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Review article

Deciphering hERG channels: Molecular basis of the rapid component of the delayed rectifier potassium current

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ABSTRACT

The rapid component of the delayed rectifier potassium current (I_{Kr}), encoded by the ether-a-go-go-related gene (*ERG1*, officially denominated as *KCNH2*), is a major contributor to repolarization in the mammalian heart. Acute (e.g. drug-induced) and chronic (e.g. inherited genetic disorder) disruptions of this current can lead to prolongation of the action potential and potentiate occurrence of lethal arrhythmias. Many cardiac and non-cardiac drugs show high affinity for the I_{Kr} channel and it is therefore extensively studied during safety pharmacology. The unique biophysical and pharmacological properties of the I_{Kr} channel are largely recapitulated by expressing the human variant (hERG1a) in overexpressing systems. hERG1a channels are tetramers consisting of four 1159 amino acid long proteins and have electrophysiological properties similar, but not identical, to native I_{Kr}. In the search for an explanation to the discrepancies between I_{Kr} and hERG1a channels, two alternative hERG1 proteins have been found. Alternative transcription of *hERG1* leads to a protein with a 56 amino acid shorter N-terminus, known as hERG1b. hERG1b can form channels alone or coassemble with hERG1a. Alternative splicing leads to an alternate C-terminus and a protein known as hERGuso. hERGuso and hERG1b regulate hERG1a channel trafficking, functional expression and channel kinetics. Expression of hERGuso leads to a reduced number of channels at the plasma membrane and thereby reduces current density. On the contrary, co-assembly with hERG1b alters channel kinetics resulting in more available channels and a larger current. These findings have implication for understanding mechanisms of disease, acute and chronic drug effects, and potential gender differences.

Contents

1. Introduction

Repolarization in the human heart is largely dependent on the cardiac delayed rectifier potassium current, consisting of a slow (I_{Ks}) and a rapid (I_{Kr}) component that differ from one another in terms of sensitivity to drugs, rectification characteristics and kinetic properties [1]. The molecular basis of I_{Kr} is the $K_v11.1$ ion channel protein, encoded by the ether-a-go-go-related gene (*ERG1*, officially denominated as *KCNH2*) [2,3]. I_{Kr} is a major contributor during repolarization of the action potential (AP) in most mammalian (except for rodents) heart

Abbreviations: AP, action potential; CNB domain, cyclic nucleotide binding domain; ER, endoplasmatic reticulum; *ERG*, ether-a-go-go-related gene; HEK cells, human embryonic kidney cells; I_{Kr}, delayed rectifier potassium current; PAS domain, Per-Arnt-Sim domain.

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cells and its unique biophysical and pharmacological properties are largely recapitulated by expressing the human variant (hERG1a) in overexpression systems such as HEK (human embryonic kidney) cells [4]. The unique and unusual kinetics of the hERG channel are characterized by a relatively slow activation and rapid inactivation, which limits I_{Kr} current amplitude during the rapid depolarization of the AP. During subsequent repolarization, hERG channels rapidly recover from inactivation and then slowly deactivate, giving rise to a large tail current [5–7].

Acute (e.g. drug-induced) and chronic (e.g. inherited genetic disorder) disruptions of this current can lead to prolongation of the action potential and potentiate the occurrence of lethal arrhythmias. Many cardiac and non-cardiac drugs show high affinity for the I_{Kr} channel and it is therefore extensively studied during safety pharmacology screening [8,9]. It has been known for a while that hERG1a channels have electrophysiological properties similar, but not identical, to native I_{Kr} [1]. Like most other potassium channels, hERG channels are tetrameres and thus formed through assembly of four proteins. Two independent reports in 1997 showed the first evidence that I_{Kr} channels comprise (at least) two different protein subunits and provided evidence that channels with heteromeric subunit expression fitted wild type I_{Kr} better than homomeric channels. [10,11]. London et al. and Lees-Miller et al. both identified a second isoform of mouse ERG1 – mERG1b – which is largely identical to mERG1a but with a modified N-terminus. mERG1a is homologous to hERG1a and expressed in heart, brain and testes whereas mERG1b is abundantly found in heart but only in trace amounts in brain.

A year later, Kupershmidt et al. [12] identified a third variant of hERG1, also identical to hERG1a but with a modified C-terminus, termed hERGuso. mRNA levels for hERGuso were twice that of hERG1a but transfection did not lead to functional channels. Co-transfection of hERGuso with hERG1a, however, altered channel kinetics. Additionally, a combination of hERG1b and hERGuso, hERG1buso, has been reported by one group but is not extensively studied [13]. Alternative isoforms of ERG are also expressed in other tissues, such as the brain [14], though that will not be discussed here. This review provides a concise summary of the research and progress made at understanding hERG1 expression and channel function in the heart over the past 15 years. Important to mention is that hERG channel function is also regulated by accessory subunits such as MiRP1, but that coexpression with hERG1a is not sufficient to recapitulate native I_{Kr} properties [15]. For a review on the impact of ancillary subunits on potassium channels see Ref. [16].

2. Molecular biology

The human *KCNH2* gene is located on the long (q) arm of chromosome 7 at position 36.1 (between base pairs 150,642,043 to 150,675,401) and consists of 15 exons (Fig. 1A). The $K_v11.1$ protein is initially synthesized in the endoplasmatic reticulum (ER) as the core-glycosylated precursor form and becomes fully glycosylated in the Golgi apparatus from where the so-called mature form is translocated to the plasma membrane [7]. Much of the increased protein size results from the addition of complex oligo-saccharides by N-linked glycosylation, and allows for transportation of the channel complexes to the plasma membrane. The hERG1a protein consists of 1159 amino acids and when expressed in HEK cells this results in an apparent molecular mass of 135 kDa when the protein is core-glycosylated and 155 kDa when fully glycosylated [7,17,18]. The hERG1b isoform has a shorter N-terminus (of 56 amino acid residues) of which the initial 36 are unique. This results in a total protein length of 819 amino acids [10,11] and apparent molecular masses of 85 and 95 kD for the core glycosylated and mature glycosylated forms respectively [17,18]. A completely unglycosylated hERG1b protein with a molecular mass of 80 kD was also reported [17]. hERGuso has an alternative C-terminus where amino acids 800–1159 are replaced by an alternative 88-residues [12], resulting in an 888 amino acid long protein with a molecular mass of 99 kDa. The fourth reported form, hERG1buso, where both the N- and the C-terminus are altered, is 548 amino acids long and has a molecular mass of 62 kDa [13]. Fig. 1B shows a schematic representation of the different proteins and Table 1 lists the molecular masses in different species.

The expression of hERG1a in the human heart has been known since 1995 [19]. Though two reports showed expression of ERG1b in mice already in 1997, other reports were unable of identifying ERG1b protein expression in hearts from rat, mouse, horse or human hearts [18,20]. In 2004, however, Jones et al. [17] presented evidence that the ERG1b isoform is expressed on protein level in ventricular myocytes of human, rat and dogs. They additionally showed that hERG1a and hERG1b proteins associate in ventricular preparations and localize at the T-tubulus. In 2008, Luo et al. [21] identified different transcription start sites and promoter regions for hERG1a and hERG1b, resulting in independent transcripts. This suggests that hERG1a and hERG1b are different isoforms rather than splice variants of the same transcript.

Contrary to hERG1b that is formed through independent transcription, evidence shows that hERGuso is generated by alternative splicing and polyadenylation of hERG1a pre-mRNA [22]. Approximately two-thirds of all hERG1a pre-mRNA is processed to hERGuso in the heart. This suggests a post-transcriptional mechanism that modulates expression and function of hERG1 channels since, on their own, neither hERGuso nor hERG1buso gives rise to functional channels but are retained in the ER [12,13,23].

Though hERG1a, hERG1b, hERGuso and hERG1buso have all been found in the human heart, the (relative) distribution throughout the four chambers is disputed. One publication revealed similar expression of hERG1a and hERG1b but overall higher expression in the right side of the heart compared to the left side [21]. Another report states that hERG1b ranged from 3 to 30% of total hERG1 expression and that as little as 3% was sufficient to alter deactivation kinetics of the channel [24]. The large difference in ratio of hERG1a versus hERG1b expression in different individuals can have important clinical implications (see further under “Implications for disease” and “Implications for pharmacology and gender” below).

The different hERG proteins coassemble as either homo- or heteromeres containing four α-subunits that each contain six transmembrane domains of which S1–S4 form the voltage sensing domain and S5 and S6 the pore (see Fig. 1B) [3]. Both the N-terminus and the C-terminus are located intracellularly and play important roles in channel kinetics and regulation.

In contrast to other mammalian ion channels, the N-terminal region contains the signal sensor Per-Arnt-Sim (PAS) domain that is important both for protein trafficking to the plasma membrane and deactivation of the hERG channel [25–27]. During translation, prior to glycosylation, hERG1a and hERG1b subunits assemble in the ER through interaction of the N-termini [50]. This interaction prevents oligomerization of hERG1b proteins and masks an exposed ER retention signal present as a $R^{15}XR$ motif (two arginines separated by any single residue) in the unique N-terminus of this subunit that otherwise prevents efficient expression of hERG1b homomeric channels at the plasma membrane [28]. Interestingly, the $R^{15}XR$ motif is not conserved across species whereas another RXR motif ($N^{22}XN$) found in the ERG1b N-terminus is conserved but does not cause ER retention. One study shows that selective deletion of *ERG1b* in mice eliminates all I_{Kr} in adult ventricular cardiomyocytes [29]. In mice, where repolarization is mainly dependent on I_{to}, this did not result in a prolonged QT interval but did predispose the mouse heart to episodic abrupt sinus bradycardias. One may speculate that the outcome in humans would be much more severe.

The C-terminus contains a cyclic nucleotide binding (CNB) domain which also plays a role in protein trafficking [30]. The C-terminus regulates protein forward trafficking and stability, though the exact