Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 554 (2007) 98–105
www.elsevier.com/locate/ejphar

The action of the novel gastrointestinal prokinetic prucalopride on the HERG K^+ channel and the common T897 polymorph

Hugh Chapman*, Michael Pasternack [1]

Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Received 28 June 2006; received in revised form 3 October 2006; accepted 6 October 2006
Available online 18 October 2006

Abstract

The *human ether-à-go-go related gene* (HERG) encodes the α-subunit of a delayed rectifier potassium channel important in the repolarisation of the cardiac action potential. Excessive action potential prolongation through HERG channel inhibition is associated with a risk of torsade de pointes arrhythmias and is a major challenge for drug development. The acute effects of the novel prokinetic prucalopride were examined on heterologously expressed HERG channels in human embryonic kidney (HEK) 293 cells using the whole-cell patch–clamp technique. Prucalopride inhibited HERG channels in a concentration-dependent manner with an IC_{50} of 4.1 μM. Prucalopride significantly slowed channel deactivation and recovery from inactivation, accelerated and altered the extent of inactivation. Similar concentration-dependency and kinetic changes were observed with the minor T897 polymorphic HERG variant. Prucalopride block was frequency-independent due to rapid state-dependent block, with binding occurring in the open and inactivated states. Though prucalopride blocks HERG channels this is unlikely to be significant at clinically relevant concentrations.

Keywords: Prucalopride; HERG [human ether-à-go-go related gene]; K^+ channel; Polymorphism; Patch–clamp

1. Introduction

Prucalopride is a potent, selective 5-HT_4 receptor agonist intended for the treatment of gastrointestinal tract motility disorders (Briejer et al., 2001). In healthy human subjects prucalopride was shown to be well tolerated, accelerating colonic transit and affecting both stool frequency and consistency (Emmanuel et al., 1998; Bouras et al., 1999; Poen et al., 1999). Subsequent studies have focused on patients with chronic idiopathic constipation with similar results obtained (Bouras et al., 2001; Emmanuel et al., 2002), as well as an enhancement of visceral sensitivity and reduction in bloating and pain (Emmanuel et al., 2002).

A number of drugs from a wide range of structural classes have had labelling revised or, as in the case of the gastrointestinal prokinetic cisapride, been withdrawn from markets due to reported adverse cardiac events (Yap and Camm, 2003; Roden, 2004), typical of the long QT syndrome. Long QT syndrome is characterised by a prolonged rate corrected QT interval which can predispose an individual to torsade de pointes (Roden et al., 1996; Viskin, 1999). This is a ventricular tachycardia, with QRS complexes of changing amplitude that appear to twist around the isoelectric line, which can give rise to syncope and following degeneration to ventricular fibrillation, cardiac arrest. Whether acquired or inherited, long QT syndrome originates from the delayed repolarisation of the myocardium increasing the duration of the ventricular action potential. Virtually all the drugs that prolong the QT interval and induce torsade de pointes preferentially inhibit the rapid component of the cardiac delayed rectifier potassium current, I_{Kr} (Roden et al., 1996; Lacerda et al., 2001; Redfern et al., 2003). The basis for this phenomenon has been attributed to various unique structural features of the α-subunit of I_{Kr} (Mitcheson et al., 2000), which is encoded by the *human*

* Corresponding author. Current address: Department of General Pharmacology, Orion Pharma, Tengströminkatu 8, P.O. Box 425, FI-20101 Turku, Finland. Tel.: +358 10 426 7455; fax: +358 10 426 7403.
E-mail address: hugh.chapman@orionpharma.com (H. Chapman).
[1] Current address: Scientific Affairs, Orion Pharma, P.O. Box 65, FI-01210 Espoo, Finland.

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.10.019

ether-à-go-go related gene (*HERG* or *KCNH2*; Sanguinetti et al., 1995). HERG mutations resulting in a loss- or gain-of-function cause congenital type 2 long QT syndrome and one form of the short QT syndrome respectively (Curran et al., 1995; Brugada et al., 2004).

Numerous studies have investigated the potential of prokinetics to inhibit HERG channels (Mohammad et al., 1997; Rampe et al., 1997; Walker et al., 1999; Drolet et al., 2000; Potet et al., 2001; Furuta et al., 2004; Claassen and Zunkler, 2005). In this study we examined the effect of prucalopride on the HERG current and characterised the biophysical mechanism of action.

Though blockade of the HERG current and QT prolongation are perceived as a marker for a drug's torsadogenic potential the exact relationship is more complex (Redfern et al., 2003; Finlayson et al., 2004; Roden, 2004). The unpredictability may arise in part from the drug itself, its interaction with the HERG channel and multiplicity of pharmacological actions. Moreover drug-induced torsade de pointes is commonly associated with identified risk factors such as female gender, overdose, drug–drug interactions and pre-existing pathological conditions (Zeltser et al., 2003; Roden, 2004). Another factor emerging as underlying the susceptibility of certain individuals are allelic variations (polymorphisms and mutations) of the genes responsible for congenital long QT syndrome (Roden, 2001; Vos and Paulussen, 2004). Therefore we also investigated whether prucalopride inhibition and its properties differed in a common HERG polymorphism. A lysine (K) 897 threonine (T) amino acid polymorphism within HERG has been identified in several populations with the minor allele (2690C corresponding to T897) present at frequencies ranging from 2–25% (Iwasa et al., 2000; Laitinen et al., 2000; Yang et al., 2002; Bezzina et al., 2003; Anson et al., 2004).

2. Materials and methods

2.1. Cell culture and expression of HERG

HEK-293 cells were maintained in Dulbecco's Modified Eagles Medium supplemented with 10% foetal calf serum, 2 mM L-glutamine and 1% penicillin–streptomycin solution at 37 °C in a humidified atmosphere of 5% CO_2. HERG cDNA (K897 HERG; U04270) in the plasmid pcDNA3 was kindly provided by Dr. Gail Robertson (University of Wisconsin, Madison, Wisconsin, USA). The T897 HERG cDNA construct was made using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, California, USA). The HEK-293 cells were transiently transfected with HERG cDNA, either the K897 or T897 isoforms, and enhanced green fluorescent protein cDNA using the calcium phosphate precipitation method (Paavonen et al., 2003). The cells were plated on poly-D-lysine coated coverslips for electrophysiological recordings.

2.2. Electrophysiological recordings and analysis

HERG currents were measured 36–72 h after transfection using the whole-cell configuration of the patch–clamp technique (Hamill et al., 1981). Patch pipettes were fabricated from borosilicate glass capillaries (Harvard Apparatus, Kent, UK) and had resistances of 1.5 to 4 MΩ when filled with a solution containing (mM): KCl 150, $MgCl_2$ 2, BAPTA 5, HEPES 10 and MgATP 5 (pH 7.2 with KOH). The extracellular solution contained (mM): NaCl 150, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1 and HEPES 5 (pH 7.4 with NaOH). Recordings were performed at room temperature (22–24 °C) using an EPC9 amplifier (HEKA Elektronik, Lambrecht, Germany) and began 5 min after rupture of the cell to allow dialysis. Capacitance and series resistance compensation were optimised, with the latter attaining ≥70%.

Data acquisition and analysis was performed with Pulse+ Pulsefit (HEKA) and Origin 4.1 (OriginLab Corporation, Northampton, Massachusetts, USA). Data are given as mean±s.e. mean of n experiments. The voltage-dependence of current activation was determined by fitting a Boltzmann equation to the data of the form:

$$y = 1/(1 + \exp^{(V - V_{1/2})/k})$$

where $V_{1/2}$ is the half-maximal activation voltage, k is the slope factor and y is the degree of activation. The concentration-response curves were fitted with a Hill equation:

$$I_{\text{prucalopride}}/I_{\text{control}} = 1/[1 + (C/\text{IC}_{50})^n]$$

Where C is the prucalopride concentration, IC_{50} is the concentration required for 50% block and n is the Hill coefficient. Statistical significance was ascertained using Student's t-test for paired or independent data when studying the differences between control and prucalopride conditions (as in Fig. 1), and between the HERG isoforms (as in Fig. 4C) respectively. P values < 0.05 were considered significant. Statistical calculations were performed with SPSS 9.0 (SPSS Inc, Chicago, Illinois, USA).

2.3. Drug

Prucalopride (4-amino-5-chloro-2,3-dihydro-*N*-[1-(3-methoxypropyl)-4-piperidinyl]-7-benzofuran carboxamide monohydrochloride; kindly provided by Johnson and Johnson Pharmaceutical Research and Development, Beerse, Belgium) was dissolved in dimethyl sulphoxide (DMSO) to obtain a stock solution of 10^{-2} M, from which the final drug concentrations were prepared daily by dilution with extracellular solution. A new stock solution was prepared weekly. All prucalopride solutions contained DMSO at a concentration (0.3%) equivalent to the highest drug dilution investigated. Prior to the introduction of drug baseline measurements were gained during perfusion with extracellular solution containing 0.3% DMSO (control).

3. Results

3.1. Inhibition of HERG potassium currents by prucalopride

Initially HEK-293 cells were transfected with the prevalent K897 isoform of HERG generating the distinctive current profile observed previously (Zhou et al., 1998; Paavonen et al., 2003). The HERG current was elicited from the holding potential of −80 mV by test pulses from −80 mV to +40 mV

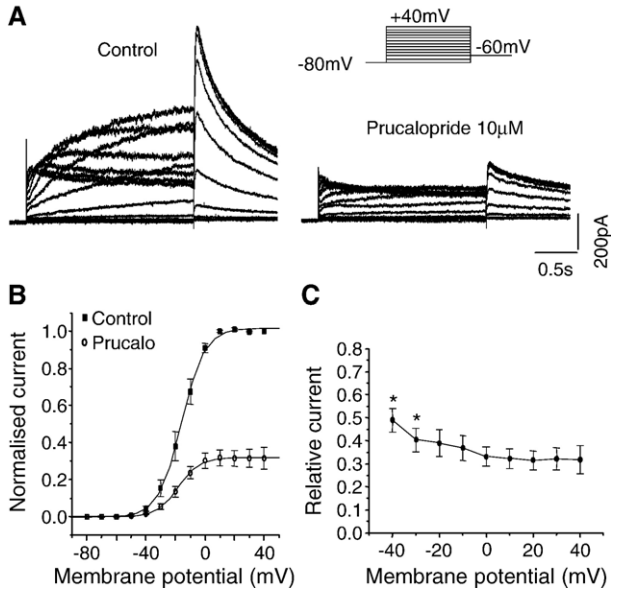

Fig. 1. Effect of prucalopride on HERG K^+ channel current. (A) HERG (K897) currents elicited in control conditions and with 10 μM prucalopride by a protocol in which 2 s depolarising steps from −80 to +40 mV were applied every 10 s from the holding potential of −80 mV. Tail currents were produced by subsequent repolarisation to −60 mV. (B) The voltage-dependence of activation of the HERG (K897) current. The peak tail current amplitudes during control and prucalopride exposure were measured, normalised for each cell to the control value at +40 mV and then averaged (n=7). These values are plotted against the preceding voltage step and fit with a Boltzmann function. (C) The voltage-dependence of tail current inhibition. The relative current (peak tail current in prucalopride divided by peak tail current in control) plotted as a function of the preceding test pulse potential (n=7). At −40 and −30 mV the relative current was significantly different (* P<0.05) from that at either +30 or +40 mV.

of 2 s duration that were applied every 10 s. The test pulse was followed by a repolarisation step to −60 mV evoking large, slowly decaying outward tail currents. Currents were recorded first in control conditions and then prucalopride (10 μM) was washed into the bath for 10 min with the cell kept at the holding potential, before commencing the current recording in the presence of drug (Fig. 1A). Prucalopride reduced both the HERG current during the test potentials and the tail current (Fig. 1A), the latter inhibited by 68.6±4.2% (n=7) after a test pulse to +20 mV (Fig. 1B). The voltage-dependent effects of prucalopride on the K897 HERG tail current are shown in Fig. 1B and C. The voltage required to half-maximally activate HERG was shifted slightly from −16.2±2.4 mV in control to −18.1 ±1.8 mV with exposure to prucalopride (P<0.05, n=7; Fig. 1B). The slope factor was not significantly affected (6.7 ±0.2 mV in control and 6.5±0.3 mV with prucalopride). Prucalopride inhibited the tail current at all potentials, though voltage-dependence was evident with a significantly weaker block at more negative potentials (Fig. 1C).

3.2. Concentration-dependence of block

Prucalopride blocked in a concentration-dependent manner the HERG tail current of both the K897 and T897 variants. The tail currents were recorded at −40 mV after a depolarising step to +40 mV for 2 s from the holding potential of −80 mV, a voltage protocol repeated at 15 s intervals (Fig. 2A). Fig. 2B shows the time course of a typical experiment, with reversibility of prucalopride block evident though not complete. The peak tail current amplitude at each prucalopride concentration was normalised to the control value for K897 (n=3–9) and for T897 (n=4–10) at prucalopride concentrations of 0.3, 1, 3, 10 and 30 μM. There was no significant difference in the relative current amplitude between the HERG variants at any of these prucalopride concentrations. The concentration-response curves gave IC_{50} values of 4.1 and 2.7 μM and Hill coefficients of 1.0 and 1.1 for block of K897 and T897 HERG channels respectively (Fig. 2C). The Hill coefficients indicating a single binding site on the HERG channel for this drug.

3.3. Effects of prucalopride on HERG channel kinetics

The time course of HERG current activation was investigated at +20 mV using an envelope of tail currents protocol (Paavonen et al., 2003). The rate of activation was similar before and after exposure to prucalopride (10 μM) for both K897 and T897 HERG isoforms (data not shown). However 10 μM prucalopride did significantly change the deactivation rate and the time course of recovery from inactivation. HERG tail currents were recorded at a number of test potentials (from

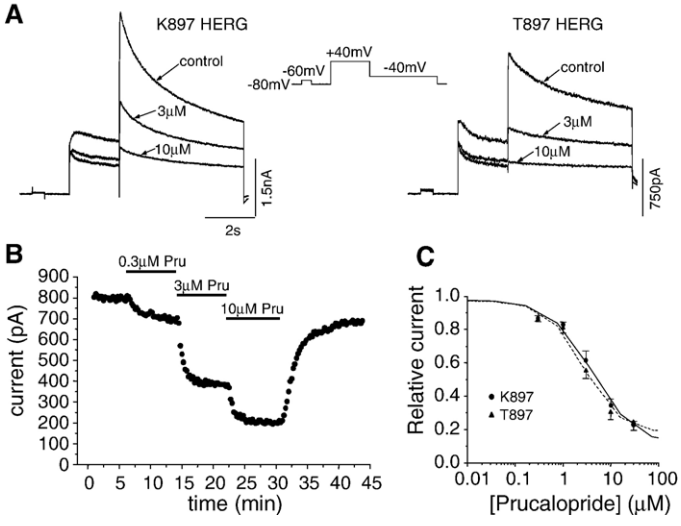

Fig. 2. Concentration-dependent inhibition of K897 HERG and the T897 variant by prucalopride. (A) HERG currents recorded in control conditions and in the presence of 3 and 10 μM prucalopride. From −80 mV HERG currents were evoked by a 2 s step to +40 mV and the tail current recorded with a subsequent step to −40 mV. The protocol was repeated every 15 s. The brief (500 ms) initial step to −60 mV in the protocol enabled an estimate of the leak current to be ascertained which was then subtracted from the peak tail current amplitude. (B) Plot of the leak subtracted peak tail current versus time from a T897 HERG expressing cell exposed to rising concentrations of prucalopride followed by washout. (C) The concentration-response for the effect of prucalopride on K897 (n=3–9) and T897 (n=4–10) HERG peak tail current fitted with the Hill equation. Relative current was calculated from the steady-state tail current amplitude in prucalopride to that from linear extrapolation of the control data over the period of the experiment as a predictor of rundown.

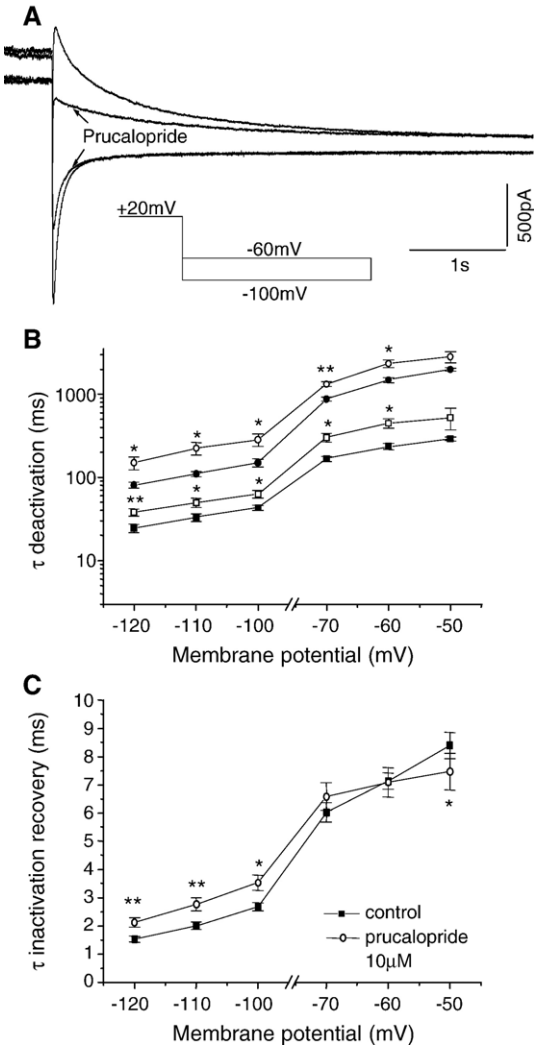

Fig. 3. Prucalopride slows the rate of deactivation and recovery from inactivation of K897 HERG channel. (A) Representative HERG tail currents recorded in control conditions and during incubation with 10 μM prucalopride. The voltage protocol consisted of a 2 s depolarising step to +20 mV, from the holding potential of −80 mV, followed by a 5 s test pulse to voltages between −50 and −120 mV. For clarity only the currents from the test potentials −60 and −100 mV are shown. (B) The voltage-dependence of the fast (■/□) and slow (●/○) deactivation time constants in control conditions (closed symbol) and with 10 μM prucalopride (open symbol) (n=5–6). The time constants were obtained from a double exponential fit of the decaying tail current. (C) The voltage-dependence of the time constant of recovery from inactivation during control and 10 μM prucalopride perfusion (n=5–6). This time constant was determined by fitting a single exponential to the hook (the initial rise in tail current amplitude) that precedes deactivation. Note that data for membrane potentials close to potassium reversal potential are excluded. * P<0.05 and ** P<0.005.

−50 mV to −120 mV) after a depolarising pulse to +20 mV for 2 s from a holding potential of −80 mV (Fig. 3A). The rate of recovery from inactivation was obtained by fitting a single exponential to the initial rise in tail current amplitude, whereas the time constants of deactivation were ascertained by fitting a double exponential to the decay of the tail current. Both the fast and the slow deactivation time constants of the K897 HERG current were significantly slower with prucalopride (Fig. 3B). Furthermore the time constant of recovery from inactivation was also significantly slower, at the more negative membrane potentials, in the presence of prucalopride (Fig. 3C). These properties were similarly altered in T897 HERG channels. At −100 mV both the time constants of the fast component of deactivation and recovery from inactivation were significantly increased from means in control of 39.4±1.2 ms and 2.8±0.1 ms to 53.3±2.5 ms and 3.6±0.2 ms when perfused with prucalopride respectively (P<0.05, n=4).

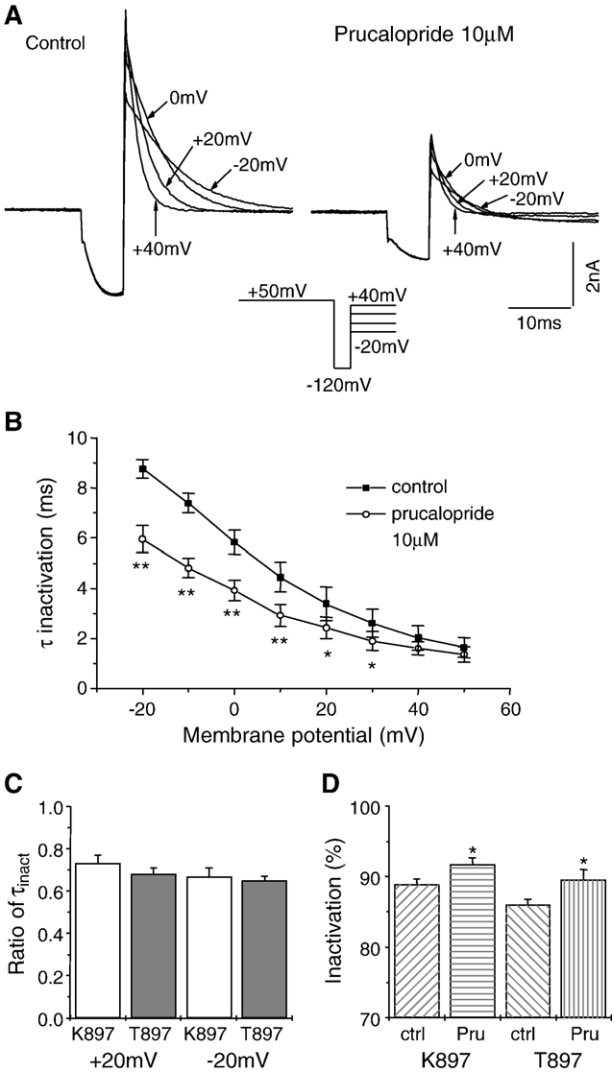

Fig. 4. Effect of prucalopride on the inactivation properties of K897 and T897 HERG channels. (A) Typical inactivating currents from a K897 HERG transfected cell in control and in the presence of 10 μM prucalopride. Currents were elicited with a three step protocol comprising of a 2 s pulse to +50 mV from the holding potential of −80 mV, a 7 ms step to −120 mV and finally another depolarising step to potentials between +50 and −20 mV. For clarity not all the current traces are shown. (B) The voltage-dependence of the time constant of inactivation for control and with 10 μM prucalopride of K897 HERG (n=5). The time constant of inactivation was obtained by fitting a single exponential to the inactivating current of the second depolarising step. (C) Ratio of the time constants of inactivation in 10 μM prucalopride versus control for K897 and T897 HERG channels at +20 and −20 mV (n=5 for both variants of HERG at both membrane potentials). (D) The effect of 10 μM prucalopride on the extent of inactivation of K897 and T897 HERG channels at a membrane potential of −20 mV (n=5 for both). The percentage of inactivation was determined from peak current and steady-state current amplitudes during the third step of the voltage protocol. * P<0.05 and ** P<0.005.

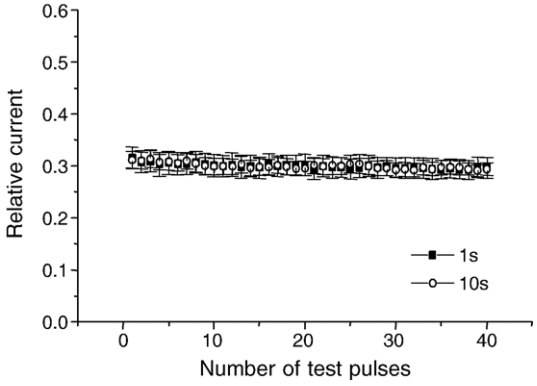

Fig. 5. The lack of frequency-dependence of prucalopride (10 μM) blockade of HERG channels. HERG (K897) channels were activated by a 300 ms pulse to +40 mV from a holding potential of −80 mV and tail currents were produced during a 300 ms step to −40 mV before returning to the holding potential. This protocol was repeated 40 times at intervals of 10 and 1 s. The peak tail current amplitudes were then expressed as relative current and plotted against the pulse number (n=4 for each frequency).

The effect on inactivation of prucalopride was investigated using a three pulse protocol (Zhou et al., 1998). From the holding potential of −80 mV the HERG channels were opened and inactivated by a 2 s step to +50 mV. This was followed by a brief (7 ms) step to −120 mV that relieved inactivation and then a step to various test potentials (from +50 mV to −20 mV) resulting in large, outward inactivating currents. These inactivating currents in control and during exposure to 10 μM prucalopride (Fig. 4A) were fit with a single exponential to determine the time constant of inactivation. Prucalopride significantly accelerated this time constant in K897 HERG, an effect which lessened with increasing depolarisation (Fig. 4B). This pattern was duplicated by prucalopride on T897 HERG channels (data not shown), with there being no significant difference in the degree of quickening of the inactivation time constant between this variant and K897 HERG (P>0.05, n=5; Fig. 4C). Additionally the extent of current inactivation for both the K897 and T897 HERG channels was altered significantly by prucalopride (Fig. 4D). This was calculated from the ratio of the steady-state current during the second depolarisation step to the peak current at the steps initiation.

3.4. The blockade of the HERG (K897) channel by prucalopride

The frequency-dependence of HERG (K897) channel block by prucalopride was evaluated with a protocol repeated 40 times at intervals of either 1 s or 10 s. The protocol comprised of a depolarising step to +40 mV for 300 ms from the holding potential of −80 mV followed by a step to −40 mV for 300 ms to record the tail current. After control data was obtained, 10 μM prucalopride was washed into the bath for 10 min while the cell was held at −80 mV. The train of pulses was then instigated again, with each cell only used at one frequency. The peak tail current amplitude was reduced by 69% with the first pulse with no further accumulation of block, i.e. no use-dependency, for both 10 s and 1 s interval conditions (n=4 for both) (Fig. 5). So neither the development of nor the amount of steady-state block was frequency-dependent, suggesting that prucalopride either blocked the closed and activated (open and/or inactivated) states with similar affinity or block was state-dependent with rapid kinetics.

The state-dependence of HERG (K897) channel block by prucalopride was investigated with two similar protocols (Zitron et al., 2002, 2004). The first was a single pulse from the holding potential (−80 mV) to 0 mV for 7 s, at which HERG channels would exist in the open and inactivated states. While the second examined the role of inactivation with an initial step to +80 mV for 3 s, that would inactivate virtually all the channels, followed by a step to 0 mV for 3.5 s. Fig. 6A and C show representative current recordings evoked by these protocols in control conditions and after 10 min incubation in 10 μM prucalopride at the holding potential, i.e. the channels were kept in the closed state during introduction of prucalopride. With the single pulse to 0 mV prucalopride block developed rapidly, a time constant of ~0.2 s, from zero at 0 s (Fig. 6B) suggesting activated state as opposed to closed state block. Moreover, as evident in Fig. 6D, little further block was obtained at the same membrane potential after visitation to the inactivated state suggesting that maximal block was already attained. Therefore prucalopride exhibits affinity primarily for the open and inactivated HERG channel states but not the closed state.

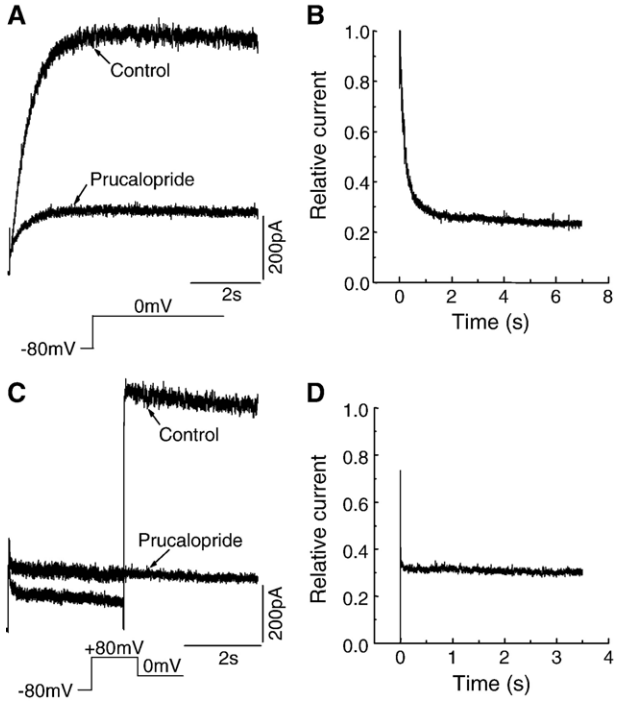

Fig. 6. State-dependent block of HERG channels by prucalopride. (A) HERG (K897) current activated in the absence and presence of 10 μM prucalopride by a 7 s pulse from the holding potential of −80 mV to 0 mV. (B) The relative current (prucalopride current/control current) during the step to 0 mV of the protocol used in A. The data represents the average of 3 cells. (C) HERG (K897) current traces in control and 10 μM prucalopride elicited by a 3.0 s pulse from the holding potential of −80 mV to +80 mV and subsequent 3.5 s step to 0 mV. (D) The relative current (prucalopride current/control current) during the step to 0 mV after the inactivating step to +80 mV. The data represents the average of 3 cells.

As seen in Fig. 6 prucalopride block occurred rapidly so we next examined the kinetics of prucalopride unblock of the HERG (K897) channel. For this cells were held at +60 mV, where HERG channels are predominantly inactivated, then a repolarising step of variable duration (4 to 128 ms) to −100 mV was applied before returning back to +60 mV eliciting a large outward current (Zhang et al., 2001). This current initially increased in amplitude as the duration of the repolarising step lengthened allowing more channels to recover from inactivation and reopen (Fig. 7A). However the peak current amplitude then decreased with a repolarising step beyond 20 ms through channel deactivation. The rate of recovery of the HERG current from prucalopride (10 μM) block at −100 mV was determined as the ratio of the peak current at +60 mV in prucalopride to control at the same recovery interval. The HERG current rapidly recovered at −100 mV from prucalopride block, with the data over this duration period adequately described by a linear fit (Fig. 7B).

4. Discussion

In the present report we describe the effect that the novel prokinetic prucalopride has on the HERG channel expressed in HEK-293 cells. We show that it blocks the HERG channel with an IC_{50} of 4.1 μM, a value in close agreement to that previously determined (Potet et al., 2001). Within the micromolar range prucalopride was recently also reported to act as a partial agonist, via the 5-HT_4 receptor, of the L-type calcium current in human atrial myocytes (Pau et al., 2005). In humans the therapeutic serum concentration is however approximately a thousand fold lower (Emmanuel et al., 1998, 2002), with the free plasma concentration likely to be lower still due to protein binding. Such a large safety margin would indicate little risk of serious ventricular arrhythmias and sudden death from the HERG blocking activity (Redfern et al., 2003; De Bruin et al., 2005).

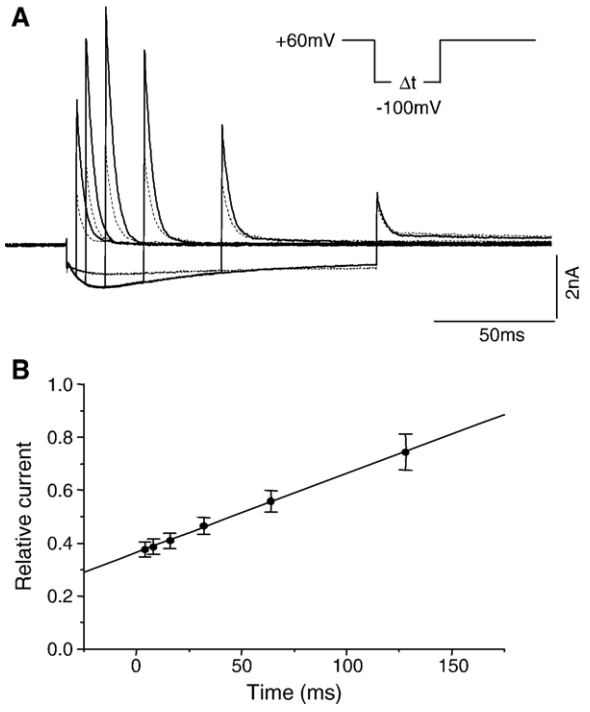

Fig. 7. The unblock of HERG (K897) channels with repolarisation in the presence of prucalopride. (A) Current traces in control conditions (solid lines) and with 10 μM prucalopride (dashed lines) elicited by a protocol in which the cells are held at +60 mV and then undergo a hyperpolarising step to −100 mV of duration 4 to 128 ms before returning back to +60 mV. (B) Time-dependent recovery of the HERG current from block. The peak current amplitudes following the hyperpolarisation step were expressed as relative current and the averaged data fit by linear regression (n=4).

Different drugs exhibit different affinities for the HERG channel states; here we suggest that prucalopride, in common with most drugs, binds preferentially to the open and/or inactivated channel states. Within these states it is probable that from the intracellular aspect drugs have access to the inner cavity of the channel and the residues that for many form the putative binding site (Mitcheson et al., 2000; Sanchez-Chapula et al., 2003; Zitron et al., 2004). A crucial component of this binding site, Y652, is also a key determinant of HERG channel voltage-dependent block (Sanchez-Chapula et al., 2003). Prucalopride exhibited voltage-dependent channel inhibition, with the enhancement of block at positive potentials coinciding with the activation of the channel. Further evidence of prucalopride interaction with the open and inactivated states, as opposed to the closed state, was found with the time-dependent increase in prucalopride block beginning only after the initiation of a depolarising step (Fig. 6B). Moreover given sufficient time at a negative potential recovery from block of the HERG current was readily achieved (Fig. 7B), also indicating a much lower affinity of prucalopride for the closed state. Interaction with the inactivated state was evident in Fig. 6D as little additional block occurred at 0 mV following the step to +80 mV, suggesting that pronounced inhibition of the HERG current had taken place during the step where inactivation predominates. The effects of prucalopride on channel kinetics: acceleration of inactivation, slowing of the recovery from inactivation and alteration of the extent of channel inactivation would also be consistent with affinity for the inactivated state. Stabilisation of the inactivated state of the HERG channel was similarly reported for the antipsychotic haloperidol and the local anaesthetics levobupivacaine, ropivacaine and bupivacaine (Suessbrich et al., 1997; González et al., 2002). However an unequivocal distinction between prucalopride interaction with the HERG channel open and inactivated states cannot be made.

In contrast to the frequency-independence of block by ketoconazole or canrenoic acid which results from binding to the closed state (Dumaine et al., 1998; Caballero et al., 2003), that by prucalopride is a consequence of the rapid kinetics of block development in the open/inactivated state. A similar mechanism has been reported for various drugs including carvedilol, cocaine, bertosamil and trazodone (Karle et al., 2001; Zhang et al., 2001; Zitron et al., 2002, 2004). Moreover the recovery of HERG channels from block by prucalopride displayed rapid kinetics (Fig. 7B). The quickness is unusual, for example recovery from cisapride blockade has a time constant in the order of seconds (Walker et al., 1999), and is comparable to that exhibited by cocaine (Zhang et al., 2001). The time-dependent unbinding of prucalopride from HERG channels may explain, as proposed for other drugs (e.g. Zhang et al., 1999;

Ferreira et al., 2001), the apparent slowing of the deactivation kinetics where channels are prevented from deactivating until reverting to the open unblocked state. Also as unbinding from the HERG channel by prucalopride was rapid the assumption would be for a fast full reversal of block, however that did not occur (Fig. 2B). This discrepancy may arise from the difference in voltage protocols, and it should be noted that the presence of a slower component of recovery from block cannot be excluded since at the longest interval relative current was still ~0.75.

Furthermore we compared the prucalopride effects seen with the wild-type HERG channels to those with the common T897 polymorphism. The K897T change did not alter the binding affinity for prucalopride, as with previous findings on cisapride (Anson et al., 2004) and the antihistamine terfenadine (Scherer et al., 2002). This is in contrast to the sulfamethoxazole sensitivity of the MiRP1, a putative β-subunit of the HERG channel, T8A polymorphism (Sesti et al., 2000). We also show that the K897T variants exhibit similar drug-induced changes in HERG channel kinetics. Subtle, sometimes contradictory, electrophysiological as well as *in vivo* phenotypic differences have been demonstrated arising from the HERG K897T polymorphism (Laitinen et al., 2000; Pietilä et al., 2002; Bezzina et al., 2003; Paavonen et al., 2003; Anson et al., 2004; Crotti et al., 2005; Pfeufer et al., 2005). The most consistent functional difference found is a decrease in the current density (Paavonen et al., 2003; Anson et al., 2004; Crotti et al., 2005), though not sufficient to a cause long QT syndrome alone the diminution of I_{Kr} and the repolarisation reserve may contribute to inherited or acquired torsade de pointes susceptibility. Similarly another HERG polymorphism R1047L, with which there is also no change in drug sensitivity (Anson et al., 2004; Sun et al., 2004), may be associated, through exacerbation of the underlying functional deficit, with a higher incidence of drug-induced torsade de pointes (Sun et al., 2004). An association with drug-induced torsade de pointes has not been found with the K897T polymorphism (Yang et al., 2002) yet, in an analogous setting, this polymorphism was recently shown to promote the clinical expression of a latent type 2 long QT syndrome mutation (Crotti et al., 2005).

In conclusion, at therapeutic concentrations of prucalopride a large safety margin in regard to acute HERG channel inhibition exists. The sensitivity to block is unaltered by the HERG K897T polymorphism as are the prucalopride induced changes in the biophysical properties of the channel.

Acknowledgements

We are grateful for the skilful assistance of Mrs. Mari Palviainen. The study was in part supported by Johnson & Johnson Pharmaceutical Research and Development.

References

Anson, B.D., Ackerman, M.J., Tester, D.J., Will, M.L., Delisle, B.P., Anderson, C.L., January, C.T., 2004. Molecular and functional characterization of common polymorphisms in HERG (KCNH2) potassium channels. Am. J. Physiol. 286, H2434–H2441.

Bezzina, C.R., Verkerk, A.O., Busjahn, A., Jeron, A., Erdmann, J., Koopmann, T.T., Bhuiyan, Z.A., Wilders, R., Mannens, M.M.A.M., Tan, H.L., Luft, F.C., Schunkert, H., Wilde, A.A.M., 2003. A common polymorphism in *KCNH2* (HERG) hastens cardiac repolarization. Cardiovasc. Res. 59, 27–36.

Bouras, E.P., Camilleri, M., Burton, D.D., McKinzie, S., 1999. Selective stimulation of colonic transit by the benzofuran $5HT_4$ agonist, prucalopride, in healthy humans. Gut 44, 682–686.

Bouras, E.P., Camilleri, M., Burton, D.D., Thomforde, G., McKinzie, S., Zinsmeister, A.R., 2001. Prucalopride accelerates gastrointestinal and colonic transit in patients with constipation without a rectal evacuation disorder. Gastroenterology 120, 354–360.

Briejer, M.R., Bosmans, J.P., Van Daele, P., Jurzak, M., Heylen, L., Leysen, J.E., Prins, N.H., Schuurkes, J.A.J., 2001. The in vitro pharmacological profile of prucalopride, a novel enterokinetic compound. Eur. J. Pharmacol. 423, 71–83.

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

Caballero, R., Moreno, I., González, T., Arias, C., Valenzuela, C., Delpón, E., Tamargo, J., 2003. Spironolactone and its main metabolite, canrenoic acid, block human ether-a-go-go-related gene channels. Circulation 107, 889–895.

Claassen, S., Zunkler, B.J., 2005. Comparison of the effects of metoclopramide and domperidone on HERG channels. Pharmacology 74, 31–36.

Crotti, L., Lundquist, A.L., Insolia, R., Pedrazzini, M., Ferrandi, C., De Ferrari, G.M., Vicentini, A., Yang, P., Roden, D.M., George Jr., A.L., Schwartz, P.J., 2005. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. Circulation 112, 1251–1258.

Curran, M.E., Splawski, I., Timothy, K.W., Vincent, G.M., Green, E.D., Keating, M.T., 1995. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 80, 795–803.

De Bruin, M.L., Pettersson, M., Meyboom, R.H.B., Hoes, A.W., Leufkens, H.G.M., 2005. Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. Eur. Heart J. 26, 590–597.

Drolet, B., Rousseau, G., Daleau, P., Cardinal, R., Turgeon, J., 2000. Domperidone should not be considered a no-risk alternative to cisapride in the treatment of gastrointestinal motility disorders. Circulation 102, 1883–1885.

Dumaine, R., Roy, M.-L., Brown, A.M., 1998. Blockade of HERG and Kv1.5 by ketoconazole. J. Pharmacol. Exp. Ther. 286, 727–735.

Emmanuel, A.V., Kamm, M.A., Roy, A.J., Antonelli, K., 1998. Effect of a novel prokinetic drug, R093877, on gastrointestinal transit in healthy volunteers. Gut 42, 511–516.

Emmanuel, A.V., Roy, A.J., Nicholls, T.J., Kamm, M.A., 2002. Prucalopride, a systemic enterokinetic, for the treatment of constipation. Aliment. Pharmacol. Ther. 16, 1347–1356.

Ferreira, S., Crumb Jr., W.J., Carlton, C.G., Clarkson, C.W., 2001. Effects of cocaine and its major metabolites on the HERG-encoded potassium channel. J. Pharmacol. Exp. Ther. 299, 220–226.

Finlayson, K., Witchel, H.J., McCulloch, J., Sharkey, J., 2004. Acquired QT interval prolongation and HERG: implications for drug discovery and development. Eur. J. Pharmacol. 500, 129–142.

Furuta, S., Kamada, E., Omata, T., Sugimoto, T., Kawabata, Y., Yonezawa, K., Wu, X.C., Kurimoto, T., 2004. Drug–drug interactions of Z-338, a novel gastroprokinetic agent, with terfenadine, comparison with cisapride, and involvement of UGT1A9 and 1A8 in the human metabolism of Z-338. Eur. J. Pharmacol. 497, 223–231.

González, T., Arias, C., Caballero, R., Moreno, I., Delpón, E., Tamargo, J., Valenzuela, C., 2002. Effects of levobupivacaine, ropivacaine and bupivacaine on HERG channels: stereoselective bupivacaine block. Br. J. Pharmacol. 137, 1269–1279.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch–clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391, 85–100.

Iwasa, H., Itoh, T., Nagai, R., Nakamura, Y., Tanaka, T., 2000. Twenty single nucleotide polymorphisms (SNPs) and their allelic frequencies in four genes that are responsible for familial long QT syndrome in the Japanese population. J. Hum. Genet. 45, 182–183.

Karle, C.A., Kreye, V.A., Thomas, D., Rockl, K., Kathöfer, S., Zhang, W., Kiehn, J., 2001. Antiarrhythmic drug carvedilol inhibits HERG potassium channels. Cardiovasc. Res. 49, 361–370.

Lacerda, A.E., Kramer, J., Shen, K.-Z., Thomas, D., Brown, A.M., 2001. Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. Eur. Heart J. 3 (suppl K), K23–K30.

Laitinen, P., Fodstad, H., Piippo, K., Swan, H., Toivonen, L., Viitasalo, M., Kaprio, J., Kontula, K., 2000. Survey of the coding region of the HERG gene in long QT syndrome reveals six novel mutations and an amino acid polymorphism with possible phenotypic effects. Hum. Mutat. 15, 580–581.

Mitcheson, J.S., Chen, J., Lin, M., Culberson, C., Sanguinetti, M.C., 2000. A structural basis for drug-induced long QT syndrome. Proc. Natl. Acad. Sci. U. S. A. 97, 12329–12333.

Mohammad, S., Zhou, Z., Gong, Q., January, C.T., 1997. Blockage of the HERG human cardiac K^+ channel by the gastrointestinal prokinetic agent cisapride. Am. J. Physiol. 273, H2534–H2538.

Paavonen, K.J., Chapman, H., Laitinen, P., Fodstad, H., Piippo, K., Swan, H., Toivonen, L., Viitasalo, M., Kontula, K., Pasternack, M., 2003. Functional characterization of the common amino acid 897 polymorphism of the cardiac potassium channel KCNH2 (HERG). Cardiovasc. Res. 59, 603–611.

Pau, D., Workman, A.J., Kane, K.A., Rankin, A.C., 2005. Electrophysiological effects of prucalopride, a novel enterokinetic agent, on isolated atrial myocytes from patients treated with β-adrenoceptor antagonists. J. Pharmacol. Exp. Ther. 313, 146–153.

Pfeufer, A., Jalilzadeh, S., Perz, S., Mueller, J.C., Hinterseer, M., Illig, T., Akyol, M., Huth, C., Schöpfer-Wendels, A., Kuch, B., Steinbeck, G., Holle, R., Näbauer, M., Wichmann, H.-E., Meitinger, T., Kääb, S., 2005. Common variants in myocardial ion channel genes modify the QT interval in the general population: results from the KORA study. Circ. Res. 96, 693–701.

Pietilä, E., Fodstad, H., Niskasaari, E., Laitinen, P.J., Swan, H., Savolainen, M., Kesäniemi, Y.A., Kontula, K., Huikuri, H.V., 2002. Association between HERG K897T polymorphism and QT interval in middle-aged Finnish women. J. Am. Coll. Cardiol. 40, 511–514.

Poen, A.C., Felt-Bersma, R.J.F., Van Dongen, P.A.M., Meuwissen, S.G.M., 1999. Effect of prucalopride, a new enterokinetic agent, on gastrointestinal transit and anorectal function in healthy volunteers. Aliment. Pharmacol. Ther. 13, 1493–1497.

Potet, F., Bouyssou, T., Escande, D., Baró, I., 2001. Gastrointestinal prokinetic drugs have different affinity for the human cardiac human ether-à-go-go K^+ channel. J. Pharmacol. Exp. Ther. 299, 1007–1012.

Rampe, D., Roy, M.-L., Dennis, A., Brown, A.M., 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.

Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., MacKenzie, I., Palethorpe, S., Siegl, P.K.S., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J., Hammond, T.G., 2003. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc. Res. 58, 32–45.

Roden, D.M., 2001. Pharmacogenetics and drug-induced arrhythmias. Cardiovasc. Res. 50, 224–231.

Roden, D.M., 2004. Drug-induced prolongation of the QT interval. New Engl. J. Med. 350, 1013–1022.

Roden, D.M., Lazzara, R., Rosen, M., Schwartz, P.J., Towbin, J., Vincent, G.M., 1996. Multiple mechanisms in the long-QT syndrome. Current knowledge, gaps, and future directions. Circulation 94, 1996–2012.

Sanchez-Chapula, J.A., Ferrer, T., Navarro-Polanco, R.A., Sanguinetti, M.C., 2003. Voltage-dependent profile of human *ether-a-go-go*-related gene channel block is influenced by a single residue in the S6 transmembrane domain. Mol. Pharmacol. 63, 1051–1058.

Sanguinetti, M.C., Jiang, C., Curran, M.E., Keating, M.T., 1995. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 81, 299–307.

Scherer, C.R., Lerche, C., Decher, N., Dennis, A.T., Maier, P., Ficker, E., Busch, A.E., Wollnik, B., Steinmeyer, K., 2002. The antihistamine fexofenadine does not affect I(Kr) currents in a case report of drug-induced cardiac arrhythmia. Br. J. Pharmacol. 137, 892–900.

Sesti, F., Abbott, G.W., Wei, J., Murray, K.T., Saksena, S., Schwartz, P.J., Priori, S.G., Roden, D.M., George Jr., A.L., Goldstein, S.A.N., 2000. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc. Natl. Acad. Sci. U. S. A. 97, 10613–10618.

Suessbrich, H., Schönherr, R., Heinemann, S.H., Attali, B., Lang, F., Busch, A.E., 1997. The inhibitory effect of the antipsychotic drug haloperidol on HERG potassium channels expressed in *Xenopus* oocytes. Br. J. Pharmacol. 120, 968–974.

Sun, Z., Milos, P.M., Thompson, J.F., Lloyd, D.B., Mank-Seymour, A., Richmond, J., Cordes, J.S., Zhou, J., 2004. Role of a KCNH2 polymorphism (R1047L) in dofetilide-induced *Torsades de Pointes*. J. Mol. Cell. Cardiol. 37, 1031–1039.

Viskin, S., 1999. Long QT syndromes and torsade de pointes. Lancet 354, 1625–1633.

Vos, M.A., Paulussen, A.D., 2004. Genetic basis of drug-induced arrhythmias. Ann. Med. 36 (suppl 1), 35–40.

Walker, B.D., Singleton, C.B., Bursill, J.A., Wyse, K.R., Valenzuela, S.M., Qiu, M.R., Breit, S.N., Campbell, T.J., 1999. Inhibition of the human ether-a-go-go-related gene (HERG) potassium channel by cisapride: affinity for open and inactivated states. Br. J. Pharmacol. 128, 444–450.

Yang, P., Kanki, H., Drolet, B., Yang, T., Wei, J., Viswanathan, P.C., Hohnloser, S.H., Shimizu, W., Schwartz, P.J., Stanton, M., Murray, K.T., Norris, K., George Jr., A. L., Roden, D.M., 2002. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. Circulation 105, 1943–1948.

Yap, Y.G., Camm, A.J., 2003. Drug induced QT prolongation and torsades de pointes. Heart 89, 1363–1372.

Zeltser, D., Justo, D., Halkin, A., Prokhorov, V., Heller, K., Viskin, S., 2003. Torsade de pointes due to noncardiac drugs: most patients have easily identifiable risk factors. Medicine 82, 282–290.

Zhang, S., Zhou, Z., Gong, Q., Makielski, J.C., January, C.T., 1999. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. Circ. Res. 84, 989–998.

Zhang, S., Rajamani, S., Chen, Y., Gong, Q., Rong, Y., Zhou, Z., Ruoho, A., January, C.T., 2001. Cocaine blocks HERG, but not KvLQT1+minK, potassium channels. Mol. Pharmacol. 59, 1069–1076.

Zhou, Z., Gong, Q., Ye, B., Fan, Z., Makielski, J.C., Robertson, G.A., January, C.T., 1998. Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Biophys. J. 74, 230–241.

Zitron, E., Karle, C.A., Wendt-Nordahl, G., Kathöfer, S., Zhang, W., Thomas, D., Weretka, S., Kiehn, J., 2002. Bertosamil blocks HERG potassium channels in their open and inactivated states. Br. J. Pharmacol. 137, 221–228.

Zitron, E., Kiesecker, C., Scholz, E., Lück, S., Bloehs, R., Kathöfer, S., Thomas, D., Kiehn, J., Kreye, V.A.W., Katus, H.A., Schoels, W., Karle, C.A., 2004. Inhibition of cardiac HERG potassium channels by the atypical antidepressant trazodone. Naunyn-Schmiedebergs Arch. Pharmacol. 370, 146–156.