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The amiodarone derivative KB130015 activates hERG1 potassium channels via a novel mechanism

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Abstract

Human *ether à go-go* related gene (hERG1) potassium channels underlie the repolarizing I_{Kr} current in the heart. Since they are targets of various drugs with cardiac side effects we tested whether the amiodarone derivative 2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran (KB130015) blocks hERG1 channels like its parent compound. Using patch-clamp and two-electrode voltage-clamp techniques we found that KB130015 blocks native and recombinant hERG1 channels at high voltages, but it activates them at low voltages. The activating effect has an apparent EC_{50} value of 12 μ M and is brought about by an about 4-fold acceleration of activation kinetics and a shift in voltage-dependent activation by -16 mV. Channel activation was not use-dependent and was independent of inactivation gating. KB130015 presumably binds to the hERG1 pore from the cytosolic side and functionally competes with hERG1 block by amiodarone, E4031 (N-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl] carbonyl] phenyl] methanesulfonamide dihydrochloride), and sertindole. Vice versa, amiodarone attenuates hERG1 activation by KB130015. Based on synergic channel activation by mallotoxin and KB130015 we conclude that the hERG1 pore contains at least two sites for activators that are functionally coupled among each other and to the cavity-blocker site. KB130015 and amiodarone may serve as lead structures for the identification of hERG1 pore-interacting drugs favoring channel activation vs. block.

Keywords

K^+ channel opener; Human *ether à go-go* related gene; KB130015; Amiodarone; Patch clamp

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1. Introduction

The human *ether à go-go* related gene 1 (hERG1, K_v11.1, KCNH2) encodes the pore-forming subunit of the K⁺ channel that carries the rapid component of the delayed rectifier current (I_{Kr}) in the heart (Sanguinetti et al., 1995; Trudeau et al., 1995). Since the function of I_{Kr} is to terminate the cardiac action potential, an impaired expression or function of hERG1 channels clinically manifests as type-2 long-QT syndrome (LQTS-2) (Sanguinetti and Tristani-Firouzi, 2006). Acquired forms of LQTS-2 typically arise from undesired pharmacological block of I_{Kr} (Fermini and Fossa 2003; Thomas et al., 2006). Supported by high-resolution data on the structural architecture of K⁺ channels (Doyle et al., 1998; Long et al., 2005; Zhou et al., 2001) it became apparent that size and shape of the central cavity, which constitutes the binding site for most blockers (Thomas et al., 2006), are the major determinants for hERG1 inhibition. Aromatic residues in the pore-lining S6 helices (Y652 and F656), their spatial orientation and dynamics during channel gating are of particular importance for the binding of most compounds (Chen et al., 2002; Mitcheson et al., 2000). Based on this knowledge, rational drug design needs to minimize intense contact with this proposed drug-binding pocket (Fermini and Fossa, 2003) in order to avoid undesired hERG1-specific side effects.

Much less is known about the mode of action of such drugs that *activate* hERG1 channels and, hence, might be useful for the treatment of certain forms of LQTS-2 (Seeböhm, 2005). Several substances were found recently to enhance hERG1 activity (Casis et al., 2006; Hansen et al., 2006; Kang et al., 2005; Zeng et al., 2006; Zhou et al., 2005) by slowing down hERG1 channel deactivation or by reducing the channel's voltage-dependent inactivation.

Amiodarone is an effective antiarrhythmic drug with multiple effects, including class III antiarrhythmic (i.e., K⁺ channel blocking) action (Kamiya et al., 2001; Kodama et al., 1999). Its derivative 2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran (KB130015, Carlsson et al., 2002, Suppl. Fig. 1) was initially developed to obtain similar antiarrhythmic action but without extra-cardiac side effects. This compound already has been shown to exhibit class III antiarrhythmic activity (Carlsson et al., 2002) and to affect cardiac ion channels (Mubagwa et al., 2003). Here we show that KB130015 opens hERG1 channels via a novel mechanism involving accelerated activation without slowing of deactivation or impairing inactivation. Because of a strong functional link of the hERG1 pore structure and the process of activation, and the herein demonstrated mutual functional competition of KB130015 with pore blockers, it becomes apparent that blockers and activators share similar molecular mechanisms despite their contrary functional consequences. In addition, we observed synergic hERG1 activation by KB130015 and mallotoxin. We conclude that hERG1 channels contain at least two binding sites for channel openers, functionally coupled among each other and to the blocker-binding site within the inner cavity.

This information will be valuable for hERG1-related drug design and may help to shed light on the gating mechanisms of potassium channels.

2. Materials and Methods

2.1. Chemicals and solutions

E4031 (N-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl] carbonyl] phenyl] methanesulfonamide dihydrochloride) was obtained from Waco Chemicals (Neuss, Germany). Sertindole was kindly provided by Knoll AG (Ludwigshafen, Germany). All other chemicals were of high grade from Sigma (Taufkirchen, Germany). KB130015 (kindly provided by Karo Bio AB, Huddinge, Sweden) was dissolved to 50 mM in dimethylsulfoxide (DMSO) and stored at -20°C. All hERG1 blockers were stored as 10 mM DMSO stock solutions at -20°C. Compounds were diluted to the final concentration immediately before the experiment.

2.2. Molecular biology

Expression vectors for hERG1 (K_v11.1, KCNH2, Sanguinetti et al., 1995, NM_000238) and hEAG1 (human *ether à go-go* channel 1, K_v10.1, KCNH1, Schönherr et al., 2000, NM_002238) were used. Single-point mutants were generated via overlap extension PCR. To create the hEAG-p(ERG) chimera, a PCR fragment encoding the pore region of hERG1 (residues 540–694) was fused to a DNA fragment covering the S1–S4 region of hEAG1 by overlap extension PCR and the end product was subcloned into hEAG1-pGEM-HE. All PCR-derived fragments were confirmed by DNA sequencing. Wild-type and mutated channel clones were transferred into pGEM-HE and pcDNA3 for expression in *Xenopus* oocytes or HEK 293 cells, respectively, as described previously (Schönherr and Heinemann, 1996; Schönherr et al., 2000). The investigations conformed to local laws, based on European guidelines relating to the care and use of laboratory animals.

2.3. Electrophysiological recordings

Patch-clamp recordings were performed at room temperature (20–23°C) using an EPC 9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Patch pipettes were formed from borosilicate (HEK 293 cells) (Kimble Glass, Vineland, NJ) or aluminum silicate (*Xenopus* oocytes) (Hilgenberg, Malsfeld, Germany) glass with tip resistances of ~1 MΩ. Pipette tips were fire-polished before use. For whole-cell recordings from HEK 293, the internal solution contained (in mM) 140 KCl, 10 EGTA, 10 HEPES, pH 7.4. The external solution consisted of (in mM) 4 KCl, 136 NaCl, 2 CaCl₂, 2 MgCl₂, 10 HEPES, pH 7.4. To adjust KCl concentrations, KCl was replaced for NaCl. A series resistance compensation of up to 80% was applied. For excised-patch recordings from *Xenopus* oocytes, the external solution was composed of (in mM) 140 Na-aspartate, 5 KCl, 1 MgCl₂, 1.8 CaCl₂, 10 HEPES, pH 7.4. The internal solution contained (in mM) 140 K-aspartate, 1 MgCl₂, 1.8 EGTA, and 10 HEPES, pH 7.4.

Two-electrode voltage-clamp recordings from *Xenopus* oocytes were performed using a TurboTEC 10 CD amplifier (NPI electronics, Tamm, Germany). Electrodes, filled with 2 M KCl, had resistances of 0.4–0.9 MΩ. The bath solution was composed of (in mM) 115 NaCl, 2.5 KCl, 1.8 CaCl₂, 10 HEPES, pH 7.2. All experiments were carried out under constant perfusion (2–3 ml / min).

Stimulation and data acquisition were controlled by Pulse or PatchMaster software (HEKA Elektronik). A P/n method was used to compensate for leakage and capacitive currents. Data were analyzed with FitMaster (HEKA Elektronik) and IgorPro (WaveMetrics Inc., Lake Oswego, OR) software.

To quantify the voltage dependence of hERG1 activation, peak tail-current amplitudes (I_{tail}) at a constant voltage were measured following 1-s prepulses to various voltages. Data were plotted against the voltage and fitted with a Boltzmann function:

$$I_{tail} = I_{tail,max} / (1 + \exp((V_a - V) / k_a)) \quad (1)$$

with the maximal tail-current amplitude $I_{tail,max}$, the voltage for half-maximal activation V_a , and k_a characterizing the steepness of the I_{tail} / V relationship.

To assay compound effects, repetitive depolarizations were applied until the current response reached a steady state. Substances were applied in increasing concentrations via complete exchange of the bath solution (exchange times <20 s for whole-cell patch-clamp recordings and <5 s for two-electrode voltage-clamp recordings) or via placing the tip of the patch pipette into the laminar flow of a 250-μm perfusion polyamide tubing (AutoMate Scientific, San

Francisco, CA). To estimate channel inhibition, steady-state current amplitudes were normalized to the control value before blocker application, plotted against the blocker concentration, and fitted with a Hill function:

$$I/I_{control} = \frac{1}{1 + \left(\frac{[compound]}{IC_{50}} \right)^{n_H}} \quad (2)$$

where IC_{50} is the compound concentration resulting in 50% inhibition and n_H the Hill coefficient. To quantify channel activation, a modified Hill function was used:

$$I/I_{control} = (1 - Act_{max}) + \frac{Act_{max}}{1 + \left(\frac{[compound]}{EC_{50}} \right)^{n_H}} \quad (3)$$

where EC_{50} is the compound concentration resulting in 50% of the maximal activation (Act_{max}).

Fits were performed for individual membrane patches/cells and the resulting fit parameters were averaged. All data are presented as mean $\pm$ S.E.M. (n) where n is the number of independent experiments. Statistical differences between groups of data were tested using two-sided Student's t-tests with unequal variances.

3. Results

3.1. KB130015 activates and blocks hERG1 channels independently of inactivation gating

Amiodarone is a potent blocker of hERG1 channels (Kiehn et al., 1999; Ridley et al., 2004). In order to test whether the amiodarone derivative KB130015 (2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran, Carlsson et al., 2002) blocks hERG1 channels similarly, we expressed hERG1 in HEK 293 cells and measured whole-cell currents using the patch-clamp technique. Increasing KB130015 concentrations enhanced hERG1 outward current amplitudes at -20 mV (Fig. 1). Under repetitive pulsing, hERG1 enhancement by $10 \mu\text{M}$ KB130015 reached a plateau at $264 \pm 2\%$ ($n=3$) of the control current. Subsequent application of $30 \mu\text{M}$ KB130015 led to an initial fast increase followed by a slow decrease in current amplitude, suggesting an overlap of activating and blocking activity of KB130015. Owing to this overlap the estimated EC_{50} value of $12.2 \mu\text{M}$ (Fig. 1C) has to be considered a lower limit for the activating potency of KB130015.

To test whether hERG1 inactivation gating is required for the KB130015 effects, we repeated this experiment with the non-inactivating mutant hERG1-S620T (Ficker et al., 1998). Mean current enhancement by $10 \mu\text{M}$ KB130015 was to $299 \pm 41\%$ ($n=3$) of control and subsequent application of $30 \mu\text{M}$ KB130015 still revealed the dual effect of initial activation and subsequent block (Suppl. Fig. 2). We thus conclude that intact inactivation gating is a requirement for neither the activation nor the block of hERG1 channels by KB130015.

3.2. KB130015 affects hERG1 voltage dependence

In the following experiments we focused on the molecular mechanism of wild-type hERG1 modulation by KB130015. A concentration of $10 \mu\text{M}$ was used to maximize the activating while minimizing the inhibiting effect on hERG1. Voltage dependence of hERG1 gating was analyzed in response to 1-s depolarizing steps in membrane potential in 10 mM external $[\text{K}^+]$

(Fig. 2). Due to fast inactivation of hERG1 channels, strong depolarizations elicited rather small outward currents, whereas subsequent repolarization to -120 mV led to large slowly deactivating inward tail currents (Fig. 2A). KB130015 blocked currents at positive potentials and enhanced currents at more negative potentials (Fig. 2B, C), thus shifting the current-voltage relationship to the left. The strength of activation at -20 mV (Fig. 2D) is in good agreement with the data obtained in 4 mM external $[K^+]$ (Fig. 1). To quantify both effects, the tail current vs. voltage data were fitted with Boltzmann functions. The voltage for half-maximal activation (V_a) was shifted ($n=14$, $P<0.005$) from -3.3 ± 1.4 mV (control) to -19.5 ± 1.5 mV (KB130015), the maximal tail-current amplitude was reduced ($P<0.005$) from -7.09 ± 0.99 nA (control) to -3.91 ± 0.38 nA, and the steepness of the voltage dependence was unaffected ($P=0.28$) with k_a values of 8.7 ± 0.2 mV and 9.2 ± 0.4 mV, respectively.

3.3. KB130015 accelerates hERG1 activation gating

Since overlapping inactivation masks the hERG1 activation time course we applied an envelope-of-tails protocol (Fig. 3A) to quantify hERG1 activation kinetics. As shown in Fig. 3A and B, hERG1 activation is significantly accelerated by KB130015 in a voltage-dependent manner (e.g., 3.8-fold at -20 mV compared to 2.4-fold at $+40$ mV). hERG1 deactivation was analyzed by fitting a biexponential function to the current decay at various voltages for hERG1 channels that were recovered from inactivation by a 10-ms step to -150 mV (Fig. 3C). KB130015 slightly (<3 -fold) reduced at least the dominating deactivation time constants and in part increased the relative contribution of the fast exponential component (Fig. 3D).

Compatible with our results on non-inactivating hERG1-S620T channels, KB130015 did not affect hERG1 inactivation gating (Suppl. Fig. 3). We conclude that KB130015 activates hERG1 channels via a novel mechanism that is not directed to removal of inactivation or to slow-down of deactivation but rather involves an acceleration of hERG1 activation.

Similar effects of KB130015 were observed for hERG channels endogenously expressed in SH-SY5Y neuroblastoma cells (Suppl. Fig. 4); $10 \mu\text{M}$ KB130015 shifted V_a by -13.0 ± 2.5 mV ($n=5$), accelerated hERG activation at 0 mV 2.7-fold, and inhibited hERG at $+50$ mV by 23%.

3.4. The hERG1 pore residue Y652 influences the effect of KB130015

For identification of molecular determinants for the interaction between KB130015 and the hERG1 channel we expressed hERG1 and mutants thereof in *Xenopus* oocytes and measured currents with the two-electrode voltage-clamp technique, because it allows stable long-term recordings. As shown in Fig. 4A, $50 \mu\text{M}$ KB130015 robustly enhanced hERG1 currents at negative voltages. The underlying shift in the voltage dependence was -9.1 ± 0.7 mV ($n=5$ batches, with 4–8 oocytes each), compared to -0.9 ± 0.2 mV for control application of 0.1% DMSO, which was used as vehicle. In agreement with data obtained from HEK 293 cells, partial (S631A, data not shown) or complete (S620T, Fig. 4) attenuation of inactivation by channel mutagenesis did not abolish the activating potency of KB130015 but slightly reduced its hERG1 inhibiting effect.

The closely related non-inactivating hEAG1 channel was almost unaffected by KB130015 (Fig. 4). In contrast, current through the chimera hEAG-p(ERG), consisting of the hERG1 pore in the background of hEAG1, was enhanced at -45 mV to $213 \pm 27\%$ and strongly blocked to $36 \pm 2\%$ ($n=10$) at $+30$ mV (Fig. 4B). The chimera hEAG-p(ERG) undergoes rapid inactivation like hERG1 wild type. Since inactivation has been ruled out as a requirement for KB130015 action these data suggest that the pore structure of hERG1 channels is essential for the KB130015 effects. We further tested this hypothesis by assaying mutant hERG1-Y652A, which was previously shown to disturb the binding of drugs in the channel's cavity (Mitcheson

et al., 2000; Thomas et al., 2006). As shown in Fig. 4, the pore mutation Y652A abolished hERG1 activation by 50 μ M KB130015 at negative voltages, but the channels were blocked in an almost voltage-independent manner to $63 \pm 4\%$ ($n=9$). These data collectively suggest that the hERG1 pore contains an interaction site for KB130015. The lack of activation of hERG1-Y652A channels by KB130015 may be interpreted in a way that Y652 is part of a binding site important for channel opening by KB130015. It has to be noted, though, that mutations heavily affecting channel gating may similarly modulate KB130015 effects on hERG1. For example, truncation of the N-terminus ($\Delta 2-373$) strongly accelerates channel activation and deactivation (Schönherr and Heinemann, 1996) and with respect to KB130015 this mutant has a phenotype similar to hERG1-Y652A exhibiting block to $61 \pm 6\%$ ($n=4$) at +30 mV without activation at negative voltages (Fig. 4B). Notably, KB130015 still can bind to $\Delta 2-373$ channels, as suggested by a right-shift in V_a by 16.2 ± 0.8 mV.

3.5. KB130015 functionally competes with inner cavity blockers of hERG1

Assuming the pore forms the binding site for KB130015, one might expect competition between KB130015 and known hERG1 cavity blockers. We therefore performed competition assays, monitoring the onset of block for various hERG1 inhibitors. Measurements were performed under control conditions and in the presence of KB130015. To assure maximal blocking efficiency, hERG1 channels were held in the open/inactivated state at 0 mV (for 5 s) and maximal outward tail currents were measured upon steps to -50 mV in 6-s intervals. Using that long depolarization, hERG1 channels are assumed to reach an equilibrium open probability of >90%. The relative left-shift in V_a by 50 μ M KB130015 from -27.7 ± 1.5 mV to -42.0 ± 1.2 mV is comparable to that observed for 1-s depolarization. After establishment of a steady state, blocker was applied and hERG1 currents were monitored for 5–20 min. As shown in Fig. 5B, 1 μ M amiodarone blocked hERG1 channels of oocytes pretreated with the vehicle (0.1% DMSO) to $55.6 \pm 5.5\%$ ($n=6$) of the control current. Preceding application of 5 μ M KB130015, a concentration too low to significantly affect channel gating, did not change the strength of block (remaining current: $56.1 \pm 2.0\%$, $n=6$, $P=0.93$). However, pretreatment with 50 μ M KB130015 significantly ($P<0.005$) attenuated hERG1 inhibition by amiodarone with a remaining current of $87.1 \pm 0.6\%$ ($n=6$). Assuming a Hill coefficient of unity, effective IC_{50} values were estimated and attenuation of block was quantified as ratio of IC_{50} values in the presence and absence of 50 μ M KB130015: $rel. IC_{50} = IC_{50(KB130015)} / IC_{50(DMSO)}$. KB130015 (50 μ M) attenuated channel block by amiodarone almost 4-fold ($rel. IC_{50} = 3.9 \pm 0.1$). Slightly weaker, yet still significant attenuation of block by KB130015 was observed for E4031 ($rel. IC_{50} = 2.7 \pm 0.3$, $n=4$) and sertindole ($rel. IC_{50} = 2.4 \pm 0.2$, $n=5$). Thus the activator site for KB130015 and the inhibitor site are either overlapping or functionally coupled. This is further supported by our observation that KB130015 and cavity blockers compete in both directions, i.e. preincubation with amiodarone attenuated hERG1 activation by KB130015 (Fig. 5C, D). It is worth mentioning that KB130015-mediated attenuation of block by sertindole was also observed for $\Delta 2-373$ channels with a $rel. IC_{50}$ of 2.0 ± 0.3 ($n=5$). Hence, attenuation of block does not simply result from the KB130015 effect on the channel's voltage dependence, shifting V_a of wild-type and $\Delta 2-373$ channels in opposite directions.

3.6. The activating effect of KB130015 differs from facilitation

Activation of hERG1 channels by hERG1 antagonists has already been described for azimilide (Jiang et al., 1999) and nifekalant (Hosaka et al., 2007). KB130015, however, obviously differs in its effects from both drugs in several aspects.

First, azimilide was proposed to act from the extracellular side of the membrane, whereas our data show that KB130015 exerts its effect from the inside. The sidedness of the KB130015 effect was assayed with mutant hERG1-S620T expressed in *Xenopus* oocytes. The wild-type channel could not be used here because of almost complete run-down upon patch excision.

While bath application of 10 μM KB130015 accelerated channel activation and left-shifted the voltage dependence in the inside-out patch-clamp configuration, it was without effect in the outside-out configuration (Fig. 6), arguing for a binding site of KB130015 accessible from the cytosolic side.

Second, the inhibiting and activating potency of nifekalant, but not of KB130015, were directly coupled: disruption of the proposed binding site within the inner cavity, e.g. by the mutation Y652A, abolished both the facilitating and the blocking effect of nifekalant. In contrast, mutation Y652A only abolished the activating effect of KB130015 on hERG1 channels, while block of the channels by KB130015 was enhanced. It should be mentioned that mutation Y652A renders hERG1 channels insensitive to 10 μM amiodarone and strongly attenuates block by sertindole (not shown), suggesting Y652 contributes to the binding site for both, amiodarone and sertindole, but not for KB130015.

Third, the facilitating effect of azimilide and nifekalant required a preceding strong depolarization and diminished upon repetitive stimulation. In contrast, activation by KB130015 did not require preceding channel activation, persisted upon repetitive stimulation (Fig. 5D), and in fact occurred with the first test depolarization (data not shown) even if hERG1 channels were locked in the closed state by holding the cells at -90 mV during 5 min-preincubation in 50 μM KB130015. We conclude that activation of hERG1 channels by KB130015 does not resemble prepulse-dependent, transient facilitation as observed for particular pore blockers.

3.7. Mallotoxin modulates KB130015 effects on hERG1 channels

Similar to KB130015, mallotoxin (MTx) activates the hERG1 channel by shifting its voltage dependence to the left and by accelerating activation (Zeng et al., 2006). In contrast to KB130015, mallotoxin slows down hERG1 deactivation, suggesting a different mode of action. To test for possible synergic effects, we analyzed hERG1 channels upon co-application of both activators (Fig. 7). Pre-activation of hERG1 channels by 4 μM MTx was accompanied by a left-shift of V_a by 9.0 ± 1.3 mV ($n=7$) and a 1.5 ± 0.1 –fold acceleration of activation at 0 mV. Concomitant application of 10 μM KB130015 left-shifted V_a by additional 14.2 ± 0.1 mV and further accelerated hERG1 activation kinetics 1.8 ± 0.1 –fold. The total shift in V_a (ΔV_a) by -23.3 ± 1.6 mV and the overall 2.7 ± 0.1 –fold acceleration of activation closely resembled the effect of the sole application of 15 μM MTx on hERG1 channels ($\Delta V_a = -19.4 \pm 3.4$ mV, $n=7$; 2.4 ± 0.3 –fold acceleration, $n=5$). Unexpectedly, the KB130015 effect on deactivation kinetics of MTx-pretreated hERG1 channels was opposite to that on untreated channels. Fast (τ_{fast}) and slow (τ_{slow}) deactivation time constants at -120 mV increased from 17 ± 1 and 74 ± 5 ms (control, $n=10$) over 30 ± 2 and 126 ± 19 ms (4 μM MTx) to 50 ± 7 and 280 ± 63 ms (4 μM MTx + 10 μM KB130015), respectively. The relative contribution of the fast exponential component (A_{fast}) decreased from $73.2 \pm 2.7\%$ over $68.9 \pm 2.6\%$ to $61.5 \pm 2.6\%$. For comparison, deactivation parameters in 15 μM MTx were: $\tau_{fast} = 57 \pm 5$ ms, $\tau_{slow} = 235 \pm 15$ ms, and $A_{fast} = 47.3 \pm 5.0\%$. In addition, hERG1 current inhibition by KB130015 at high voltages was almost completely abolished. Again, the effect of 4 μM MTx and 10 μM KB130015 closely resembled the effect of application of 15 μM MTx; i.e., application of KB130015 to hERG1 channels preactivated by MTX elicits an effect phenotypical of MTX rather than of KB130015. We conclude that MTx and KB130015 synergically activate hERG1 channels, requiring the existence of at least two functionally coupled activator sites.

4. Discussion

4.1. KB130015 is a novel type of hERG1 channel activator

While reduction of undesired block of hERG1 channels to prevent acquired LQTS has become an important incentive in drug development, direct application of hERG1 activators may be a feasible strategy, too. Potential candidates of such compounds have been recently discovered (Casis et al., 2006; Hansen et al., 2006; Kang et al., 2005; Zeng et al., 2006; Zhou et al., 2005). As far as known, these activators act either via a deceleration of deactivation (e.g., mallotoxin) or a reduction of effective inactivation (e.g., NS1643). Here we present data on a novel type of hERG1 activator, the amiodarone derivative KB130015. This compound accelerates hERG1 activation and deactivation and shifts the voltage dependence of hERG1 activation to the left, resulting in an up to 6-fold current increase at low voltages. The effective concentration in the micromolar range of hERG1 activation by KB130015 is comparable to that of RPR260243, NS1643, and PD-118057, whereas mallotoxin, the only known natural hERG1 activator, is active even in the sub-micromolar range.

Designating KB130015 a novel type of hERG1 opener, it has to be noted that a similar mode of action has been reported for fenamates (Malykhina et al., 2002). Here, however, effects on hERG1 current amplitude (< 2 -fold increase) and voltage dependence ($\Delta V_a \approx -5$ mV) were substantially smaller than we observed for KB130015 and required more than 10-fold higher concentrations. Fenamates, clinically used as anti-inflammatory drugs, are rather unspecific. They have been shown to activate large-conductance calcium-activated potassium (BK) channels (Ottolia and Toro, 1994) and the heteromultimeric KCNQ1/KCNE1 (I_{Ks}) channels (Abitbol et al., 1999). KB130015 has to be considered unspecific as well, since it also activates BK channels (Gessner et al., 2007) and it has numerous effects on various other ion channel types (Mubagwa et al., 2003).

4.2. KB130015 putative binding sites and mode of action

Two lines of evidence show that KB130015 acts from the intracellular side: its lack of effectiveness in the outside-out configuration and its reduced effectiveness in *Xenopus* oocytes, where drug binding to the yolk protein may reduce the effective intracellular drug concentration. Hence, the external pore entrance, proposed to bind the hERG1 activators NS1643 and PD307243 (Xu et al., 2008), can be excluded as binding site. Because we measured functional effects instead of direct binding, loss of KB130015-mediated activation by channel mutagenesis does not allow a clear conclusion whether for instance the hERG1 N-terminus or residue Y652 contribute to KB130015 binding. Similarly, the competition experiments not unequivocally prove physical competition for the central cavity binding site. Nevertheless, the herein demonstrated *functional* competition is of interest in particular for clinical settings, where such drug-drug interactions may manifest in clinically relevant changes of the QT interval.

We may speculate that the cytoplasmic ends of S5 and S6 helices form the binding site, as proposed for RPR260243 (Perry et al., 2007). This site should be accessible from the intracellular side in the closed channels state. Competition with pore blockers – binding from inside the pore to S6 – might result from activator binding to S6 from outside the pore. The presence of this site in quadruplicate agrees well with the observed dual effect of KB130015 (Fig. 1) and the synergic effects of mallotoxin and KB130015 (Fig. 7), both requiring at least two binding sites. While we cannot exclude that KB130015 binding in the central cavity partly underlies the inhibitory effects of KB130015 and its competition with pore blockers, the lack of use-dependence for hERG1 activation by KB130015 requires an “activator” site outside the inner cavity. Accordingly, mutation Y652A, which severely attenuates hERG1 block by amiodarone and sertindole, likely diminishes hERG1 activation by KB130015 in an allosteric

manner. We assume that KB130015-mediated hERG1 activation depends on conformational transitions that require a tyrosine at position 652. A similar mechanism has been proposed for the process of hERG1 activation by RPR260243 (Perry et al., 2007), where residue Y652 may be critical for coupling between RPR260243 binding to a cytoplasmic site and altered P-type inactivation at the external vestibule of the channel (Sanguinetti and Tristani-Firouzi 2006). In fact, “long-range” modulation of drug action seems to be a general phenomenon in K⁺ channel/drug interactions, as exemplified by mutual interactions of drugs from opposite sides of the membrane: (1) internal dofetilide and external PD307243 on hERG1 channels (Xu et al., 2008) and (2) internal dehydrosoyasaponin-1 and external charybdotoxin on BK channels (McManus et al., 1993).

Assuming channel activation by KB130015 is mediated *allosterically* – may this apply to channel inhibition as well? Reduction of hERG1 currents under different conditions does not necessarily result from the same molecular mechanism. On the one hand, the slow and possibly use-dependent block of hERG1 channels as depicted in Fig.1 as well as the functional competition with cavity blockers (Fig. 5) may result from KB130015 binding within the inner cavity. On the other hand, KB130015-mediated reduction of hERG1 inward tail currents was stronger than the corresponding reduction of outward currents before repolarization (e.g. $40 \pm 3\%$ vs. $23 \pm 5\%$ block at +40 mV, $P=0.007$ see Fig. 2) and, thus, partly results from the accelerated deactivation gating. This effect can be directly observed during the recovery phase of the triple-pulse protocol as shown in Sup. Fig. 3. A similar mechanism may also underlie the “block” of $\Delta 2$ -373, Y652A and hEAG-p(ERG) channels (Fig. 4). It is conceivable that KB130015 binding to hERG1 induces either activation or inhibition, depending on the “gating status” of the channel, which may be affected by mutation (Y652A / $\Delta 2$ -373) or drug (maltotoxin) binding. This would imply that the terms “activator” or “inhibitor” sites are not strictly correct and may be replaced by, e.g., “modulator” sites.

In this report we show for the first time that two different activator molecules can synergically activate hERG1 channels. Moreover, our data clearly show that structurally related compounds can act as hERG1 inhibitors (amiodarone) or activators (KB130015). Future experiments have to clarify which chemical substituents differing between amiodarone and KB130015 (Suppl. Fig. 1) are responsible for this fundamental change in effect on hERG1 channels. It will be informative to know whether such a “transformation” of *blockers* into *activators* can be achieved for other drugs as well, and – consequently – whether this may become a novel strategy in drug design. In any case, the hERG1 pore has to be considered more than a mere binding site for cavity blockers, it rather integrates binding of multiple ligands to promote either channel block or activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

KB130015 activates and blocks hERG1 channels expressed in HEK 293 cells. A: Representative whole-cell currents elicited by repetitive (6-s interval) depolarizations to -20 mV before and after application of KB130015 at the indicated increasing concentrations. B: Maximal outward current amplitudes at the end of the depolarizations were normalized to the control level, plotted against the time with KB130015 concentrations indicated. C: Concentration dependence of the relative maximal current increases induced by KB130015 ($n=3$). The continuous curve is the result of a Hill fit described by a maximal current increase of 470%, an EC_{50} value of 12.2 μM , and a Hill coefficient of 1.1. Extracellular solutions contained 4 mM K^+ .

Fig. 2. KB130015 shifts the voltage dependence of hERG1 channel activation to the left. A: Representative whole-cell currents of hERG1-expressing HEK 293 cells before (left) and after (right) application of 10 μ M KB130015 elicited by the indicated voltage protocol. B, C: Current amplitudes at the end of the depolarizations (B) and maximal inward tail-current amplitudes (C) in the absence (open symbols) and presence (filled symbols) of KB130015, as a function of voltage. Solid lines in (C) are Boltzmann fits to the mean tail-current/voltage data, fit parameters were -2.7 mV (V_a) and 9.3 mV (k_a) for control compared to -19.6 mV (V_a) and 9.5 mV (k_a) in the presence of KB130015. D: The relative current normalized to control data before KB130015 application highlights activation of hERG1 currents in the negative voltage

range for both, outward (circles) and tail (squares) currents. $n=14$ (B–D). Extracellular solutions contained 10 mM K^+ .

Fig. 3.

KB130015 accelerates hERG1 activation and deactivation kinetics. hERG1 activation time course was quantified by an envelope-of-tails analysis as shown in (A). Peak inward tail-current amplitudes (symbols) were fitted with monoexponential functions (superimposed). B: Activation time constants as a function of voltage for control (open) and 10 μ M KB130015 conditions (filled symbols). C: Typical current responses to the indicated triple-pulse protocol at -120 mV in the absence or presence of KB130015 (10 μ M). Channel deactivation was analyzed by biexponential fits (superimposed) to the current decay. D: Fast (circles) and slow (squares) deactivation time constants, and the relative contributions of the fast component (A_{fast}) as a function of voltage. (B, D): $n=5-10$; asterisks indicate statistically significant differences ($P < 0.05$). Extracellular solutions contained 10 mM K^+ .

Fig. 4.

KB130015 activation of hERG1 channels depends on hERG1 gating. A: Representative current recordings from whole *Xenopus* oocytes expressing the indicated channel constructs in the absence (thin black traces) and presence of 50 μM KB130015 (thick gray traces). S620T, Y652A and Δ2-373 are mutant hERG1 channels, hEAG-p(ERG) is a chimeric channel with a hERG1 pore in the background of the hEAG1 channel, a close relative of hERG1. Scale bars: 2 μA, 400 ms. B: Current amplitudes at the end of the depolarizations were normalized to the control current, averaged ($n=4-6$) and plotted against the voltage.

Fig. 5.

Functional competition between KB130015 and amiodarone. A,B: KB130015 competes with hERG1 block by amiodarone. Block of hERG1 channels expressed in *Xenopus* oocytes was assessed via repetitive (6-s interval) 5-s depolarizations to 0 mV followed by a pulse to -50 mV to measure tail currents. Traces are shown before (control) and 4 minutes after application of 1 μ M amiodarone (A). Peak outward tail currents were normalized to the control current before blocker application (start at Time=0 s), averaged ($n=6$), and plotted against the time in (B) for pretreatment with 0.1% DMSO, 5 μ M KB130015, or 50 μ M KB130015, as indicated. C,D: Amiodarone competes with hERG1 activation by KB130015. Time course of hERG1 activation by 50 μ M KB130015 was monitored by 0.1 Hz repetitive 0.5-s depolarizations to -30 mV. C: Representative current recordings before (control) and 10 min after application of KB130015. The current at the end of the depolarizations was normalized to the control current, averaged among 4 oocytes and plotted against time (D) for oocytes pretreated with 0.1% DMSO or 10 μ M amiodarone, as indicated. KB130015 application starts at Time=0 s. Gray bars in B and D indicate application of amiodarone and KB130015, respectively.

Fig. 6. KB130015 affects hERG1 channels from the intracellular side only. Representative current traces from excised patches of hERG1-S620T expressing oocytes before (black traces) and after (gray traces) addition of 10 μ M KB130015 in the outside-out and inside-out configuration, as indicated. Similar results were obtained from >7 further membrane patches in both recording modes.

Fig. 7.

Synergistic activation of hERG1 channels by mallotoxin and KB130015. A: Representative whole-cell currents in HEK 293 cells elicited by 1-s depolarizations to 0 mV under control conditions (con, black) and after application of 4 μ M mallotoxin (MTx, dark gray) or co-application of 4 μ M mallotoxin and 10 μ M KB130015 (MTx + KB130015, light gray). Note the slowdown in deactivation by mallotoxin that is even more pronounced upon co-application of KB130015. B: Maximal inward tail-current amplitudes upon repolarization from a family of 1-s depolarizations are plotted against voltage in B and fitted with a Boltzmann function (superimposed). C: The relative tail currents normalized to control data before application highlight the synergistic effect of mallotoxin and KB130015. Note that the strength of activation upon co-application closely resembles the effect by 15 μ M mallotoxin (dotted line) but clearly deviates from the theoretical sum (dashed line) of both effects upon single application of mallotoxin (dark gray) or KB130015 (see Fig. 2D), in particular at positive voltages, where KB130015 alone would reduce tail current. Extracellular solutions contained 10 mM K^+ .