The electric heart of hERG

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Potassium channels encoded by the human ether-á-go-go–related gene (hERG) are of crucial importance for the repolarization of the cardiac action potential, as they constitute the main component of the cardiac delayed rectifier current, I_{Kr} (Sanguinetti et al., 1995). The primary sequence of these channels has high similarity to other voltage-gated potassium (Kv) channels, such as the prototypical *Drosophila melanogaster Shaker* and the mammalian Kv1.2. This similarity suggests that their quaternary structure will closely resemble the structure determined from Kv1.2 (Long et al., 2005), and that they will share the same or similar determinants of channel behavior.

In spite of this presumed structural similarity, the particular gating processes in hERG channels lead to unusual current behavior under voltage clamp. Both channel opening and closing are steeply voltage dependent but, upon depolarization, these channels mediate small amplitude, slowly activating currents. This is mainly because activation is intrinsically slow and inactivation occurs in a much faster time scale. In contrast, large inward currents are generated during a repolarizing voltage pulse, because inactivation recovers very quickly and channel closing is slower than recovery from inactivation. This behavior is reflected in a macroscopic inwardly rectifying current–voltage relationship.

One expectation based on its sequence similarity with the canonical Kv channels has been that hERG should undergo similar voltage-dependent conformational changes leading to the opening and closing of the channels. For example, the inactivation process in hERG (Schönherr and Heinemann, 1996; Smith et al., 1996) occurs through a very similar mechanism to that of the C-type inactivation phenomenon described in *Shaker* and other potassium channels (Hoshi et al., 1991). Also resembling *Shaker* channels, the six positive charges located in the S4 transmembrane helix seem to be the main carriers of the gating charge and are thought to establish electrostatic interactions with countercharges in the S2 and S3 transmembrane segments (Zhang et al., 2005). Some evidence is even consistent with a sequence alignment where the first charge in *Shaker* S4, R1, is equivalent to the first lysine in the S4 of hERG (Wang et al., 1997).

The first experiments in hERG that explored the involvement of the S4 transmembrane segment in channel gating and its voltage dependence also demonstrated that, as occurs in Kv channels, this part of the protein responds to changes in membrane potential with movements that could be tracked by voltage-clamp fluorometry (VCF) (Smith and Yellen, 2002). These early experiments could correlate the measured fluorescence-quenching signal with the equilibrium and kinetic properties of the conductance changes. Although the VCF signal was complex, a good correlation was found between the slow current activation and a slow component of the fluorescence, which also had the same voltage dependence as the conductance change. This was strong evidence that the speed of voltage-sensor movement and its voltage dependence were the rate-limiting step in channel activation. This is opposed to the situation found in Kv channels like *Shaker*, where voltage-sensor movement occurs rapidly and at voltages more negative than the voltages that produce channel opening. Consistently, models for Kv channel activation contain several transitions among closed states, which correspond to voltage-sensor movement, and one or few concerted transitions before the open state, which constitute the rate-limiting step (Zagotta et al., 1994; Schoppa and Sigworth, 1998). Intriguingly, early recording and detailed characterization of hERG currents (Wang et al., 1997) showed that the time course of activation was sigmoidal and consistent with the presence of several transitions among closed states before the final opening step. These recordings also suggested that the early transitions, presumably associated with movement of the voltage sensor, were not rate limiting.

Recording the movement of the voltage sensor can be performed directly by measuring the nonlinear capacitive currents that result from the displacement of the fixed charges of the S4 voltage sensor. As this rearrangement occurs only when the voltage across the membrane is changed, these currents are transient. The magnitude and time course of these displacement currents, also known as gating currents, are dependent on both the amount of charge associated with the transition and the rate at which this charge is translocated.

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The first gating currents from hERG were reported by the Sanguinetti laboratory in 2003 (Piper et al., 2003). These currents were obtained from experiments in *Xenopus laevis* oocytes using the cut-open oocyte voltage-clamp technique and showed a series of unusual characteristics. Consistent with the VCF recordings of Smith and Yellen (2002), hERG gating currents showed two components of charge movement, vastly diverging in time course by almost two orders of magnitude. The fast component had a very depolarized voltage dependence and, although reminiscent of fast inactivation, did not correlate with it. The slow component correlated with the time course of current activation but, contrary to the slow component of VCF recordings, had a voltage dependence that was shifted to more negative voltages than the conductance change, just as it is observed in other Kv channels.

These gating current recordings were consistent with both components of charge movement being associated with movement of the S4 voltage sensor. The fast component could be associated at least in part with the inactivation process, whereas the slow component was thought more likely to represent transitions occurring before channel opening. Given that the slow component had almost the same time course as ionic current activation, the experiments also suggested that the rate-limiting step in channel activation is the movement of the voltage sensor.

An important piece of information came more recently (Es-Salah-Lamoureux et al., 2010) with VCF experiments demonstrating that the original fluorescence measurements of Smith and Yellen (2002) might have been contaminated with fluorescence signals arising from endogenous cysteines. These cysteine residues became modified by the fluorophore and also underwent voltage-dependent exposure changes. The experiments of Es-Salah-Lamoureux et al. (2010) demonstrated that, in the absence of two cysteines present in the S1–S2 linker, the fluorescence signal retained fast and slow kinetic components but, importantly, the fast component now developed at more negative voltages than the opening of the channels, suggesting that S4 movement is indeed faster than the conformational change associated with pore opening, as has been known to happen in Kv channels such as *Shaker*.

This sets the stage for the results reported by Wang et al. in this issue. This paper reports on new recordings of gating currents from hERG channels expressed in a mammalian cell line along with cysteine-accessibility measurements of S4 movement. These experiments were performed using patch-clamp techniques in the whole-cell configuration. The recordings in this paper show that, as is the case in the earlier gating current recordings of Piper et al. (2003), charge movement in hERG is distributed among two main components that are distinct in their kinetics and voltage dependence.

The fast component of gating charge starts moving at very negative voltages and appears as small and rapid gating currents with a time constant at 0 mV of ∼5 ms. This earlier fast component seems to be responsible for approximately one third of the total charge movement and is almost two orders of magnitude faster than channel activation.

The slower component is so slow that it is very difficult to resolve during depolarization pulses (recall that the magnitude of gating currents is also a function of the speed of charge movement: slow charge movement gives rise to small amplitude components of gating current). For this reason, Wang et al. (2013) use an ad hoc protocol to resolve slow charge movement. As the charge returns after a depolarizing pulse (off-gating currents), the slow component can be resolved as the appearance of extra charge that reaches a steady-state magnitude only after long depolarizing pulses. This slow charge movement accounts for two thirds of the total charge movement.

The most important finding reported in these experiments is that both components of gating currents precede channel opening in time and voltage. To substantiate this finding, Wang et al. (2013) perform estimates of the movement of the voltage sensor by measuring the kinetics and voltage dependence of modification of cysteines introduced in the S4 segment. The result of these experiments is that the changes in exposure of S4 are entirely consistent with the gating current measurements in that they are comparable in speed to charge movement and have a voltage dependence that precedes the conductance change.

The picture that emerges from these new experiments and other available data is that, just as in canonical Kv channels, charge movement in hERG occurs before channel opening, and the rate-limiting step for current activation may be caused by other processes different from S4 translocation. There is considerable evidence in the literature documenting the participation of the S4–S5 linker as well as the N terminus in electromechanical coupling (Chen et al., 2001; Gustina and Trudeau, 2011). Thus, it is entirely possible that processes associated with this linker are responsible for the slow activation of hERG currents.

Overall, these results suggest that in hERG channels, just as in canonical Kv channels, charge movement may be strictly coupled in a sequential manner to pore opening. This hypothesis could now be tested by carefully measuring the limiting slope of current activation and comparing it to the voltage dependence of gating currents, although these measurements could be complicated by the presence of multiple open states in hERG channels.

Although the paper by Wang et al. (2013) clarifies a series of details regarding charge movement in hERG, there are still puzzling questions to be solved: Is there an intrinsic voltage dependence to inactivation, or is this derived solely from its coupling to channel opening? Is hERG opening allosteric or linearly coupled to charge movement? These are important problems that should be tackled in the near future.

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