Investigating the utility of adult zebrafish *ex vivo* whole hearts to pharmacologically screen hERG channel activator compounds

Christina M. Hull, Christine E. Genge, Yuki Hobbs, Kaveh Rayani, Eric Lin, Marvin Gunawan, Sanam Shafaattalab, Glen F. Tibbits, Tom W. Claydon[1]

Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, B.C., Canada

Running title: hERG activator screening in zebrafish *ex vivo* whole hearts

Key words: hERG, zebrafish, activators, zERG, optical mapping, *zkcnh6*

[1]Address correspondence to Dr. Thomas W. Claydon, Department of Biomedical Physiology and Kinesiology, Simon Fraser University, 8888 University Drive, Burnaby, B.C., V5A 1S6, Canada. Tel: (778) 782-8514. Fax (778) 782-3040. E-mail: thomas_claydon@sfu.ca

Final version published as:
Am J Physiol Regul Integr Comp Physiol. 2019 Oct 30. DOI: 10.1152/ajpregu.00190.2019

Abstract

There is significant interest in the potential utility of small molecule activator compounds to mitigate cardiac arrhythmia caused by loss-of-function of hERG1a voltage-gated potassium channels. Zebrafish (*Danio rerio*) have been proposed as a cost effective, high throughput drug-screening model to identify compounds that cause hERG1a dysfunction. However, there are no reports on the effects of hERG1a activator compounds in zebrafish, and consequently on the utility of the model to screen for potential gain-of-function therapeutics. Here, we examined the effects of hERG1a blocker, and type 1 and type 2 activator, compounds on isolated *zkcnh6a* (zERG3) channels in the *Xenopus* oocyte expression system, as well as action potentials recorded from *ex vivo* adult zebrafish whole hearts using optical mapping. Our functional data from isolated *zkcnh6a* channels show that under the conditions tested these channels are blocked by hERG1a channel blockers (dofetilide and terfenadine), and activated by type 1 (RPR260243) and type 2 (NS1643, PD-118057) hERG1a activators with higher affinity than *hKCNH2a* channels (except NS1643), with differences accounted for by different biophysical properties in the two channels. In *ex vivo* zebrafish whole hearts, two of the three hERG1a activators examined caused abbreviation of the APD, while hERG1a blockers caused APD prolongation. These data represent, to our knowledge, the first pharmacological characterization of isolated *zkcnh6a* channels and the first assessment of hERG enhancing therapeutics in zebrafish. Our findings lead us to suggest that the zebrafish *ex vivo* whole heart model serves as a valuable tool in the screening of *hKCNH2a* blocker and activator compounds.

Introduction

The human ether-à-go-go related gene (hERG, or *KCNH2*) encodes the voltage-gated potassium (Kv) channel underlying the rapid cardiac delayed rectifier current, I_{Kr} (43, 44, 48). The unique properties of *KCNH2* channels allow this current to play a critical role in cardiac repolarization and the termination of the ventricular action potential (43, 44, 48). Compared with other Kv channels, *KCNH2* channels display unusual gating, with slow activation and deactivation and rapid inactivation and recovery from inactivation. The disruption of this current, due to inherited loss-of-function mutations in *KCNH2* or pharmacological blockade of the channel, can lead to a disorder known as long QT syndrome (LQTS). LQTS is characterized by delayed cardiac repolarization and consequently prolongation of the QT-interval and this may lead to fatal cardiac arrhythmias (10, 43). As such, there is significant interest in the development of strategies to enhance *KCNH2* channel function, as well as the establishment of translatable screening models that account for the complexities of electrical activity in cardiomyocytes, such as the presence of multiple cardiac ion currents and individual genomic variability.

There has been increasing interest in the use of zebrafish as a translatable screening model for LQTS, in part because the cardiac electrophysiology of this teleost species more closely resembles that of human than some mammals (e.g. murines). For example, the intrinsic heart rate in zebrafish, 110-130 bpm, is similar to human (47), while the typical resting heart rate in the mouse is 500-700 bpm (11, 22, 49). Morphological differences of the murine cardiac action potential, specifically the lack of a plateau phase and relatively short AP (28), further complicate the translation of findings to the human heart, particularly for the study of phase 3 repolarization disorders, such as LQTS. In contrast, the zebrafish ventricular action potential exhibits a robust plateau phase and an action potential duration (APD) that more closely reflects that observed in human ventricular myocytes (4, 25, 34, 50). Furthermore, zebrafish ventricular myocytes possess many of the same ion currents found in the human ventricle with repolarization in the zebrafish strongly reliant on I_{Kr} (1, 3, 15, 18, 24, 34, 41, 45).

The utility of zebrafish to study repolarization disorders such as LQTS caused by hERG channel dysfunction can be well demonstrated by previous phenotypic characterization of native or engineered mutant zebrafish strains (1, 18, 23, 24, 52). Several zebrafish *kcnh* genes (zERG) have been identified (*zkcnh2a*, *zkcnh2b*, *zkcnh6a* and *zkcnh7*), with *zkcnh6a*, and to a lesser extent *zkcnh2a*, being the predominant transcript in the heart (26, 50). The *breakdance* mutant results from a mutation in the *zkcnh6a* gene, and displays 2:1 atrioventricular block (8), which has been observed with LQTS (2). The *reggae* mutant shortens QT interval as a result of a mutation that resulted in altered gating of *zkcnh6a* channels (18). Other loss-of-function *zkcnh6a* mutations result in a silent ventricle phenotype and one of these mutants, *zkcnh6a* M521K, was found to prolong the APD_{90} in a heterozygous embryo (1). In addition, *zkchn6a*, and to a lesser extent *zkcnh2a*, knockdown using morpholinos (33) resulted in altered QT duration and bradycardia (19, 24). Similarly, application of a range of *hKCNH2*-blocking compounds to WT zebrafish embryos results in bradycardia (24, 33) and some *hKCNH2*-blockers have been shown to cause QT prolongation in zebrafish larvae (35) and adult zebrafish *in vivo* (31). These electrophysiological characteristics of zebrafish hearts make them well suited for the investigation of human cardiac electrical disorders, and in particular I_{Kr} (hERG) related phase 3 repolarization-related disorders.

Despite the prominent expression of *zkcnh6a* and significant interest in using zebrafish as a drug screening platform (31, 33, 37), the pharmacological properties of *zkcnh6a* channels have not been examined. Furthermore, since there is significant interest in the screening of hERG activator compounds, investigation of the facilitatory effects of hERG activators on *zkcnh6a* channels is warranted. Here, we have characterized the effects of several *hKCNH2a* blocker and activator compounds to investigate the pharmacological properties of *zkcnh6a* channels in a heterologous expression system. We examine a type 1 activator, which increase repolarizing current primarily by slowing channel deactivation, and type 2 activators, which increase *hKCNH2a* tail current primarily by reducing inactivation. We then use optical mapping of whole *ex-vivo* zebrafish hearts to demonstrate prolongation of the APD following treatment with

hKCNH2a-blocking compounds and APD shortening in the presence of some *hKCNH2a* activator compounds. These data highlight the utility of the zebrafish whole heart model as an attractive translational model for studying *hKCNH2a* function and pharmacology. This model also provides insight into the complex action of *hKCNH2a*-activator compounds on both APD and action potential morphology that are useful in validating their therapeutic potential.

Materials and Methods

Heterologous expression of zkcnh6a in Xenopus laevis oocytes

hKCNH2a (hERG1a) and *zkcnh6a* (zERG3a) channel constructs were expressed in a pBluescript SKII vector as described previously (9). *zkcnh6a* cDNA (Accession No. NM_212837) was synthesized commercially (Genewiz, New Jersey, USA) and subcloned into the pBluescript vector. cRNA was transcribed from each construct using the mMessagemMachine T7 Ultra cRNA transcription kit (Ambion, Austin, TX) following cDNA linearization with *Xbal* restriction endonuclease.

Oocytes were isolated from female *Xenopus laevis* frogs and *hKNCH2a* or *zkcnh6a* cRNA was injected in accordance with the Simon Fraser University Animal Care Committee, and Canadian Council on Animal Care protocols and procedures as described previously (9). Following cRNA injection, oocytes were incubated in SOS+ media (in millimolar: 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 2.5 sodium pyruvate, 100 mg/L gentamycin sulfate and 5% horse serum, titrated to pH 7.4) for 2-7 days at 19 °C prior to voltage clamp experiments.

Membrane currents were recorded from *hKNCH2a* or *zkcnh6a* channels using the two-electrode voltage clamp technique with an Axoclamp 900A amplifier (Axon Instruments, Foster City, CA) and computer-driven voltage protocols (pClamp 10 software and Digidata 1440 interface; Axon Instruments). Recordings were obtained in ND96 solution (in millimolar: 96 NaCl, 3 KCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 5 HEPES, titrated to pH 7.4). Microelectrodes had a resistance of 0.2-2.0 MΩ when filled with 3 M KCl. Signals were

acquired at a sampling rate of 10 kHz with a 4 kHz low-pass filter. Experiments were performed at 20-22 °C.

Conductance-voltage (G-V) relations were calculated from peak tail current amplitudes. Curves were fitted with a single Boltzmann function: $y = 1/\{1+\exp[(V_{1/2}-V)/k]\}$, where y is the conductance normalized with respect to the maximal conductance, $V_{1/2}$ is the half-activation potential, V is the test voltage, and k is the slope factor. The late phase of activation, from 50% of G_{max}, was fitted with a single exponential using $f(t) = A*\exp(t/\tau) + C$, where A is the amplitude of the fit, t is time, τ is the time constant of activation and C is the residual current. Deactivating currents were fitted with a double exponential using $f(t) = A_{slow}*\exp(t/\tau_{slow}) + A_{fast}*\exp(t/\tau_{fast}) + C$, where A_{fast} and A_{slow} are the amplitudes of the two components of the fit, t is time, τ_{fast} and τ_{slow} are the time constants of the two components of deactivation, and C is the residual current. The τ_{deact} values presented represent the sum of the two components (τ_{fast} and τ_{slow}) weighted by the relative amplitude of each component. Rectification factor was calculated as previously described (43) using: $R = I/Gn(V - E_{rev})$, where R is the rectification factor, I is the membrane current, G is the slope conductance calculated from the fully activated current-voltage relationship, n is the activation variable (which was set at 1 as our data were collected from fully activated channels), V is the test voltage and E_{rev} is the measured reversal potential. The current voltage relationship in response to an action potential protocol were plots to examine the voltage of peak current as previously described (5). The concentration-response relationship of drug block of *hKCNH2a* and *zkcnh6a* channels was described by the Hill equation: $y = 1 - x^b/(c^b + x^b)$, where y is the fractional conductance, c is the IC_{50} value, and b is the Hill coefficient. The concentration-response relationship of drug enhancement of *hKCNH2a* and *zkcnh6a* channels was described by the Hill equation: $y = 1 + ax^b/(c^b + x^b)$, where y is the fractional conductance, a is (I_{max} - I_{min}), c is the EC_{50} value, and b is the Hill coefficient. Data throughout the text and figures are shown as the mean ± SE. In all figures, arrows mark the zero-current level.

The effects of *hKCNH2*-blockers, dofetilide and terfenadine, as well as *hKCNH2*-activators, NS1643, PD-118057, and RPR260243, were examined. Dofetilide, PD-118057

and terfenadine were purchased from Sigma-Aldrich (Oakville, ON), NS1643 was purchased from Tocris Bioscience (Oakville, ON) and RPR260243 was purchased from Aobious (Gloucester, MA). Drug solutions were prepared immediately prior to experiments by dilution of stock solutions: 25 mM NS1643 in DMSO; 10 mM dofetilide, PD-118057 or RPR260243 in DMSO; 10 mM terfenadine in EtOH.

Zebrafish ex vivo whole heart optical mapping

Zebrafish whole-hearts were isolated from adult zebrafish in accordance with the Simon Fraser University Animal Care Committee, and Canadian Council on Animal Care protocols and procedures, and as described previously (27). Calcium Tyrode's solution was used for all procedures. Fish were euthanized by cold shock followed by decapitation, and hearts were dissected from a tissue wedge excised from the region between the gills and pectoral fins. Isolated hearts were cannulated through the bulbus arteriosus using a 34-gauge needle. Hearts were labeled with the potentiometric dye, RH-237 (20 µM; AAT Bioquest, Sunnyvale, CA) in Ca^{2+} Tyrode's solution at room temperature for 30 min. This was followed by treatment with the myosin II inhibitor, blebbistatin (20 µM; TRC, North York, ON) in Ca^{2+} Tyrode's solution for 60 min. Imaging and analysis were carried out as described in Lin et al. (27). Briefly, cannulated hearts were suspended in in Ca^{2+} Tyrode's solution supplemented with 20 µM blebbistatin in a temperature-controlled chamber. RH-237 fluorophores were excited using a 350 mW 530 nm LED module (Thorlabs) via a 630 nm long pass dichroic mirror (XF2021, 630 DRLP, Omega Optical, Brattleboro, VT). A 700 nm long pass filter (Omega 700LP) was used to isolate the fluorescence emission and the signal was detected using a GE680 CCD camera (Allied Vision Technologies, Burnaby, BC, Canada) at a rate of 205 frames per second. All hearts had an intrinsic rate above 60 bpm prior to the stimulation protocols. Hearts were paced at rates greater than the intrinsic heart rate (pacing rates ranging from 100 - 180 bpm) and all experiments were conducted at 28 °C. Following control recordings, hearts were superfused with a *hKCNH2a*-modifying compound and recordings were taken every 20 min. Recordings represent average AP traces from a region of interest defined by the experimenter. Typically, this included the majority of the ventricle. Ventricular APs were recorded

immediately prior to (control condition) and 60 min post superfusion with a *hKCNH2*-modifying compound (drug condition). The ventricular APD from 50% upstroke to either 25% downstroke (APD_{25}) or 75% downstroke (APD_{75}) was calculated from hearts in control and drug conditions. The ΔAPD_{75} represents the drug-induced in measured APD_{75} values ($\Delta APD_{75} = APD_{75\ drug} - APD_{75\ control}$).

Statistical Methods

Statistical differences between *hKCNH2a* and *zkcnh6a* were evaluated by a two-tailed independent *t*-test ($\alpha = 0.05$) for the functional characterization, pharmacological block, and fold change in peak current in response to an action potential protocol (Fig. 1, 2, & 5). Statistical tests could not be performed to assess differences between the EC_{50} values for the pharmacological activators tested in *hKCNH2a* compared with *zkcnh6a* (Fig. 3 & 4), since it was not always possible to collect data at all drug concentrations in each oocyte. Concentration-response curves for activator compounds and EC_{50} values therefore reflect fits to the mean data without statistical comparison. Statistical comparisons were made between mean data describing the effect of near saturating concentrations, i.e the highest concentration tested that induced a near maximal effect) of each activator using a two-tailed independent *t*-test. Statistical differences in APD following drug treatment (compared to control) were evaluated by a two-tailed one-way repeated measures ANOVA ($\alpha = 0.05$) for the zebrafish *ex vivo* whole hearts (Fig. 6).

Results

Functional Characterization of zkcnh6a Channels

A recent study shows the presence of several *zkcnh* transcripts in the adult zebrafish ventricle; *zkcnh2a*, *zkcnh2b*, *zkcnh6a* and *zkcnh7*; of which *kcnh6a* was by far the most predominant (50). Looking to use whole hearts to screen hERG blocker and activator compounds, we conducted a biophysical and pharmacological characterization of *zkcnh6a* channels expressed in a heterologous system. Our biophysical characterization of *zkcnh6a* (zERG3a) channels expressed in *Xenopus laevis* oocytes, alongside that of *hKCNH2a* (hERG1a) channels, is shown in Fig. 1. The *zkcnh6a* channel had a right-shifted $V_{1/2}$ of activation (-8.6 ± 1.2 mV) compared with that of *hKCNH2a* (-31.1 ± 1.5 mV, $p <$

0.001) with no change in the slope (8.2 ± 0.2 and 8.0 ± 0.3, respectively, p = 0.672) consistent with previous biophysical measurements (45) (Fig. 1B). The time constant of activation (τ_{act}) in response to a +60 mV depolarizing pulse was greater for *zkcnh6a* than *hKCNH2a* (Fig. 1C), with τ_{act} being 74.1 ± 2.8 ms and 54.1 ± 4.2 ms, respectively (p = 0.004). To assess inactivation properties, we compared the rectification of the two channels. Fig. 1 E shows that *zkcnh6a* channels display a left-shifted voltage dependence of inactivation (-76.9 ± 1.2 mV) compared to *hKCNH2a* channels (-50.6 ± 2.1 mV, p < 0.001). We also examined the kinetics of deactivation (τ_{deact}) by fitting tail currents to a biexponential function. The *zkcnh6a* channel displayed accelerated τ_{deact} compared to the *hKCNH2a* channel at all voltages examined (Fig. 1 F, p < 0.001). To assess how the differences in *zkcnh6a* channel gating affect the morphology of the current contributing to an action potential, we recorded current traces in response to a ventricular action potential stimulus waveform (Fig. 1 G). The overall morphology of the *zkcnh6a* channel current mimicked that of the *hKCNH2a* channel current, with limited outward current during the initial phases followed by a large outward current peaking during the repolarization phase of the voltage protocol. However, there was a reduction in the outward current passed during the plateau phase of the action potential in *zkcnh6a* channels. Quantification of the current-voltage relationship (Fig. 1 H) shows that *zkcnh6a* channels display a negative shift in the voltage at which peak current occurred (-38.8 ± 0.5 mV) compared to *hKCNH2a* channels (-28.9 ± 1.4 mV, p < 0.001).

Effects of hKCNH2a Channel Blockers, Dofetilide and Terfenadine, on zkcnh6a Channels

Both dofetilide and terfenadine have been shown to be high-affinity *hKCNH2a* channel blockers. To assess the concentration-dependence and efficacy of drug block by dofetilide and terfenadine, oocytes were subjected to a drug diary protocol which consisted of a repeated 500 ms depolarizing pulse to +60 mV followed by a -110 mV repolarizing pulse (pulse frequency, 0.1 Hz) to elicit tail currents. Peak tail currents in response to the -110 mV pulse were used to assess steady-state drug block of *hKCNH2a* channels and *zkcnh6a* channels (Fig. 2). Drug block of *zkcnh6a* channels by dofetilide was greater than the block of *hKCNH2a* channels with IC_{50} values of 4.3 ± 0.7 µM and 2.6 ± 0.4

μM, respectively (p = 0.045). Terfenadine showed a greater affinity for *zkcnh6a* channels compared with *hKCNH2a* channels, with IC_{50} values of 6.7 ± 1.0 μM for *hKCNH2a* and 0.7 ± 0.2 μM for *zkcnh6a* (p < 0.001).

Effects of a Type 2 Activator on zkcnh6a Channels

NS1643 is a type 2 activator compound (which primarily modify hERG inactivation) that has been shown to increase hERG1a (*hKCNH2a*) channel tail currents. NS1643 leads to accelerated activation, slowed inactivation, a left-shifted voltage-dependence of activation and a right-shifted voltage-dependence of inactivation, the combined influence of which leads to an increase in peak tail current amplitude (6, 17). Here we measured the fold increase in the peak tail current (during a -60 mV pulse) following a 2 s depolarizing step to +10 mV to compare the effects of NS1643 on *hKCNH2a* and *zkcnh6a*. Both *hKCNH2a* channels and *zkcnh6a* channels showed an increase in outward current during the depolarizing +10 mV step as well as the -60 mV repolarizing step in response to treatment with 30 μM NS1643 (Fig. 3 A). At this concentration, which was close to saturation of the NS1643 effect, the current was enhanced 1.5 ± 0.0-fold in *hKCNH2a* and 1.6 ± 0.1-fold in *zkcnh6a* (*t*-test; p = 0.383), with EC_{50} values of 4.9 μM and 8.7 μM, respectively (Fig. 3 B).

PD-118057 is another type 2 activator compound that has been shown to enhance hERG1a (*hKCNH2a*) channel function (38, 57). Application of PD-118057 leads to an increase in peak tail current as well as a right-shifted voltage-dependence of inactivation (38, 57). Both *hKCNH2a* channels and *zkcnh6a* channels displayed increased tail current amplitude and a right-shift in the voltage-dependence of inactivation following treatment with 10 μM PD-118057 (Fig. 3C). At a near saturating concentration (30 μM), PD-118057 increased peak tail current amplitude 1.8 ± 0.1-fold in *hKCNH2a* and 2.9 ± 0.1-fold in *zkcnh6a* (*t*-test, p < 0.001), with EC_{50} values of 4.0 μM and 11.5 μM, respectively. The $V_{1/2}$ of inactivation was shifted by +14.6 ± 1.0 mV in *hKCNH2a* and by +27.4 ± 1.5 mV in *zkcnh6a* (*t*-test, p < 0.001), with EC_{50} values of 1.8 μM and 6.8 μM, respectively (Fig. 3 D).

Effects of a Type 1 Activator on zkcnh6a Channels

RPR260243 is a type 1 activator compound (primarily modifies hERG deactivation)

that has been shown to dramatically slow hERG1a channel (*hKCNH2a*) deactivation gating as well as right-shift the voltage-dependence of inactivation (20, 39). We observed a marked slowing of deactivation upon application of 10 μM RPR260243 for both *hKCNH2a* and *zkcnh6a* channels (Fig. 4 A). Since the effects of RPR260243 on deactivation kinetics are complex at higher RPR260243 concentrations, changes in deactivation were quantified by measuring the fold increase of the integrated tail current (integral I_{tail}) as performed previously (39). At 30 μM, which was close to saturation of the effect on deactivation, RPR260243 increased the integral I_{tail} 3.6 ± 0.4-fold in *hKCNH2a* and 10.5 ± 1.8-fold in *zkcnh6a* (*t*-test, p = 0.016), with EC_{50} values of 6.7 μM and 26.1 μM, respectively (Fig. 4 B).

Effects of hKCNH2a Channel Blockers and Activators on zkcnh6a During an Action Potential

Since many drugs that interact with *hKCNH2a* channels have multiple mechanisms of action, we sought to describe the composite effect of each drug tested on the current in response to an action potential voltage protocol (Fig. 5). As expected, we observed a large reduction in the current in all phases of the action potential protocol for both *hKCNH2a* channels and *zkcnh6a* channels treated with 5 μM dofetilide (Fig. 5 A). Conversely, we observed enhanced current throughout the action potential protocol for both *hKCNH2a* channels and *zkcnh6a* channels treated with 30 μM NS1643 (Fig. 5 A). The fold change in *zkcnh6a* and *hKCNH2a* peak current during an action potential protocol in response to dofetilide ($p = 0.221$), terfenadine ($p = 0.100$) and NS1643 ($p = 0.324$) were not different, whereas fold increase in peak current in response to PD-118057 ($p < 0.001$) and RPR260243 ($p < 0.001$) was greater in *zkcnh6a* compared with *hKCNH2a* channels (Fig. 5 B). A *zkcnh6a* homology model constructed based on the cryo-EM structure of hERG (51) using SWISSMODEL (Fig. 5 C) and a sequence alignment of *hKCNH2a* and *zkcnh6a* with residues previously identified (14, 16, 30, 38–40, 53–55) to be involved in activator binding/coordination highlighted (Fig. 5 D) suggests that the binding site for the

activators examined would be expected to be conserved between *zkcnh6a* and *hKCNH2a* channels.

Evaluation of the effects of hKCNH2a-modifying compounds on Zebrafish ex vivo Whole Hearts

We aimed to use zebrafish whole hearts to study the potential of the model to screen for *hKCNH2a* blocker-induced arrhythmogenicity as well as for testing the therapeutic potential of *hKCNH2a* activator compounds. We examined the gross electrical activity of isolated zebrafish whole-hearts by optically mapping voltage changes in excised cannulated hearts in the absence and presence of *hKCNH2a* blockers or activators (Fig. 6 A). Superfusion of the *hKCNH2a* channel blockers, dofetilide (50 nM) or terfenadine (200 nM), resulted in a prolongation of the APD_{75} (Fig. 6 B). The drug-induced ΔAPD_{75} indicated significant prolongation ($p < 0.05$) at all stimulation rates tested for both dofetilide and terfenadine (Fig. 6 C).

We next investigated the effects of *hKCNH2a*-activating compounds. Superfusion of RPR260243 (30 µM) or PD-118057 (40 µM) resulted in abbreviation of the APD_{75} (Fig. 6 B). The RPR260243- and PD-118057-induced shortening of APD_{75} was significantly different ($p < 0.05$) at all stimulation rates tested, with the effects of RPR260243 being particularly robust, while there were no significant changes in APD_{25} (Fig. 6 C). In the presence of NS1643 (20 µM), we did not observe shortening of the APD_{75} (Fig. 6 B and C); however, APD_{25} was significantly abbreviated ($p < 0.05$) at all but the highest stimulation rate tested (Fig. 6 C).

Discussion

One of the main goals of the present study was to evaluate the use of adult zebrafish hearts as a translational model for pharmacological investigation of both *hKCNH2a* channel blockers and activators. To do this, we firstly conducted a biophysical and pharmacological characterization of the *zkcnh* isoform, *zkcnh6a*, the predominant transcript expressed in adult zebrafish at 28℃. Detailed characterization showed some differences in the biophysical properties of *zkcnh6a* compared with *hKCNH2a* that

manifest in a delay in repolarizing current during an action potential waveform. Our pharmacological characterization shows that the blockers tested reduce *zkcnh6a* and *hKCNH2a* current during a stylized ventricular action potential waveform to a similar degree, while activators increased *zkcnh6a* current to the same degree or greater than *hKCNH2a* current. Translating these findings to the zebrafish *ex vivo* whole heart model we show that all *hKCNH2a* activator compounds tested abbreviate the ventricular action potential, although in at least one instance the mechanism by which this occurs appears complex. These findings lead us to suggest that *zkcnh6a* channel currents play a comparable role to *hKCNH2a* in the cardiac action potential, contributing primarily to phase 3 repolarization and support the potential utility of the zebrafish whole-heart model in screening for *hKCNH2a* channel activator compounds with some caveats discussed below.

We found the biophysical properties of *zkcnh6a* channels to be consistent with those described previously (45), with a right-shifted voltage dependence of activation and a left-shifted voltage dependence of inactivation compared with *hKCNH2a*. *zkcnh6a* also displayed slowed activation kinetics and accelerated deactivation kinetics compared with *hKCNH2a*. These differences appear to be of some potential functional significance as they produced differences in the morphology of *zkcnh6a* current and earlier repolarization in response to an action potential waveform voltage protocol, although the gross phenotype closely resembled that of *hKCNH2a* current. There are limitations to the interpretation of these observations in that our experiments, which were conducted at 20-22℃, cannot account for differential effects of temperature between *hKCNH2a* (37℃) and *zkcnh6a* (28℃) in their native *in vivo* environment. In addition, *zkcnh6a* and *hKCNH2a* channel currents were recorded in response to a stylized ventricular action potential, which may not fully represent zebrafish or human action potential morphology at physiological temperatures or at different rates.

Our pharmacological characterization showed that the IC_{50} values describing dofetilide and terfenadine drug block of *zkcnh6a* were reduced compared to those with *hKCNH2a*. The impact of these differences was assessed by measuring the effect of each

compound on *zkcnh6a* or *hKCNH2a* current during an action potential waveform. We found that the blockers dofetilide and terfenadine reduced *zkcnh6a* and *hKCNH2a* current during an action potential waveform to the same extent. When examining *hKNCH2a* activator compounds, we found that type 1 and type 2 *hKCNH2a* activator compounds enhanced *zkcnh6a* channel currents. The effect size, or potency, of an activator on the specific gating parameter measured was in some cases enhanced in *zkcnh6a* channels compared with *hKCNH2a* channels (e.g. NS1643 and RPR260243) and the EC_{50} values for all activators tested showed a trend to be higher in *zkcnh6a* compared with *hKCNH2a*. *zkcnh6a* and *hKCNH2a* current during an action potential waveform was again used to assess the overall significance of these gating alterations. We found that NS1643 enhanced *zkcnh6a* current during an action potential waveform to the same extent that *hKCNH2a* current was enhanced, while RPR260243 and PD-118057 had a greater enhancing effect on *zkcnh6a* than *hKCNH2a* currents during an action potential waveform. This may be due to a faster initial deactivation rate and a more negative initial voltage-dependence of inactivation in *zkcnh6a* channels than *hKCNH2a* channels that allow for a larger effect size of the action of RPR260243 (which slows deactivation) and PD-118057 (which right-shifts the voltage-dependence of inactivation), respectively. Overall, these data lead us to suggest that *zkcnh6a* and *hKCNH2a* respond similarly to the blockers tested, and that the effect of activators may be similar or enhanced in *zkcnh6a* channels compared with *hKCNH2a* channels depending on their mechanism of action. Given that channel biophysical properties and the action potential morphology may be different under physiological temperatures in zebrafish and human, further studies evaluating drug binding under these conditions would be valuable in assessing the utility of the zebrafish heart translational model.

Using optical mapping of zebrafish whole-hearts we examined the effects of *hKCNH2a*-modifying compounds at the adult zebrafish whole organ level at physiological temperature. Our selected *hKCNH2a* activator compounds have well characterized mechanisms of action (6, 12, 13, 17, 20, 21, 29, 36, 38, 39, 55, 57) and have been shown to lead to abbreviation of the APD (12, 17, 20, 57). We found that both RPR260243 and

PD-118057 caused abbreviation of the action potential duration. Both activators abbreviated APD_{75} (representative of the repolarization phase) without significant alteration to early phases of the action potential (assessed by changes to APD_{25}). These abbreviations are likely to be *zkcnh6a*-mediated given the prominent role of I_{Kr} in zebrafish ventricular repolarization (1, 18, 24, 34). This is consistent with APD shortening observed in other models as a direct result of activator-induced gain of function of *hKCNH2a* channels. For example, PD-118057 and RPR260243 both shorten APD in guinea pig cardiomyocytes following dofetilide-induced APD prolongation (20, 57). Interestingly, although NS1643 shortened APD in a manner that could be reversed by E-4031 (17), and also prevented triggered activity in hypokalemic Langendorff perfused murine hearts (21), we did not observe APD shortening in the presence of NS1643. The NS1643 *hKCNH2a* activator did however alter the morphology of the plateau and reduce the APD_{25} (Fig. 6 A, B). In zebrafish, both I_{CaL} and I_{CaT} contribute to the plateau phase of the action potential and have a relatively larger current density when compared to I_{CaL} in the human heart (56). Evidence from canine cardiomyocytes suggests that I_{CaL} is inhibited by NS1643 with a higher potency and a greater sensitivity (EC_{50} of 2.9 ± 0.4 µM; (46) than activation of I_{Kr} in the zebrafish heart. Thus, a larger I_{CaL} that is inhibited by NS1643 could account for the action potential morphology changes we observed.

The *hKCNH2* blockers tested, dofetilide and terfenadine, prolonged APD as anticipated. Interestingly, both drugs prolonged APD_{75} and APD_{25} suggesting that *zkcnh6a* channels contribute to repolarization throughout much of the AP. Our findings of impaired repolarization are consistent with previous observations that terfenadine, cisapride, and E-4031 block caused bradycardia in three-day-old zebrafish embryos (24), and terfenadine produced prolongation of the QTc interval, premature ventricular contractions, ventricular tachycardia, and AV block in isolated hearts (7). Terfenadine also restored WT-like function in *reggae* mutant embryos (18), while dofetilide prolonged APD in hearts isolated from WT zebrafish (15) and *breakdance* zebrafish (32).

Perspectives and Significance

Both electrophysiological examination of *zkcnh6a* channels and optical mapping of adult zebrafish whole-hearts highlight the reliance on I_{Kr} current in zebrafish cardiac repolarization. Despite the differences in ion channel expression (i.e., *hKCNH2a* versus *zkcnh6a*) in human and zebrafish cardiac tissue, our data lead us to suggest that *ex vivo* hearts provide an informative model for drug screening of *hKCNH2a*-blocker compounds, as well as *hKCNH2a*-activator compounds. The apparent reliance on zERG channels for cardiac repolarization and effect of hERG-specific activators may afford a pre-screening model for further evaluation in other more sophisticated translational disease models, such as hiPSC-derived cardiomyocytes. Furthermore, our findings highlight the complexity of pharmacological action in a whole organ physiologically relevant system and the importance of screening blockers and activators in a whole-heart or similar complex system. Continued expansion of our understanding of zebrafish cardiac electrophysiology will enable the use of this translational model to study more complex cardiac physiology and pathology.

Acknowledgements

This research was supported by a Canadian Institutes of Health Research Project grant (TWC) and a Natural Sciences and Engineering Research Council of Canada Discovery grant (TWC). C.M.H. was supported by a Natural Sciences and Engineering Research Council of Canada Alexander Graham Bell Canada Graduate Scholarship.

References

1. **Arnaout R**, **Ferrer T**, **Huisken J**, **Spitzer K**, **Stainier DYR**, **Tristani-Firouzi M**, **Chi NC**. Zebrafish model for human long QT syndrome. *Proceedings of the National Academy of Sciences* 104: 11316–11321, 2007. doi: 10.1073/pnas.0702724104
2. **Aziz PF**, **Tanel RE**, **Zelster IJ**, **Pass RH**, **Wieand TS**, **Vetter VL**, **Vogel RL**, **Shah MJ**. Congenital long QT syndrome and 2:1 atrioventricular block: An optimistic outcome in the current era. *Heart Rhythm* 7: 781–785, 2010. doi: 10.1016/j.hrthm.2010.02.035
3. **Baker K**, **Warren KS**, **Yellen G**, **Fishman MC**. Defective “pacemaker” current (Ih) in a zebrafish mutant with a slow heart rate. *Proceedings of the National Academy of Sciences* 94: 4554–4559, 1997. doi: 10.1073/pnas.94.9.4554
4. **Brette F**, **Luxan G**, **Cros C**, **Dixey H**, **Wilson C**, **Shiels HA**. Characterization of isolated ventricular myocytes from adult zebrafish (Danio rerio). *Biochemical and Biophysical Research Communications* 374: 143–146, 2008. doi: 10.1016/j.bbrc.2008.06.109
5. **Butler A**, **Zhang Y**, **Stuart AG**, **Dempsey CE**, **Hancox JC**. Action potential clamp characterization of the S631A hERG mutation associated with short QT syndrome. *Physiol Rep* 6: e13845, 2018. doi: 10.14814/phy2.13845
6. **Casis O**, **Olesen S-P**, **Sanguinetti MC**. Mechanism of action of a novel human ether-a-go-go-related gene channel activator. *Molecular Pharmacology* 69: 658–665, 2006. doi: 10.1124/mol.105.019943
7. **Chaudhari GH**, **Chennubhotla KS**, **Chatti K**, **Kulkarni P**. Optimization of the adult zebrafish ECG method for assessment of drug-induced QTc prolongation. *Journal of Pharmacological and Toxicological Methods* 67: 115–120, 2013. doi: 10.1016/j.vascn.2013.01.007
8. **Chen J-N**, **Haffter P**, **Odenthal J**, **Vogelsang E**, **Brand M**, **van Eeden FJM**, **Furutani-Seiki M**, **Granato M**, **Hammerschmidt M**, **Heisenberg C-P**, **Jiang Y-J**, **Kane DA**, **Kelsh RN**, **Mullins MC**, **Nüsslein-Volhard C**. Mutations affecting the cardiovascular system and other internal organs in zebrafish. *Development* 123: 293–302, 1996.

9. **Cheng YM**, **Hull CM**, **Niven CM**, **Qi J**, **Allard CR**, **Claydon TW**. Functional interactions of voltage sensor charges with an S2 hydrophobic plug in hERG channels. *The Journal of General Physiology* 142: 289–303, 2013. doi: 10.1085/jgp.201310992
10. **Curran ME**, **Splawski I**, **Timothy KW**, **Vincent GM**, **Green ED**, **Keating MT**. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 80: 795–803, 1995. doi: 10.1016/0092-8674(95)90358-5
11. **D'Angelo DD**, **Sakata Y**, **Lorenz JN**, **Boivin GP**, **Walsh RA**, **Liggett SB**, **Dorn GW**. Transgenic Gαq overexpression induces cardiac contractile failure in mice. *Proceedings of the National Academy of Sciences* 94: 8121–8126, 1997. doi: 10.1073/pnas.94.15.8121
12. **Diness TG**, **Hansen RS**, **Olesen S-P**, **Grunnet M**. Frequency-dependent modulation of KCNQ1 and HERG1 potassium channels. *Biochemical and Biophysical Research Communications* 343: 1224–1233, 2006. doi: 10.1016/j.bbrc.2006.03.072
13. **Diness TG**, **Yeh Y-H**, **Qi XY**, **Chartier D**, **Tsuji Y**, **Hansen RS**, **Olesen S-P**, **Grunnet M**, **Nattel S**. Antiarrhythmic properties of a rapid delayed-rectifier current activator in rabbit models of acquired long QT syndrome. *Cardiovascular Research* 79: 61–69, 2008. doi: 10.1093/cvr/cvn075
14. **Gardner A**, **Sanguinetti MC**. C-Linker Accounts for Differential Sensitivity of ERG1 and ERG2 K+ Channels to RPR260243-Induced Slow Deactivation. *Molecular Pharmacology* 88: 19–28, 2015. doi: 10.1124/mol.115.098384
15. **Genge CE**, **Lin E**, **Lee L**, **Sheng XY**, **Rayani K**, **Gunawan M**, **Stevens CM**, **Li AY**, **Shafaattalab S**, **Claydon TW**, **Hove-Madsen L**, **Tibbits GF**. The zebrafish heart as a model of mammalian cardiac function. *Rev Physiol Biochem Pharmacol* 171: 99–136, 2016. doi: 10.1007/112_2016_5
16. **Grunnet M**, **Abbruzzese J**, **Sachse FB**, **Sanguinetti MC**. Molecular determinants of human ether-a-go-go-related gene 1 (hERG1) K+ channel activation by NS1643. *Molecular Pharmacology* 79: 1–9, 2011. doi: 10.1124/mol.110.067728

17. **Hansen RS**, **Diness TG**, **Christ T**, **Demnitz J**, **Ravens U**, **Olesen S-P**, **Grunnet M**. Activation of human ether-a-go-go-related gene potassium channels by the diphenylurea 1,3-Bis-(2-hydroxy-5-trifluoromethyl-phenyl)-urea (NS1643). *Molecular Pharmacology* 69: 266–277, 2006. doi: 10.1124/mol.105.015859

18. **Hassel D**, **Scholz EP**, **Trano N**, **Friedrich O**, **Just S**, **Meder B**, **Weiss DL**, **Zitron E**, **Marquart S**, **Vogel B**, **Karle CA**, **Seemann G**, **Fishman MC**, **Katus HA**, **Rottbauer W**. Deficient zebrafish ether-à-go-go-related gene channel gating causes short-QT syndrome in zebrafish reggae mutants. *Circulation* 117: 866–875, 2008. doi: 10.1161/CIRCULATIONAHA.107.752220

19. **Jou CJ**, **Barnett SM**, **Bian J-T**, **Weng HC**, **Sheng X**, **Tristani-Firouzi M**. An in vivo cardiac assay to determine the functional consequences of putative long QT syndrome mutations. *Circulation Research* 112: 826–830, 2013. doi: 10.1161/CIRCRESAHA.112.300664

20. **Kang J**, **Chen X-L**, **Wang H**, **Ji J**, **Cheng H**, **Incardona J**, **Reynolds W**, **Viviani F**, **Tabart M**, **Rampe D**. Discovery of a Small Molecule Activator of the Human. *Molecular Pharmacology* 67: 827–836, 2005. doi: 10.1124/mol.104.006577

21. **Killeen MJ**, **Thomas G**, **Olesen S-P**, **Demnitz J**, **Stokoe KS**, **Grace AA**, **Huang CL-H**. Effects of potassium channel openers in the isolated perfused hypokalaemic murine heart. *Acta Physiologica* 193: 25–36, 2008. doi: 10.1111/j.1748-1716.2007.01773.x

22. **Kramer K**, **van Acker SABE**, **Voss H-P**, **Grimbergen JA**, **van der Vijgh WJF**, **Bast A**. Use of telemetry to record electrocardiogram and heart rate in freely moving mice. *Journal of Pharmacological and Toxicological Methods* 30: 209–215, 1993.

23. **Langenbacher AD**, **Dong Y**, **Shu X**, **Choi J**, **Nicoll DA**, **Goldhaber JI**, **Philipson KD**, **Chen J-N**. Mutation in sodium-calcium exchanger 1 (NCX1) causes cardiac fibrillation in zebrafish. *Proceedings of the National Academy of Sciences* 102: 17699–17704, 2005. doi: 10.1073/pnas.0502679102

24. **Langheinrich U**, **Vacun G**, **Wagner T**. Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe

arrhythmia. *Toxicology and Applied Pharmacology* 193: 370–382, 2003. doi: 10.1016/j.taap.2003.07.012

25. **Leong IUS**, **Skinner JR**, **Shelling AN**, **Love DR**. Zebrafish as a model for long QT syndrome: The evidence and the means of manipulating zebrafish gene expression. *Acta Physiologica* 199: 257–276, 2010. doi: 10.1111/j.1748-1716.2010.02111.x
26. **Leong IUS**, **Skinner JR**, **Shelling AN**, **Love DR**. Identification and expression analysis of kcnh2 genes in the zebrafish. *Biochemical and Biophysical Research Communications* 396: 817–824, 2010. doi: 10.1016/j.bbrc.2010.04.157
27. **Lin E**, **Craig C**, **Lamothe M**, **Sarunic M V**, **Beg MF**, **Tibbits GF**. Construction and use of a zebrafish heart voltage and calcium optical mapping system, with integrated electrocardiogram and programmable electrical stimulation. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 308: R755–R768, 2015. doi: 10.1152/ajpregu.00001.2015
28. **London B**. Cardiac arrhythmias: from (transgenic) mice to men. *Journal of Cardiovascular Electrophysiology* 12: 1089–1091, 2001. doi: 10.1046/j.1540-8167.2001.01089.x
29. **Lu HR**, **Vlaminckx E**, **Hermans AN**, **Rohrbacher J**, **Van Ammel K**, **Towart R**, **Pugsley M**, **Gallacher DJ**. Predicting drug-induced changes in QT interval and arrhythmias: QT-shortening drugs point to gaps in the ICHS7B Guidelines. *British Journal of Pharmacology* 154: 1427–1438, 2008. doi: 10.1038/bjp.2008.191
30. **Mao H**, **Lu X**, **Karush JM**, **Huang X**, **Yang X**, **Ba Y**, **Wang Y**, **Liu N**, **Zhou J**, **Lian J**. Pharmacologic Approach to Defective Protein Trafficking in the E637K-hERG Mutant with PD-118057 and Thapsigargin. *PLoS ONE* 8, 2013. doi: 10.1371/journal.pone.0065481
31. **Milan DJ**. In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation. *AJP: Heart and Circulatory Physiology* 291: H269–H273, 2006. doi: 10.1152/ajpheart.00960.2005
32. **Milan DJ**, **Kim AM**, **Winterfield JR**, **Jones IL**, **Pfeufer A**, **Sanna S**, **Arking DE**, **Amsterdam AH**, **Sabeh KM**, **Mably JD**, **Rosenbaum DS**, **Peterson RT**, **Chakravarti A**,

Kääb S, **Roden DM**, **MacRae CA**. A drug-sensitized zebrafish screen identifies multiple genes, including GINS3, as regulators of myocardial repolarization. *Circulation* 120: 553–559, 2009. doi: 10.1161/CIRCULATIONAHA.108.821082

33. **Milan DJ**, **Peterson TA**, **Ruskin JN**, **Peterson RT**, **MacRae CA**. Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. *Circulation* 107: 1355–1358, 2003. doi: 10.1161/01.CIR.0000061912.88753.87
34. **Nemtsas P**, **Wettwer E**, **Christ T**, **Weidinger G**, **Ravens U**. Adult zebrafish heart as a model for human heart? An electrophysiological study. *Journal of Molecular and Cellular Cardiology* 48: 161–171, 2010. doi: 10.1016/j.yjmcc.2009.08.034
35. **van Opbergen CJM**, **Koopman CD**, **Kok BJM**, **Knöpfel T**, **Renninger SL**, **Orger MB**, **Vos MA**, **van Veen TAB**, **Bakkers J**, **de Boer TP**. Optogenetic sensors in the zebrafish heart: a novel in vivo electrophysiological tool to study cardiac arrhythmogenesis. *Theranostics* 8: 4750–4764, 2018. doi: 10.7150/thno.26108
36. **Patel C**, **Antzelevitch C**. Cellular basis for arrhythmogenesis in an experimental model of the SQT1 form of the short QT syndrome. *Heart Rhythm* 5: 585–590, 2008. doi: 10.1016/j.hrthm.2008.01.022
37. **Peal DS**, **Mills RW**, **Lynch SN**, **Mosley JM**, **Lim BSE**, **Ellinor PT**, **January CT**, **Peterson RT**, **Milan DJ**. Novel chemical suppressors of long QT syndrome identified by an in vivo functional screen. *Circulation* 123: 23–30, 2011. doi: 10.1161/CIRCULATIONAHA.110.003731
38. **Perry M**, **Sachse FB**, **Abbruzzese J**, **Sanguinetti MC**. PD-118057 contacts the pore helix of hERG1 channels to attenuate inactivation and enhance K+ conductance. *Proceedings of the National Academy of Sciences* 106: 20075–20080, 2009. doi: 10.1073/pnas.0906597106
39. **Perry M**, **Sachse FB**, **Sanguinetti MC**. Structural basis of action for a human ether-a-go-go-related gene 1 potassium channel activator. *Proceedings of the National Academy of Sciences* 104: 13827–13832, 2007. doi: 10.1073/pnas.0703934104
40. **Perry M**, **Sanguinetti MC**. A single amino acid difference between ether-a-go-go-related gene channel subtypes determines differential sensitivity to a small

molecule activator. *Molecular Pharmacology* 73: 1044–1051, 2008. doi: 10.1124/mol.107.043018

41. **Rayani K**, **Lin E**, **Craig C**, **Lamothe M**, **Shafaattalab S**, **Gunawan M**, **Li AY**, **Hove-Madsen L**, **Tibbits GF**. Zebrafish as a model of mammalian cardiac function: Optically mapping the interplay of temperature and rate on voltage and calcium dynamics. *Progress in Biophysics and Molecular Biology* 138: 69–90, 2018. doi: 10.1016/j.pbiomolbio.2018.07.006
42. **Sanguinetti MC**. HERG1 channel agonists and cardiac arrhythmia. *Current Opinion in Pharmacology* 15: 22–27, 2014. doi: 10.1016/j.coph.2013.11.006
43. **Sanguinetti MC**, **Jiang C**, **Curran ME**, **Keating MT**. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell* 81: 299–307, 1995. doi: 10.1016/0092-8674(95)90340-2
44. **Sanguinetti MC**, **Jurkiewicz NK**. Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents. *The Journal of General Physiology* 96: 195–215, 1990. doi: 10.1085/jgp.96.1.195
45. **Scholz EP**, **Niemer N**, **Hassel D**, **Zitron E**, **Bürgers HF**, **Bloehs R**, **Seyler C**, **Scherer D**, **Thomas D**, **Kathöfer S**, **Katus HA**, **Rottbauer WA**, **Karle CA**. Biophysical properties of zebrafish ether-à-go-go related gene potassium channels. *Biochemical and Biophysical Research Communications* 381: 159–164, 2009. doi: 10.1016/j.bbrc.2009.02.042
46. **Szabó G**, **Farkas V**, **Grunnet M**, **Mohácsi A**, **Nánási PP**. Enhanced repolarization capacity: New potential antiarrhythmic strategy based on hERG channel activation. *Current Medicinal Chemistry* 18: 3607–3621, 2011. doi: 10.2174/092986711796642382
47. **Taggart P**, **Sutton PMI**, **Boyett MR**, **Lab M**, **Swanton H**. Human ventricular action potential duration during short and long cycles. *Circulation* 94: 2526–2534, 1996.
48. **Trudeau M**, **Warmke J**, **Ganetzky B**, **Robertson G**. HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* 269: 92–95, 1995. doi: 10.1126/science.7604285

49. **Uechi M**, **Asai K**, **Osaka M**, **Smith A**, **Sato N**, **Wagner TE**, **Ishikawa Y**, **Hayakawa H**, **Vatner DE**, **Shannon RP**, **Homcy CJ**, **Vatner SF**. Depressed heart rate variability and arterial baroreflex in conscious transgenic mice with overexpression of cardiac G(sα). *Circulation Research* 82: 416–423, 1998. doi: 10.1161/01.RES.82.4.416
50. **Vornanen M**, **Hassinen M**. Zebrafish heart as a model for human cardiac electrophysiology. *Channels* 10: 101–110, 2016. doi: 10.1080/19336950.2015.1121335
51. **Wang W**, **MacKinnon R**. Cryo-EM structure of the open human ether-à-go-go-related K+ channel hERG. *Cell* 169: 422-430.e10, 2017. doi: 10.1016/j.cell.2017.03.048
52. **Warren KS**, **Baker K**, **Fishman MC**. The slow mo mutation reduces pacemaker current and heart rate in adult zebrafish. *American Journal of Physiology - Heart and Circulatory Physiology* 281: H1711–H1719, 2001. doi: 10.1152/ajpheart.2001.281.4.H1711
53. **Wu W**, **Gardner A**, **Sanguinetti MC**. Concatenated hERG1 tetramers reveal stoichiometry of altered channel gating by RPR-260243. *Molecular pharmacology* 87: 401–9, 2015. doi: 10.1124/mol.114.096693
54. **Wu W**, **Sachse FB**, **Gardner A**, **Sanguinetti MC**. Stoichiometry of altered hERG1 channel gating by small molecule activators. *The Journal of General Physiology* 143: 499–512, 2014. doi: 10.1085/jgp.201311038
55. **Xu X**, **Recanatini M**, **Roberti M**, **Tseng G-N**. Probing the binding sites and mechanisms of action of two human ether-a-go-go-related gene channel activators, 1,3-bis-(2-Hydroxy-5-trifluoromethyl-phenyl)-urea (NS1643) and 2-[2-3,4-Dichloro-phenyl)-2,3-dihydro-1H-isoindol-5-ylamino]-nicotinic acid (PD30. *Molecular Pharmacology* 73: 1709–1721, 2008. doi: 10.1124/mol.108.045591
56. **Zhang P-C**, **Llach A**, **Sheng XY**, **Hove-Madsen L**, **Tibbits GF**. Calcium handling in zebrafish ventricular myocytes. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 300: R56–R66, 2011. doi: 10.1152/ajpregu.00377.2010

57. **Zhou J**, **Augelli-Szafran CE**, **Bradley JA**, **Chen X**, **Koci BJ**, **Volberg WA**, **Sun Z**, **Cordes JS**. Novel potent human ether-a-go-go-related gene (hERG) potassium channel enhancers and their in vitro antiarrhythmic activity. *Molecular Pharmacology* 68: 876–884, 2005. doi: 10.1124/mol.105.014035

Figure Legends

Figure 1. Characterization of the *zkcnh6a* K^+ channel. A., D., G. Typical current traces recorded from *hKCNH2a* channels (black) and *zkcnh6a* channels (blue) in response to the voltage protocols shown. **B.** Conductance-voltage relationships calculated from peak tail currents from *hKCNH2a* (n = 13) and *zkcnh6a* (n = 14) channels. **C.** Normalized peak tail current amplitudes from *hKCNH2a* (n = 5) and *zkcnh6a* (n = 5) channels recorded during an envelope of tails voltage protocol (inset) to measure the time course of activation. Representative current traces from *zkcnh6a* are shown in the inset. **E.** Rectification factor for *hKCNH2a* (n = 13) and *zkcnh6a* (n = 14) calculated from the data in D as a measure of the voltage-dependence of inactivation. **F.** Weighted τ of deactivation following a 500 ms step to +40 mV for *hKCNH2a* (n = 13) and *zkcnh6a* (n = 14). Representative tail current traces at -110 mV are shown for *hKCNH2a* (black) and *zkcnh6a* (blue) in the inset. **G.** Typical currents in response to an action potential protocol show that the characteristic morphology of *hKCNH2a* action potential current is well preserved in the current from *zkcnh6a*. **H.** Representative plot of the current voltage relationship in response to an action potential protocol. The box and whisker plot represents the voltage of peak normalized current of *hKCNH2a* (n = 26) and *zkcnh6a* (n = 28). Asterisks (*) denote a statistically significant difference between *hKCNH2a* and *zkcnh6a* ($p < 0.05$, two-tailed independent *t*-test) and error bars represent ± SE.

Figure 2. Effect of *hKCNH2a* channel blockers on *zkcnh6a* channels. A. Concentration-response relationship for the blocker action of dofetilide (left) and terfenadine (right) on *hKCNH2a* (black) and *zkcnh6a* (blue) channels (n = 5-9). Concentration-response data were fitted with the Hill equation (see Material and Methods). Asterisks (*) denote a statistically significant difference in the IC_{50} concentration between *hKCNH2a* and *zkcnh6a* ($p < 0.05$, two-tailed independent *t*-test) and error bars represent ± SE.

Figure 3. Effects of type 2 *hKCNH2a* channel activators on *zkcnh6a* channel. A. Typical current traces recorded from *hKCNH2a* and *zkcnh6a* channels before and after NS1643

(30 µM) treatment. **B.** Effect of NS1643 on peak tail current. Concentration-response data were fitted with the Hill equation (see Material and Methods) for *hKCNH2a* (n = 5-6) and *zkcnh6a* (n = 5-7) **C.** Fully-activated current-voltage relationships following a 500 ms depolarizing step to +40 mV before and after treatment of *hKCNH2a* channels or *zkcnh6a* channels with 10 µM PD-118057. Fully activated I-V relationships were normalized to peak outward control tail current (left) and voltage-dependence of inactivation determined from rectification of the fully activated I-V relationships (right) (43) from *hKCNH2a* (n = 6) and *zkcnh6a* (n = 6) channels before and after PD-118057 (10 µM) treatment. **D.** Effect of PD-118057 on peak tail current (*hKCNH2a*: black, n = 5-6; *zkcnh6a*: blue, n = 5-6) and change in $V_{1/2}$ of inactivation (*hKCNH2a*: grey, n = 5-6; *zkcnh6a*: light blue, n = 5-6). As in B, EC values were derived from the fit to the mean data. Error bars represent ± SE.

Figure 4. Effects of a type 1 *hKCNH2a* channel activator on *zkcnh6a* channels. A. Typical current traces recorded from *hKCNH2a* and *zkcnh6a* channels before and after RPR260243 (10 µM) treatment. A 500 ms depolarizing pulse to +60 mV was used to activate channels and this was followed by a 10 s pulse to -60 mV to elicit tail currents. **B.** Concentration-response relationships of RPR260243 with *hKCNH2a* (n = 3-6) and *zkcnh6a* (n = 3-5). Concentration-response data were fitted with the Hill equation (see Material and Methods). EC values reflect the fit to the mean data as in Fig. 3. The effect of RPR260243 on deactivation was quantified by the integral of $I_{tail\ drug}/I_{tail\ control}$ (integral I_{tail}) at -60 mV during a 10 s pulse. Error bars represent ± SE.

Figure 5. *zkcnh6a* current in response to a ventricular action potential voltage waveform. A. Typical action potential current traces recorded before and after treatment with dofetilide (a *hKCNH2a* channel blocker: red) or NS1643 (a *hKCNH2a* channel activator: green). **B.** Plot of fold change in *hKCNH2a* (n = 5, 5, 4, 6, 6) and *zkcnh6a* (n = 5, 5, 6, 6, 6) peak current from control (absence of drug) following treatment with *hKCNH2a* channel blockers (dofetilide: 5 µM; terfenadine: 5 µM) and activators (NS6143: 30 µM;

PD-118057: 10 µM; RPR260243: 10 µM). Asterisks (*) denote a statistically significant difference in the fold change between *hKCNH2a* and *zkcnh6a* ($p < 0.05$, two-tailed independent *t*-test). **C.** *zkcnh6a* homology model (blue) showing from the S4 segment to the CNBHD overlaid on the *hKCNH2a* cryo-EM structure (grey) (51). **D.** Sequence alignment of the S5 segment, pore helix, S6 segment, C-linker and αA helix of the CNBHD (labelled as CNBHD) of *hKCNH2a* and *zkcnh6a* channels. Circles highlight residues involved in the agonist action of the *hKCNH2a* channel activators NS1643 (purple), PD-118057 (teal), and RPR260243 (olive) (42) and boxes highlight non conserved residues.

Figure 6. Optically recorded action potentials from paced adult zebrafish whole-hearts treated with *hKCNH2a* channel blockers and activators. A. Mean ventricular voltage responses (action potentials) of adult zebrafish whole-hearts paced at 120 bpm before and after treatment with *hKCNH2a* channel blockers or activators. Control averages are shown in black. *hKCNH2a* channel blocker averages are shown in red (top: 50 nM dofetilide, n = 6; bottom: 200 nM terfenadine, n = 5), and *hKCNH2a* channel activator averages are shown in green (top: 20 µM NS1643, n = 4; middle: 40 µM PD-118057, n = 6; bottom: 30 µM RPR260243, n = 5). **B.** Mean APD_{75} before and after treatment with *hKCNH2a* channel blockers or activators (n = 4-6). **C.** Change in APD (ΔAPD_{75} closed circles; ΔAPD_{25} open circles) following treatment with *hKCNH2a* channel blockers or activators (n = 4-6). Asterisks (*) denote a statistically significant difference in APD_{75} and double barred crosses (‡) denote a statistically significant difference in APD_{25} following drug treatment compared to control ($p < 0.05$, two-tailed one-way repeated measures ANOVA). Error bars represent ± SE.