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PERSPECTIVE

Refining Insights into High-Affinity Drug Binding to the Human *Ether-à-go-go*-Related Gene Potassium Channel

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ABSTRACT

hERG (*human ether-à-go-go-related gene*) potassium (K^+) channels play a crucial role in electrophysiological activity in the heart, exerting a profound influence on ventricular action potential repolarization and on the duration of the QT interval of the electrocardiogram. hERG channels are strongly implicated in the acquired form of long QT syndrome in that they exhibit a unique susceptibility to pharmacological inhibition by therapeutically and chemically diverse drugs. Investigations over a number of years provide compelling evidence that a comparatively large inner cavity and the presence of particular aromatic amino acid residues (Tyr652 and Phe656) on the inner (S6) helices of the channel are important features that allow hERG to accommodate and bind disparate drugs. However, whereas functional hERG channels are composed of four identical subunits, blocking molecules may not interact equally with aromatic residues from each of the four subunits. In this issue of *Molecular Pharmacology*, Myokai et al. (p. 1643) report for the first time the use of tandem dimers incorporating mutations to Tyr652 and Phe656 to elucidate asymmetric binding of the high affinity hERG inhibitor cisapride. Not only has this approach provided increased information on spatial arrangements involved in cisapride binding to the channel, but it offers a powerful means of refining the wider understanding of hERG channel structure-function in relation to drug binding.

Torsades de pointes (TdP) is a polymorphic ventricular tachycardia that is strongly associated with delayed ventricular repolarization in the congenital and acquired (drug-related) forms of long QT syndrome (LQTS) (Yap and Camm, 2003; Modell and Lehmann, 2006; Sanguinetti and Tristani-Firouzi, 2006). Although TdP can spontaneously resolve back into a normal cardiac rhythm it can also degenerate into potentially fatal ventricular fibrillation. It is the spectre of TdP that makes QT interval prolongation by either clinically used drugs or drugs in development a matter for some concern. Although the incidence of TdP in patients receiving a particular drug usually tends to be rather low (Yap and Camm, 2003), the attendant risk may nevertheless be unacceptable for many drugs, particularly in the case of medications used to treat non–life-threatening conditions. Two examples serve as useful illustrations here. In 1997, the United States Food & Drug Administration announced withdrawal of approval for antihistamines incorporating terfenadine as a result of cardiac safety issues (Cruzan, 1997; Josefson, 1997). In 2000, the Food & Drug Administration announced the voluntary withdrawal of US marketing of a treatment for severe heartburn called cisapride (Propulsid; Janssen Pharmaceutica, New Brunswick, NJ) as a result of multiple reports of heart-rhythm abnormalities, including fatalities, primarily among patients either taking other medications or with conditions predisposing toward cardiac arrhythmia (Henney, 2000).

In principle, delayed ventricular repolarization (and thereby QT interval prolongation) could result from drug-induced modulation of a number of different cardiac ion channels. It is remarkable, however, that virtually all drugs that delay ventricular repolarization share a common feature: an ability to inhibit the functional activity of ion channels responsible for the 'rapid' delayed rectifier potassium current (I_{Kr}), which plays a crucial role in ventricular action potential repolarization (Witchel and Hancox, 2000; Sangui-

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ABBREVIATIONS: TdP, *Torsades de pointe;* LQTS, long QT syndrome; hERG, human *ether-à-go-go*-related gene; WT, wild type.

netti and Tristani-Firouzi, 2006). The association between I_{Kr} channel inhibition and drug-induced QT interval prolongation is sufficiently strong that relevant nonclinical drug-safety testing guidelines (the International Conference on Harmonization document ICH S7B) recommend use of an in vitro I_{Kr} assay as part of an integrated risk assessment of the propensity of pharmaceuticals to delay ventricular repolarization (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005).

"hERG" came to prominence when the *hERG* (*human ether-à-go-go-related gene*, alternative nomenclature *KCNH2*) product was shown to be a functional potassium channel that mediates ionic current with properties close to native I_{Kr}, and mutations to the gene were implicated in the familial LQTS (Curran et al., 1995; Sanguinetti et al., 1995; Trudeau et al., 1995). A link between hERG and acquired (drug-induced) LQTS was also suggested (Sanguinetti et al., 1995). Since that time numerous hERG mutations have been implicated in congenital LQTS (Modell and Lehmann, 2006), and a gain-of-function hERG mutation has been found to mediate one form of the recently identified short QT syndrome (Brugada et al., 2004). To return to our earlier drug examples of terfenadine and cisapride, despite differences in therapeutic class and chemical structure, both were found to be potent hERG K^+ channel inhibitors (Roy et al., 1996; Suessbrich et al., 1996; Rampe et al., 1997). Moving forward to the current day, many structurally and therapeutically diverse drugs have now been identified as inhibitors of the hERG K^+ channel including both cardiac (class I and III antiarrhythmic drugs) and numerous non-cardiac drugs (Vandenberg et al., 2001; Sanguinetti and Mitcheson, 2005). Although many of these drugs have in common a central basic nitrogen group together with several hydrophobic/aromatic groups, it is the diversity of structures producing hERG K^+ channel blockade that is particularly striking. Given the strong association between I_{Kr}/hERG block, QT interval prolongation, and risk of TdP, an obvious and important question arises: what makes I_{Kr}/hERG uniquely susceptible to pharmacological inhibition by chemically and therapeutically diverse drugs? Considerable insight into the nature of drug-hERG interactions has emerged since 2000, and in this issue of *Molecular Pharmacology*, Myokai et al. (2008) further this field. The brief historical perspective below aims to place this important new study in context.

hERG is a six-transmembrane domain voltage-gated K^+ channel that normally functions as a homotetramer (Fig. 1A) and the majority of I_{Kr}/hERG-blocking drugs appear to cross the cell membrane and enter the channel from the cytosolic side when it opens. Recovery from blockade by high-affinity methanesulfonanilide drugs is slow, and data from experiments using a hERG channel mutant (D540K) that opens on hyperpolarization and a relatively bulky molecule, MK-499, provided compelling evidence that slow recovery results from drug retention ("trapping") in the inner cavity once the channel has closed (Mitcheson et al., 2000b). The structural correlate here is a larger inner cavity of hERG than of other Kv channels because of the absence in hERG of a proline (P-x-P) motif that otherwise restricts inner cavity size, probably by causing a kink in the inner (S6) helices (del Camino et al., 2000; Mitcheson, 2003). A second key structural determinant of hERG's drug-sensitivity is the presence of particular aromatic amino acid residues in on S6 helices facing the inner cavity. In 2000, a phenylalanine residue at position 656 was found to be critically important for high-affinity binding by the methanesulfonanilides, dofetilide and MK-499 (Lees-Miller et al., 2000; Mitcheson et al., 2000a). Alanine scanning mutagenesis, coupled with homology modeling based on the bacterial KcsA channel (Mitcheson et al., 2000a), mapped the methanesulfonanilide binding site (for MK-499) to the S6 and pore helices implicating Phe656, a nearby tyrosine (Tyr652), as well as several other residues in MK-499 binding (Fig. 1B). It is noteworthy that Tyr652 and Phe656 were found also to be critical for binding of terfenadine and cisapride (Mitcheson et al., 2000a), suggesting that these residues may be consensus determinants of high-affinity hERG channel blockade, via cation-π and π stacking interactions with the basic tertiary nitrogen and aromatic groups of the blocking drug.

While Phe and Tyr at positions analogous to 656 and 652, respectively, are common to hERG and the related EAG channel, other voltage-dependent K^+ channels lack aromatic amino acid residues at these positions (Sanguinetti and Mitcheson, 2005). hERG's uniquely fast 'C-type' inactivation (Smith et al.,

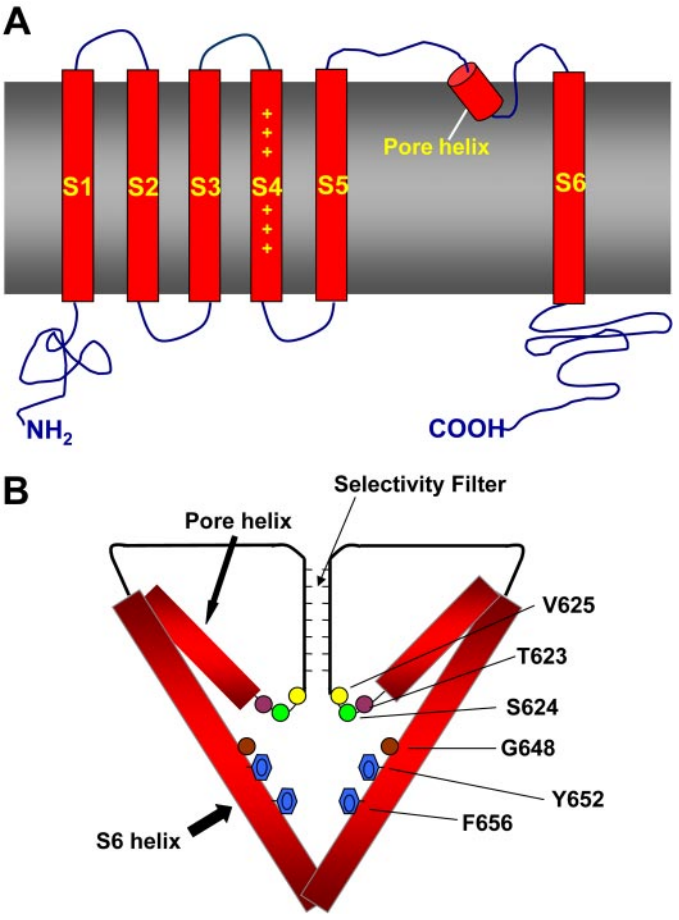

Fig. 1. A, schematic diagram of a single hERG channel subunit, showing transmembrane domains (red; labeled S1-S6 in yellow), lipid membrane (shaded gray) and intracellular N (NH_2) and C (COOH) termini. Four similar subunits combine to produce a functional hERG channel. B, schematic diagram illustrating selected amino acid residues (not to scale) found to be important for drug binding to the hERG channel. A vertical section shows the pore and inner (S6) helices from two of the four channel subunits; the position of the selectivity filter is also shown. The aromatic residues shown in blue (Tyr652 and Phe656) are key determinants of high affinity binding for a number of drugs including cisapride.

1996) is also likely to participate in high-affinity blockade, since introduction of C-type inactivation into the normally noninactivating bovine EAG channel increased by 30-fold the channel's sensitivity to dofetilide (Ficker et al., 2001). For at least some compounds, including cisapride, it may be that conformational changes during hERG's unusually rapid 'C type' inactivation optimizes the positioning of these aromatic residues for drug binding, because repositioning of Tyr652 and Phe656 on the S6 helix of hERG has been shown to reduce cisapride sensitivity, whereas repositioning of the analogous residues in EAG induced cisapride sensitivity (Chen et al., 2002). Although there may be some exceptions (e.g., Milnes et al., 2003; Ridley et al., 2004), the majority of drugs that have been examined in detail effect hERG blockade via interactions with one or both of Tyr652 and Phe656 (Fig. 1B; for reviews, see Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006). Progressively more detailed knowledge of drug-binding mechanisms from structure-function analysis (Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006) and from in silico modeling of hERG-drug interactions (Recanatini et al., 2008) may ultimately facilitate removal of potential hERG blockade early during the drug design process.

Cisapride has figured repeatedly in structure-function analyses of hERG channel inhibition (Mitcheson et al., 2000a; Chen et al., 2002; Fernandez et al., 2004). Systematic mutation of Tyr652 and Phe656 to other residues has provided evidence that potency of blockade is well correlated with hydrophobicity, particularly of the side chain of residue 656, whereas for residue 652, high-affinity inhibition is contingent upon an aromatic side group, implicating cation-π interactions with the drug's basic tertiary nitrogen (Fernandez et al., 2004). Functional hERG channels are tetrameric and to-date, structure function analyses have compared wild-type and mutant homotetrameric channels, which will presumably therefore have a rotational symmetry about an axis through the center of the pore. Drug structures may be asymmetric, however, and it follows that such molecules will not necessarily interact equally with particular residues on all four subunits. In this volume of *Molecular Pharmacology*, Myokai et al. (2008) have investigated this issue for cisapride, using tandem dimers of hERG incorporating wild-type (WT) and/or mutant subunits in which one or both of Tyr652 and Phe656 had been mutated to alanine. The use of tandem dimers is not without potential problems, because linking monomers could potentially influence conformational changes during channel gating and alter channel kinetics. However, even with a relatively short linking sequence, the voltage-dependent kinetics of WT hERG current (I_{hERG}) were little affected for WT tandem dimers, although the deactivation time course was accelerated. Substitution of one tyrosine at position 652 on adjacent subunits markedly reduced the affinity of the channel for cisapride, suggesting that both Tyr652 residues on adjacent subunits were required for high-affinity drug binding. In contrast, mutation of a single Phe656 on adjacent subunits did not necessarily result in a reduction in affinity. Most notably, the double mutants, in which (in addition to one of the Tyr652s) one Phe656 on each tandem subunit had also been mutated, has provided the first direct experimental evidence of the importance of the particular arrangement of the aromatic residues for high affinity binding: tandem dimers in which Phe656 had been mutated on a subunit with an intact Tyr652, whereas the adjacent subunit contained Y652A and an intact Phe656, had a significantly lower binding affinity than tandem dimers consisting of a WT subunit in tandem with a subunit containing both Y652A and F656A. The authors employed a thermodynamic double mutant cycle analysis of changes in binding energy, in which each double mutant can be regarded as the consequence of two successive single mutations through different routes, to demonstrate that mutation of Phe656 on a subunit containing a mutated Tyr652 produced comparatively little further change in binding energy, whereas mutation of Phe656 on a subunit with intact Tyr652 but with Y652A on the adjacent subunit produced a marked reduction in binding affinity. The data are interpreted as suggestive of additive interactions between Tyr652 and Phe656 on the same subunit, but cooperative interactions between these residues on adjacent subunits. The findings of Myokai et al. (2008) are also broadly consistent with a recent simulation study involving simulation of cisapride docking to the hERG K^+ channel tetramer (using a template based on KvAP), which suggested T-shaped π-π stacking interactions between cisapride and diagonally opposite Tyr652s and a parallel displaced interaction with a Phe656 (Farid et al., 2006). In summary, this important study not only provides unprecedented insight into the nature of cisapride blockade of the hERG channel but also establishes an exciting approach that can be expected to be invaluable in refining ideas as to how drugs may bind to functional tetrameric hERG channels.

References

Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Sheng Wu Y, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, and Antzelevitch C (2004) Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation* **109:**30–35.

Chen J, Seebohm G, and Sanguinetti MC (2002) Position of aromatic residues in the S6 domain, not inactivation, dictates cisapride sensitivity of HERG and eag potassium channels. *Proc Natl Acad Sci U S A* **99:**12461–12466.

Cruzan SM (1997) FDA proposes to withdraw seldane approval. FDA talk paper T97–3. United States Food & Drug Administration, Rockville, MD. Available at http://www.fda.gov/bbs/topics/ANSWERS/ANS00780.html. Accessed March 27, 2008.

Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, and Keating MT (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* **80:**795–803.

del Camino D, Holmgren M, and Yellen G (2000) Blocker protection in the pore of a voltage-gated K^+ channel and its structural implications. *Nature* **403:**321–325.

Farid R, Day T, Friesner RA, and Pearlstein RA (2006) New insights about HERG blockade obtained from protein modeling, potential energy mapping, and docking studies. *Bioorg Med Chem* **14:**3160–3173.

Fernandez D, Ghanta A, Kauffman GW, and Sanguinetti MC (2004) Physicochemical features of the hERG channel drug binding site. *J Biol Chem* **279:**10120–10127.

Ficker E, Jarolimek W, and Brown AM (2001) Molecular determinants of inactivation and dofetilide block in ether a-go-go (EAG) channels and EAG-related K^+ channels. *Mol Pharmacol* **60:**1343–1348.

Henney JE (2000) From the Food and Drug Administration. *JAMA* **283**:2228-.

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005) ICH S7B guideline (step 5 implementation): the nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland. Available at http://www.ich.org/cache/compo/502-272-1.html#S7B, accessed March 27, 2008.

Josefson D (1997) Hay fever drug to be banned by the FDA. *Brit Med J* **314:**248.

Lees-Miller JP, Duan Y, Teng GQ, and Duff HJ (2000) Molecular determinant of high-affinity dofetilide binding to HERG1 expressed in *Xenopus* oocytes: involvement of S6 sites. *Mol Pharmacol* **57:**367–374.

Milnes JT, Crociani O, Arcangeli A, Hancox JC, and Witchel HJ (2003) Blockade of HERG potassium currents by fluvoxamine: incomplete attenuation by S6 mutations at F656 or Y652. *Br J Pharmacol* **139:**887–898.

Mitcheson JS (2003) Drug binding to HERG channels: evidence for a 'non-aromatic' binding site for fluvoxamine. *Br J Pharmacol* **139:**883–884.

Mitcheson JS, Chen J, Lin M, Culberson C, and Sanguinetti MC (2000a) A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci U S A* **97:**12329–12333.

Mitcheson JS, Chen J, and Sanguinetti MC (2000b) Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. *J Gen Physiol* **115:**229–240.

Modell SM and Lehmann MH (2006) The long QT syndrome family of cardiac ion channelopathies: a huge review. *Genet Med* **8:**143–155.

Myokai T, Ryu S, Shimizu H, and Oiki S (2008) Topological mapping of the asymmetric drug binding to the HERG potassium channel by use of tandem dimers. *Mol Pharmacol* **73:**1643–1651.
Rampe D, Roy M-L, Dennis A, and Brown AM (1997) A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett* **417:**28–32.
Recanatini M, Cavalli A, and Masetti M (2008) Modeling hERG and its interactions with drugs: recent advances in light of current potassium channel simulations. *ChemMedChem*, in press.
Ridley JM, Milnes JT, Witchel HJ, and Hancox JC (2004) High affinity HERG K^+ channel blockade by the antiarrhythmic agent dronedarone: resistance to mutations of the S6 residues Y652 and F656. *Biochem Biophys Res Commun* **325:**883–891.
Roy M-L, Dumaine R, and Brown AM (1996) HERG, a primary human ventricular target of the nonsedating antihistamine terfenadine. *Circulation* **94:**817–823.
Sanguinetti MC, Jiang C, Curran ME, and Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell* **81:**299–307.
Sanguinetti MC and Mitcheson JS (2005) Predicting drug-hERG channel interactions that cause acquired long QT syndrome. *Trends Pharmacol Sci* **26:**119–124.
Sanguinetti MC and Tristani-Firouzi M (2006) hERG potassium channels and cardiac arrhythmia. *Nature* **440:**463–469.
Smith PL, Baukrowitz T, and Yellen G (1996) The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* **379:**833–836.
Suessbrich H, Waldegger S, Lang F, and Busch AE (1996) Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole. *FEBS Lett* **385:**77–80.
Trudeau MC, Warmke JW, Ganetzky B, and Robertson GA (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* **269:**92–95.
Vandenberg JI, Walker BD, and Campbell TJ (2001) HERG K^+ channels: friend and foe. *Trends Pharmacol Sci* **22:**240–246.
Witchel HJ and Hancox JC (2000) Familial and acquired long QT syndrome and the cardiac rapid delayed rectifier potassium current. *Clin Exp Pharmacol Physiol* **27:**753–766.
Yap YG and Camm AJ (2003) Drug induced QT prolongation and torsades de pointes. *Heart* **89:**1363–1372.

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