Influence of Amino-Terminal Structures on Kinetic Transitions between Several Closed and Open States in Human erg K⁺ Channels

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Abstract. Gating kinetics of human ether-a-go-go (eag)-related gene (HERG) K⁺ channel expressed in Xenopus oocytes was studied using non-inactivating channel variants carrying different structural modifications in the amino terminus. A kinetics model was elaborated to describe the behavior of full-length channels, that includes at least three open states besides the three closed states previously proposed. Deletion of the HERG-specific proximal domain (HERG Δ138–373) accelerated all individual forward transitions between closed states. Whereas relatively large amplitude depolarizations were required to drive full-length HERG channels to more distal open states, these were reached more easily in channels without proximal domain. Alteration of the initial eag/PAS domain by introduction of a short amino acid sequence at the beginning of the amino terminus did not alter transitions between closed states, but prevented the channels from reaching the farthest open states that determine slower deactivation rates. This indicates that the presence of specific amino-terminal structures can be correlated with the occurrence of distinctive molecular transitions. It also demonstrates that both proximal and eag/PAS domains in the amino terminus contribute to set the gating characteristics of HERG channels.

Key words: HERG — Potassium channel — Kinetic model — Human heart — Xenopus oocytes

Introduction

Human ether-a-go-go-related gene (HERG) and HERG-like potassium channels play a key role setting the electrical behavior of a variety of cell types (Barros et al., 1994, 1997; Arcangeli et al., 1997; Zhou et al., 1998; Akbarali et al., 1999; Bauer et al., 1999; Schäfer et al., 1999; Cherubini et al., 2000; Emmy et al., 2000; Overholt et al., 2000; Rosati et al., 2000). The best documented implication of HERG relates to cardiac ventricular cells, in which this channel mediates the repolarizing current IKr. This current participates in the repolarization phase of the cardiac action potential, and subsequently in control of the spike and interspike intervals (Smith, Baukrowitz & Yellen, 1996; Zhou et al., 1998). Hence mutations in the h-erg gene have been recognized as the cause of certain forms of familial long-QT syndrome (Curran et al., 1995; Sanguinetti et al., 1995; Spector et al., 1996a), and pharmacological blockade of HERG is also the determinant of acquired forms of this syndrome (Rodé et al., 1996; Spector et al., 1996a). Crucial kinetic determinants of HERG physiological roles are the existence of a particularly slow activation rate that overlaps with a rapid and voltage-dependent inactivation process, limiting the level of outward current upon depolarization, and a particularly slow deactivation rate following the fast recovery of inactivation upon repolarization (Sanguinetti et al., 1995; Trudeau et al., 1995; Schönherr & Heinemann, 1996; Smith et al., 1996; Spector et al., 1996b; Wang et al., 1997). This makes HERG functionally appear as an inward rectifier, although its molecular structure of six membrane-spanning domains and an S4 charged region corresponds to that of a typical depolarization-activated channel (Warmke & Ganetzky, 1994).
The amino-acid sequence of HERG shows that it has the longest amino terminus of any potassium channel, mainly due to the presence of a long stretch of residues extending from about position 135 to about position 366 (the “proximal” domain; see Viloria et al., 2000) that follows an initial domain (the eag or PAS domain) conserved in the eag family. Although the best described functional role of K+ channel N-terminal domain(s) is to provide the ball for N-type inactivation (Hoshi et al., 1990), recent work has implicated the N-terminus of different channels in regulation not only of channel assembly, but also of voltage dependence and/or activation kinetics (Schönherr & Heinemann, 1996; Spector et al., 1996b; Marten & Hoshi, 1997; Pascual et al., 1997; Terlau et al., 1998; Cushman et al., 2000; Minor et al., 2000). Previous work from several laboratories indicated that the interaction of the eag/PAS domain with the channel core acts as a molecular brake that determines the slow HERG deactivation kinetics (Cabral et al., 1998; Wang et al., 1998; Sanguinetti & Xu, 1999). However, the molecular determinant(s) of HERG activation slowness are still poorly defined. The crystal structure of the eag/PAS domain has been resolved (Cabral et al., 1998). Interestingly, the initial 25 residues most conserved in this domain that remain disordered in the crystal seem to constitute a key factor to specifically stabilize the open state and thus slow HERG closing (Wang, Myers & Robertson, 2000). Nevertheless, the exact nature of the interactions between this domain and either the channel core or the remaining amino-terminal sequences is not known. In a recent work, we showed that the presence of the proximal domain exclusive of HERG constitutes an essential determinant of its slow activation gating (Viloria et al., 2000). Our results suggested that the need to adequately modify the conformation of the proximal domain acts as a constraining factor on efficient progress through the activation pathway, limiting or slowing down the interaction of the eag domain with the channel.

In this report, the activation and deactivation gating characteristics of HERG are deeply explored using non-inactivating S620T channel variants carrying different structural modifications in the amino terminus. This led us to elaborate a kinetics model to describe the behavior of full-length channels, which includes at least three open states along with the three closed states previously proposed (Wang et al., 1997; Kiehn, Lacerda & Brown, 1999). Such a model reproduced not only the sigmoid time course of HERG activation, but also the complex time course of deactivation, including the dependence of closing rates on the magnitude and/or duration of previous depolarization (Viloria et al., 2000). Performance of similar kinetic analysis with channels without proximal domain indicates that all individual transitions between closed states are accelerated by such a domain removal and that proximal domain-deleted channels move more easily through the activation pathway up to the more distal open states. However, whereas a variant carrying an alteration in the eag/PAS domain shows transitions between closed states similar to those of wild-type channels, it seems unable to reach the farthest open states that determine slower deactivation rates. This allows us to associate the presence of specific amino-terminal structures with the occurrence of distinctive molecular transitions. It also emphasizes the relevance of both proximal and eag/PAS domains of the amino terminus setting HERG gating characteristics, and hence its physiological role in cardiac, neuronal and neuroendocrine cells.

Materials and Methods

Generation of Channel Mutants

Unless indicated, all experiments were performed on HERG channels made incapable of inactivation by substitution of serine with threonine in position 620 (S620T; Ficker et al., 1998; Viloria et al., 2000). We always use “wild-type” in the graphs and throughout the text to refer to full-length S620T channels, and “Δ136–373” or “H3HA” for S620T channels carrying additional modifications in the amino terminus. The procedure for generation of the Δ136–373 channel lacking the proximal domain has been detailed elsewhere (Viloria et al., 2000). To construct the H3HA variant, a nine-amino-acid hemagglutinin (HA) epitope tag recognized by the monoclonal antibody 12CA5 was placed at the beginning of the amino terminus by inserting in the HindIII site of the HERG clone a double-stranded oligonucleotide (5'-AG CTT AGG ATG TAC CCT TAC GAC GTT CCT GAC TAC GCT A-3') that contains the recognition site for HindIII and an ATG followed by the HA epitope sequence.

Plasmids and Preparation of CRNA

The plasmid containing the cDNA for the HERG channel was a generous gift of Dr. E. Wanke (University of Milano, Milano, Italy). Plasmids were linearized and capped cRNA was in vitro synthesized from the linear cDNA templates by standard methods using SP6 RNA polymerase as described (de la Peña et al., 1992).

Oocyte Expression and Solutions

Procedures for frog anaesthesia and surgery, oocyte obtaining and microinjection have been detailed elsewhere (Barros et al., 1998). Oocytes were maintained in OR-2 medium (in mm: NaCl 82.5, KCl 2, CaCl2 2, MgCl2 2, Na2HPO4 1, HEPES 10, at pH 7.5). Cyttoplasmic microinjections were performed with 30–50 nl of in vitro synthesized cRNA per oocyte. HERG currents were studied in manually defolliculated oocytes (de la Peña et al., 1992; Barros et al., 1998). Recordings were obtained in standard OR-2 or in high-K+ medium in which 50 mm KCl substituted an equivalent amount of NaCl. Functional expression was typically assessed 2–3 days after microinjection.