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The effective use of blebbistatin to study the action potential of cardiac pacemaker cells of zebrafish (*Danio rerio*) during incremental warming

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ABSTRACT

Blebbistatin potently inhibits actin-myosin interaction, preventing contractile activity of excitable cells including cardiac myocytes, despite electrical excitation of an action potential (AP). We collected intracellular microelectrode recordings of pacemaker cells located in the sinoatrial region (SAR) of the zebrafish heart at room temperature and during acute warming to investigate whether or not blebbistatin inhibition of contraction significantly alters pacemaker cell electrophysiology. Changes were evaluated based on 16 variables that characterized the AP waveform. None of these AP variables nor the spontaneous heart rate were significantly modified with the application of 10 μM blebbistatin when recordings were made at room temperature. Compared with the control group, the blebbistatin-treated group showed minor changes in the rate of spontaneous diastolic depolarization ($P = 0.027$) and the 50% and 80% repolarization ($P = 0.008$ and 0.010, respectively) in the 26°C–29°C temperature bin, but not at higher temperatures. These findings suggest that blebbistatin is an effective excitation-contraction uncoupler that does not appreciably affect APs generated in pacemaking cells of the SAR and can, therefore, be used in zebrafish cardiac studies.

1. Introduction

The zebrafish (*Danio rerio*) is a well-established vertebrate model to study cardiac development, its electrophysiology, and cardiac arrhythmia (Arnaout et al., 2007; Bakkers, 2011; Genge et al., 2016; Jensen et al., 2013; Liu and Stainier, 2012; Zon and Peterson, 2005). Yet, little is known about cardiac pacemaking mechanisms even though cardiac excitation and the propagation of electrical signals have been extensively investigated in the zebrafish (Lin et al., 2014, 2015; Stoyek et al., 2016). As in all teleosts, the zebrafish action potential (AP) is initiated in pacemaker cells located in the sinoatrial region (SAR) located around the margins of the sinoatrial valves (Capillo et al., 2021; Saito, 1973; Stoyek et al., 2015; Tessadori et al., 2012; Vornanen et al., 2010). However, direct electrophysiological recordings of pacemaker APs in isolated, spontaneously beating hearts are scarce in the teleost literature, in part due to the small number of pacemaker cells in the fish heart as well as the technical challenges associated with mechanical movement of this region of cardiac tissue when contraction occurs after the AP (Haverinen and Vornanen, 2007). Fluorescent dyes and calcium indicators have been used for high-resolution optical recordings of pacemaker APs, but these indicators, like microelectrode recordings, are also sensitive to motion artifacts that distort the optical signals (Fedorov et al., 2007; Li and Nattel, 2007; Swift et al., 2012). Reducing motion artifacts using pharmacological agents, such as 2,3-butanedione monoxime and cytochalasin D can improve optical and electrophysiological recordings. However, besides altering Ca^{2+} handling to inhibit contraction, these two agents alter ion channel kinetics and AP characteristics in a species-dependent manner (Jou et al., 2010; Kettlewell et al., 2004; Liu et al., 1993; Rueckschloss and Isenberg, 2001; Watanabe et al., 2001). Myocardial contractions can cause the intracellular recording microelectrodes to damage the cell membrane, resulting in depolarization of the membrane potential (ion leak) or cell membrane rupture (Arnaout et al., 2007; Chi et al., 2008; Milan et al., 2009).

Another option to facilitate intracellular recordings from pacemaker cells in isolated cardiac tissue is to stop myocyte contraction with blebbistatin, a highly specific myosin II inhibitor that readily crosses the cell membrane and leaves actin in a detached state (Kovács et al., 2004). While blebbistatin appears not to affect the mammalian cardiac AP, $[Ca^{2+}]_i$ transients, or cardiac electrical activity including ECG

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Abbreviations

AP	Action potential
ECF	Extracellular fluid
f_H	Heart rate
SAR	Sinoatrial region

parameters and refractory periods of atria or ventricles (Fedorov et al., 2007; Lou et al., 2012), its effects on the electrical properties of teleost pacemaking cells in general, and zebrafish specifically, are unknown. Therefore, the primary goal of this study was to uncouple excitation-contraction with blebbistatin in an isolated and reduced zebrafish heart preparation that was spontaneously beating. We sought to validate the use of this agent as an excitation-contraction uncoupler for intracellular recording of pacemaker activity and electrophysiological investigations of the zebrafish heart more broadly. We evaluated the effect of blebbistatin on the AP waveform recorded in adult zebrafish pacemaker cells at room temperature (20°C–23°C) and during acute warming. Our results suggest that blebbistatin uncouples cardiac excitation-contraction without significantly altering the AP waveform at room temperature and can, therefore, be reliably used in electrophysiological recordings of zebrafish pacemaker cells.

2. Materials and methods

2.1. Fish maintenance

Adult (12–18 months post-fertilization) AB wild-type zebrafish were obtained from a local pet store (Noah's Arc, Vancouver, BC, Canada). Fish were kept under standard laboratory conditions (28°C, 14:10 light: dark photoperiod) in recirculating aquaria, and were fed daily with tropical fish flakes (Nutrafin max; A6702, Rolf C. Hagen Inc). All experiments were carried out in accordance with the guidelines of the Canadian Council for Animal Care and the University of British Columbia Animal Care Committee (protocol: A18-0014).

2.2. Heart isolation and tissue preparation

Zebrafish were sacrificed by lethal overdose of buffered MS-222 (100 mg l^{-1}), followed by severing of the spine and pithing of the brain. Fish were transferred to a dissection dish at room temperature and submerged in extracellular fluid (ECF) solution containing (in mM): 124.1 NaCl, 5.1 KCl, 2.9 Na_2HPO_4, 1.9 $MgSO_4$–$7H_2O$, 1.4 $CaCl_2$–$2H_2O$, 11.9 $NaHCO_3$; pH 7.2 (Stoyek et al., 2015). The heart was exposed through a ventral midline incision and was excised as a block consisting of the ventricle, atrium, sinus venosus, and ducts of Cuvier. This was transferred to a recording chamber (1 ml) containing ECF solution at room temperature. The ventricle and the majority of the atrium distal to the SAR were removed, leaving a reduced preparation of the SAR, proximal atrium, and sinus venosus. This tissue was pinned to the Sylgard-coated bottom of the recording chamber. The endocardial surface was oriented upward to expose pacemaker cells for microelectrode access. The tissue was then left undisturbed for a 5 min recovery period during which forceful contractions of regular rhythm resumed.

2.3. Action potential recording

Microelectrodes were pulled from borosilicate glass capillaries (Sutter Instruments; BF-150-110-7.5, Novato, CA, USA) using a micropipette puller (Sutter Instruments; P-97; mean resistance 35 MΩ when filled with 3 M KCl). Manipulation of the microelectrode was performed using a Sutter micromanipulator (MPC-200; Sutter Instrument Novato, CA, USA) to impale SAR cells for recording transmembrane potential. Electrical zero was established by correcting the pipette offset before impaling a cell. Potentials were recorded in current-clamp mode with an Axopatch 200B amplifier and were digitized using a Digidata 1320 digitizer (Axon Instruments; San Jose, CA, USA), acquired with Axoscope software and stored on a computer hard drive for later analysis.

Pacemaker cells were identified by slow diastolic depolarization during phase 4 of the AP. Once a signal was obtained, the pipette was left undisturbed for the duration of the recording. Stable APs were recorded for a minimum of 20 s before the micropipette was removed from the cell and the electrical zero was verified. APs were deemed stable when the maximum hyperpolarization voltage and AP threshold potential were consistent over consecutive beats without drifting toward 0 mV, and the amplitude of consecutive APs did not differ. The bath solution was then replaced with fresh saline containing 10 μM blebbistatin (Cayman Chemicals catalog #13013, Burlington, ON, Canada). Blebbistatin was dissolved in DMSO (10 mM stock solution) and stored at −20°C until used. On the day of the experiment, this stock solution was then diluted with room temperature ECF (final bath concentration 10 μM) and mixed with a vortex mixer for 30 s before being applied to the preparation in a stop-bath mode. Tissue was left undisturbed until the complete cession of contraction before APs were recorded from pacemaker cells. After application of blebbistatin, APs could easily be recorded continuously from individual pacemaker cells for over 1 min without signal decay. To avoid photoinactivation of blebbistatin by ambient light during these experiments, the recording chamber containing blebbistatin was protected by covering it with an aluminum dome.

2.4. Action potential recording with increasing temperature

Pacemaker APs were recorded before (control) and during blebbistatin exposure with the bath at room temperature (20°C–23°C). Bath temperature was then increased over the experimental temperature range (23°C–33°C) by progressively heating in discrete steps (1°C every 5 min) using an in-line heater attached to a water jacket (Warner Instruments, Hamden, CT, USA). APs were recorded from different pacemaker cells at each temperature setting before and during blebbistatin exposure. The effect of blebbistatin was evaluated over the 20°C–33°C temperature range to include room temperature (20°C–23°C) commonly used for ex vivo tissue and cellular investigation, common zebrafish rearing temperature (~28°C) and the upper cardiac thermal limit of 33°C (Marchant and Farrell, 2019).

2.5. Data analysis

Stored digital records of APs were analyzed offline using Clampfit software (Axon Instruments). The quality of the recordings was first verified: any recordings with a spontaneous AP firing rate lower than 50 beats min^{-1} or with a baseline that depolarized over time were excluded from the analysis. Mean values were calculated from six consecutive APs per recording, and 16 variables were extracted from each AP as shown in Fig. 1. Temperature bins were used to parse data into a room temperature group (20°C–23°C) and groups at three elevated temperature intervals (23°C–26°C, 26°C–29°C, and 29°C–33°C) during the acute warming protocol. Each temperature bin contained data pooled from at least three fish. References to the term "rate" indicate the change of voltage per unit time (mV ms^{-1}) of measured membrane potentials, with the exception of heart rate (f_H; beats min^{-1}).

2.6. Statistical analysis

The effects of blebbistatin on the pacemaker AP variables at room temperature were determined by comparing variable means before (control) and during blebbistatin treatment.

At room temperature, blebbistatin effects on the pacemaker AP variables were determined by comparing control values before and

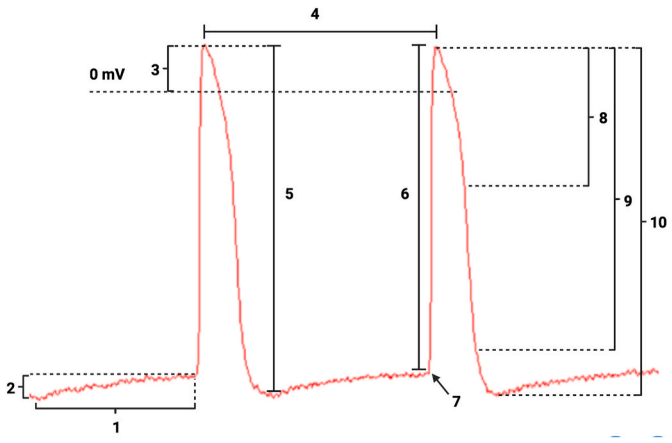

Fig. 1. A schematic protocol of action potential (AP) variable extraction from raw AP traces recorded from pacemaker cells. Numbers correspond to the following AP variables: 1, spontaneous depolarization duration (ms); 2, spontaneous depolarization potential (mV); 3, overshoot potential (mV); 4, beat to beat period (ms; heart rate in beats min^{-1} was calculated as the inverse of the period); 5, AP amplitude (mV); 6, depolarization potential (mV); depolarization duration was taken as time from threshold to peak depolarization (ms); 7, threshold potential (mV); 8, 9, 10: total repolarization potential (10) was the measured difference between peak AP potential and the maximum hyperpolarization potential (mV). 50% AP repolarization potential (8) was calculated as half the total repolarization potential (mV) and 50% AP duration was taken as the time between this potential and the equivalent point on the depolarizing phase of the AP. 90% AP repolarization potential (9, mV) was that at 90% of the total repolarization potential; 90% repolarization duration (ms) was the time (ms) between this potential and the equivalent point on the depolarizing phase of the AP.

treatment values during blebbistatin exposure. For each temperature bin, data were first tested for normality (Kolmogorov-Smirnov test) then analyzed for statistical significance between control and treatment means with an unpaired two-tailed *t*-test. Data across the thermal range were compared using linear regression. Temperature matched data points were obtained using the linear regression equation of the measured data points to predict the values that were not recorded at any given temperature. The predicted values were plotted with the recorded values and a linear regression analysis was performed. Statistical differences in the mean values for each temperature bin were then determined using a one-way ANOVA with a Bonferroni multiple comparison test, thereby reducing the risk of type 2 statistical error.

3. Results

Perfusion with 10 μM blebbistatin stopped cardiac contractions after 5–20 min in all reduced heart preparations. By removing the contraction, the complete elimination of motion artifacts enabled longer and more stable AP recordings.

At room temperature, blebbistatin did not significantly affect the AP firing rate (i.e., f_H) of pacemaker cells or the rates of spontaneous depolarization (Table 1), rate of depolarization, or rate of repolarization (Fig. 2), nor the voltage (diastolic depolarization amplitude, AP amplitude, or repolarization amplitude) or time (diastolic duration, depolarization duration, or repolarization duration) variables used to calculate these rates (Table 1). Furthermore, there was no difference in any of the voltage variables (AP threshold potential, overshoot potential, maximum hyperpolarization potential; Table 1) that might indicate a voltage shift or a change in the AP waveform.

Acute warming accelerated f_H as well as the rate-dependent AP variables, including the rates of spontaneous depolarization, AP depolarization, and AP repolarization (Fig. 2). Blebbistatin did not significantly affect the majority of the AP variables within each temperature bin, although minor changes in the rate of spontaneous diastolic depolarization ($P = 0.027$; Fig. 2) and the 50% and 80% repolarization ($P = 0.008$ and 0.010, respectively) in the 26°C–29°C temperature bin were observed, but not at higher temperatures (Table 1). A linear regression analysis across the entire thermal range revealed no significant differences for any of the AP variables before and after blebbistatin treatment. Also, confidence intervals for the linear regressions completely overlapped for the rates of spontaneous, AP depolarization, and AP repolarization depolarization in the two groups (Fig. 2). Temperature did not significantly affect the voltage-dependent variables between control and blebbistatin-treated heart preparations at any temperature, with the exception of the overshoot potential, which was significantly different in blebbistatin-treated preparations compared to control between 23°C and 29°C (Table 1). As pacemaker action potentials were not recorded at all temperature for both treatment groups, the missing values were predicated using the equation of the linear regressions for each of the variables. Linear regression analysis of the temperature matched datasets showed that there was no significant difference between the control and blebbistatin-treated pacemaker cells for each of the variables (Fig. 3).

4. Discussion

We investigated the effect of blebbistatin, a myosin II uncoupler, on the AP of adult zebrafish cardiac pacemaker cells located in the SAR. Uncoupling excitation-contraction with blebbistatin eliminated motion artifacts caused by myocyte contraction, rendered pacemaker APs more stable during recording, and prolonged cellular recordings. This is the first study to test for the effects of blebbistatin on in situ pacemaker cells of any adult fish species, although embryonic zebrafish have been studied previously (Jou et al., 2010). Our reduced and spontaneously beating preparation preserved intercellular communication and the electrical continuity between the pacemaker cells and the adjacent atrial tissue. These connections are important for the initiation of rhythmical pacemaker discharge to establish the beating rate of cardiac myocytes (Bakker et al., 2010; Masahito et al., 1994; Shiels, 2017).

Blebbistatin had no significant effect on any of the AP variables recorded at room temperature, including f_H. Previous studies using isolated heart preparation of adult zebrafish at room temperature have reported similar f_H (Stoyek et al., 2016). Therefore, blebbistatin likely does not alter the electrophysiological properties or the waveform of pacemaker cell APs at room temperature. Furthermore, the lack of major changes in transmembrane potentials and AP properties in our study during exposure to blebbistatin suggests that ion channel dynamics that generate the pacemaker AP are not significantly affected by blebbistatin. Similarly, AP variables during acute warming of the isolated zebrafish hearts up to 33°C were largely unchanged by blebbistatin. The only exceptions were the rate of spontaneous diastolic depolarization and the 50% and 80% repolarization in the 26°–28.9°C temperature bin, which likely occurred due to the lower number of APs obtained from fewer fish at this temperature bin than other temperature bins. Even so, no effect of blebbistatin occurred at a higher temperature (29°C–33°C). Consequently, the present study with adult zebrafish is entirely consistent with previous studies in rats, rabbits (Fedorov et al., 2007; Lou et al., 2012), and embryonic zebrafish (Jou et al., 2010), where blebbistatin had no significant effect on ion channel dynamics, calcium handling, or cardiac electrophysiological variables, such as ECG variables, atrial and ventricular effective refractory periods, or atrial and ventricular activation patterns, regardless of the concentration tested (1, 5 or 10 μM). However, given the considerable interspecific and intraspecific variability in the electrophysiological responses to blebbistatin reported in the literature among mammals (Brack et al., 2013; Fedorov et al., 2007) care should be used when extrapolating our data for zebrafish to other fish species.

Blebbistatin effectively uncoupled cardiac excitation-contraction in embryonic (48 h post-fertilization) zebrafish hearts (Jou et al., 2010)

Table 1

Pairwise comparison of AP variables from control and blebbistatin-treated cells with increasing temperature. Within each temperature group, control (pre-blebbistatin) and treated means were tested for statistical significance using an unpaired two-tailed *t*-test. Statistically significant differences between means at room temperature (RT) and elevated temperatures (T1, T2, and T3) were determined by one-way ANOVA; where significant f-values occurred, differences between pairs of means were determined using post hoc Bonferroni multiple-means comparison tests; $P \leq 0.05$ was taken as the level of significance. Significant differences are indicated in **bold** text. The number of hearts for data in each row is indicated as (n =). f_H (heart rate), depol (depolarization), hyperpol (hyperpolarization), repol (repolarization), RT (room temperature.

Temperature (°C)	Treatment	f_H (beats min^{-1})	AP period (ms)	AP amplitude (mV)	Overshoot potential (mV)	Maximum hyperpol potential (mV)	AP threshold potential (mV)	AP depol potential (mV)	AP depol time (ms)	Rate of depol (mV/ms)	AP repol time (ms)	Rate of repol (mV/ms)	AP duration 50% (ms)	AP duration 80% (ms)	Diastolic depol amplitude (mV)	Diastolic depol duration (ms)	Rate of diastolic depol (mV/ms)
20°C - 22.9°C Room temperature (RT)	**Control (n = 12)**	73	859	50	8	−43	−36	42	46	0.94	162	0.33	76	94	8	658	0.01
	SD	15	177	11	3	9	5	9	14	0.21	34	0.06	19	22	3	168	0.005
	Blebbistatin (n = 12)	75	821	55	9	−46	−38	47	45	1.08	165	0.34	69	88	8	611	0.01
	SD	16	147	7	4	5	6	7	12	0.24	27	0.07	12	14	3	159	0.006
	***P*-Value**	>0.999	0.998	0.402	0.573	0.584	0.573	0.298	0.966	0.150	0.656	>0.999	0.370	0.500	>0.999	0.887	>0.999
23°C -25.9°C (T1)	**Control (n = 9)**	84	741	48	5	−44	−32	39	36	1.19	143	0.38	66	80	10	563	0.02
	SD	17	130	11	3	11	10	11	10	0.39	56	0.13	26	30	5	106	0.012
	Blebbistatin (n = 9)	95	663	52	5	−45	−35	40	35	1.19	116	0.44	51	63	10	513	0.02
	SD	35	176	13	3	12	10	12	8	0.20	29	0.08	14	11	4	181	0.013
	***P*-Value**	0.993	0.951	0.828	0.826	0.831	0.826	0.910	0.735	0.963	0.216	0.876	0.153	0.121	>0.999	0.910	0.983
Blebbistatin RT *vs.* T1	***P*-Value**	0.781	0.253	>0.999	**0.042**	>0.999	>0.999	0.261	**0.019**	>0.999	**<0.001**	0.985	0.112	**0.008**	0.937	0.346	**0.036**
26°C - 28.9°C (T2)	**Control (n = 6)**	143	464	48	6	−38	−31	34	21	1.86	102	0.42	72	80	10	365	0.03
	SD	54	143	15	4	7	8	8	14	0.60	14	0.11	14	15	4	129	0.004
	Blebbistatin (n = 7)	136	483	45	4	−47	−41	45	29	1.47	96	0.51	36	49	6	348	0.02
	SD	46	149	11	4	12	10	11	11	0.57	13	0.11	24	21	3	154	0.004
	***P*-Value**	>0.999	>0.999	0.255	0.410	0.143	0.410	0.063	0.087	0.244	0.284	0.812	**0.008**	**0.010**	0.397	0.999	0.370
Blebbistatin RT *vs.* T2	***P*-Value**	**<0.001**	**<0.001**	0.114	**0.010**	>0.999	>0.999	>0.999	**<0.001**	**0.063**	**<0.001**	**0.007**	**0.003**	**<0.001**	0.870	**0.006**	0.212
29°C – 33°C (T3)	**Control (n = 9)**	160	404	50	8	−46	−38	42	27	1.58	73	0.55	47	52	8	314	0.03
	SD	33	92	11	4	13	15	12	9	0.42	15	0.10	11	11	4	75	0.016
	Blebbistatin (n = 6)	136	441	49	6	−42	−37	45	21	1.85	98	0.47	43	54	7	322	0.02
	SD	25	68	5	3	8	10	8	7	0.31	18	0.09	30	27	2	60	0.005
	***P*-Value**	0.778	0.999	0.289	0.333	0.530	0.333	0.519	0.384	0.212	0.540	0.798	0.670	0.812	0.999	>0.999	>0.999
Blebbistatin RT *vs.* T3	***P*-Value**	**<0.001**	**<0.001**	0.785	0.203	>0.999	>0.999	>0.999	**<0.001**	**<0.001**	**<0.001**	**0.028**	**0.026**	**0.002**	>0.999	**0.005**	**0.017**

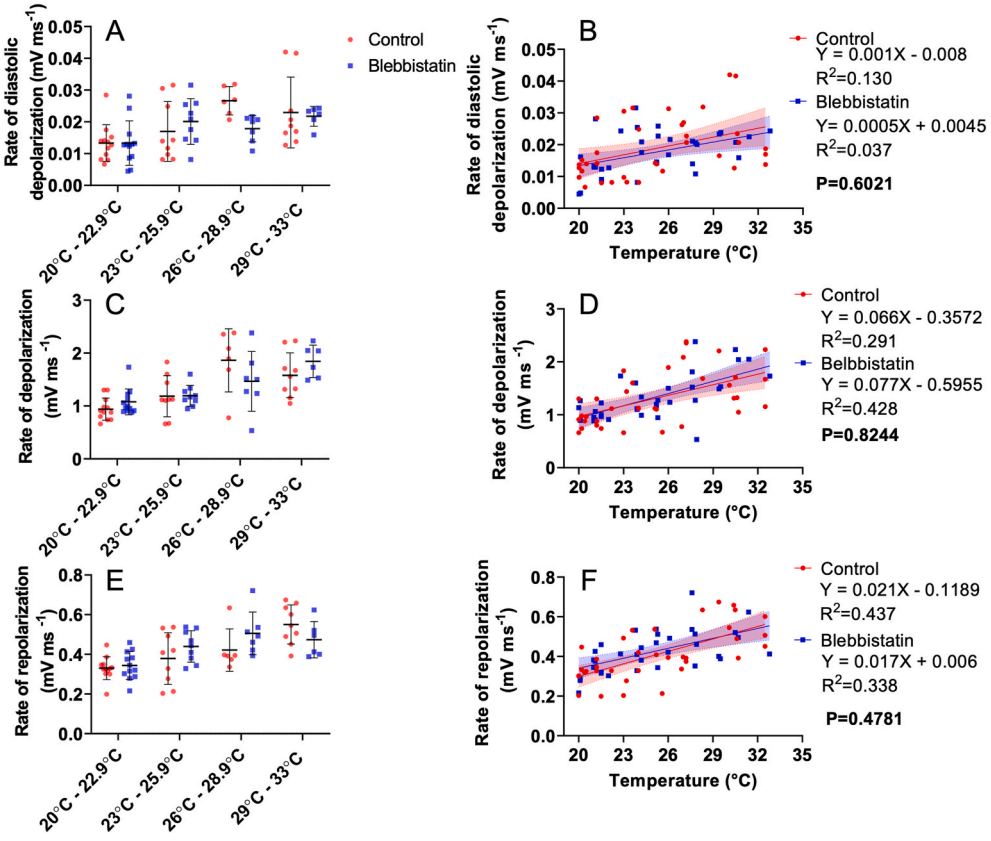

Fig. 2. The effect of blebbistatin and temperature on the rate of diastolic depolarization (A, B), the rate of depolarization (C, D), and the rate of repolarization (E, F). Pairwise comparison of AP variables within different temperature bins is illustrated in panels A, C, and E. Linear regression analysis over the range of warming is shown in panels B, D, and F. Data points represent individuals. Mean data values are presented with their respective standard deviations. Statistical significance of pairwise comparisons was determined using a *t*-test ($P \leq 0.05$).

without significant changing the AP waveform or the characteristics of atrial and ventricular spontaneous APs (i.e., cycle length, maximum diastolic potential, maximum upstroke velocity, and the AP duration) and regardless of the blebbistatin concentration (1, 5, or 10 μM). The embryonic and adult waveforms differ somewhat for these two studies. The embryonic f_H and AP repolarization rates were faster than in the adult zebrafish of the present study and the embryonic maximum diastolic potential was more negative (−56 mV compared with −43 mV to −46 mV in adults). Therefore, blebbistatin does not appear to adversely affect the electrophysiological properties of the heart cells of either adult or embryonic zebrafish.

In mice, blebbistatin did not significantly affect AP duration, ventricular activation, or conduction velocity, but it reduced myosin Ca^{2+} sensitivity and arrhythmia susceptibility (Baudenbacher et al., 2008). In the rat, blebbistatin was reported not to have any effect on the electrophysiological properties of the Ca^{2+} transient or the AP (i.e., amplitude, duration, upstroke velocity, and time of decay) heart (Fedorov et al., 2007), nor modify Ca^{2+} handling in isolated rat myocytes (Farman et al., 2008). Moreover, blebbistatin has been used to immobilize human hearts, with no reported effect on the AP (Fedorov et al., 2010, 2011; Glukhov et al., 2010).

While the electrophysiological properties of most mammalian hearts (Fedorov et al., 2007; Lou et al., 2012) are unaffected by blebbistatin, ventricular cells of New Zealand white rabbits are an important exception (Brack et al., 2013). Blebbistatin (5 μM) reportedly prolonged the left ventricular apical and basal monophasic action potential duration in Langendorff preparations, increased the maximal slope of restitution while significantly reducing the heart's susceptibility to ventricular fibrillation (Brack et al., 2013), and prolonged the AP duration (Kappadan et al., 2020). Similarly, blebbistatin (10 μM) prolonged the AP duration in Langendorff-perfused pig hearts (Lee et al., 2019), but time-paired control was lacking. The species-specific effects of blebbistatin therefore must be considered in designing further experiments.

A difficulty with studying intact hearts is that uncoupling of excitation-contraction imposes different consequences on the metabolic demand of each tissue which might shorten the AP due to activation of ATP-sensitive potassium channels caused by elevated ATP concentrations (Garrott et al., 2017; Lee et al., 2019). In rats, high blebbistatin concentrations (10 – 100 μM) have been reported to disrupt intracellular calcium dynamics as spontaneous excitation and triggered activities (Kanlop and Sakai, 2010). However, these elevated concentrations may induce spontaneous excitation and triggered activities. Therefore, the effects of blebbistatin may be both species- and concentration-dependent and its potential effects on the components of the AP warrant further investigation. It is worth noting here that the intracellular mechanisms of blebbistatin action in the heart have not been completely established. In addition, high blebbistatin concentrations lead to crystallization which may alter the final concentration available at the tissue. However, once precipitates have formed, they do not readily go back into solution upon heating in unstirred solutions (Swift et al., 2012), maintaining a constant tissue concentration across warming profiles.

In the adult zebrafish heart, blebbistatin effectively uncouples excitation-contraction without significantly modifying any of the AP variables investigated across the thermal range of 20°C to 33°C. Blebbistatin is therefore a useful pharmacological agent for cardiac investigation involving motion-sensitive techniques including AP recordings of in situ pacemaker cells in ex vivo heart preparations.

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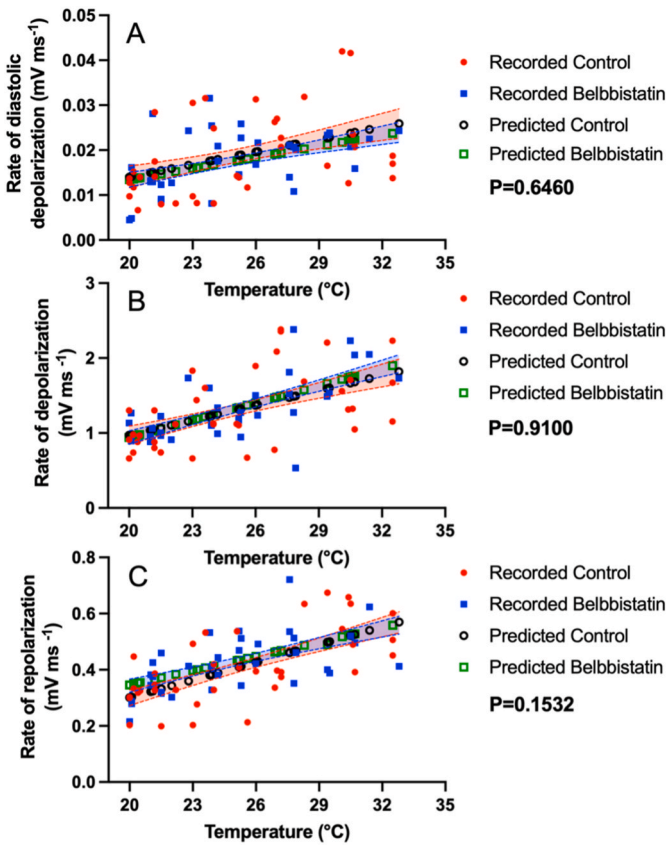

Fig. 3. Linear regression analysis of the effect of blebbistatin and temperature on the rate of diastolic depolarization (A), the rate of depolarization (B), and the rate of repolarization (C). Data points represent individuals where solid-colored points represent recorded measures and open data points represent predicted values.

Summary statement

Blebbistatin inhibits cardiac contraction without significantly modifying the sinoatrial pacemaker action potentials in the zebrafish.

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Credit Author Statement

JLM and FMS designed and performed the experiments. JLM carried out the data analysis and wrote the manuscript. FMS and APF provided edits.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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