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Original article

Molecular determinants of hERG potassium channel inhibition by disopyramide

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ABSTRACT

The Class Ia antiarrhythmic drug disopyramide (DISO) causes QT interval prolongation that is potentially dangerous in acquired Long QT Syndrome but beneficial in short QT syndrome, through inhibition of the *hERG*-encoded channels responsible for rapid delayed rectifier K^+ current (I_{Kr}). In this study, alanine mutants of hERG S6 and pore helix residues and MthK-based homology modelling and ligand docking were used to investigate molecular determinants of DISO binding to hERG. Whole-cell hERG current (I_{hERG}) recordings were made at 37 °C from HEK-293 cells expressing WT or mutant hERG channels. WT outward I_{hERG} tails were inhibited with an IC_{50} of 7.3 μM, whilst inward I_{hERG} tails in a high $[K^+]_e$ of 94 mM were blocked with an IC_{50} of 25.7 μM. The IC_{50} for the Y652A mutation was ~55-fold that of WT I_{hERG}; this mutation also abolished a leftward shift in voltage-dependent I_{hERG} activation present for WT hERG. The IC_{50} for F656A I_{hERG} was ~51 fold its corresponding WT control. In contrast to previously studied methanesulphonanilide hERG inhibitors, neither the G648A S6 nor the T623A and S624A pore helical mutations modified DISO IC_{50}. Computational docking with the hERG model showed that DISO did not exhibit a single unique binding pose; instead several low energy binding poses at the lower end of the pore cavity favoured interactions with Y652 and F656. In the WT hERG model DISO did not interact directly with residues at the base of the pore helix, consistent with the minimal effect of mutation of these residues on drug block.

1. Introduction

Repolarization of cardiac action potentials (APs) is controlled by a number of potassium (K^+) channels [1]. The rapid delayed rectifier K^+ channel current (I_{Kr}) plays a key role in ventricular AP repolarization and, thereby, in setting the duration of the QT interval of the electrocardiogram [1,2]. *hERG* (*human Ether-à-go-go Related Gene*; alternative nomenclature *KCNH2*) is responsible for the pore-forming subunit of the I_{Kr} channel [3,4] and *hERG* mutations are responsible for the LQT2 form of Long QT Syndrome (LQTS) and for the SQT1 form of the short QT syndrome (SQTS) [5–8]. Both native I_{Kr} and recombinant hERG channels are sensitive to pharmacological inhibition by a wide range of clinically used drugs; this action, on the one hand, mediates delayed repolarization produced by Class Ia and III antiarrhythmic drugs and, on the other, can give rise to drug-induced (acquired) LQTS (aLQTS) and *torsades de pointes* (TdP) arrhythmia [8–10]. The pharmacological promiscuity of hERG appears in part to be attributable to a relatively large inner cavity [11] and in part to the presence of aromatic amino-acids in the S6 helices, which facilitate drug interactions [8,10,12].

Disopyramide (4-(diisopropylamino)-2-phenyl-2-(pyridin-2-yl) butanamide) is a Class Ia antiarrhythmic drug that is known to inhibit both native I_{Kr} and hERG [13–16]. Its clinical use is associated with a risk of acquired LQTS and TdP [17–19]. In contrast, in the setting of hERG-linked SQT1 disopyramide may help to normalise abbreviated repolarization [20]. The principal form of SQT1 arises due to a gain-in-function hERG mutation (N588K, located in the channel's S5-Pore linker) that impairs the channel's inactivation process [21]. In contrast with methanesulphonanilide hERG inhibitors, disopyramide binding to the hERG channel exhibits relatively little dependence on channel inactivation [14,15,21–24], and this may account for its efficacy both in vitro in inhibiting SQT1 mutant hERG channels and in SQT1 patients [15,16,20]. However, despite disopyramide's relevance both to aLQTS and SQT1, obligatory molecular determinants of disopyramide binding to hERG have not yet been identified. Consequently, the present study was performed in order to investigate the molecular basis of disopyramide's inhibition of hERG.

2. Materials and methods

2.1. Alanine mutants

Cell lines stably expressing S6 aromatic residue mutations of hERG (F656A or Y652A) were employed as described previously (e.g. [25–27]). Alanine mutants at the base of the pore helix (T623A, S624A), the selectivity filter (V625A) and the S6 helix (G648A) [12] were constructed using QuikChange® (Stratagene) mutagenesis. The following forward primer sequences were used: 5′CAG CAG CCT CGC CAG TGT GGG3′ for T623A; 5′CAG CAGCCTCACCGCTGTGGGCTTCGGC3′

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for S624A, 5′CCTCACCAGTGCGGGCTTCGG C3′ for V625A; 5′ CGTCATGCTCATTGCCTCCCTCATGTATG 3′ for G648A. In all cases, DNA was sequenced for the full length of the hERG insert to ensure that only the correct mutation had been made (Eurofins MWG Operon).

2.2. Maintenance of cells and cell transfection

Most experiments employed Human Embryonic Kidney (HEK-293) cells stably or transiently expressing HERG constructs. Cells were passaged using a non-enzymatic agent (Enzyme Free, Chemicon® International) and maintained as previously described [25–28]. HEK-293 stable cell lines either expressing wild-type (WT) hERG (provided by Professor Craig January; [29]) or hERG mutants F656A and Y652A [25] were employed. For transient transfection experiments, 24 h after plating cells out, cells were transiently transfected with 1 μg of each HERG construct using Lipofectamine™ LTX (Invitrogen) according to the manufacturer's instructions. Expression plasmid encoding CD8 was also added (in pIRES, donated by Dr I Baró and Dr J Barhanin) to be used as a successful marker of transfection. Cells were plated onto small sterilised collagen-coated glass coverslips 6 h after transfection and recordings were made after at least 24 h incubation at 37 °C. Successfully transfected cells (positive to CD8) were identified using Dynabeads® (Invitrogen).

2.3. Electrophysiological recordings

For whole-cell patch-clamp recording cells were continuously superfused (at 37 °C) with an external solution containing (in mM): 140 NaCl, 4 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 Glucose and 5 HEPES (titrated to pH 7.45 with NaOH). For experiments employing the S6 mutants T623A, G648A, F656A and their WT control, the external solution contained 94 mM KCl (the NaCl concentration was correspondingly reduced) [26,27]. Patch-pipettes (Corning 7052 glass, AM Systems) were pulled and heat-polished (Narishige MF83) to 2.5–4 MΩ; pipette dialysate contained (in mM): 130 KCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP, 10 HEPES (titrated to pH 7.2 using KOH) [30]. Recordings of hERG current (I_{hERG}) were made using an Axopatch 200 amplifier (Axon Instruments) and a CV201 head-stage. Between 70 and 80% of pipette series resistance was compensated. Voltage-clamp commands were generated using 'WinWCP' (John Dempster, Strathclyde University).

2.4. Disopyramide

Disopyramide-phosphate powder (Sigma) was dissolved in Milli-Q water to produce an initial stock solution of 400 mM which was diluted to produce stock solutions ranging down to 1 mM. The disopyramide-phosphate stock solutions were diluted at least 1:1000-fold with Tyrode's solution to achieve concentrations stated in the Results section. External solutions were applied using a home-built, warmed and rapid solution exchange device.

2.5. Electrophysiology data analysis

The voltage dependence of I_{hERG} activation was determined by fitting the values of I_{hERG} tail currents (normalised to peak I_{hERG} tail value and plotted against voltage) with a Boltzmann equation of the form:

$$I = I_{MAX}/(1 + \exp(V_{0.5} - V_m)/k) \quad (1)$$

where I is the I_{hERG} tail amplitude following test potential V_m, I_{MAX} is the maximal I_{hERG} observed, $V_{0.5}$ is the potential at which I_{hERG} was half-maximally activated, and k is the slope factor for the relationship. Concentration–response relations were fitted with a standard Hill equation to obtain half-maximal inhibitory concentration (IC_{50}) and Hill-coefficient (n_H) values. Mean values in the text are presented either as mean ± SEM or (for IC_{50} and n_H values) as mean ± 95% confidence intervals (C.I.). Statistical analysis was performed using analysis of variance or *t*-tests as appropriate (Graphpad Prism v5). p values of less than 0.05 were taken as statistically significant.

2.6. Molecular modelling

The homology model of the hERG pore region in the open channel state used for disopyramide docking was based on the crystal structure of MthK [31], as described and used in previous docking studies of propafenone and erythromycin [32,33]. An alignment of hERG and MthK spanning the pore- and S6-helices is given in Fig. 6B. Disopyramide (S+ disopyramide) was constructed in the Biopolymer module of Insight and energy minimised using Discover. All drug binding was carried out at a nominal pH of 7.0, which defines the protonation state of ionizable groups on both drug and receptor. Docking was carried using the Flexidock module of Sybyl x1.1. Flexidock uses a genetic algorithm to identify low energy poses for binding of a ligand to a broadly specified binding pocket. In the case of the hERG model, the binding pocket was defined as the entire pore region spanning the volume from just below the bottom of the selectivity filter and pore helix to the "cytoplasmic" side of the pore approximately 4 Å below residue S660. Flexidock allows unlimited ligand and template side chain flexibility which we consider to be important for ligand binding to a "receptor" for which true side chain rotamers are unknown. During docking runs, all non-trivial bond rotations in disopyramide were allowed (a trivial rotation is one that rotates a methyl group around its three-fold axis). Side chain flexibility of residues T623, S624, V625 near and in the bottom of the selectivity filter, and Y652, F656, S660 in the S6 helix was allowed. Additional docking runs were done using a template with altered Tyr-652 side chain rotamers chosen to maximise the space for drug binding below the selectivity filter, and using mutant channels in which chosen side chains (S624, Y652 or F656) were changed to alanine; free side chain flexibility was also allowed in these runs. Further computational details and justification of the MthK homology model are described in the Supplemental Data.

3. Results

3.1. Concentration-dependent I_{hERG} inhibition by disopyramide

The sensitivity of WT I_{hERG} to disopyramide was determined by repetitive application (every 12 s) of a 2-s depolarizing voltage command from a holding potential of −80 mV to +20 mV followed by a 4-s repolarizing step to −40 mV [15,16,25,27]. We have previously characterised the concentration dependence of WT I_{hERG} by disopyramide under similar recording conditions to this study [14,15] and consequently in this study selected three drug concentrations (1, 10 and 100 μM) over the known I_{hERG} blocking range, for comparison with previously published data [14,15] and with the hERG mutant channels studied here under conditions matching those for WT I_{hERG}. Inhibition of the elicited current typically reached a steady-state within 3 min of drug perfusion. Fig. 1A shows representative traces before application (control) and in the presence of disopyramide (DISO 10 μM). For each concentration, the mean fractional block of outward I_{hERG} tail at −40 mV was calculated as previously described [15] and plotted as shown in Fig. 1B. The half maximal inhibitory concentration (IC_{50}) derived from a standard Hill equation was 7.28 μM (C.I. 5.76 μM to 9.20 μM) with a Hill coefficient (n_H) of 0.87 (C.I. 0.70 to 1.05). This is in good agreement with previously published values of 7.23 ± 0.72 μM [14] and 10.66 ± 0.02 μM [15]; simulated concentration–response curves based on these prior data are shown superimposed in Fig. 1B for comparison with data from this study.