Regulation of the voltage-insensitive step of HERG activation by extracellular pH

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Submitted 29 December 2009; accepted in final form 30 March 2010

Zhou Q, Bett GC. Regulation of the voltage-insensitive step of HERG activation by extracellular pH. Am J Physiol Heart Circ Physiol 298: H1710–H1718, 2010. First published April 2, 2010; doi:10.1152/ajpheart.01246.2009.—Human ether-a`-go-go-related gene (HERG, Kv11.1, KCNH2) voltage-gated K+ channels dominate cardiac action potential repolarization. In addition, HERG channels play a role in neuronal and smooth cell excitability as well as cancer pathology. Extracellular pH (pHo) is modified during myocardial ischemia, inflammation, and respiratory alkalosis, so understanding the response of HERG channels to changes in pH is of clinical significance. The relationship between pH and HERG channel gating appears complex. Acidification has previously been reported to speed, slow, or have no effect on activation. We therefore undertook comprehensive analysis of the effect of pHo on HERG channel activation. HERG channels have unique and complex activation gating characteristics with both voltage-sensitive and voltage-insensitive steps in the activation pathway. Acidosis decreased the activation rate, suppressed peak current, and altered the sigmoidicity of gating near threshold potentials. At positive voltages, where the voltage-insensitive transition is rate limiting, pHo modified the voltage-insensitive step with a pKₐ similar to that of histidine. Hill coefficient analysis was incompatible with a coefficient of 1 but was well described by a Hill coefficient of 4. We derived a pHo-sensitive term for a five-state Markov model of HERG channel gating. This model demonstrates the mechanism of pHo sensitivity in HERG channel activation. Our experimental data and mathematical model demonstrate that the pHo sensitivity of HERG channel activation is dominated by the pHo sensitivity of the voltage-insensitive step, in a fashion that is compatible with the presence of at least one proton-binding site on each subunit of the channel tetramer.

THE HUMAN ETHER-À-GO-GO-RELATED GENE (HERG, Kv11.1, KCNH2) encodes the α-subunit of the human inwardly rectifying voltage-gated K+ channel underlying rapid delayed rectifying K+ current (I_K) in the heart (22, 30, 34). The inwardly rectifying HERG current plays a critical role in the timing of action potential repolarization in the heart, and modulation of HERG activity can result in arrhythmogenesis. In addition to its central role in the heart, the HERG channel is also present in neurons, where it plays a role in spike frequency adaptation, neuritogenesis, and differentiation (7), and in smooth muscle, where it controls motility patterns (8). Expression of the HERG channel has also been implicated in cancer pathophysiology (2). Changes in extracellular pH (pHo) can arise after myocardial ischemia, inflammation, and respiratory alkalosis (12, 36).

Determining the effect of pH on HERG channel gating is therefore of great clinical and physiological significance. Previously published reports have presented a conflicting view of the effect of pHo on HERG activation. The complex gating kinetics of HERG, in which inactivation is not only voltage dependent but is also faster than activation at positive potentials (27, 28, 33), means that activation cannot be studied directly, and oblique protocols must be used (17). In the existing published studies, the differences in experimental strategy and the range of voltages and pHo used to measure activation may account for some of the observed differences in pHo sensitivity. Acidification has been reported to speed activation (3), slow activation (14, 29), have no effect (13), or have mixed results (1). Some of these studies used direct fit of currents to determine activation (rather than envelope of tails analysis), and some used only very limited pHo and voltage ranges. We therefore undertook a comprehensive analysis of the effect of pHo on HERG activation at a wide range of voltages and pHos. We used an envelope of tails protocol, which takes advantage of the fact that although at potentials positive to approximately +20 mV a small and brief transient component of inactivation overlaps with activation (17), recovery from inactivation is rapid (25) and deactivation is relatively slow (10). The degree of channels activated by a given depolarization can therefore be determined by following activation/inactivation with a step to a potential at which recovery from inactivation is rapid and there is significant current flow through the activated channels before they slowly deactivate. Biophysical analysis of our experimental results suggests that much of the complexity of regulation of HERG can be accounted for by the pHo sensitivity of an intermediate voltage-insensitive step in the activation process.

We used quantitative analysis of the pHo sensitivity of our experimental data to derive a pHo-sensitive term to modify a Markov model of HERG gating (17, 32). The model has three closed states, one open state, and one inactivated state, and transitions between the closed states include both voltage-sensitive and voltage-insensitive steps (see scheme 1). The introduction of pHo sensitivity to the voltage-insensitive step in this model of HERG activation enabled the model to recapitulate our experimental results. This indicates that the pHo sensitivity of activation of HERG is dominated by the effects of pHo on the voltage-insensitive transition.

MATERIALS AND METHODS

All animals were cared for by standards approved by the Institutional Animal Care and Use Committee of the State University at New York, University at Buffalo. Mature female Xenopus laevis (Xenopus Express) were anesthetized by immersion in a solution containing 1 g/l tricaine (Sigma). Oocytes were removed by partial oварiectomy...
and digested in a collagenase-containing Ca\(^{2+}\)-free OR2 solution containing (in mM) 82.5 NaCl, 2 KCl, 1 MgCl\(_2\), and 5 HEPES (pH 7.4) with 1 mg/ml \(^1\) collagenase type I, Sigma. Oocytes were gently shaken for 1.5–2 h, and the enzyme solution refreshed at 1 h. Defolliculated oocytes (stages V–VI) were injected with \(\leq 50\) ng mRNA for HERG using the Nanoject microinjection system (Drummond Scientific). Oocytes were voltage clamped in the whole cell mode configuration using a two-microelectrode oocyte clamp amplifier (CA-1B, Dagan, Minneapolis, MN), and currents were recorded at room temperature (25–27°C). Microelectrodes with resistances of 0.5–1.5 MΩ were fabricated from 1.5-mm outer diameter borosilicate glass tubing (TW150–4, World Precision Instruments) using a two-stage puller (Kopf Instruments) and filled with 3 M KCl. The extracellular solution contained (in mM) 96 NaCl, 2 KCl, 1 MgCl\(_2\), 1.8 CaCl\(_2\), 10 HEPES, and 5 MES. The high-K\(^+\) concentration solution contained (in mM) 98 KCl, 1 MgCl\(_2\), 1.8 CaCl\(_2\), 10 HEPES, and 5 MES. The pH was adjusted to between 5.5 and 8.2, as noted in the text. Exposure to extremes of pH\(_e\) was checked for rapid reversibility, and exposure was limited to <5 min to guard against the accumulation of intracellular pH changes with time. This is well within the 50-min safety limit for pH\(_e\) being manipulated independently of intracellular pH (1). The voltage-clamp protocols used are described as appropriate in the text.

Data were digitized and analyzed using pCLAMP 10.0 (Axon Instruments). Further analysis was performed using Clampfit 10 (Axon Instruments), Excel (Microsoft), and Origin (Microcal Software). Data were filtered at 2 kHz. Data are shown as means ± SE. Confidence levels were calculated using Student’s paired \(t\)-test.

The mathematical model of HERG channel gating was based on the previously published model of Wang et al. (32). This model was the initial quantitative model of HERG in which it was experimentally determined that there were three closed states, with a voltage-insensitive step surrounded on either side by a voltage-sensitive step. All subsequent quantitative models of multistep HERG activation are based on this structure and analysis. The main modification of this model occurred as a result of the report of Kiehn et al. (16), who analyzed single channel data and proposed a direct connection to the inactivated state from a closed state and no access to the inactivated state from the open state. This model has a specific few time constants (in ms) for specific voltages rather than a modelled voltage dependency for the appropriate transitions. It was based on single channel analysis only, without any missed event corrections. It did not have a voltage-independent step, which is required to recapitulate the whole cell data. The lack of a transition to the inactivated state directly from the open state means that this model cannot be used to represent the whole cell current. A recent attempt to combine the Wang et al. model with the Kiehn et al. data comes from the action potential model of Mazhari et al. (18), who merged a version of the Wang et al. whole cell model with the Kiehn et al. single channel analysis to arrive at a channel with a three-step activation sequence including the voltage-independent step of Wang et al. Mazhari et al. (18) included transitions to the inactivated state from both the preactivated closed state and the open state. Unlike the Wang et al. model, this model has not been experimentally validated against macroscopic currents. In addition, at \(+20\) mV, the Mazhari et al. model predicts that the channel is \(~3,000\) times more likely to transition from the closed state to the open state than it is to transition directly to the inactivated state. At \(+50\) mV, it is over \(~6,000\) times more likely to transition from the closed state to the open state rather than the inactivated state, rendering the direct closed to inactivated transition effectively zero and reducing the model to the original Wang et al. model.

HERG was modelled as a five-state model with three closed states (C), one open and conducting state (O), and one inactivated state (I), as follows (scheme 1):

\[
\begin{align*}
\alpha_i & \equiv \alpha \equiv \kappa_i \\
\beta_1 & \equiv \beta_1 \\
\beta_2 & \equiv \beta_2 \\
\end{align*}
\]

where the voltage-dependent rate constants \(\alpha\) and \(\beta\) and voltage-insensitive rate constants \(\kappa_i\) and \(\kappa_o\) were as follows:

\[
\begin{align*}
\alpha_i & = 0.02234Q_{10}\exp(0.01176V_m)\text{ ms}^{-1} \\
\beta_1 & = 0.047002Q_{10}\exp(-0.0631V_m)\text{ ms}^{-1} \\
\alpha_2 & = 0.01373\exp(0.038198V_m)\text{ ms}^{-1} \\
\beta_2 & = 0.0000689\exp(-0.04178V_m)\text{ ms}^{-1} \\
K_i & = 0.023761Q_{10}\text{ ms}^{-1} \\
K_o & = 0.036778Q_{10}\text{ ms}^{-1} \\
\alpha_i & = 0.090821Q_{10}\exp(0.023391V_m)\text{ ms}^{-1} \\
\beta_1 & = 0.006497Q_{10}\exp(-0.03268V_m)\text{ ms}^{-1} \\
\end{align*}
\]

where \(V_m\) is membrane potential and \(Q_{10}\) is a temperature scalar to account for the fact that our experimental data were obtained at relatively high room temperatures (25–27°C) compared with the lower room temperature (21–23°C) of the original modeled data (32). Deactivation is the only transition in scheme 1 that is not electrically silent, and so these transitions were additionally modified by a factor of six to fit our experimental data. This is consistent with the findings of Vandenbergh et al. (31), who determined that multiple scaling factors are required to describe the temperature dependence of HERG gating.

Previously, we (5) have shown that the conductance of HERG is sensitive to pH\(_e\), in a manner consistent with proton block of the pore that is distinct from its effects on gating kinetics. We therefore incorporated a term into the conductance \(G\) of HERG as follows:

\[
G_{HERG} = G_{HERG,0}\left[\frac{1}{1 + \left(\frac{\left[H^+\right]}{5.5}\right)}\right]
\]

Model simulations were calculated using a fourth-order Runge-Kutta algorithm with a variable step size and implemented in Microsoft Visual C++ 2008. Numerical accuracy was confirmed by demonstrating an insensitivity to step size. All computations were performed on a Dell Precision T7500 with 2 Intel Xeon CPU E5520.

RESULTS

The HERG voltage-gated K\(^+\) channel has gating kinetics distinct from most other voltage-gated channels. HERG activation is relatively slow, whereas HERG inactivation is relatively rapid, particularly at positive potentials, which results in strong inward rectification (27, 28, 33). It is not therefore possible to measure activation from direct fit of the time course of the current elicited in response to a single depolarizing step. At negative potentials, recovery of HERG from inactivation is rapid (25). This, combined with the fact that HERG deactivation is relatively slow (10), means that the degree of activation for a given depolarization stimulus can be determined using an “envelope of tails” protocol (17, 32). The initial depolarization will activate the channels, which will then rapidly transition to the inactivated state. A second step to \(-40\) mV is sufficient to initiate rapid recovery from inactivation and reveal the degree of channel activation before the channel slowly proceeds to deactivation.

We used the envelope of tails protocol to study the heterologous expression of HERG channels in Xenopus oocytes. We determined the activation rate at test potentials between \(-20\...
and +80 mV and at pHo values from 5.5 to 8.2. Given that the existing literature on the effect of pHo on HERG is conflicting, we used a broad range of pHo concentrations and voltages to determine the physiological properties of the HERG channel and to establish the saturation values for parameters. Understanding of the complex behavior of the HERG channel in the more narrow range of physiological parameters can only be done by understanding the broad behavior of the channel across the biophysical spectrum.

Representative traces of ionic currents elicited by the envelope of tails protocol at +50 and 0 mV with pHo values of 7.4 and 6.0 are shown in Fig. 1. In both cases, acidification of pHo from 7.4 to 6.0 clearly resulted in a slowing of the rate of activation. In addition, the magnitude of the outward current was reduced and the rate of deactivation was speeded, which is consistent with our previous results (5). The effects of pHo on the channel were rapid and reversible.

We repeated the envelope of tails protocol in the presence of 98 mM extracellular K+ concentration ([K+]o). The voltage protocol was amended to measure the tail current elicited on repolarization to −90 mV. This results in an inward tail current, as increasing [K+]o moves the reversal potential close to 0 mV. Figure 2 shows representative traces from depolarizing steps to +50 and 0 mV at pHo values of 7.4 and 6.0. The effects of pHo on activation were similar in high (98 mM) K+ with an inward tail current, as they were in low (2 mM) K+ with an outward tail current. In both cases, acidification from pHo from 7.4 to 6.0 slowed the rate of activation and reduced the peak current. The effect of pHo on activation is therefore independent of the direction of the current flow through the pore. This suggests that the binding site that mediates HERG sensitivity to pHo is exposed to little or none of the transmembrane electrical field (35).

We used the envelope of tails protocol in 2 mM K+ concentration to determine the pHo sensitivity of activation over a range of voltages. Figure 3 shows averaged data at pH values of 6.0 and 7.4 for steps to +80, +50, +20, and −10 mV in the presence of 2 mM [K+]o. In all cases, acidification reduced the peak outward current. This is consistent with our previous findings (5) and is likely to be the result of proton block of the pore (35). In addition to the decrease in peak current, acidification also slowed the rate of activation. At lower potentials, when the depolarizing activation step was to voltages near the threshold for activation, acidification increased the degree of sigmoidicity in the early phase of activation (see Fig. 3, C and D). The activation of HERG is complex and has two distinct types of transitions: voltage-sensitive and voltage-insensitive steps (see scheme 1). At voltages near the threshold for activation, HERG activation is sigmoid, which indicates the channel transitions through a number of closed states. At more positive voltages, the rate of activation reaches a maximum value that is voltage independent and rate limiting. Figure 3 shows that the effect of pHo is larger at voltages closer to the activation threshold than at higher voltages. At higher voltages, there is a reduction in the peak outward current and a modest reduction in the apparent rate of activation. At voltages closer to the threshold for activation, there is not only a reduction in the peak outward current and a slowing of the rate of activation but also a clear increase in sigmoidicity in activation. As pHo makes the transition to the open state more difficult at voltages close to the activation threshold, this suggests that pHo has an unequal effect on the voltage-dependent and voltage-independent transitions in the HERG activation pathway.

The transition of HERG through closed states during activation, before channel opening, has both voltage-sensitive and voltage-insensitive components. These are well described by the model shown in scheme 1. At lower voltages, the voltage-sensitive steps (α1 and α2) dominate the activation process, as those transitions are slower than the voltage-insensitive step (Ki). The result is activation that has a pronounced sigmoid shape. At more positive voltages, the voltage-dependent transitions become faster and the voltage-insensitive step eventually becomes rate limiting. The presence of two components of activation means that even using the envelope of tails protocol, the separate components of activation cannot be studied directly, as the peak tail currents are not well fit with an

**Fig. 1.** Activation of HERG is extracellular pH (pHo) sensitive. Representative traces from an envelope of tails protocol are shown. Membrane voltage was depolarized from the holding potential of −90 mV to the test potential for durations between 20 and 500 ms in 60-ms increments. The membrane potential was then returned to −40 mV, which elicited an outward tail current, the peak of which indicates the degree of activation at the end of the depolarizing pulse. A: depolarization to +50 mV with pH 7.4. B: depolarization to +50 mV with pH 6.0. C: depolarization to −10 mV with pH 7.4. D: depolarization to −10 mV with pH 6.0. All traces were recorded with 2 mM extracellular K+ concentration ([K+]o). Currents are truncated for clarity. **Insets:** voltage protocols.
increase the asymptote value at lower pHo. The rate of activation is consistent over a range of depolarizing potentials between -10 and +80 mV. For both pH values, the relationship between activation voltage and the late phase of activation is well fit by a single exponential. For both pHo values, the apparent activation rate is constant over a range of depolarizing potentials between -10 and +80 mV. However, during the late phase of activation, after the sigmoid portion of the relationship, the rate of activation is well fit by a single exponential. We therefore truncated the first 80 ms of activation tail currents and a single exponential was fit to the late phase of activation (11). Figure 4 shows the relationship between the time constant of the late phase of activation at +50 mV over the range of pHo values between 5.5 and 8.2. There is a steep transition in the relationship between pHo and the activation rate between pHo values of 6.0 and 6.5. As these data were obtained with an activation step to +50 mV, a voltage at which the voltage-insensitive step is rate limiting, this again suggests that pHo affects the voltage-insensitive step. Hill coefficient analysis of the pHo dependence was incompatible with a Hill coefficient constrained to one or two but was well described by a Hill coefficient of at least four. This suggests that modulation of HERG activation by pHo involves more than the interaction of a single proton with the channel and is likely to be the result of four extracellular binding sites, i.e., one for each subunit. The Hill function fits indicated that the approximate pHo of this process is compatible with the pKa of histidine, i.e., ~6.4. This suggests that a histidine side group may mediate the relationship between pHo and changes in activation, but other interactions are also possible (13). Taken together, these data suggest the voltage-insensitive step of HERG activation is affected by pHo at specific extracellular binding sites on the channel.

In addition to the abrupt transition centered around pH 6.4, there was a modest decrease in the rate of activation at pHo values more basic to pH 7.4. This likely reflects a process distinct from the effect of acidification on activation, and this minor effect is not part of our analysis. It does, however, resolve some of the previous apparently conflicting data on the relationship between pHo and activation. The data points at pH 7.8 and 8.2 were not included in the Hill function analysis.

Although acidification of HERG reduces the rate of activation at all voltages, the activation of HERG is complex, and the relationship between pHo and activation depends on both the voltage and the degree of acidification. To examine this complexity, we compared the time course of activation at different potentials and different pHo values. Figure 6 shows the relative time course of activation with pH 6.0 compared with pH 7.4. The peak current elicited at a given time point in the course of activation with pH 6.0 was plotted as a fraction of the peak current elicited by the same depolarization duration with pH 7.4. Data for activating depolarizations to -10, +20, +50, and +80 mV are shown. When the activation depolarization was to -10 mV, the fully activated peak current with pH 6.0 was only 35.4 ± 1.9% of that elicited in pH 7.4. This reduction in final current reflects a combination of both a shift of steady-state activation and the proton-mediated reduction of conductance (5). In contrast, when the activation depolarization was to +50 mV, the fully activated peak current at pH 6.0 was 58.5 ± 3.2% of that elicited by pH 7.4.

At positive potentials (+50 and +80 mV), the fraction of current elicited with pH 6.0 is consistently around two-thirds of the current elicited in pH 7.4. At these voltages, the channels are fully activated, and the current reduction reflects reduced conductance alone (5). The relative current recorded with activation voltages of +50 and +80 mV was very nearly identical. This reflects the fact that at these voltages, the voltage-sensitive steps are very fast relative to the voltage-insensitive step. The pHo sensitivity in the activation rate can be seen only in the shorter duration steps, i.e., in the early portion of the fraction curves for +50 and +80 mV of Fig. 6.

Fig. 2. HERG pHo dependence is independent of [K+]o. Representative traces from an envelope of tails protocol performed with 98 mM [K+]o are shown. Membrane voltage was depolarized from -90 mV to the test potential for a duration between 20 and 500 ms in 60-ms increments. The potential was then returned to -90 mV to elicit an inward tail current, the peak of which indicates activation during the depolarizing step. A: depolarization to +50 mV with pH 7.4. B: depolarization to +50 mV with pH 6.0. C: depolarization to 0 mV with pH 7.4. D: depolarization to 0 mV with pH 6.0. Insets: voltage protocols.
The later flat component reflects the fact that steady state has already been reached.

At the negative extreme, when the activation depolarization was $-10 \text{ mV}$, close to the activation threshold, changes in the fraction of peak current between pH 6.0 and 7.4 with time was relatively shallow (Fig. 6), with the peak current in pH 6.0 approximately one-third of that seen with pHo 7.4. This reflects the slowing of the voltage-dependent steps by the reduced driving force on the voltage sensor. As the voltage-sensitive steps become slower, they dominate the time course. A slight “bend” in the ratio reflects the complex interaction between multiple steps and the fact that no one step is entirely rate limiting.

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At the intermediate activation potential of $+20 \text{ mV}$, there was a complex interaction among pH o, voltage, and time.
There was a considerable difference in the fraction of current at pHo 6.0 versus 7.4 seen with longer-duration depolarizations compared with shorter-duration pulses. This complex relationship reflects the multistep process of activation and the effects of pHo on at least one step in the activation process. This complex behavior is not intuitively obvious and provides a test for the validity of our interpretation that the pHo sensitivity of the voltage-insensitive step dominates the relationship between acidification and activation of HERG channels.

**DISCUSSION**

The α-subunit encoded by HERG (KCNH2, Kv11.1) is the major molecular component of I_Kr (22, 30, 34), which plays a critical role in atrial and ventricular action potential repolarization (19, 21). In addition to its role in the heart, HERG plays important roles in neurones (7), smooth muscle (8), and cancer pathophysiology (2). HERG has a typical voltage-gated channel topology, with six membrane-spanning segments (S1–S6) and four subunits forming a functional channel. S4 has a series of positive charges that is thought to be responsible for voltage-dependent activation (6, 23). HERG inactivates via a “C-type” mechanism, which is common in K+ channels, and may be related to “slow” inactivation in Na+ and Ca2+ channels (24, 26, 37).

HERG gating kinetics are unusual: inactivation is faster than activation at positive potentials, which leads to rectification (17, 27, 32). HERG deactivation is slow (10, 25), and recovery of HERG from inactivation at negative potential is rapid (25). These unusual properties give HERG special importance in action potential repolarization. The timing of HERG current may indirectly lead to Ca2+ loading and early afterdepolarizations (19). Of more direct consequence to this study, changes in pHo are associated with changes in the shape and duration of the action potential (4, 15). Our data demonstrated that the pHo sensitivity of the HERG channel is complex and is dominated by the pHo sensitivity of the voltage-insensitive step.

Previously published studies have presented conflicting data on the effect of pHo on HERG. Acidification has been reported to speed activation (3), slow activation (14, 29), have no effect (13), or have mixed results (1). This is in contrast to the well-established consensus that the reverse process, i.e., deactivation, in which the channel transitions from the open state to the closed state in response to repolarization, is strongly pHo sensitive and is speeded with increasing proton concentration (1, 3, 5, 13, 14, 29). Our data indicate that acidification over the range of pHo 7.4 to pH 5.5 slows the rate of HERG activation. However, in transitioning from pHo from 7.4 to more basic pH 8.2, there is a modest decrease in the rate of activation. These data, obtained over a large range of pH values, reconcile all of the previously apparently conflicting data on the relationship between pH and activation rate (see Fig. 5). If studies are performed on a limited range around pH 7.4, no changes will be observed. Over the range of pHo from 7.4 to 8.2, there is a modest increase in the apparent activation rate with relative acidification. Finally, over the range of pH 5.5–7.0, there is a sharp decrease in the rate of activation with acidification. Thus, our data remove the apparent conflict of the results from the previously published studies (1, 3, 13, 14, 29). The modest change in activation seen in response to alkalosis is relatively small and is unlikely to be related to the major changes in activation observed in response to acidification. The functional significance of this weaker component of pH sensitivity is unclear at best, but it does address some apparent controversies in the literature. We will not address it further in this study.

The dominant effect of pHo on HERG activation is slowing of the activation rate with acidification. To quantitatively analyze the late phase of activation only, the first 80 ms of activation tail currents were truncated, and a single exponential was fit to the late phase of activation (11). The steepest part of the relationship between activation rate and pHo occurs around pH 6.4. This transition is very steep and is not well described by a first-order or even second-order process. It is, however, well described by a Hill coefficient with n = 4. The pKα for the underlying proton binding reaction of 6.4 is close to the pKα for histidine (∼6.5), although other possibilities for proton binding to side chains are also possible (13). The prediction from these data is that there should be at least one pHo-sensitive site for each subunit of the HERG tetramer.

Activation of HERG is sigmoid, especially at depolarizations close to the activation threshold, indicating the channel transitions through a number of closed states before reaching the open state. Our experimental data suggest that the pHo sensitivity of HERG activation is dominated by the pHo sensitivity of the voltage-insensitive step. To assess the validity of this conclusion, we developed a model of the pHo sensitivity of the activation process of HERG. The HERG channel is well represented by a Markov model with three closed states, one open state, and one inactivated state, with transitions between the closed states being voltage sensitive and voltage insensitive (see scheme 1) (32). Some variations on this model have also been proposed (9, 16, 20, 31, 38), with most differences involving transitions into and out of the inactivated state. The linear activation scheme of scheme 1 is generally applicable to all of these models, and so the model of Wang et al. (32) was used as the basis for our model for pHo analysis.

Our experimental data suggest that the pHo dependence of activation is dominated by modification of the voltage-insensitive transitions. As shown in scheme 1, the voltage-insensitive step is a closed state to closed state transition, bounded on
either side by voltage-dependent steps. The voltage-sensitive step differs from the idea of this step being a chemically driven reaction in which binding and unbinding close and open the channel directly. In a chemically driven reaction, proton binding and unbinding would be represented by the following formalism (scheme 2):

\[
C \rightarrow \overset{p}{\overset{\text{H}^+}{\text{O}}}
\]

In this formalism, the binding and unbinding of protons moves the channel in the open or closed direction in a fashion directly proportional to the proton concentration. The main consequence of such a mechanism is that as the proton concentration approaches zero the backwards rate approaches zero. Such a scheme is clearly not consistent with our data. The equilibrium for opening and closing would be strongly dependent on pH, which is not what was experimentally observed (5). Instead, we propose that the binding or unbinding of protons changes the free energy levels of the C1-C2 transition of scheme 1. Addition or subtraction of a proton will change the charge and lipophilicity of residues on the channel and the relative free energy between the two states, and the transition barrier height is changed depending on whether protons are bound or unbound. Thus, for a single binding site the probability of a single subunit being unbound \(P_{\text{unbound}}\) should follow the following simple first-order relationship given by the Eq. 9, with \(n = 1\):

\[
P_{\text{unbound}} = \frac{1}{1 + \left(\frac{[\text{H}^+]}{K_D}\right)^n}
\]

Our data suggest that \(n = 1\) does not fit the data well, and a higher-order form is required. Given the tetrameric symmetry of the HERG channel, we chose to set \(n = 4\), which provides a good fit of the data (see Fig. 5). We used this equation to modify the voltage-insensitive step by adding or subtracting a specific amount of free energy from the voltage-insensitive rate constants \(K_f\) and \(K_b\), as expressed in the following equations:

\[
K_f = K_{f,0}B_1\left[1 + \frac{A_1}{1 + \left(\frac{[\text{H}^+]}{K_D}\right)^4}\right]
\]

Fig. 7. Model analysis. Simulated traces were obtained using the same protocol as shown in Fig. 1. A: depolarization to +50 mV with pH 7.4. B: depolarization to +50 mV with pH 6.0. C: depolarization to −10 mV with pH 7.4. D: depolarization to −10 mV with pH 6.0. All traces were simulated with 2 mM [K\(^+\)]. Insets: voltage protocols. E: activation rate versus pH, determined with a depolarizing step to +50 mV (cf. Fig. 5). F: the activation rate saturated at positive potentials at both pH 7.4 and 6.0. Solid lines are exponential fit of data (cf. Fig. 4). G: the time course of the fraction of current elicited in pH 6.0 versus 7.4 is voltage dependent. Data are shown for depolarizations to +80, +50, +20, and −10 mV (cf. Fig. 6).
that the relationship between the time constant of activation and voltage-insensitive step is the dominant mediator of HERG pH sensitivitiy. We have shown that the shift in the saturating late component can be observed experimentally was also reproduced by our model and results in a dose-response relationship similar to the experimental results of Fig. 5. The more complex behavior that we observed experimentally was also reproduced by our model. Figure 7F shows that the model accounts for the saturation of the apparent late component of activation at positive potentials and that the rate constant at which saturation occurs is pH sensitive, as was observed experimentally (cf. Fig. 4). This validates our interpretation of the experimental data that the shift in the saturating late component can be explained by a single voltage-insensitive step. Finally, our model reproduces the complex behavior resulting from the interaction between pH and voltage experimentally reported in Fig. 6. The link between this complex behavior and the introduction of a pH-sensitive step was not intuitively obvious, and so the ability of our model to simulate these results (Fig. 7G) is a strong indicator that the voltage-insensitive step is the dominant mediator of HERG pH sensitivity.

Physiologically, the gating of HERG channel during the cardiac action potential is of critical importance. Any manipulation or intervention that results in a decrease in HERG current is thought to reduce repolarization reserve. This can be a static phenomenon, such as the reduction of total current associated with drug block of the channel, or trafficking defects, which result in reduced channel expression. Alternatively, HERG current can be reduced by a dynamic phenomenon, in which altered kinetics yield a current that fails to activate quickly enough and results in a reduction in repolarizing currents, which can alter the restitution relationship. The pH sensitivity of the activation steps in HERG described here indicates a steep threshold for slowing and decreasing the steady-state activation with acidosis. This sharp dependence is due to the fourth-order nature of the binding of protons. This pH-dependent reduction in functional current occurs at the extreme ranges of pH encountered physiologically during ischemic injury and may be important during irreversible ischemic injury. However, it is not reasonable to extrapolate the precise quantitative values for parameters such as pKa obtained in a heterologous expression system to the pathophysiology of in situ mammalian cardiac myocytes. There are likely to be some quantitative differences in pH affinity and rate constants between our data and native cells, but the fourth-order pH sensitivity of the voltage-independent activation step is likely to be retained in the native current.

In summary, our data show that there is a complex relationship between HERG activation and pH. We have shown that this complex gating behavior can be reproduced by the introduction of a pH-sensitive term in the voltage-insensitive step of HERG gating. There is a weak component of pH sensitivity of activation at alkaline pH that is not accounted for by our model that likely occurs due to a distinct mechanism and that may, in the future, provide additional information on the gating processes of the HERG channel. Our data and simulations confirm that changes in free energy induced by proton binding to domains involved in mediating the voltage-insensitive step of activation dominate the pH sensitivity of HERG activation.

**GRANTS**

This work was funded in part by a Scientist Development Grant from the American Heart Association (to G. C. L. Bett).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**

hERG ACTIVATION AND pH


