 charge recovery. A third possible interpretation of the results from Fig. 5 is that the two charge systems recover in parallel, but simply at the same rate. However, if the Q1 and Q2 charge systems can recover independently, the voltage dependence of the recovery rates should differ on the basis of the strong disparity between the voltage dependence of activation of Q1 and Q2 (Fig. 3 and Table 1). Again, to avoid the problem of rate-limiting transitions around opening, a three-pulse protocol was used to test this idea in the presence of 1 mM 4-AP. The first pulse was either to +80 mV to move both Q1 and Q2 or to −20 mV to move Q1 alone. This conditioned the channels, and then variable duration repolarizations to three potentials allowed charge system recovery, before the third pulse to −20 or +80 mV to assess the recovery of the Q1 and Q2 charge systems, respectively. The voltage dependence of the recovery rate of the two charge systems was assessed by varying the repolarization potential (−120, −80, and −40 mV) during the second pulse.

After a repolarizing pulse to very negative potentials (−120 mV), recovery of Q1 alone, Q1 after Q2 movement, and Q2 proceeded relatively quickly and with a similar time course (Fig. 6A). However, after a −40-mV repolarizing pulse, recovery of the charge systems was much slower with the exception of Q1 alone, which continued to recover at a relatively rapid rate (Fig. 6B). The data in Fig. 6C represent a summary of the recovery time constants of Q1, Q2 after movement, and Q1 alone at the three repolarization potentials studied. Recovery rates of Q1 and Q2 from a state in which both Q1 and Q2 have moved were essentially indistinguishable at all three recovery potentials and showed some voltage dependence. Recovery of Q1 alone from a channel state in which Q2 was not moved was much faster, with a shallow voltage dependence, and stayed rapid at all three potentials. These results suggest a system in which recovery of Q2 during channel deactivation is strongly coupled and not one in which Q1 and Q2 movement can operate in parallel.

Modeling of Q1 and Q2 charge systems. The properties of Kv1.5 gating currents described to this point have guided the construction of a model of on-gating currents in which Q1 and Q2 are first modeled as separate three-state systems and then integrated into a five-state sequential model (Fig. 7A). An overall scheme in which two transitions were involved in each of the Q1 and Q2 charge systems provided very good fits to Kv1.5 on-gating currents. The rising and decay phases of gating currents are reproduced as well as the marked plateau phases at intermediate potentials in Fig. 7B, left. Here, original experimental data (noisy) have been overlain by model tracings. The model also gives an indication of Q1 gating currents at higher potentials (greater than −20 mV), where they cannot be isolated from Q2 experimentally (Fig. 7B, middle). Q1 is predicted to move progressively faster at higher depolariza-
recovery rate ($Q_{r}$), $r_{n}$, and recovery ($Q_{n}$) in absence of $Q_{1}$ and $Q_{2}$. At more positive potentials $Q_{2}$ movement accelerates dramatically and accounts for a large part of the composite waveform (Fig. 7B, left). The disparity between $Q_{1}$ and $Q_{2}$ kinetics has been shown experimentally by Bezanilla et al. (3) through kinetic separation of $Q_{1}$ and $Q_{2}$, a process that was not possible with Kv1.5 gating currents.

A further examination of the relationship between the $Q_{1}$ and $Q_{2}$ charge systems illustrated by the model is shown in Fig. 7C, which represents simulations of the experiments shown in Fig. 4B. In accordance with the data in Fig. 4B, test pulses from a $-80$-mV holding potential showed gating currents with a small rising phase before the peak, followed by a complex decay phase. Test currents after a $-20$-mV prepulse showed a rapid rise to peak, followed by a significant reduction in the complexity of the decay phase. Curves in each set of traces shown in Fig. 7C reflect contributions of the $Q_{1}$ and $Q_{2}$ charge systems to the overall simulated gating current waveform for a depolarization from $-80$ mV. The relatively rapid decay of $Q_{1}$ is complemented by the emergence of $Q_{2}$ after movement of the $Q_{1}$ charge system. Although experimentally obtained $Q_{2}$ traces (after a $-20$-mV prepulse) show rapid kinetics (Fig. 4B), the constraint that $Q_{2}$ may only move after $Q_{1}$ imparts slower kinetics on the dominant $Q_{2}$ charge system. Within the combined system then, as illustrated by the model, $Q_{2}$ shows a relatively long time to reach peak and slow decay to baseline (Fig. 7C).

The model predicts that this delayed emergence of the $Q_{2}$ charge system, following from the relatively rapid

**Fig. 6.** Voltage dependence of $Q_{1}$ and $Q_{2}$ recovery. (A): rate of $Q_{2}$ recovery ($\bullet$, $n=5$), $Q_{1}$ recovery after $Q_{2}$ and $Q_{2}$ movement ($\Delta$, $n=6$), and $Q_{1}$ recovery in absence of $Q_{2}$ movement ($\square$, $n=5$) during a $-120$-mV repolarizing pulse. Inset: pulse protocol used to measure voltage dependence of $Q_{1}$ and $Q_{2}$ recovery kinetics. B: rate of $Q_{2}$ recovery ($\bullet$, $n=5$), $Q_{1}$ recovery after $Q_{2}$ and $Q_{2}$ movement ($\Delta$, $n=4$), and $Q_{1}$ recovery in absence of $Q_{2}$ movement ($\square$, $n=5$) during a $-40$-mV repolarizing pulse. C: summary of voltage dependence of $Q_{2}$ recovery rate ($\bullet$), $Q_{1}$ recovery rate in presence of $Q_{2}$ movement ($\Delta$), and $Q_{1}$ recovery in absence of $Q_{2}$ movement ($\square$) at 3 repolarization potentials. 4-AP (1 mM) was present continuously in all experiments.

**Fig. 7.** Model of $Q_{1}$ and $Q_{2}$ charge systems. A: linear sequential scheme used to model Kv1.5 gating. $C_{0}$ refers to closed state most distal to open state, whereas $C_{4}$ refers to closed state most proximal to open state; $C_{1}$, $C_{2}$, and $C_{3}$ are intermediate states. Transitions corresponding to $Q_{1}$ and $Q_{2}$ are bracketed; $z_{l}$ to $z_{i}$ refer to effective valence sensed by gating particle associated with each transition. B, left: superimposed model fits (solid lines) through experimental points for $I_{g_{ss}}$ elicited by depolarizing pulses from 0 mV to $+80$ mV in steps of 20 mV; middle and right: modeled $Q_{1}$ and $Q_{2}$ components, respectively, of model waveform at left. C: model of effect of a $-20$-mV prepulse on currents at $-4$ mV (left) and $+12$ mV (right). Protocol is same as that used to obtain experimental data in Fig. 3B. Dotted lines indicate modeled $Q_{1}$; dashed lines indicate modeled $Q_{2}$ for composite solid line showing total charge movement without a prepulse.
Q1 charge system, accounts for the biphasic $I_{\text{gon}}$ waveforms evident in Fig. 4B.

**DISCUSSION**

$I_{\text{gon}}$ waveforms from cardiac Kv1.5 share several features in common with gating currents from other voltage-gated ionic channels, including the general kinetic properties of the waveforms and the presence of two distinct gating charge systems \( (3, 16) \). However, the voltage-dependent parameters of the Q1 and Q2 gating charge systems are different between these channels, as exemplified by the biphasic waveforms characteristic of Kv1.5-W472F $I_{\text{gon}}$, at intermediate potentials. These two charge systems appear to move in a sequential manner similar to that described in ShB H4-I-R-W434F \( (3) \). Modeling of Q1 and Q2 in a simple linear sequential scheme demonstrates the important interactions between the two charge systems and how this results in the overall $I_{\text{gon}}$ waveform.

General kinetic features of $I_{\text{gon}}$ are invariable among different channels. Many examples of gating current have been published from a variety of voltage-gated ion channels including Drosophila Shaker K+ \( (3, 21, 23) \), Kv1.5 \( (8) \), Kv2.1 \( (29) \), squid axon Na+ \( (30, 31) \), and L-type cardiac Ca2+ channels \( (11, 15, 16) \). Despite the genetic diversity between these ion channel families, $I_{\text{gon}}$ waveforms consistently have a rising phase followed by a decay phase with kinetics that become faster with depolarization. Kv1.5 $I_{\text{gon}}$ waveforms are no exception (Fig. 2A), and these similarities highlight a conserved voltage-dependent gating mechanism whereby initial transitions are slower and/or carry less charge than subsequent gating transitions. An additional conserved feature of $I_{\text{gon}}$ waveforms is the presence of two gating charge systems that contribute to the overall $I_{\text{gon}}$ waveform in L-type Ca2+ and Shaker K+ channels \( (3, 16) \). We have demonstrated the existence of two charge systems in a human cardiac delayed rectifier K+ channel. The smaller Q1 component is activated at more hyperpolarized potentials and is less voltage dependent than the larger Q2 component (Fig. 3) in all three voltage-gated ion channels. This suggests that Q1 and Q2 charge systems have a conserved role in voltage-dependent gating.

Charge movement after a \(-20\text{-mV prepulse} is much faster, as shown by the rapid time course of isolated Q2 traces compared with the sigmoidal form of total Qgon movement (compare Fig. 2 and Fig. 3B, left inset). If movement of the two charge systems is coupled so that Q2 only moves after Q1, prepulses can remove this restriction and speed up the Q2 kinetics. Another interpretation is that prior movement of Q1 allosterically causes the channel to enter a state that is more permissive to Q2 movement. This interpretation does not require a strict sequential relationship between the two charge systems but does suggest at least indirect interactions. This acceleration of Q2 kinetics likely contributes to the Cole-Moore shift of ionic currents \( (9) \) and has also been demonstrated in Shaker gating currents, which show a more rapid decay and a less pronounced rising phase \( (28) \). The early closed-closed transitions are traversed during movement of Q1 charge, so Q1 movement may be the gating mechanism underlying the Cole-Moore shift.

Voltage-dependent parameters of Q1 and Q2 differ among channel subtypes. In Kv1.5, the half-activation potentials (V0.5) for Q1 and Q2 are approximately \(-30 \text{ and } -2 \text{ mV} \), respectively, which reflect the separation of the two charge systems along the voltage axis. L-type Ca2+ channels show a separation of \(-60 \text{ mV} \) \( (16) \), whereas charge system separation in Shaker channels is less than that in Kv1.5, at \(-19 \text{ mV} \) \( (3) \). The steepness, K1 and K2, of the Q-V curves for Q1 and Q2 is determined by the valence associated with the individual charge system multiplied by the fraction of the electric field sensed. In Kv1.5, the z' values for Q1 and Q2 are 1.87 and 5.53 \( \text{e}^- \), respectively, quite similar to the values for Shaker \( (2.4 \text{ and } 5.04 \text{ e}^- \) for Q1 and Q2, respectively), whereas Q1 and Q2 in L-type Ca2+ channels are less voltage dependent with z' values of \(-1.6 \text{ and } -1.7 \text{ e}^- \), respectively. Both V0.5 separation and z' values dictate whether the two charge systems can be separated with prepulses. L-type Ca2+ channels show a strong V0.5 separation but have a Q1 system with a shallow voltage dependence, so saturation of Q1 is far from complete at potentials at which Q2 appears, and separation becomes impossible. In Shaker, although the voltage dependencies of Q1 and Q2 are relatively steep, their V0.5 values are not separated sufficiently along the voltage axis to allow steady-state separation. In Kv1.5, steady-state separation of Q1 and Q2 can be obtained by using a \(-20\text{-mV prepulse} (Fig. 3, left and right insets). This method has also been used to separate charge systems in R365C Shaker mutants \( (1) \).

Biphasic nature of $I_{\text{gon}}$ at intermediate potentials. One striking difference between $I_{\text{gon}}$ from Kv1.5 and those from other channels is the biphasic waveform at intermediate depolarizations (from \(-4 \text{ mV} \) to \(+12 \text{ mV} \)). These deviate from the rising phase, sharp peak, and exponential decay of $I_{\text{gon}}$ from other channels \( (3, 16, 19, 25) \). The \(-4\text{-mV} I_{\text{gon}} \) waveform has a clear rising phase, so charge movement cannot be described by a single transition. The rapid decay phase, corresponding to a subsequent faster transition (or one that carries more charge), is interrupted by a slower decay that substantially slows the decay of the overall trace (Fig. 4A). Because Q2 appears at \(-20\text{ mV} \) and is \(<50\% \) available at \(-4 \text{ mV} \) (Fig. 3), this emerging slow phase is probably Q2, and the coexistence of the two charge components with widely different kinetics accounts for the biphasic $I_{\text{gon}}$ waveform. Because isolated Q2 traces appear to rise and decay quickly (Fig. 4B), it is sequential coupling (or allosterism) that creates biphasic waveforms at intermediate potentials by imposing a delay on Q2 movement until Q1 has moved. As the kinetics of the nascent Q2 system become faster at more positive potentials, this notch vanishes, replaced by a plateau phase as the two charge systems merge more closely in time with each other. In other channels, sequential charge movement does not result in biphasic $I_{\text{gon}}$ waveforms. Shaker $I_{\text{gon}}$ waveforms show merged biexponential decays at intermediate potentials \( (3) \), and
this is due to the unique kinetics and voltage-depen-
dence of the Shaker Q1 and Q2 systems. This biexponen-
tial nature of Igon decay has greatly facilitated the study of Shaker Q1 at more positive potentials, whereas the biphasic shape of K(V1.5) Igon waveforms has made separation by exponential fitting impossible at these intermediate voltages.

Q1 and Q2 recover at the same rate on deactivation. Recent experimental models of K+ channel gating assume that the channel deactivates along the same pathway as that of activation (3, 19, 26, 33), with the exception of transient sojourns in closed states that carry no charge and are not part of the main deactiva-
tion pathway (26, 33). If the two charge systems were to recover at different rates, a strong argument could be put forth for independent movement of the two systems on both activation and deactivation. The experiments in Fig. 5, A–C, demonstrated equivalent recovery rates of Q1 and Q2 at −80 mV, which suggests sequential coupling. However, other transitions that carry little charge but are closely associated with channel opening must also be reversed on deactivation. Recovery from inactivation may also retard voltage sensor recovery on activation (8). Any of these processes can be rate limiting and may dictate recovery of both charge sys-
tems. Early rate-limiting transitions are consistent with equal Q1 and Q2 recovery rates for either a sequential or parallel system.

4-AP blocks some voltage-gated K+ channels by inhibiting transitions that occur later in the activation pathway leading to channel opening and inactivation (4, 5, 10, 19). Igon waveforms are not appreciably affected by 4-AP (7, 10), but 4-AP accelerates the off-gating current (compare panels A and D of Fig. 5) (10) and also the time course of recovery of Q1 and Q2 (compare panels C and F of Fig. 5). Again, though, the recovery rates of Q1 and Q2 do not differ significantly, arguing against independent charge system gating (Fig. 5F). Still, these data do not exclude the possibility that the charge systems may simply recover at the same rate and need not be sequentially coupled. The time course of off-gating current waveforms on repolariza-
tion is known to be highly voltage dependent in
Shaker channels, becoming progressively faster at more hyperpolarized potentials (3). Q1 carries less charge than Q2 (Fig. 3), and, as such, its recovery rate should be less dependent on the repolarization potential. If the two systems recover sequentially, Q2 recovery before Q1 should impart a steep voltage dependence to Q1 recovery, similar to that of Q2 recovery. A steep decline in the recovery rate between repolarization potentials of −80 and −40 mV was evident for both Q1 and Q2 in Fig. 6C. As a control, the recovery rate of Q1 in the absence of Q2 movement showed minor voltage dependence, because it represented intrinsic Q1 recovery. This provides strong evidence for sequential recovery of the two charge systems. After Q1 and Q2 movement, Q1 recovery must follow Q2 recovery and, as such, adopts its stronger voltage dependence.

Q1 and Q2 movement can be modeled with a simple linear sequential scheme. Many models of K+ channel gating have been based on a strict sequential relation-
ship between the various gating transitions (1, 3, 26). Other models have used a branched sequential scheme in which, at many points along the activation path, the channel has more than one possible route it may take (19, 33). To minimize the number of free parameters, Q1 and Q2 charge systems were modeled using a simple linear sequential scheme (Fig. 7A). We have not at-
ttempted to model off-gating currents because their kinetics are known to be affected by transitions that occur on or after channel opening. These transitions do not affect the on-charge movement because they carry little charge, but they strongly limit the time course of charge return (Fig. 2A, inset, and Fig. 5).

Q1 Igon waveforms measured in isolation negative to −20 mV showed a small rising phase as indicated by the delayed rise of the Q1 charge traces (Fig. 3). At least two transitions were required to describe this Q1 charge movement, connecting three discrete Markov states (C0–C2). The first transition had a faster forward rate (data not shown) but carried less charge than the subsequent transition (Fig. 7A), which accounted for the rising phase of Igon. At more depolarized voltages the rate of the second transition exceeded that of the first transition and produced a more pronounced rising phase (Fig. 7B, middle). The third state in the model, C2, is an intermediate state connecting the Q1 and Q2 charge systems and was previously identified by fluorescence measurements of S4 movement (1). Q2 moves once the channel reaches C2 and there is sufficient energy provided by the voltage pulse to surmount the energy barrier for the third transition. Isolated Q2 was also modeled with two gating transitions. The second transition associated with Q2 movement has slower kinetics than the first transition throughout the volt-
age range over which Q2 moves (data not shown). However, the first transition only carries one-third of total Q2 charge, so a modest rising phase was repro-
duced.

Modeled Q2 charge, when moved in the presence of Q1 (from a negative holding potential), had very slow kinetics (Fig. 7B, right), in agreement with the slow Q2 kinetics observed in Shaker Igon waveforms when fit with a double exponential function (3). However, it is the association of Q2 with Q1 that produces the marked rising phase of Q2, because the isolated kinetics of Q1 and Q2 Igon waveforms are fast (Fig. 3, insets). This suggests that intrinsic Q2 kinetics cannot be obtained from exponential fits of Igon decay if Q2 follows Q1 according to a sequential gating scheme. At −4 and +12 mV, at which Q1 and Q2 contribute similar amounts of charge to the overall Igon, the rapid rise of Q2 Igon after a −20-mV prepulse (Fig. 4B) is well reproduced in the model (Fig. 7C). The kinetic disparity between isolated Q2 Igon and Q1 Igon, moving with Q1 is evident, and it is clear that delayed Q2 movement is important in slowing Igon decay and makes a significant contribu-
tion to the rising phase of Igon.

The knowledge that K(V1.5) activation gating is accom-
plished by sequential movement of two charge systems will be useful in the further analysis of drug actions on cardiac K+ channels that involve resting or closed-state block. Future studies of the biochemical basis of the Q2
charge system will need to take sequential coupling with Q₁ into account, because independent Q₂ transitions are much faster than evident from kinetic analysis of overall Iₙa waveforms.

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REFERENCES


