Chapter 5

Excitation and Excitation-Contraction Coupling of the Zebrafish Heart: Implications for the Zebrafish Model in Drug Screening

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Additional information is available at the end of the chapter

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Abstract

There are several similarities and differences in electrical excitability between zebrafish and human ventricles. Major ion currents generating ventricular action potentials are largely the same in human and zebrafish hearts with some exceptions. A large T-type calcium current is unique to the zebrafish ventricle (absent in human ventricle), and two potassium currents (I_{Ks} and I_{to}) may be absent in zebrafish ventricular myocytes. However, there are substantial differences among alpha subunit isoforms of the ion channel families or subfamilies (e.g. zebrafish Kv11.2 vs. human Kv11.1; zebrafish Kir2.4 vs. human Kir2.1) between human and zebrafish hearts. Contraction of zebrafish ventricle is strongly dependent on extracellular calcium, while human ventricle relies heavily on calcium stores of the sarcoplasmic reticulum. These differences may affect the use of zebrafish as a model in drug screening and safety pharmacology.

Keywords: cardiac action potential, ion currents, ion channels, drug screening, e-c coupling

1. Introduction

Zebrafish (*Danio rerio*), a tropical freshwater fish species, is a popular vertebrate model and widely used to resolve diverse research questions in developmental biology and genetics, human diseases, environmental toxicology and several other disciplines. The advantages of the zebrafish model are research technical (e.g. well-annotated and easily modifiable genome,

transparency of embryos), economical (cost and ease of maintenance, large number of offspring and short generation time) and ethical (replacement of mammalian models—3R principle) [1, 2]. Those qualifications have made zebrafish an interesting object in studies, where new molecules are searched and selected for drug development programs [3–5]. Potentially zebrafish could be a high throughput and relatively inexpensive *in vivo* model for screening therapeutically effective and nontoxic candidate molecules for drug development programs. Indeed, great expectations are set on the zebrafish model, which is sometimes regarded as an ideal system for preclinical screening of cardiovascular drugs [6]. The expectations are based on the conserved properties of cardiac physiology between humans and zebrafish, such as the similarities in the shape of ventricular action potential (AP) and heart rate [7–9]. The documented responses of zebrafish hearts (e.g. bradycardia, atrioventricular block, prolongation QT interval of electrocardiogram) to the inhibitors of human e*ther-à-go-go*-related (KCNH2) channel provide some credence to those expectations, even though sensitivity and specificity of the responses are not optimal [9–13]. In the screening of cardiovascular drugs, the *in vivo* zebrafish model has the advantage that all cardiac ion channels are simultaneously exposed to the compound, thereby allowing phenotype-based screening. However, in order to provide an accurate mode for the human heart, molecular composition, voltage-dependence and gating kinetics of ion channels of the zebrafish heart should closely match those of the human heart. Unfortunately, the ionic and molecular bases of electrical excitability of the zebrafish heart are still unsatisfactorily known. This is a significant shortcoming, since the requirements set for effective and safe drugs are extremely rigorous, and safety evaluation necessitates exact knowledge about the mode of drug action [14]. Those requirements are delineated in Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative for cardiac safety evaluation of new drugs, which provides an accurate mechanistic-based assessment of proarrhythmic potential [14–16]. Rational evaluation of drug toxicity in the zebrafish model is not possible before ion currents and channels of the zebrafish cardiac myocytes are known in sufficient detail. The present overview compares ion current and ion channel compositions of zebrafish and human ventricles in order to indicate similarities and differences between the fish model and the human heart, and gaps in our knowledge of the zebrafish cardiac excitability and excitation-contraction (e-c) coupling. These issues have also been discussed in other recent reviews [17–19].

2. Cardiac action potential

Contraction of cardiac myocytes is triggered by electrical excitation of myocyte sarcolemma in the form of cardiac AP. Propagation of AP through the heart can be recorded as electrocardiogram (**Figure 1A**). Each functionally different cardiac tissue has a characteristic AP shape generated by the tissue-specific ion currents and ion channel compositions. The five different phases of the mammalian ventricular AP—with the exception of phase 1 fast repolarization—are readily discernible in the zebrafish ventricular AP (**Figure 1B**). Similar to the human ventricular AP (but unlike the murine AP), the zebrafish ventricular AP has a distinct plateau phase (phase 2) at positive voltages [8, 9, 20] (**Figure 1B**). Indeed, the only major difference between zebrafish and human ventricular APs is the absence of the fast phase 1 repolarization

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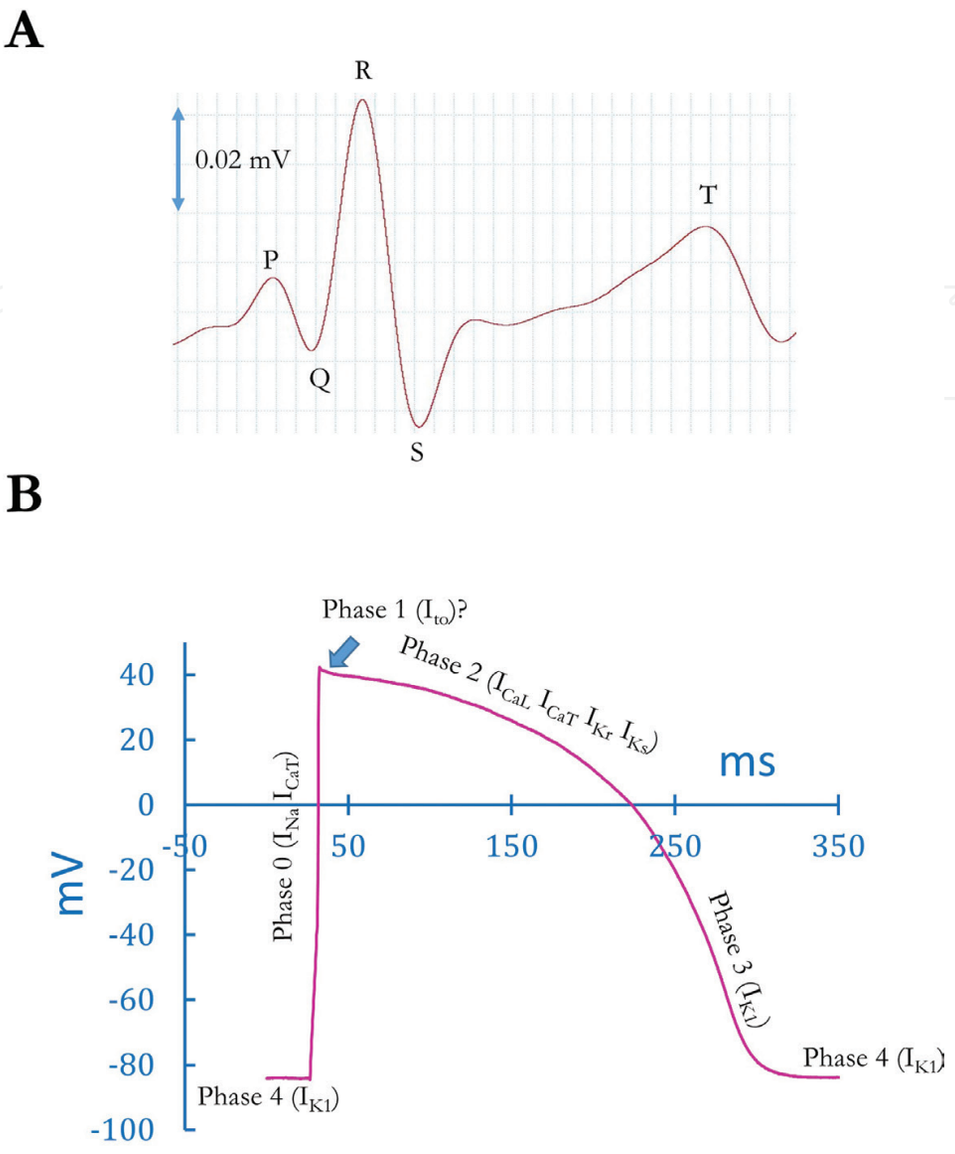

Figure 1. Electrocardiogram (A) and ventricular action potential (B) of the zebrafish heart at 26°C. Electrocardiogram was recorded from surface of spontaneously beating heart *in vitro*. Ventricular action potential was recorded from an enzymatically isolated cardiac myocyte with patch-clamp technique. The main ion currents responsible for different phases of ventricular action potential are also shown. I_{Na}, Na^+ current; I_{CaL}, L-type Ca^{2+} current; I_{CaT}, T-type Ca^{2+} current; I_{Kr}, the fast component of delayed rectifier K^+ current; I_{Ks}, the slow component of the delayed rectifier K^+ current; I_{K1}, the inward rectifier K^+ current.

in the zebrafish AP [19]. This may be due to the absence of the transient outward K^+ current (I_{to}) in zebrafish ventricular myocytes [8].

Zebrafish are ectothermic vertebrates and therefore their AP characteristics may change depending on the rearing temperature of the fish, as has been reported for several teleost fish species [21, 22]. In the adult zebrafish, reared at 28°C, the duration of ventricular AP (APD_{50}; at 28°C) is about 30% shorter than that of the human ventricular AP at 37°C (**Table 1**). At 36°C, the duration of zebrafish ventricular AP is only 80 ms, i.e. about 25% of the duration of human ventricular AP at 37°C. The shorter AP of the zebrafish heart may be associated with

	Zebrafish	Reference	Human	Reference
Relative heart size (% of body mass)	0.1	[48]	0.64	[50]
Diastolic/systolic blood pressure (mm Hg)	0.42/2.51	[70]	70/125	[71]
Myocyte size (ventricle) (pF)	26–33	[8, 39]	117–227	[72, 73]
T-tubuli	No	[20, 48]	Yes	[67, 74]
Role of CICR[1] in e-c coupling (%)	15	[48]	~70	[75, 76]
Myofibril location	Subsarcolemmal	[47]	Throughout the myocyte	
Ventricular AP duration (ms)	~240 at 28°C ~80 at 37°C	[19]	~330 at 37°C	[77]
Resting heart rate (bpm)	120–130 at 28°C ~287 at 37°C	[8, 19, 23]	60–80 bpm at 37°C	[71]

[1]CICR, Ca^{2+}-induced Ca^{2+} release.

Table 1. Basic characteristics of the zebrafish heart and cardiac myocytes in comparison to those of the human heart.

the higher heart rate of the fish, which at 28°C is about double (120–130 beats per minute) and at 37°C about quadruple (287 beats per minute) the human resting heart rate [19, 23] (**Table 1**). Temperature is an important environmental factor for an ectothermic vertebrate, which modifies cardiac gene expression and ion channel function [24–26]. Therefore, rearing and experimental temperatures should be carefully controlled and reported in zebrafish studies.

3. Ion currents and ion channels

Density and kinetics of ion currents must be such that chamber-specific APs are generated and can be adjusted to heart rates according to the circulatory demands. This is reflected in the composition of ion channel assemblies and their abundances in different cardiac chambers and in a species-specific manner [21]. This overview is limited to ventricular myocytes, since atrial ion currents/channels of the zebrafish heart are still relatively poorly known.

3.1. Sodium currents and channels

Sodium influx through the voltage-gated Na^+ channels initiates the all-or-none action potential (AP) of atrial and ventricular myocytes, when the current flow from the upstream cell depolarizes the membrane of the downstream cell to the threshold level (about −55 mV). At the threshold voltage, the density of inward Na^+ current (I_{Na}) exceeds the total density of the outward K^+ currents (I_K). The rapid opening of Na^+ channels generates a fast upstroke (depolarization) of AP and an overshoot to the level of about +40 mV (phase 0) [19, 20] (**Figure 1B**). Then I_{Na} quickly inactivates due to the closing of the inactivation gate of the channel.

The density of I_{Na} is the main determinant for the rate of AP propagation over the heart. The rate of AP upstroke in zebrafish ventricular myocytes at 28°C is about 130 V s^{-1} (RMP ~ −84 mV) (Haverinen et al., submitted), which is less than half of the rate of AP upstroke in human ventricular myocytes at 37°C [27]. These findings suggest that the density of ventricular I_{Na} is lower and the rate of AP propagation slower than in human ventricles at the species-specific temperatures (28°C vs. 37°C). However, a thorough analysis of the zebrafish I_{Na} is needed to reveal to what extent these differences are due to RMP (availability of Na^+ channel for opening), Na^+ channel density and kinetic properties of the cardiac Na^+ channels.

The zebrafish heart expresses eight different Na^+ channel alpha subunits. The main isoforms are $Na_v1.5Lb$ (83.1% of the transcripts) and $Na_v1.4b$ (16.2%), which are orthologues to the human cardiac $Na_v1.5$ (71.1% in the right ventricle) and skeletal $Na_v1.4$ channels, respectively [28] (**Table 2**). $Na_v2.1$ is abundantly expressed in the human right ventricle (27.8%), but seems to be absent in zebrafish ventricular myocytes. Unlike the mammalian cardiac I_{Na}, which is tetrodotoxin-resistant (IC_{50} about 1 μM), the zebrafish I_{Na} is more than two orders of magnitude more sensitive to tetrodotoxin (IC_{50} about 6 nM) (Haverinen et al., submitted), similar to the I_{Na} of other fish species [29, 30]. Thus, there is a remarkable difference in tetrodotoxin-sensitivity and some minor differences in Na^+ channel composition between zebrafish and human hearts.

3.2. Calcium currents and channels

The vertebrate heart usually has two main types of Ca^{2+} currents, a high-threshold or long-lasting L-type current (I_{CaL}) and a low-threshold or transient T-type (I_{CaT}) current. The former is activated at voltages more positive than −40 mV and with the peak amplitude at about +10 mV, while the latter is generated already at −60 mV and with the peak current amplitude at about −30 mV [31, 32].

I_{CaL} is the main I_{Ca} of atrial and ventricular myocytes. It has a significant physiological function in maintaining the long plateau (phase 2) of the cardiac AP and mediating Ca^{2+} influx into the myocyte (**Figure 1B**). I_{CaL} contributes to the Ca^{2+} transient, which sets cardiac contraction in motion, either directly by increasing cytosolic Ca^{2+} concentration or triggering a further release of Ca^{2+} from the sarcoplasmic reticulum (SR) (for more details see excitation-contraction coupling). The mean density of I_{CaL} in ventricular myocytes of the zebrafish heart at 28°C is 6–8 pA pF^{-1}, which is about double the density of the human ventricular I_{CaL} at 35°C (3–4 pA pF^{-1}) [8, 20, 33, 34]. This difference may signify a larger role of sarcolemmal Ca^{2+} influx in e-c coupling of the zebrafish heart [33]. In the human ventricle, the main L-type Ca^{2+} channel isoform is $Ca_v1.2$, which represents 98.4% of the total Ca^{2+} channel transcripts in the right ventricle [35] (**Table 2**). In the zebrafish ventricle, seven different L-type Ca^{2+} alpha subunits are expressed, among them three paralogue pairs [36]. Similar to the human ventricle, $Ca_v1.2$ is the most abundant L-type Ca^{2+} channel isoform in zebrafish ventricle consisting of 38.3% of the all Ca^{2+} channels transcripts.

In vertebrate hearts I_{CaT} is a sizeable current in nodal myocytes and it may be also present in atrial myocytes, but it is usually absent in ventricular myocytes. In this respect, the zebrafish is clearly different. A characteristic feature for zebrafish ventricular myocytes is a large I_{CaT} with a

Ion current	Ion channels*	Ion channels**
	Zebrafish	**Human**
I_{Na}	**Na_v1.5Lb (83.1%)**	**Na_v1.5 (71.1%)**
	Na_v1.4b (16.2%)	Na_v2.1 (27.8%)
	Na_v1.1b (0.48%)	Na_v1.3 (7.1%)
	Na_v1.6b (0.12%)	Na_v1.1 (2.7%)
	Na_v1.6a (0.03%)	Na_v1.7 (1.4%)
	Na_v1.1a (0.02%)	
	Na_v1.4a (0.01%)	
	Na_v1.5La (0.01%)	
I_{Ca}		
I_{CaL}	**Ca_v1.2 (38.3%)**	**Ca_v1.2 (98.4%)**
	Ca_v1.3a (0.07%)	Ca_v1.3 (0.039%)
	Ca_v1.1a (2.63%)	
I_{CaT}	**Ca_v3.1 (54.8%)**	Ca_v3.1 (0.14%)
	Ca_v3.2a (0.06%)	**Ca_v3.2 (1.45%)**
	Ca_v3.2b (0.03%)	
$I_{CaP/Q}$	Ca_v2.1b (3.84%)	
I_K (voltage-gated)		
I_{Kr}	K_v11.1a (0.1%)	**K_v11.1[2] (54.3%)**
	K_v11.1b (0.1%)	
	K_v11.2a[1] (84.6%)	
	K_v11.2b[1] (0.3%)	
	K_v11.3 (0.2%)	
I_{to}		K_v1.5 (26.1%)
I_{Ks}	K_v7.1 (14.6%)	K_v7.1 (15.7%)
I_{Kur}	Not examined	**K_v4.3 (12.1%)**
I_{K1} (inward rectifying)		
	Kir2.1a (0.6%)	**Kir2.1 (46.5%)**
	Kir2.1b (0.005%)	
	Kir2.2a (6.3%)	Kir2.2 (28.9%)
	Kir2.2b (0.1%)	
	Kir2.3 (0.04%)	Kir2.3 (24.5%)
	Kir2.4 (93.0%)	

[1]KCNH6 (Zebrafish erg).

[2]KCNH2 (Human erg).

*Zebrafish results are from [19, 36] and unpublished results of Hassinen et al.

**Human results are from [35].

Table 2. Major ion currents and ion channel transcripts of zebrafish ventricle in comparison to those of the human ventricle.

current density almost equal to that of I_{CaL} [8, 37]. T-type Ca^{2+} channels pass Ca^{2+} influx at more negative voltages than L-type Ca^{2+} channels. Therefore, they may contribute to upstroke and early plateau of the ventricular AP. Although I_{CaT} inactivates faster than I_{CaL}, it allows a significant sarcolemmal Ca^{2+} entry into zebrafish ventricular myocytes. During a 300 ms depolarizing pulse to −30 mV the Ca^{2+} influx through T-type Ca^{2+} channels is about 35% of the Ca^{2+} influx of L-type Ca^{2+} channels during 300 ms pulse to +10 mV [36]. Therefore, I_{CaT} may have a significant role in e-c coupling of zebrafish ventricular myocytes. T-type Ca^{2+} channels are abundantly expressed in the zebrafish ventricle constituting majority of the transcripts (about 55%) of the total Ca^{2+} channel population (**Table 2**). Altogether five alpha subunits of the T-type (Ca_v3) family are expressed in the zebrafish ventricle. $Ca_v3.1$ (alpha1G) is clearly the dominant isoform, not only among T-type Ca^{2+} channels, but also among all the cardiac Ca^{2+} channel types with the transcript abundance of 54.8%. The other T-type alpha subunits are expressed only in trace amounts. In human ventricular myocytes, the T-type Ca^{2+} channels are very weakly expressed. $Ca_v3.1$ and $Ca_v3.2$ alpha subunits constitute together less than 2% of the Ca^{2+} channels transcripts in the human right ventricle [35] (**Table 2**). Taken together the prominent expression of I_{CaT} in zebrafish ventricular myocytes is one of the most striking differences in ion channel composition between zebrafish and human hearts. The exact role of I_{CaT} in excitation and e-c coupling of zebrafish ventricular myocytes needs to be examined in detail.

Overall, the diversity of Ca^{2+} channels in zebrafish ventricle is larger than in the human ventricle. Most notably, T-type Ca^{2+} channels are more abundant than L-type Ca^{2+} channels.

3.3. Potassium currents and channels

Outward potassium currents (I_K) are repolarizing, i.e. they maintain negative resting membrane potential (RMP) and limit the duration of cardiac AP.

3.3.1. Inward rectifier K^+ currents and channels

The inward rectifier K^+ current (I_{K1}) maintains RMP of atrial and ventricular myocytes and provides K^+ efflux for the final phase 3 repolarization of AP [38] (**Figure 1B**). This current is generated by the Kir2 subfamily channels in vertebrate hearts. Characteristic for the inward rectifier K^+ channels of the Kir2 family is that they pass outward I_{K1} in the voltage range from about −80 to 0 mV with the peak current at −59 mV in zebrafish ventricular myocytes [39]. At RMP, the net K^+ flux at the RMP is almost zero, but the outward I_{K1} activates instantaneously on depolarization of sarcolemma and generates a fast outward surge of I_{K1} at the rising phase of AP. At the plateau voltage, K^+ efflux is small due to the voltage-dependent block of the channel pore by intracellular polyamines and Mg^{2+} ions. When membrane potential starts to repolarize (due to the activation of I_{Kr} and I_{Ks} and inactivation of I_{Ca}), the polyamine block of Kir2 channels relaxes and K^+ efflux through Kir2 channel accelerates repolarization at phase 3. Different members of the Kir2 family differ in the ease with which they allow K^+ efflux through the sarcolemma. Large difference in Ba^{2+}-sensitivity between Kir2 isoforms suggests that their interaction with potential medicinal drug molecules might also differ [39].

The main Kir2 channel isoform of the zebrafish ventricle is Kir2.4, as in the most teleost fishes studied thus far [39]. In the zebrafish atrium Kir2.2a channels are abundantly expressed in

addition to Kir2.4 channels. There are striking differences in isoform composition between zebrafish and human ventricles. The main isoform in the human ventricles is Kir2.1 (46.5% of the transcripts in the right ventricle), which appears only in trace amounts (0.8%) in the zebrafish ventricle. Kir2.3 is abundantly expressed in human ventricle (24.5%), but almost totally absent in the zebrafish ventricle (**Table 2**). Since the Kir2 isoforms differ in their rectification properties those differences in isoform composition are likely to have functional consequences for repolarization of AP.

3.3.2. Voltage-gated K^+ currents and channels

Voltage-gated K^+ currents provide sarcolemmal K^+ efflux for repolarization of AP. Several different voltage-gated K^+ currents are expressed in human ventricle including fast and slow components of the delayed rectifier current (I_{Kr} and I_{Ks}, respectively) and transient outward current (I_{to}) [27, 40, 41]. Transcripts for the ultra-rapid component of the delayed rectifier channel ($K_v1.5$) are expressed in the human ventricle, but the current seems to be specific for atrial myocytes and not expressed in the ventricles [42]. Much less is known about the voltage-gated K^+ currents of the zebrafish heart. Similar to the human ventricle, I_{Kr} seems to be the main repolarizing current also in zebrafish ventricle [8, 37, 43]. Until now, I_{Ks} has not been recorded in zebrafish cardiac myocytes, even though transcripts of the $K_v7.1$ (KCNQ1) channel are expressed in the zebrafish ventricle (Hassinen et al., unpublished) (**Table 2**). Neither has been I_{to} found in zebrafish cardiac myocytes [8]. These findings suggest that repolarization of zebrafish ventricular myocytes might be more strongly dependent on single voltage-gated K^+ current, I_{Kr}, than human ventricular myocytes. However, a closer examination of the K^+ ion composition of zebrafish ventricular myocytes is needed to verify/falsify these assumptions.

I_{Kr} is the dominant repolarizing current in human and zebrafish ventricular myocytes. Notably the current is generated by different isoforms in human and zebrafish heart. In the human cardiac myocytes, I_{Kr} flows through the erg1 (Herg or KCNH2 or $K_v11.1$) channels (**Table 2**). In zebrafish myocytes I_{Kr} is generated almost exclusively by KCNH6 ($K_v11.2a,b$ or zebrafish erg) channels [19, 44], although it is often referred to as an orthologue to human KCNH2 channels [9, 11, 45]. Indeed, the expression of $K_v11.1a,b$ transcripts in the zebrafish ventricle is only 0.2%, while $K_v11.2a,b$ channel transcripts constitute 84.9% of the total voltage-gated K^+ channel alpha subunit population. Transcripts of the $K_v7.1$ channels form about 15% of the total transcripts of the voltage-gated K^+ channels in both human and zebrafish ventricles (**Table 2**). $K_v1.5$ channels are expressed in the human ventricle, but no reports exist about any zebrafish orthologues.

4. Excitation-contraction coupling

There are prominent differences in size, shape and fine structure between zebrafish and human ventricular myocytes (**Table 1**). Zebrafish ventricular myocytes are 5–10 times smaller (26.1–33.3 pF) than human ventricular myocytes (117–227 pF). Zebrafish ventricular myocytes are almost as long as human ventricular myocytes, but are much thinner [20, 46]. In human ventricular myocytes, myofibrils are evenly distributed throughout the myocyte, while in

zebrafish ventricular myocytes myofibrils locate immediately under the sarcolemma [47]. Due to the small diameter of ventricular myocytes and cortical location of myofibrils T-tubuli are unnecessary for cellular signaling and probably therefore completely absent in zebrafish myocytes [20, 48].

The marked differences in myocyte size and structure appear as significant differences in the excitation-contraction (e-c) coupling between zebrafish and human (mammalian) cardiac myocytes (**Figure 2**). The contraction of human ventricular myocyte mainly relies on intracellular Ca^{2+} stores of the sarcoplasmic reticulum (SR) in generating cytosolic Ca^{2+} transients. A small Ca^{2+} influx through L-type Ca^{2+} channels triggers a large Ca^{2+} release via ryanodine-sensitive Ca^{2+} release channels of the SR so that about 77% of the cytosolic Ca^{2+} transient originates from the SR [49, 50]. In ventricular myocytes of the zebrafish heart Ca^{2+} release from the SR makes only about 15% of the total Ca^{2+} transient [48]. Voltage-dependence of cell shortening and Ca^{2+} transients also suggest that sarcolemma Ca^{2+} influx is the main source of cytosolic Ca^{2+} in zebrafish cardiomyocytes [51]. In human cardiac myocytes, voltage dependence of the Ca^{2+} transients is bell-shaped reflecting the voltage dependence of the trigger for Ca^{2+} release from the SR, I_{CaL} [52]. In zebrafish myocytes both I_{CaL} and Na^+-Ca^{2+}-exchange directly contribute to

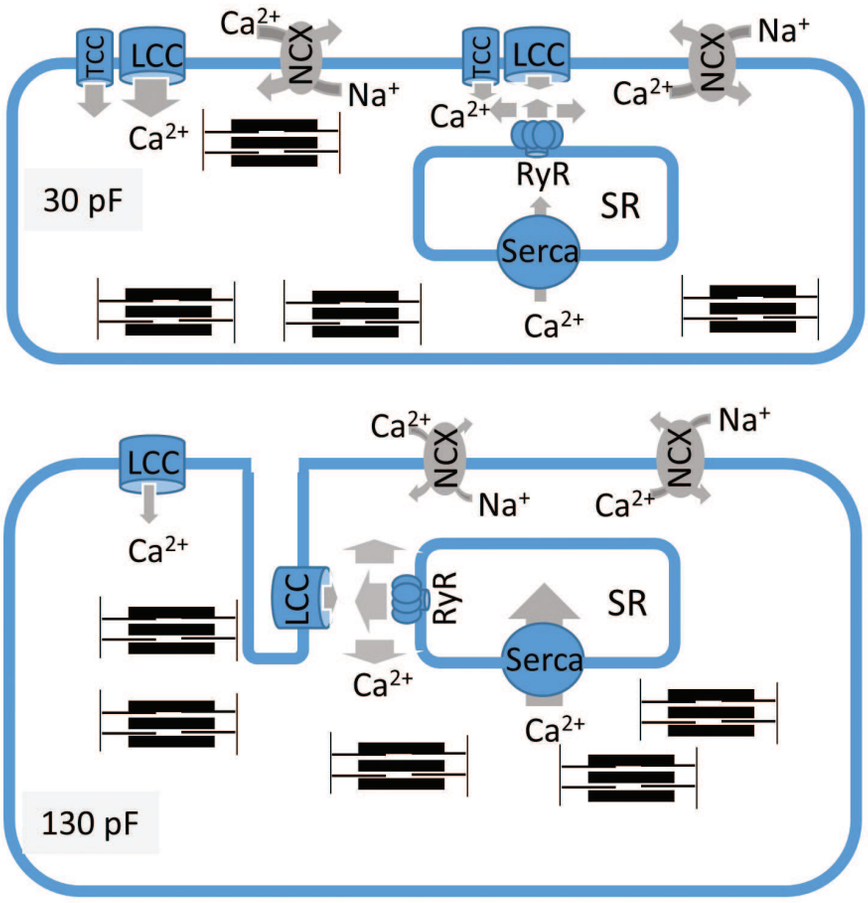

Figure 2. A simplified scheme about excitation-contraction coupling of the zebrafish ventricle (top) in comparison to that of the human ventricle (bottom). Main influx and efflux pathways of Ca^{2+} during contraction and relaxation of the ventricular myocyte. LCC, L-type Ca^{2+} channel; TCC, T-type Ca^{2+} channel; NCX, Na^+-Ca^{2+} exchange; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; Serca, sarco-endoplasmic reticulum Ca^{2+}-ATPase.

cytosolic Ca^{2+} transient resulting in monophasic voltage dependence of cell shortening and Ca^{2+} transients [51]. Sarcolemmal Ca^{2+} influx via I_{CaL} and I_{CaT} during a single twitch is almost 130 μM L^{-1} from which about 32% occurs through T-type Ca^{2+} channels [36]. These differences in Ca^{2+} handling are associated with 71% lower expression of ryanodine receptors in the zebrafish ventricle in comparison to mammalian (rabbit) ventricle, whereas little differences exist in the SR Ca^{2+} content [48]. Ca^{2+} sensitivity of ryanodine receptors of the fish heart is often low in comparison to that of mammalian cardiac ryanodine receptors [53], which might also contribute to the small SR Ca^{2+} release in zebrafish cardiomyocytes [48]. Overall, the main differences in e-c coupling between zebrafish and human ventricular myocytes are the smaller role of intracellular Ca^{2+} stores of the SR, the presence of large I_{CaT} and the absence of T-tubuli in the zebrafish myocytes.

5. Implications for the use of zebrafish in drug screening

The use of animal models for studies of human cardiac electrophysiology is based on the similarity of animal and human hearts concerning ion current densities, ion channel compositions and mechanisms of ion channel regulation by rate changes and autonomic nervous system agonists [54, 55]. However, electrophysiological properties of cardiac myocytes are species-specific and significantly different even between mammalian species (e.g. human vs. dog, rabbit and guinea pig) [54, 55]. Therefore, it is necessary to consider, whether the noticed differences in electrophysiology between zebrafish and human ventricular myocyte might affect the status of zebrafish as a model for drug screening and safety pharmacology. In this respect, quantitative differences in repolarizing currents between model and human hearts are regarded significant [54, 55]. Since ion channel isoforms often differ concerning activation and inactivation kinetics, voltage-dependence and drug affinity [39, 43, 56–58], isoform-composition of the expressed ion channels is also expected to be important for drug screening.

The human erg (KCNH2) channel is known for its propensity of being blocked by wide variety of small molecules, which may lead to AP prolongation and lethal cardiac arrhythmias. Therefore, preclinical drug screening procedures aim to assess drug-induced inhibition of I_{Kr} and prolongation of AP and QTc interval of electrocardiogram in order to remove proarrhythmic molecules from the drug development programs. However, drugs which inhibit I_{Kr} do not always produce QT prolongation due to simultaneous inhibition of the depolarizing I_{Na} and I_{Ca} currents, or since other ion channels provide a repolarization reserve to compensate for I_{Kr} inhibition. Proarrhythmic effects appear only when the drug changes the balance between inward and outward currents. To improve preclinical drug screening, the consortium of international stakeholders has recently launched an initiative called Comprehensive in-vitro Proarrhythmia Assay (CiPA). One of the central tenets of this initiative is that drug molecules are tested *in vitro* against multiple ion channels. Indeed, recent studies have indicated that the assessment of drug affinity toward multiple ionic targets improves the prediction of proarrhythmia risk in comparison to the sole I_{Kr} analysis [59, 60]. For example, if the outward I_{Kr} and inward I_{CaL} are inhibited with a similar IC_{50} value (e.g. in the case of verapamil), the proarrhythmia risk is low.

Potential confounding factors in the applicability of zebrafish as a model is the presence of large I_{CaT} and the putative absence of I_{Ks} and I_{to}. Since different channel isoforms of the same

subfamily may have different electrophysiological properties and drug affinities, the erg (human KCNH2 vs. zebrafish KCNH6) and Kir2 (human Kir2.1 vs. zebrafish Kir2.4) channel isoform compositions may be also important. Zebrafish might be a useful high-throughput drug screening platform with the advantages of both phenotype screening, if it fulfils the qualifications of the CiPA procedure. Therefore, the calibration routine of the CiPA initiative should be conducted on zebrafish cardiac myocytes [61]. This routine involves key cardiac ion channels (I_{Kr}, I_{CaL}, I_{Na}, I_{to}, I_{Ks} and I_{K1}), which should be examined under standardized voltage-clamp conditions for inhibition potency (IC_{50}) of 12 selected drugs of the "minimally acceptable" dataset [61]. These compounds are categorized into high, intermediate and low risk of torsades de pointes arrhythmia according to their currently known properties. The list of target channels should also include I_{CaT} in the zebrafish.

One more factor that may be important in regard to proarrhythmia potency of zebrafish heart is the e-c coupling of ventricular myocytes. Factors recognized as significant causes of cardiac arrhythmias in mammals include APs that are too long or too short. If APs are abnormally long, early afterdepolarizations during the AP plateau (Phase 2 or 3) may be provoked by reactivation of Ca^{2+} or Na^{+} currents in the voltage "window," where all Ca^{2+} and Na^{+} channels have not yet been inactivated and can be reactivated. In addition, early afterdepolarizations are promoted by spontaneous Ca^{2+} release from the SR that activates inward current via the reverse mode operation of Na^{+}-Ca^{2+} exchange [62]. APs that are too short can predispose the heart to delayed afterdepolarizations, which occur in early diastole (Phase 4), when spontaneous Ca^{2+} releases from the SR activate the inward Na^{+}-Ca^{2+}-exchange current. Afterdepolarizations may depolarize membrane potential to the AP threshold and induce extra systoles (triggered activity). Generation of delayed afterdepolarizations requires spontaneous Ca^{2+} release from the SR, which subsequently activates Ca^{2+} efflux via Na^{+}-Ca^{2+} exchange and membrane depolarization to the AP threshold [63]. In zebrafish heart, SR makes only a minor contribution to cardiac e-c coupling, possibly due to the low Ca^{2+} sensitivity of the ryanodine receptors. The relative independence of fish heart contraction from Ca^{2+}-induced Ca^{2+} release is expected to make the zebrafish heart relatively resistant against early and delayed afterdepolarizations and therefore less suitable as an arrhythmia model [18].

The similarities between human and zebrafish cardiac electrophysiology are often emphasized, while the differences are overlooked or neglected. However, it may be that those physiological functions and electrophysiological properties that are unique to the zebrafish heart are the most useful features for cardiac research. Perhaps the most spectacular example is the exceptional regeneration power of the zebrafish heart, which may reveal to us the molecular underpinnings needed to heal the damaged human heart [64]. Similarly, the exceptionally strong expression of the I_{CaT} in the zebrafish ventricle may provide a test system to examine the role of T-type Ca^{2+} channels in e-c coupling and its significance as a drug target. In the human heart, I_{CaT} is re-expressed and T-tubuli are lost, when the heart is subjected to pathological stressors that induce hypertrophy and failure [65–67]. Thus, zebrafish heart could be a "natural" model for testing proarrhythmic propensity of the drugs in the diseased heart. Structural (small cell size, absence of T-tubuli) and functional characteristics (minor role of SR in e-c coupling, presence of I_{CaT}) of the zebrafish ventricle are more like those of fetal or neonatal mammalian heart than those of the adult human heart [68, 69]. Therefore, the zebrafish heart might be a better model for fetal/neonatal and diseased human heart than for the adult human heart in drug screening.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] Kalueff AV, Echevarria DJ, Stewart AM. Gaining translational momentum: More zebrafish models for neuroscience. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2014;**55**:1-6

[2] Briggs JP. The zebrafish: A new model organism for integrative physiology. The American Journal of Physiology. 2002;**282**:R3-R9

[3] Chakraborty C, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: A complete animal model for in vivo drug discovery and development. Current Drug Metabolism. 2009; **10**(2):116-124

[4] Parng C, Seng WL, Semino C, McGrath P. Zebrafish: A preclinical model for drug screening. Assay and Drug Development Technologies. 2002;**1**(1):41-48

[5] MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. Nature Reviews Drug Discovery. 2015;**14**(10):721-731

[6] Qi M, Chen Y. Zebrafish as a model for cardiac development and diseases. Human Genetics & Embryology. 2015;**2015**

[7] 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 (Oxford, England). 2010 Jul 1;**199**(3):257-276

[8] 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. 2010 Jan;**48**(1):161-171

[9] Arnaout R, Ferrer T, Huisken J, Spitzer K, Stainier DY, Tristani-Firouzi M, et al. Zebrafish model for human long QT syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2007 Jul 3;**104**(27):11316-11321

[10] 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. 2003;**193**(3):370-382

[11] Milan DJ, Peterson TA, Ruskin JN, Peterson RT, MacRae CA. Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. Circulation. 2003;**107**(10):1355-1358

[12] Mittelstadt SW, Hemenway CL, Craig MP, Hove JR. Evaluation of zebrafish embryos as a model for assessing inhibition of hERG. Journal of Pharmacological and Toxicological Methods. 2008 Mar-Apr;**57**(2):100-105

[13] Tsai CT, Wu CK, Chiang FT, Tseng CD, Lee JK, Yu CC, et al. In-vitro recording of adult zebrafish heart electrocardiogram—A platform for pharmacological testing. Clinica Chimica Acta. 2011;**412**(21-22):1963-1967

[14] Cavero I, Guillon JM, Ballet V, Clements M, Gerbeau JF, Holzgrefe H. Comprehensive in vitro Proarrhythmia Assay (CiPA): Pending issues for successful validation and implementation. Journal of Pharmacological and Toxicological Methods. 2016;**81**:21-36

[15] Vicente J, Stockbridge N, Strauss DG. Evolving regulatory paradigm for proarrhythmic risk assessment for new drugs. Journal of Electrocardiology. 2016;**49**(6):837-842

[16] Crumb WJ, Vicente J, Johannesen L, Strauss DG. An evaluation of 30 clinical drugs against the comprehensive in vitro proarrhythmia assay (CiPA) proposed ion channel panel. Journal of Pharmacological and Toxicological Methods. 2016;**81**:251-262

[17] Genge CE, Lin E, Lee L, Sheng XY, Rayani K, Gunawan M, et al. The zebrafish heart as a model of mammalian cardiac function. Reviews of Physiology, Biochemistry and Pharmacology. 2016;**171**:99-136

[18] Verkerk AO, Remme CA. Zebrafish: A novel research tool for cardiac (patho)electrophysiology and ion channel disorders. Frontiers in Physiology. 2012 Jul 10;**3**:255

[19] Vornanen M, Hassinen M. Zebrafish heart as a model for human cardiac electrophysiology. Channels. 2016;**10**:101-110

[20] 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. 2008 Sep 12;**374**(1):143-146

[21] Vornanen M. Electrical excitability of the fish heart and its autonomic regulation. In: Gamperl KA, Gillis TE, Farrell PA, Brauner CJ, editors. Fish Physiology. The Cardiovascular Physiology. Morphology, Control and Function. London, UK: Elsevier; 2017. pp. 99-153

[22] Vornanen M. The temperature-dependence of electrical excitability of fish heart. The Journal of Experimental Biology. 2016;**219**:1941-1952

[23] Barrionuevo WR, Burggren WW. O_2 consumption and heart rate in developing zebrafish (*Danio rerio*): Influence of temperature and ambient O_2. The American Journal of Physiology. 1999;**276**:R505-R513

[24] Haverinen J, Vornanen M. Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. The American Journal of Physiology. 2007 Feb; **292**:R1023-R1032

[25] Haverinen J, Vornanen M. Temperature acclimation modifies Na^+ current in fish cardiac myocytes. The Journal of Experimental Biology. 2004;**207**:2823-2833

[26] Vornanen M, Ryökkynen A, Nurmi A. Temperature-dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. The American Journal of Physiology. 2002;**282**:R1191-R1199

[27] Grandi E, Pasqualini FS, Bers DM. A novel computational model of the human ventricular action potential and Ca transient. Journal of Molecular and Cellular Cardiology. 2010;**48**(1):112-121

[28] Chopra SS, Stroud DM, Watanabe H, Bennett JS, Burns CG, Wells KS, et al. Voltage-gated sodium channels are required for heart development in zebrafish. Circulation Research. 2010 Apr 30;**106**(8):1342-1350

[29] Haverinen J, Hassinen M, Vornanen M. Fish cardiac sodium channels are tetrodotoxin sensitive. Acta Physiologica. 2007;**191**(3):197-204

[30] Vornanen M, Hassinen M, Haverinen J. Tetrodotoxin sensitivity of the vertebrate cardiac Na^+ current. Marine Drugs. 2011;**9**:2409-2422

[31] Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacological Reviews. 2005 December 01;**57**(4):411-425

[32] McDonald TF, Pelzer S, Trautwein W, Pelzer DJ. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. Physiological Reviews. 1994; **74**(2):365-507

[33] Beuckelmann DJ. Contributions of Ca^{2+}-influx via the L-type Ca^{2+}-current and Ca^{2+}-release from the sarcoplasmic reticulum to $[Ca^{2+}]_i$-transients in human myocytes. Basic Research in Cardiology. 1997;**92**(Suppl. 1):105-110

[34] Mewes T, Ravens U. L-type calcium currents of human myocytes from ventricle of non-failing and failing hearts and from atrium. Journal of Molecular and Cellular Cardiology. 1994;**26**(10):1307-1320

[35] Gaborit N, Le Bouter S, Szuts V, Varro A, Escande D, Nattel S, et al. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. The Journal of Physiology. 2007 Jul 15;**582**(Pt 2):675-693

[36] Haverinen J, Dash SN, Hassinen M, Vornanen M. The large T-type Ca2+ current of the zebrafish ventricular myocytes is mainly produced by α1G ($Ca_v3.1$) channels. 2017. (submitted)

[37] Baker K, Warren KS, Yellen G, Fishman MC. Defective "pacemaker" current (I_h) in a zebrafish mutant with a slow heart rate. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**:4554-4559

[38] Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y. Inwardly rectifying potassium channels: Their structure, function and physiological role. Physiological Reviews. 2010;**90**:291-366

[39] Hassinen M, Haverinen J, Hardy ME, Shiels HA, Vornanen M. Inward rectifier potassium current (I_{K1}) and Kir2 composition of the zebrafish (*Danio rerio*) heart. Pflügers Archiv. 2015;**467**:2437-2446

[40] ten Tusscher KHWJ, Noble D, Noble PJ, Panfilov AV. A model for human ventricular tissue. The American Journal of Physiology. 2004;**286**(4):H1573-H1589

[41] Priebe L, Beuckelmann DJ. Simulation study of cellular electric properties in heart failure. Circulation Research. 1998 Jun 15;**82**(11):1206-1223

[42] Ravens U, Wettwer E. Ultra-rapid delayed rectifier channels: molecular basis and therapeutic implications. Cardiovascular Research. 2011 March 01;**89**(4):776-785

[43] Scholz EP, Niemer N, Hassel D, Zitron E, Burgers HF, Bloehs R, et al. Biophysical properties of zebrafish ether-a-go-go related gene potassium channels. Biochemical and Biophysical Research Communications. 2009 Apr 3;**381**(2):159-164

[44] Leong IUS, Skinner JR, Shelling AN, Love DR. Identification and expression analysis of kcnh2 genes in the zebrafish. Biochemical and Biophysical Research Communications. 2010 Jun 11;**396**(4):817-824

[45] Peal DS, Mills RW, Lynch SN, Mosley JM, Lim E, Ellinor PT, et al. Novel chemical suppressors of long QT syndrome identified by an in vivo functional screen. Circulation. 2011 January 04;**123**(1):23-30

[46] Gerdes AM, Capasso JM. Structural remodeling and mechanical dysfunction of cardiac myocytes in heart failure. Journal of Molecular and Cellular Cardiology. 1995; **27**(3):849-856

[47] Jopling C, Sleep E, Raya M, Martí M, Raya A, Belmonte JCI. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature. 2010; **464**(7288):606-609

[48] Bovo E, Dvornikov AV, Mazurek SR, de Tombe PP, Zima AV. Mechanisms of Ca^{2+} handling in zebrafish ventricular myocytes. Pflügers Archiv. 2013 Dec;**465**(12):1775-1784

[49] Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. The American Journal of Physiology. 1983;**245**:C1-C14

[50] Piacentino III V, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DM, et al. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. Circulation Research. 2003;**92**(6):651-658

[51] Zhang PC, Llach A, Sheng XY, Hove-Madsen L, Tibbits GF. Calcium handling in zebrafish ventricular myocytes. The American Journal of Physiology. 2011 Jan;**300**(1):R56-R66

[52] Beuckelmann DJ, Näbauer M, Erdmann E. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. Circulation. 1992 Mar; **85**(3):1046-1055

[53] Vornanen M. Temperature and Ca^{2+} dependence of [^{3}H]ryanodine binding in the burbot (*Lota lota* L.) heart. The American Journal of Physiology. 2006;**290**(2):R345-R351

[54] O'Hara T, Rudy Y. Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. The American Journal of Physiology. 2012 Mar 1;**302**(5):H1023-H1030

[55] Jost N, Virág L, Comtois P, Ördög B, Szuts V, Seprényi G, et al. Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. Journal of Physiology. 2013 September 01;**591**(17):4189-4206

[56] Wright SN, Wang SY, Kallen RG, Wang GK. Differences in steady-state inactivation between Na channel isoforms affect local anesthetic binding affinity. Biophysical Journal. 1997 Aug;**73**(2):779-788

[57] 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. 2008 Apr;**73**(4):1044-1051

[58] Hassinen M, Haverinen J, Vornanen M. Molecular basis and drug sensitivity of the delayed rectifier (I_{Kr}) in the fish heart. Comparative Biochemistry and Physiology. C. 2015 Oct-Nov;**176-177**:44-51

[59] Kramer J, Obejero-Paz CA, Myatt G, Kuryshev YA, Bruening-Wright A, Verducci JS, et al. MICE models: Superior to the HERG model in predicting Torsade de Pointes. Scientific Reports. 2013;**3**:2100

[60] Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovascular Research. 2003 Apr;**58**(1, 1):32-45

[61] Colatsky T, Fermini B, Gintant G, Pierson JB, Sager P, Sekino Y, et al. The comprehensive in vitro proarrhythmia assay (CiPA) initiative—Update on progress. Journal of Pharmacological and Toxicological Methods. 2016;**81**:15-20

[62] Choi BR, Burton F, Salama G. Cytosolic Ca^{2+} triggers early afterdepolarizations and Torsade de Pointes in rabbit hearts with type 2 long QT syndrome. The Journal of Physiology. 2002;**543**(2):615-631

[63] Kihara Y, Morgan JP. Intracellular calcium and ventricular fibrillation. Studies in the aequorin-loaded isovolumic ferret heart. Circulation Research. 1991;**68**(5):1378-89

[64] Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. Science. 2002;**298**: 2188-2190

[65] Martinez ML, Heredia MP, Delgado C. Expression of T-type Ca^{2+} channels in ventricular cells from hypertophied rat hearts. Journal of Molecular and Cellular Cardiology. 1999;**31**:1617-1625

[66] Izumi T, Kihara Y, Sarai N, Yoneda T, Iwanaga Y, Inagaki K, et al. Reinduction of T-type calcium channels by endothelin-1 in failing hearts in vivo and in adult rat ventricular myocytes in vitro. Circulation. 2003 Nov 18;**108**(20):2530-2535

[67] Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ, et al. Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. Proceedings of the National Academy of Sciences of the United States of America. 2009 Apr 21;**106**(16):6854-6859

[68] Qu Y, Boutjdir M. Gene expression of SERCA2a and L-and T-type Ca channels during human heart development. Pediatric Research. 2001;**50**(5):569-574

[69] Kim HD, Kim DJ, Lee IJ, Rah BJ, Sawa Y, Schaper J. Human fetal heart development after mid-term: Morphometry and ultrastructural study. Journal of Molecular and Cellular Cardiology. 1992;**24**(9):949-965

[70] Hu N, Yost HJ, Clark EB. Cardiac morphology and blood pressure in the adult zebrafish. Anatomical Record. 2001;**264**:1-12

[71] Guyton AC. Textbook of Medical Physiology. Narwalk, USA: Sounders Company; 1981. pp. 1-1074

[72] Li GR, Feng J, Yue L, Carrier M, Nattel S. Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. Circulation Research. 1996 Apr;**78**(4):689-696

[73] Wettwer E, Amos GJ, Posival H, Ravens U. Transient outward current in human ventricular myocytes of subepicardial and subendocardial origin. Circulation Research. 1994 Sep;**75**(3):473-482

[74] Nelson DA, Benson ES. On the structural continuities of the transverse tubular system of rabbit and human myocardial cells. The Journal of Cell Biology. 1963 Feb;**16**:297-313

[75] Bassani JWM, Bassani RA, Bers DM. Relaxation in rabbit and rat cardiac cells: Species-dependent differences in cellular mechanisms. The Journal of Physiology. 1994; **476**:279-293

[76] Bers DM. Cardiac excitation-contraction coupling. Nature. 2002;**415**:198-205

[77] Franz MR, Swerdlow CD, Liem LB, Schaefer J. Cycle length dependence of human action potential duration in vivo. Effects of single extrastimuli, sudden sustained rate acceleration and deceleration, and different steady-state frequencies. The Journal of Clinical Investigation. 1988 Sep;**82**(3):972-979

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