Can many subunits make light work of ion channel inactivation.

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Voltage-gated K^+ channels have fascinated physiologists for many decades, not just because they play important roles in a myriad of cellular functions including neurotransmission, cardiac repolarization, hormone secretion and cell volume homeostasis but also because they are exemplary nanomachines that are capable of extraordinary feats of molecular gymnastics. In recent years, the human *ether a go-go* related gene (hERG) K^+ channel has been of particular interest as mutations in *hERG* result in a markedly increased risk of cardiac arrhythmias and because these channels are the molecular target for the vast majority of drugs that have had to be withdrawn from the market due to an unacceptably high risk of drug-induced cardiac arrhythmias (Vandenberg et al., 2012).

Voltage-gated K^+ channels transition between closed, open and inactivated states. The rates and voltage dependence of these transitions vary between family members allowing for the fine-tuning of electrical signals in different tissue types. In hERG channels, the rapid voltage dependent transition between the open and inactivated states, coupled with slow open-closed state transitions, are critical for their role in cardiac repolarization and in opposing ectopic beats (Smith et al., 1996). Despite the distinctive kinetics and voltage dependence, inactivation gating in hERG K^+ channels shares many similarities with the slow "c-type inactivation" in Shaker K^+ channels (Smith et al., 1996). All voltage gated K^+ channels are tetrameric complexes with a single central ion conduction pathway, the extracellular end of which is lined by one selectivity filter from each of the four subunits. C-type inactivation is thought to involve rearrangements of the selectivity filter region of the channel. However, recently it has been shown that inactivation of hERG K^+ channels is a significantly more complex allosteric process involving sequential rearrangements of multiple domains in the channel (Wang et al., 2011). Hitherto, it has been unclear to what extent these rearrangements occur within and/or between channel subunits.

In a study published in this issue, Wu and colleagues have utilized a subunit concatemer approach to specifically investigate whether inactivation in hERG K^+ channels requires co-operative interactions between subunits. This approach allows the introduction of mutations into one or more specific subunits within the tetramer, rather than having to rely on the random association of subunits that occurs with co-expression of wildtype and mutant subunits. One issue with this approach is that three of the free N-termini and C-termini present in wildtype channels are removed in the concatemer. For channels where the N-terminus or C-terminus influences the gating transition of interest this can be a problem. Whilst deletion of the N-terminus of hERG K^+ channels can influence inactivation gating (Gustina & Trudeau, 2013) previous studies have shown that concatenation of four WT hERG subunits did not significantly affect inactivation (Wu et al., 2014).

Initially, Wu and colleagues show that the introduction of either the S620T or the G628C/S631C mutation into a single subunit of the concatenated hERG K^+ channel prevented channels from inactivating. Thus, all four subunits must be inactivation competent for the channels to inactivate. This suggests that the final, or at least a very late, step in the inactivation process requires a concerted all-or-nothing co-operative interaction between the four subunits. Interestingly, an investigation of mutants more remote from the selectivity filter showed quite distinct effects on inactivation. For example, studies of T618A (in the pore helix) and S631A (just external to the selectivity filter) suggested a

sequential model of cooperative subunit interactions, whereas studies of M645C, in the pore lining S6 domain, suggested that the subunits act independently of one another during inactivation. So the answer to the question of how subunits interact during inactivation of hERG K^+ channels depends very much on what stage of the process you are looking at.

Previous studies of C-type inactivation in other voltage-gated K^+ channels, using a similar concatemer approach, have suggested that there are co-operative interactions between subunits. However, most of these studies have only studied one or two mutations close to the selectivity filter. The study from Wu and colleagues indicates that the apparent nature of the subunit interactions is dependent on the location of the mutation studied. It would be intriguing to see if there is a similar level of variability in co-operativity for C-type inactivation in other channels. If this were the case, it would suggest that the basic process of C-type inactivation is widely conserved and that hERG K^+ channel inactivation represents one variation of a common theme. The current studies add a new chapter to our understanding of the complexity of hERG K^+ channel gating, but whether this level of complexity is conserved in all voltage-gated K^+ channels remains to be determined.

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Additional Information

Competing Interests

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