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REVIEW

The temperature dependence of electrical excitability in fish hearts

Matti Vornanen*

ABSTRACT

Environmental temperature has pervasive effects on the rate of life processes in ectothermic animals. Animal performance is affected by temperature, but there are finite thermal limits for vital body functions, including contraction of the heart. This Review discusses the electrical excitation that initiates and controls the rate and rhythm of fish cardiac contraction and is therefore a central factor in the temperature-dependent modulation of fish cardiac function. The control of cardiac electrical excitability should be sensitive enough to respond to temperature changes but simultaneously robust enough to protect against cardiac arrhythmia; therefore, the thermal resilience and plasticity of electrical excitation are physiological qualities that may affect the ability of fishes to adjust to climate change. Acute changes in temperature alter the frequency of the heartbeat and the duration of atrial and ventricular action potentials (APs). Prolonged exposure to new thermal conditions induces compensatory changes in ion channel expression and function, which usually partially alleviate the direct effects of temperature on cardiac APs and heart rate. The most heat-sensitive molecular components contributing to the electrical excitation of the fish heart seem to be Na^+ channels, which may set the upper thermal limit for the cardiac excitability by compromising the initiation of the cardiac AP at high temperatures. In cardiac and other excitable cells, the different temperature dependencies of the outward K^+ current and inward Na^+ current may compromise electrical excitability at temperature extremes, a hypothesis termed the temperature-dependent depression of electrical excitation.

KEY WORDS: Electrical excitation, Action potential, Ion currents, Ion channels, Temperature acclimation, Temperature adaptation, Heat tolerance

Introduction

There are >33,000 extant fish species (http://www.fishbase.org/home.htm), all of which – with the exception of ∼30 species – are ectotherms (Dickson and Graham, 2004). They inhabit thermally diverse aquatic habitats and their thermal tolerances vary from −2.5°C to +44°C (Somero and DeVries, 1967; Bennett and Beitinger, 1997). Based on temperature tolerance limits, fishes can be roughly divided into two groups: thermal specialists (stenotherms; see Glossary) and thermal generalists (eurytherms; see Glossary), capable of survival at a narrow and wide range of temperatures, respectively. Northern temperate fishes usually survive at temperatures close to freezing, whereas their upper thermal tolerance limit is variable (Beitinger et al., 2000). Tropical species, such as the zebrafish (*Danio rerio*), do not tolerate freezing temperatures, but can survive briefly at temperatures that are similar to or higher (+40°C) than the body temperature of endotherms (Cortemeglia and Beitinger, 2005; López-Olmeda and Sánchez-Vázquez, 2011; Johnson et al., 2014). In many habitats, fishes experience marked temperature changes over different time scales. When swimming at different depths, crossing the thermocline (see Glossary) or exposed to diurnal temperature change in tidal pools or other shallow water bodies, the body temperature of fish may acutely change from a few degrees to 20°C over a time scale of minutes to hours (Gunter, 1941; Brill et al., 1999; Nakano and Iwama, 2002; Fangue and Bennett, 2003; Cooke et al., 2008; Caudill et al., 2013; Shiels et al., 2015). Many fishes experience more sustained temperature changes of similar (or greater) magnitude between seasons.

For ectothermic fishes, temperature is the prime abiotic factor that determines the metabolic rate of the animal and sets demands on blood circulation and cardiac function. Because cardiac output (CO; see Glossary), the volume of blood pumped by the heart within a unit of time, is the product of heart rate (f_H) and stroke volume, f_H has a direct effect on the pumping function of the heart. f_H is accelerated and decelerated by acute increases and decreases in water temperature, respectively, and by this means CO is adjusted to match temperature-dependent changes in metabolic rate (Brett, 1971; Barrionuevo and Burggren, 1999; Lefrançois and Claireaux, 2003). Indeed, f_H is the main factor in the temperature-dependent regulation of circulation in fishes (Steinhausen et al., 2008; Mendonça and Gamperl, 2010). Because ionic and molecular mechanisms of electrical excitation are, in principle, similar in stenothermic and eurythermic fishes (Lim et al., 2013), better knowledge of the basic mechanisms involved could help us to formulate unifying hypotheses about temperature-dependent changes in f_H and CO and the thermal responses of fishes. In this Review, I present an overview of current data on electrical excitation in fish hearts under different temperature regimes, with an emphasis on the role of crosstalk between the major voltage-gated Na^+ and K^+ ion currents of the cardiac sarcolemma (SL) in regulating the initiation and duration of the cardiac action potential (AP). Electrical excitation of the heart stimulates cardiac contraction; therefore, investigating cardiac excitability can provide important information about the mechanisms by which cardiac function is adapted to different thermal habitats, acclimates to regular seasonal temperature fluctuations and responds to acute temperature changes. Considering that global climate models predict increases in the frequency, severity and duration of temperature extremes in the near future (Rummukainen, 2012; Seneviratne et al., 2012), studies on the temperature dependence of electrical excitability should increase our understanding of the mechanisms that limit the survival and performance of ectotherms, and thereby help to identify possible 'winners and losers' of climate change (Somero, 2010).

Furthermore, findings from the investigation of the temperature dependence of electrical excitation of cardiac myocytes are not limited to the heart muscle. Because all excitable cells share the same key characteristics – i.e. the ability to produce propagating APs via the operation of voltage-gated ion channels (Hille, 2001) –

University of Eastern Finland, Department of Environmental and Biological Sciences, PO Box 111, Joensuu 80101, Finland.

*Author for correspondence (Matti.vornanen@uef.fi)

M.V., 0000-0003-0953-1425

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List of symbols and abbreviations

AP	action potential
APD	action potential duration
APD_{50}	action potential duration at 50% repolarization level
ECG	electrocardiogram
f_H	heart rate
I_{Ca}	Ca^{2+} current
I_{CaL}	L-type Ca^{2+} current
I_{CaT}	T-type Ca^{2+} current
I_h	pacemaker current
I_{K1}	inward rectifier K^+ current
I_{Kr}	rapid component of the delayed rectifier K^+ current
I_{Ks}	slow component of the delayed rectifier K^+ current
I_{Na}	Na^+ current
I_{To}	transient outward current
NCX	Na^+–Ca^{2+} exchanger
RMP	resting membrane potential
SL	sarcolemma
TDEE	temperature-dependent depression of electrical excitability
TP	threshold potential

Glossary

Bradycardia
Slowing of heart rate.
Break point temperature
The temperature at which the rate of function starts to decrease.
Cardiac output
The volume of blood pumped by the heart in a unit of time.
Critical depolarization
The size of depolarization needed to trigger an action potential.
Depolarization
Change of resting membrane potential towards more positive values.
Diastole
The part of the cardiac cycle during which the heart fills with blood.
Eurytherm
An animal able to live at a wide range of temperatures.
P wave
A wave on an ECG that is generated by atrial depolarization.
QRS complex
A waveform on an ECG that is generated by ventricular depolarization.
Repolarization reserve
Extra capacity for K^+ currents that is activated under increased demand for repolarization.
Stenotherm
An animal able to live only within a narrow range of temperatures.
Systole
The part of the cardiac cycle when the heart contracts.
Temperature compensation
Physiological adjustments that reduce temperature effects on the rate of body functions.
Threshold potential
The value of membrane potential at which an all-or-none action potential is generated.
Thermocline
A layer of sea or lake water in which temperature changes rapidly with depth.
T wave
A wave of an ECG that is generated by ventricular repolarization.

findings on the thermal responses of cardiac myocytes can inform us about the common principles of temperature effects on excitable membranes. These principles should be applicable to neurons, skeletal muscle fibers and smooth muscle cells. Moreover, studying the heart provides certain technical advantages in comparison with other excitable tissues. Recording of electrocardiograms (ECGs) represents a convenient and easy way to analyze thermal effects on cardiac excitability *in vivo*. ECGs provide information on the rate and rhythm of the cardiac pacemaker, the rate of impulse propagation between different parts of the heart, the duration of ventricular APs and possible disturbances in excitability (De Vera and Priede, 1991; Campbell et al., 2006; Yu et al., 2012; Badr et al., 2016). The underlying molecular mechanisms of excitation can then be studied with great accuracy in enzymatically isolated myocytes, which are available in large numbers from a single heart for patch-clamp experiments (Vornanen, 1997; Hove-Madsen and Tort, 1998). Studying the electrical excitation of the heart thus provides a means to test ideas about temperature effects on organ function at different levels of biological organization.

In this Review, I will first describe the generation of APs in the fish heart, before giving an overview of (1) ion channel function in response to acute temperature changes, (2) ion channel plasticity under chronic temperature changes and (3) examples of putative evolutionary adaptations of ion channel structure and function to temperature. Finally, (4) a testable hypothesis about temperature-dependent depression of electrical excitability is discussed, with the aim of stimulating further research on the thermal responses of electrically excitable tissues.

Electrical excitation of the heart

Excitable cells are defined as cells that contain voltage-gated ion channels, which enable them to generate and transmit APs in response to depolarizing stimuli. More specifically, electrical excitability of the heart can be described as the ease with which cardiac cells undergo sequential depolarization (see Glossary) and repolarization, electrical communication with adjacent cells and the propagation of APs over the cardiac chambers (Boyet and Jewell, 1981). The firing of an AP, a small (~100 mV) voltage change of the cardiac SL, triggers a transient increase in the concentration of intracellular free Ca^{2+} and sequential contraction of atrial and ventricular chambers (Bers, 2002; Vornanen et al., 2002a,b). Cardiac excitability determines the beating rate, rhythm and contractility of the heart, and is therefore regulated to adjust circulation to meet metabolic demands. For the well-being of the fish, cardiac excitability should be such that it prevents cardiac arrhythmias under all temperature regimes that the animal may encounter in its habitat.

Cardiac APs result from precisely timed opening and closing of voltage- and ligand-gated ion channels of the myocyte SL. Ion current densities and channel compositions show regional specialization in the heart, generating morphologically distinct and functionally different APs for each cardiac chamber (Fig. 1A). Pacemaker APs are characterized by a slow depolarization of membrane potential during diastole (see Glossary), which forms the basis for the spontaneous rhythm of the heart. In contrast, atrial and ventricular APs have a stable resting membrane potential (RMP) and a fast depolarization, characteristics needed to generate a fast-propagating excitation in the contracting myocardium. The atrial AP is shorter than the ventricular AP, which is consistent with the faster atrial contraction.

The atrial and ventricular APs

Five different phases (0–4) are recognized in the atrial and ventricular APs of vertebrate animals (Fozzard, 1977), although fish cardiac myocytes typically lack phase 1 (Fig. 1B) (Vornanen and Hassinen, 2016). In atrial and ventricular myocytes, APs start

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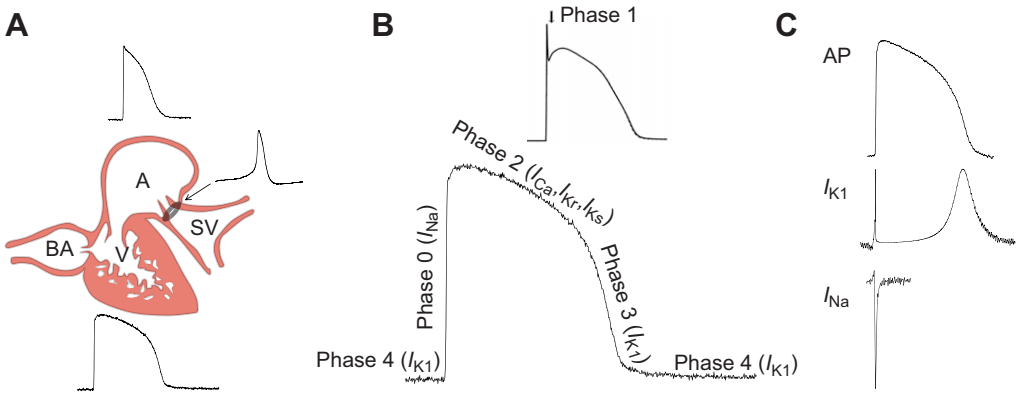

Fig. 1. Electrical excitation of the fish heart. (A) Schematic representation of the fish heart chambers and the characteristic action potentials (APs) of the sinoatrial pacemaker, atrium and ventricle. A, atrium; BA, bulbus arteriosus; SV, sinus venosus; V, ventricle. Sinoatrial pacemaker tissue is shown as a gray circle at the junction between the sinus venosus and the atrium. (B) Different phases of the fish ventricular AP. The main ion currents involved in shaping the AP waveform are shown in brackets for each phase. The upper part of the panel shows the human ventricular action potential with a clear phase 1 repolarization. Modified from Vornanen and Hassinen (2016). (C) The time course of the inward sodium current (I_{Na}) and the outward rectifier potassium current (I_{K1}) during the fish ventricular AP. The fast upstroke of the cardiac AP is produced by I_{Na} and it is opposed by a transient surge of the outward I_{K1} at the threshold potential of AP initiation. When the threshold potential is exceeded, the large I_{Na} overwhelms the smaller I_{K1}. I_{K1} is reactivated during the repolarization phase of the AP, whereby it strongly contributes to the rate of final phase-3 repolarization. I_{Na} was measured as a tetrodotoxin-sensitive current from a rainbow trout ventricular myocyte, and I_{K1} was simulated based on the inward rectification by the crucian carp Kir2 channels (Hassinen et al., 2008a,b).

and finish at a stable negative RMP (−70 to −90 mV). This period of RMP between successive APs is designated as phase 4. RMP is maintained by a small efflux of K^+ ions from the cell via the inward rectifier K^+ channels, which generate the inward rectifier K^+ current (I_{K1}). Phase 0 is the fast depolarization of atrial and ventricular myocytes from the RMP to slightly positive voltages (typically +10 to +40 mV) (Talo and Tirri, 1991; Vornanen, 1996; Molina et al., 2007; Haverinen and Vornanen, 2009; Galli et al., 2009; Ballesta et al., 2012). This rapid voltage change is produced by the opening of Na^+ channels, which generate the inward Na^+ current (I_{Na}). The density of I_{Na} is a major factor in determining the rate of AP propagation over atrial and ventricular myocardia. The phase 1 repolarization is generated by the transient outward current (I_{To}), which is mainly carried by K^+ ions; the absence of phase 1 from the AP of fish cardiac myocytes suggests that the channels generating I_{To} are not expressed in fish hearts. The long plateau phase 2 results from the balance between inward Ca^{2+} currents (I_{Ca}) and outward K^+ currents (I_K). The long duration of the cardiac AP (from several hundred milliseconds to several seconds in fish) is necessary for SL Ca^{2+} influx and cardiac contraction, and it prevents the heart from beating prematurely by delaying the recovery of Na^+ channels from the inactivated state. The final phase 3 repolarization is generated by various outward K^+ currents, particularly the rapid component of the delayed rectifier K^+ current (I_{Kr}) and the I_{K1}. In some fishes, the slow component of the delayed rectifier K^+ current (I_{Ks}) may be also involved (Hassinen et al., 2011).

The role of the pacemaker

In the fish heart, electrical excitation originates from the spontaneously active pacemaker cells of the specialized cardiac tissue at the border zone between the sinus venosus and the atrium (Yamauchi and Burnstock, 1968; Saito, 1969), which corresponds to the sinoatrial node of the mammalian heart. In contrast to the elongated shape of the mammalian sinoatrial node, the sinoatrial tissue of most fish hearts is a ring-shaped aggregation of cardiac myocytes (Laurent, 1962; Haverinen and Vornanen, 2007; Zaccone et al., 2010; Tessadori et al., 2012; Newton et al., 2014) (Fig. 1). Cells of the atrioventricular canal can also generate spontaneous APs, but with lower frequency than the sinoatrial pacemaker (spontaneously active cells of the sinoatrial junction and atrioventricular canal are collectively called 'nodal tissue') (Saito, 1969). As the fastest rhythm generator, the sinoatrial pacemaker determines f_H in fish under different temperatures and physiological states. Although temperature directly modulates the firing rate of pacemaker APs, f_H may be limited by temperature-dependent changes in the electrical excitability of atrial and ventricular myocytes, as discussed later in this Review. Pacemaker APs excite atrial myocytes to produce a fast-propagating atrial AP and vigorous contraction of the atrium. The rate of impulse propagation decreases at the junction between the atrium and the ventricle, and thereby allows more time for ventricular filling. In the wall of the ventricle, the rate of AP propagation is again fast and induces ventricular contraction (Sedmera et al., 2003).

The pacemaker AP

The shape of the pacemaker AP in fish is similar to that of other vertebrates, but its ionic basis is practically unexplored. Therefore, the information presented below on the ionic mechanisms of pacemaker cell function is based on knowledge from mammalian and frog studies (Irisawa, 1978). Unlike atrial and ventricular APs, those generated by pacemaker cells have only three clearly distinguishable phases: diastolic depolarization (phase 4), AP upstroke (phase 0) and repolarization (phase 3) (Figs. 1A, 2A). Pacemaker cells do not have a stable RMP. Instead, the activity of pacemaker cells is characterized by a slowly creeping diastolic depolarization, which results in regular firing of pacemaker APs at the threshold potential (TP; see Glossary) of the L-type Ca^{2+} current, I_{CaL}. Depolarization starts from the maximum diastolic potential, which is significantly less negative (−50 to −65 mV) than the RMP of atrial and ventricular myocytes, probably because of the absence or low density of the I_{K1}. The upstroke of the AP is slower in pacemaker cells than in atrial and ventricular myocytes, and it is generated by I_{CaT} and I_{CaL}, even though I_{Na} has been shown to be present in rodent pacemaker cells (Maier et al., 2003; Lei et al., 2004). Pacemaker APs do not have such a prominent plateau as those of ventricular myocytes, because various K^+ currents quickly repolarize the membrane.

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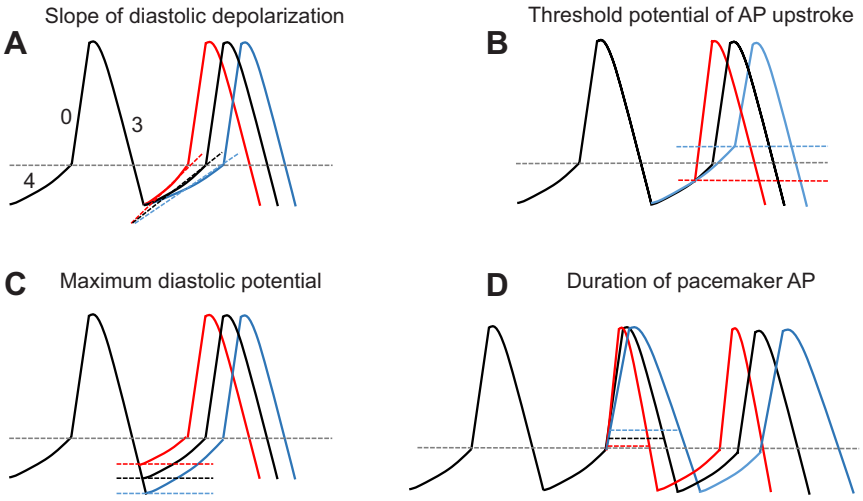

Fig. 2. Modifications of the pacemaker action potential by which temperature-dependent changes in heart rate could be achieved. Temperature-induced changes in the rate of diastolic depolarization (A), threshold potential (B), maximum diastolic potential (C) and action potential (AP) duration (D). Red and blue lines indicate high and low temperatures, respectively; the black lines represent an intermediate temperature. The dashed lines indicate temperature-dependent changes in each of the four AP parameters. The gray dashed line indicates the threshold potential. The three phases (4, 0 and 3) of the pacemaker AP are shown in A.

Pacemaker APs are produced by a crosstalk between two clock mechanisms, the membrane clock and the calcium clock (Mangoni and Nargeot, 2008; Lakatta et al., 2010). The former is exclusively a function of currents maintained by SL ion channels and ion transporters including, among others, the pacemaker current (I_h), K^+ and Ca^{2+} currents (I_{Kr}, I_{CaT}, I_{CaL}), and currents resulting from the activity of the Na^+/Ca^{2+} exchanger (NCX) and the Na^+/K^+ pump. The function of the calcium clock is produced by local, spontaneous Ca^{2+} release from the sarcoplasmic reticulum, which activates SL NCX and accelerates the diastolic depolarization. Changes in intracellular Ca^{2+} concentration link the membrane clock and the calcium clock, thus generating the intrinsic rhythm of the cardiac pacemaker. The relative significance of the membrane clock and calcium clock in the fish cardiac pacemaker is unknown.

Acute temperature effects on fish cardiac APs and ion currents

In aquatic habitats, temperature can be spatially heterogeneous; therefore, fishes experience acute temperature changes over minutes to hours. Acute temperature changes affect the rate of opening and closing of ion channels and the catalytic rates of pumps and transporters, which is reflected as changes in the rate, rhythm and amplitude of the pacemaker AP (Hille, 2001). Because CO is the product of f_H and stroke volume, the rate of the cardiac pacemaker directly affects blood circulation, which is increased during acute increases in temperature in order to match the temperature-dependent increase in metabolic rate. Temperature-induced changes in f_H can be due to four different modifications of pacemaker AP; changes in (1) the rate of diastolic depolarization, (2) the value of maximum diastolic potential, (3) the duration of pacemaker AP or (4) the TP of the AP upstroke (Fig. 2). In rainbow trout (*Oncorhynchus mykiss*), f_H is altered by temperature-induced changes in the rate of diastolic depolarization and duration of the pacemaker AP (Haverinen and Vornanen, 2007). In contrast, maximum diastolic potential and TP do not change in the physiological temperature range of rainbow trout (4–18°C). The paucity of knowledge on fish cardiac pacemakers means that we cannot make any generalizations about the effect of temperature on the function of the fish cardiac pacemaker. Finding a good 'model' species for isolating sufficiently large numbers of single pacemaker myocytes from the fish heart would open avenues for rapid progress in pacemaker research.

Although considerable differences exist in the shape and duration of cardiac APs between fish species, in all cardiac compartments the AP duration (APD) decreases as temperature acutely increases (Vornanen et al., 2002a,b, 2014; Galli et al., 2009; Haverinen and Vornanen, 2009; Ballesta et al., 2012; Shiels et al., 2015). This indicates that inward I_{Ca} and outward K^+ currents (I_{K1}, I_{Kr}, I_{Ks}) are affected differently by temperature at the AP plateau. The shortening of APs under acute increases in temperature indicates that repolarizing K^+ currents increase with a greater thermal sensitivity to warming than the depolarizing I_{Ca}. In fish cardiac myocytes, the temperature dependence of I_{K1} density is weak (Q_{10}=1.3–1.5) in comparison to that of I_{Kr} density (Q_{10}=2.3–3.2) (Vornanen et al., 2002a,b). The peak amplitude of the fish cardiac I_{Ca} has a Q_{10} value of ~1.8 in rainbow trout cardiac myocytes (Shiels et al., 2000), but the peak amplitude of this current is much larger in the ventricular myocytes of the Alaska blackfish (*Dallia pectoralis*), especially at low temperatures (Kubly and Stecyk, 2015). Temperature- and voltage-dependent changes in the opening and closing kinetics of ion channels play a large role in thermal responses. Temperature-induced changes in APD are probably largely due to temperature-dependent changes in the rate of I_{CaL} inactivation (Shiels et al., 2000; Kubly and Stecyk, 2015).

Temperature also affects the RMP and AP amplitude of atrial and ventricular myocytes. Acute temperature increases make RMP more negative and have a biphasic effect on amplitude and overshoot of the AP in brown trout (*Salmo trutta fario*) ventricular myocytes (Vornanen et al., 2014) – rising temperatures are associated with increases in AP amplitude up to 26.4°C, but above that point the amplitude steeply declines. It should be noted that, *in vitro*, APs can be elicited – if the stimulus strength is sufficiently large – much above the temperature where *in vivo* spontaneous heartbeat stops. This indicates that myocytes remain excitable at high temperatures, but in the intact heart the threshold for AP generation has risen, which prevents spontaneous excitation.

Seasonal temperature changes and thermal plasticity of cardiac excitation

In temperate and polar regions, fishes are exposed to slow seasonal temperature changes, the magnitude of which can extend from a few degrees to >35°C, depending on factors such as geographical location, water depth and type of habitat (ocean, lake or river). Many fishes possess homeostatic mechanisms that enable modification of cardiac structure and function in order to adjust CO to seasonal temperature changes (Klaiman et al., 2011). These reversible physiological adjustments help to mitigate the acute

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effects of temperature on the CO and allow the animal to survive despite the sustained temperature changes. Species-specific variability in thermal plasticity may partly determine the ability of fishes to accommodate to the predicted global warming (Somero, 2010). Unsurprisingly, the electrical excitability of the fish heart shows considerable plasticity under sustained temperature changes; at the level of the whole organ, this plasticity is evident as compensatory changes in the f_H and the duration of cardiac APs. Temperature changes have a strong effect on f_H, whereas stroke volume tends to be fairly independent of temperature. Compensatory changes in f_H in fish are therefore the only means to alleviate the effects of seasonal temperature changes on CO. To maintain a proper balance between the durations of diastole (cardiac rest) and systole (cardiac contraction), the duration of the AP must also conform to changes in f_H.

In several northern temperate fish species, thermal acclimation results in compensatory changes in f_H that involve changes in the excitability of myocytes and their neural control. Temperature compensation (see Glossary) of the f_H requires modifications in the shape of the pacemaker AP (Fig. 2). In the marine plaice (*Pleuronectes platessa*), seasonal acclimation is associated with a cold-induced increase in f_H (Harper et al., 1995). Thermal compensation of f_H in the plaice is attained by shortening of the pacemaker APD without changes in the rate of diastolic depolarization. Similarly, the elevated f_H of the cold-acclimated (4°C) rainbow trout is associated with strong shortening of the pacemaker AP, whereas the rate of diastolic depolarization, the maximal diastolic potential and TP remain unaltered (Haverinen and Vornanen, 2007). Thus, the current findings suggest that compensatory changes in pacemaker rate are primarily achieved by temperature-induced changes in APD.

Increases in f_H induced by sustained cold are often associated with a concomitant shortening of atrial and ventricular APs (Haverinen and Vornanen, 2009; Hassinen et al., 2014; Abramochkin and Vornanen, 2015). Coordinated adjustments of APD and f_H help to ensure that the relative lengths of diastole and systole are unchanged, which is necessary for proper filling of the heart with blood and for thermal compensation of CO. Thermal compensation of APD is, in most cases, only partial, i.e. after acclimation to new thermal conditions, APD still varies with temperature but less so. However, in the seasonally acclimated marine navaga cod (*Eleginus navaga*), complete compensation of ventricular AP has been reported (Abramochkin and Vornanen, 2015). In winter, navaga live at subzero temperatures in the Arctic Ocean but, in summer, in the subarctic parts of the ocean (the White Sea), these fish are exposed to temperatures that are 15°C higher. Navaga are eurytherms of cold polar seas, which explains their excellent acclimation capacity. The AP responses of polar stenotherms and tropical eurytherms to thermal acclimation are poorly elucidated, and therefore thermal plasticity of cardiac excitation in these species remains to be shown. In polar stenotherms, the capacity of cardiac function to acclimate to changes in temperature is more limited (Podrabsky and Somero, 2006; Franklin et al., 2007; Bilyk and DeVries, 2011; Egginton and Campbell, 2016); therefore, it can be anticipated that the thermal plasticity of excitability in these species is also modest.

Collectively, the current data indicate that sustained temperature changes induce compensatory adjustments in the duration of pacemaker, atrial and ventricular APs, which provide partial independence of f_H and CO from seasonal temperature changes. It should be noted, however, that there are a few fish species that become inactive or dormant in winter, such as crucian carp (*Carassius carassius*), cunner (*Tautogolabrus adspersus*), annual killifish (*Austrofundulus limnaeus*) and (perhaps) the air-breathing Alaska blackfish (Podrabsky and Hand, 1999; Vornanen et al., 2009; Costa et al., 2013; Kubly and Stecyk, 2015). These species do not need to compensate for reductions in temperature with changes in cardiac activity, which is reflected by the absence of positive compensation or even the reduction in ion current densities in the cold (i.e. reverse thermal compensation) (Vornanen and Paajanen, 2004; Kubly and Stecyk, 2015).

Upregulation of the I_{Kr}, a ubiquitous response to chronic cold

Thermal compensation of APD signifies an altered balance between depolarizing and repolarizing currents. This compensation can largely be attributed to prominent changes in the density of the rapid component of the delayed rectifier I_{Kr}, the major repolarizing current of the fish heart. Increased density of the cardiac I_{Kr} following cold acclimation has been observed in nearly all fish species studied thus far (Vornanen et al., 2002a,b; Haverinen and Vornanen, 2009; Galli et al., 2009; Abramochkin and Vornanen, 2015). Indeed, the increased I_{Kr} appears to be an almost ubiquitous response of fish hearts to chronic cold, which often (but not always) produces a compensatory shortening of the APD and makes room for a compensatory increase in f_H and, thus, CO (Haverinen and Vornanen, 2009; Galli et al., 2009) (Fig. 3A).

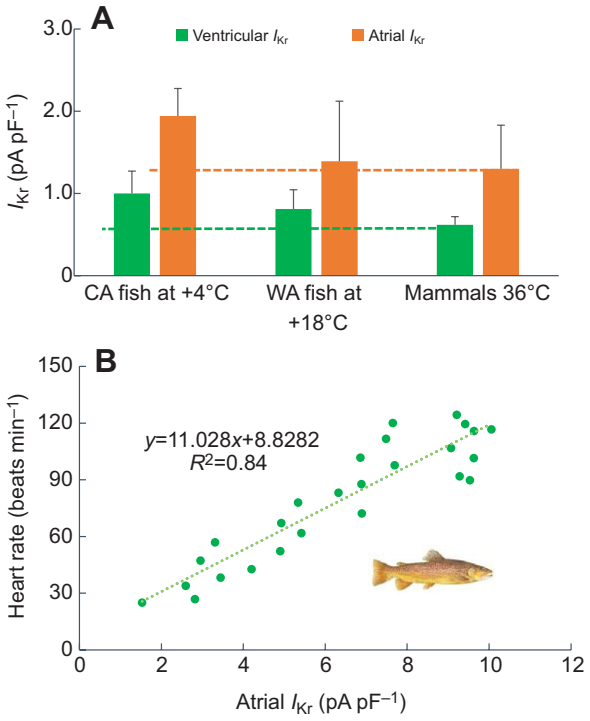

Fig. 3. The rapid component of the delayed rectifier K^+ current (I_{Kr}) of the fish hearts. (A) A comparison of I_{Kr} densities in atrial and ventricular myocytes of fish and mammalian hearts. The bars represent means±s.e.m. from five to six fish species acclimated to cold (+4°C) or warm (+18°C) temperatures (Haverinen and Vornanen, 2009; Abramochkin and Vornanen, 2015) and three to four mammalian species (guinea pig, rabbit, dog, human). Note that the density of fish cardiac I_{Kr} at +4°C and +18°C is similar to or larger than mammalian I_{Kr} at +36°C. CA, cold acclimated; WA, warm acclimated. (B) There is close correlation between the density of atrial I_{Kr} and heart rate in brown trout acclimated at +12°C. Data were modified from Vornanen and Hassinen (2016) and Vornanen et al. (2014). Mammalian data are from Sanguinetti and Jurkiewicz (1990), Lu et al. (2001), Li et al. (2001) and Melnyk et al. (2005).

Interestingly, the temperature-induced changes in I_{Kr} density and APD occur not only in atrial and ventricular myocytes, but also in the pacemaker cells. Thermal compensation of the f_H results from changes in pacemaker APD, possibly because of temperature-related changes in the density of the I_{Kr} (Haverinen and Vornanen, 2007), an important current of the cardiac pacemaker (Himeno et al., 2011). Indeed, there is a close correlation between the density of atrial I_{Kr} and f_H (Fig. 3B). Thus, a common molecular mechanism, I_{Kr}, could provide a simple means to coordinate f_H and APD.

The balance between I_{Na} and I_{K1} regulates initiation of cardiac APs

Two currents, I_{Na} and I_{K1}, are functionally closely linked and are key factors in the initiation of atrial and ventricular APs (Golod et al., 1998; Whalley et al., 1994). Cardiac APs are elicited when the membrane is depolarized to the TP, i.e. to the level at which the density of inward I_{Na} exceeds the density of outward I_{K1} (Golod et al., 1998). I_{Na} and I_{K1} function antagonistically, in that they generate fast surges of inward and outward current, respectively, upon depolarization of the SL (Li et al., 1998; Whalley et al., 1994) (Fig. 1C). At the RMP, the electrochemical driving force favors Na^+ influx, whereas depolarization of the RMP restores the driving force for K^+ efflux through the inward rectifier channels. A small depolarization of the RMP induces a fast and transient opening of Na^+ channels (creating an inward surge of current) and an immediate K^+ efflux through constitutively open inward rectifier K^+ channels (an outward surge of current). Na^+ channels are quickly closed by an intracellular inactivation gate, and K^+ efflux is temporarily prevented by intracellular Mg^{2+} ions and polyamines, which enter the K^+ channel pore in a voltage-dependent manner and block it.

To maintain constant cardiac excitability despite seasonal temperature changes, the properties of I_{K1} and I_{Na} should change in unison. Indeed, in several fish species, temperature acclimation modifies the densities of I_{K1} and I_{Na} in the same direction – for example, both currents are usually upregulated in the cold (Vornanen et al., 2002a,b; Haverinen and Vornanen, 2004, 2009). Notably, in the crucian carp, a cold-dormant and anoxia-resistant species, the density of both currents is decreased in the cold, i.e. the balance between I_{Na} and I_{K1} is still maintained but at a lower total current level (Haverinen and Vornanen, 2004; Hassinen et al., 2008a,b). In contrast, by differentially affecting the two currents, cardiac excitability can be increased or decreased by thermal acclimation. Interestingly, in the mammalian heart, inward rectifier K^+ channels and Na^+ channels are part of a common macromolecular complex within the cell and mutually regulate each other's expression (Milstein et al., 2012).

How are temperature-specific excitability phenotypes achieved?

Phenotypic changes in electrical excitability can be obtained by changes in the level of channel expression, temperature-related shifts in the expression of qualitatively different gene products or the regulation of covalent modifications that alter channel function. Over >400 million years of evolution, fishes have adapted to different temperatures, and the early duplication of the teleost genome provided abundant material for the thermal specialization of fish genes (Volff, 2005). Owing to whole genome duplications, there are several gene paralogs for Na^+, K^+ and Ca^{2+} channels in fish genomes (Wong et al., 2006; Widmark et al., 2011; Hassinen et al., 2015; Vornanen and Hassinen, 2016).

Many of the ion current changes that result from thermal acclimation in fish hearts are due to changes in gene expression either at the transcript or protein level. Cardiac K^+ currents are particularly malleable, as indicated by the almost ubiquitous upregulation of I_{Kr} in cold-acclimated fishes. Four I_{Kr} (erg) channel genes are expressed in fish hearts, including one paralog pair (erg2a, erg2b) (Vornanen and Hassinen, 2016). The cold-induced increase in I_{Kr} density seems to be mainly due to an increase in the number of α subunits of erg2a, the major erg channel of fish hearts (Hassinen et al., 2008a,b; Abramochkin and Vornanen, 2015; Vornanen and Hassinen, 2016). Temperature-induced changes in the cardiac I_{K1} involve prominent isoform shifts. Altogether, six gene products encoding channels involved in the generation of I_{K1} (Kir2 channels), including one paralog pair (Kir2.2a, Kir2.2b), are expressed in zebrafish heart (Hassinen et al., 2015). Interestingly, in the crucian carp heart, thermal acclimation is associated with a notable quantitative change in the isoform composition of the Kir2 channels. In the warm-acclimated crucian carp, expression of Kir2.2a is upregulated, while that of Kir2.2b is simultaneously reduced (Hassinen et al., 2008a,b). Thus, the ratio of Kir2.2b/2.2a in the ventricle shifts from 2 in cold-acclimated fish to 0.3 in warm-acclimated fish. The expression of Kir2.2a is also upregulated in rainbow trout under warm conditions (Hassinen et al., 2007). These findings suggest that the two Kir2.2 gene paralogs are specialized to function under different thermal regimes: Kir2.2a is the warm-adapted isoform and Kir2.2b is the cold-adapted isoform. Functionally, Kir2.2b seems to be almost completely insensitive to acute temperature changes (V. Paajanen, M. Hassinen, J. Haverinen and M.V., unpublished observations).

Thermal adaptation of cardiac excitability

Most fishes inhabit environments with temperatures lower than the typical body temperatures of endothermic animals. Therefore, it is not surprising that the ion channels of fish hearts are adapted to function at lower temperatures than those of endothermic animals. This appears as an intolerance of fish cardiac ion currents to high temperatures (+36–38°C), which are optimal for the functioning of mammalian ion channels (Vornanen et al., 2014), although this must not apply to the most heat-resistant tropical fish species.

APD must correlate inversely with f_H to allow sufficient time for the two phases of the cardiac cycle, diastole and systole; otherwise, high f_H values will obliterate diastole. Indeed, in mammalian hearts, APD scales inversely with f_H (and therefore with metabolic rate) – smaller animals have higher f_H values and shorter APs (Rosati et al., 2008). An analogous relationship between f_H and APD prevails in fish hearts under acute temperature changes; APD at the level of 50% repolarization (APD_{50}) shortens exponentially with increasing f_H, giving a linear relationship when APD_{50} is plotted against f_H on a double log scale (Fig. 4). Thus, in both fishes and mammals, APD correlates inversely with f_H and metabolic rate. The inverse correlation between f_H and APD means that athletic and active fishes (e.g. tunas, salmonid species) and tropical fishes (e.g. zebrafish) have higher f_H values and shorter ventricular APs than sluggish and less athletic fishes (e.g. crucian carp) or fishes inhabiting cold polar waters (e.g. navaga cod) (Haverinen and Vornanen, 2009; Galli et al., 2009; Hassinen et al., 2014; Vornanen and Hassinen, 2016). A comparison of piscine and mammalian APs at the same experimental temperature suggests that APD is shorter in fish than in the mammalian heart, probably as an adaptation of fishes to low temperatures. Although such comparisons are problematic, because APD is strongly dependent on body mass and f_H in mammals, and physiological body temperatures of fishes and mammals seldom overlap, some supportive evidence can be provided. For example, the APD of the brown trout ventricular

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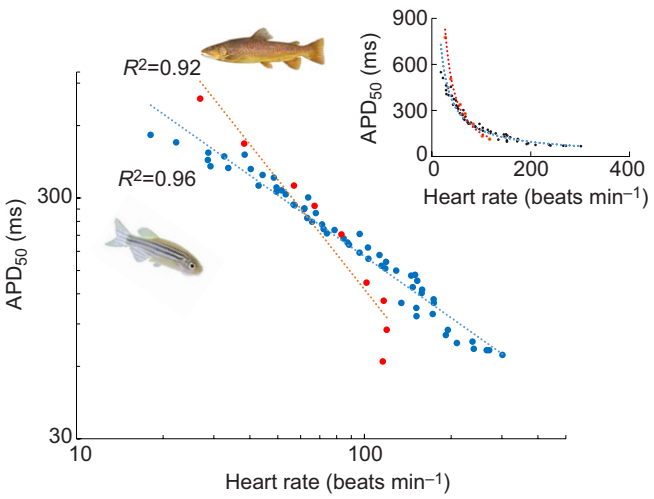

Fig. 4. The relationship between heart rate and action potential duration. The graph shows the correlation between heart rate and action potential duration at the level of 50% repolarization (APD_{50}) for zebrafish (blue) and brown trout (red) hearts *in vitro* during acute temperature changes. The inset shows the data on linear axes. The figure is modified from the existing literature (Vornanen et al., 2014; Vornanen and Hassinen, 2016).

myocytes is 85 ms at +25°C and only 26 ms at +35°C (Vornanen et al., 2014), whereas in the similar-sized guinea pig, the APD of ventricular myocytes is 496 and 250 ms at +35°C and +25°C, respectively (Hume and Uehara, 1985), despite the higher f_H in guinea pig (resting f_H of 230–300 beats min^{-1}) than brown trout (maximal f_H=124 beats min^{-1}) (Rouslin et al., 1995; Vornanen et al., 2014). Similarly, the ventricular APD_{50} of the zebrafish, a species tolerating temperatures as high as +40°C (Cortemeglia and Beitinger, 2005), is only ~60 ms at +36°C, which is much shorter than the ventricular AP of most laboratory mammals (200–300 ms at +34°C) with comparable f_H values (Rosati et al., 2008; Vornanen and Hassinen, 2016).

In ectothermic fishes, ion channel function should be flexible and should allow unperturbed excitation of the heart under variable temperature regimes. Therefore, cardiac excitability should show adaptations in ion current densities, ion channel compositions, opening and closing kinetics of channels or nervous control of ion channel function. Comparing I_{Kr} between mammalian and fish hearts indicates that the density of I_{Kr} is markedly higher in fish than in mammalian cardiac myocytes. Notably, the I_{Kr} density of fish cardiac myocytes at +4°C and +18°C is higher than the mammalian cardiac I_{Kr} density at +36°C, i.e. fish show overcompensation of cardiac I_{Kr} relative to mammals (Fig. 3A). Similarly, activation and deactivation kinetics of I_{Kr} are faster in trout cardiac myocytes than in mammalian cardiac myocytes (Hassinen et al., 2008a,b). The importance of the I_{Kr} in fish cardiac myocytes may, in fact, lie in the fast activation kinetics of this current, which makes it suitable for maintaining cardiac function at the relatively low body temperatures of the fish. In contrast to I_{Kr}, there are no systematic differences in the density of the I_{K1} or L-type Ca^{2+} current (I_{CaL}) between fish and mammalian hearts when the experimental temperature is taken into account. It has been suggested that I_{Na} is lower in fish than mammalian hearts (Brette et al., 2008), but more comparisons between fish and mammalian I_{Na} under identical ionic, thermal and voltage conditions are needed to verify this. Thus, the current evidence suggests that a major factor in the relatively short APD in fish hearts is the high density of the major repolarizing K^+ current, the I_{Kr}.

The slow component of the delayed rectifier K^+ current (I_{Ks}) provides an interesting example of kinetic adaptation of cardiac ion channels. I_{Ks} is part of the 'repolarization reserve' (see Glossary) of the heart, which is called into play during exercise and in β-adrenergic tone, and at high f_H values (Schmitt et al., 2014). In mammalian hearts, I_{Ks} is generated by channels that are heteromeric assemblies of Kv7.1 α-subunits and MinK β-subunits (Sanguinetti et al., 1996). Consistent with its name, I_{Ks} activates very slowly, and the slow activation kinetics are due to the presence of MinK in the channel assembly. Activation of the mammalian heteromeric Kv7.1/MinK channels is so slow that the I_{Ks} current would hardly make any contribution to repolarization at low body temperatures, such as those typical of many fishes. Interestingly, in the heart of crucian carp, the expression of the MinK β-subunit is very low (<3% of the α-subunit level), and the functional channel is mainly formed by homotetrameric Kv7.1 α-subunits (Hassinen et al., 2011). Thus, by simply omitting the β-subunit from the channel assembly, a kinetically faster I_{Ks} is generated, which is adequate to contribute to the repolarization phase of the AP at the low body temperatures typical of fishes (Fig. 5). How widely this adaptation occurs in fish hearts remains to be shown.

High-temperature tolerance of cardiac excitation: searching for the weakest link

Climate warming and the potential role of the cardiovascular system in the high-temperature tolerance of fishes makes the heat tolerance of cardiac excitation an important research topic (Pörtner, 2001; Eliason et al., 2011). In fishes, acute increases in temperature increase CO mainly through increases in f_H, because stroke volume responds weakly to temperature changes (Steinhausen et al., 2008; Mendonça and Gamperl, 2010). However, at extreme temperatures, CO appears to be first limited by the inability of the fish heart to increase f_H, suggesting thermal limitations in electrical excitability (Anttila et al., 2014; Vornanen et al., 2014). Although the temperature dependence of f_H has been extensively studied, little is known about the underlying mechanisms that limit increases in f_H. In several fish species, the first sign of thermodynamic limitation

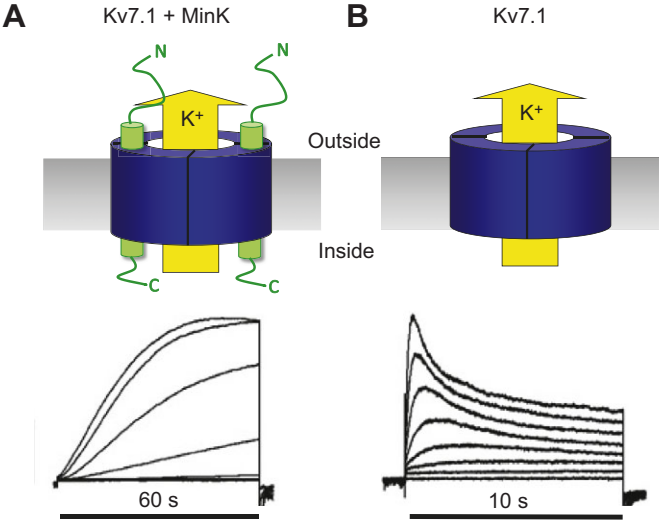

Fig. 5. Activation kinetics of the crucian carp (*Carassius carassius*) I_{Ks} current. (A) Activation kinetics of the heteromeric Kv7.1+MinK channels are much slower than (B) those of the homomeric Kv7.1 α subunit channel expressed in Chinese hamster ovary cells. The figure is modified from Hassinen et al. (2011).

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seems to be a decrease in the rate at which *in vivo* f_H increases with increasing temperature (Gollock et al., 2006; Clark et al., 2008). With further increases in temperature, f_H declines, suggesting that bradycardia (see Glossary) is the first severe form of cardiac arrhythmia – although more subtle irregularities occur at lower temperatures (Badr et al., 2016). Missing beats (atrioventricular block) and a burst of fast beating (tachyarrhythmia) are sometimes reported (Fig. 6) (Casselman et al., 2012; Vornanen et al., 2014). Finally a complete cessation of the heartbeat (asystole) ensues.

Basically, the heat-induced deterioration of excitation could be due to failure of either impulse generation or impulse conduction, even though malfunctions in the neuronal regulation of the heart cannot be excluded (Gollock et al., 2006). Conceivably, gradual deterioration of the primary cardiac pacemaker could cause slowing of the f_H and result in asystole. Unfortunately, direct recordings of pacemaker cell activity under heat stress are still missing. Another possible mechanism of the thermal failure of excitation is a break in impulse conduction. The heartbeat frequency will decrease if atrial or ventricular myocytes become unexcitable, i.e. unable to elicit APs. APs would be generated by the pacemaker, but the impulse would not be conducted to atrial or ventricular myocytes, i.e. the block could appear at the sinoatrial or at the atrioventricular junction.

Under acute increases in temperature, different ion currents of fish cardiac myocytes first increase but then start to decrease, each at a characteristic break point temperature (see Glossary). In atrial and ventricular myocytes of the cold-acclimated brown trout, the order of thermal tolerance from strongest to weakest is I_{K1} (>+32°C), I_{Ca} (+30.1°C), I_{Kr} (+27.3°C) and I_{Na} (+20.9°C) (Vornanen et al., 2014). The upper thermal tolerance limit of the brown trout is +22–25°C (Elliott and Elliott, 2010), and the upper thermal limit at which the brown trout heart can continue to beat *in vitro* is +25.8°C; thus, I_{Na} is clearly much more heat sensitive than other higher level functions. Consequently, the most heat-sensitive processes in excitability of the brown trout atrial and ventricular myocytes would be the initiation of the AP and the rate of AP upstroke (and, as a consequence of the latter, the rate of impulse propagation). Consistent with the hypothesis that failure of I_{Na} is the mechanism of the thermal failure of excitation, break point temperatures for the rate of AP upstroke in ventricular myocytes and QRS duration (see Glossary) of the *in vivo* ECG are +21.7°C and +21.9°C, respectively (Vornanen et al., 2014). In contrast, the two outward K^+ currents, I_{K1} and I_{Kr}, are relatively resistant to high temperatures and continue to increase far above the incipient lethal temperature of the brown trout.

Temperature-dependent depression of electrical excitation

Here, I suggest a testable hypothesis – the temperature-dependent depression of electrical excitation (TDEE) – based on the current data covered in this Review. The hypothesis is based on the roles of I_{K1} and I_{Na} in the initiation of atrial and ventricular APs, and the findings that the two opposing membrane currents may behave differently under acute temperature changes (Vornanen et al., 2014). I suggest that the discordant temperature dependencies of I_{Na} and I_{K1} create an imbalance between depolarizing and repolarizing power, and this mismatch may cause AP failure at extremes of high and low temperature. At the level of the working heart, this manifests as cardiac arrhythmia, including missing ventricular beats, bradycardia and, finally, complete cessation of the heartbeat (Fig. 6).

In atrial and ventricular myocytes, I_{Na} and I_{K1} are involved in the initiation of cardiac AP (Fig. 1C). I_{K1} maintains the negative RMP, and I_{Na} provides depolarizing current for the fast upstroke of the AP. When the two opposing currents are correctly balanced, a small depolarizing pulse is able to trigger an all-or-none AP in the cardiac myocyte. APs arriving from the pacemaker tissue to atrial myocytes or from the atrioventricular canal to ventricular myocytes are able to depolarize the membrane to the TP, where so many Na^+ channels are open that the density of the inward I_{Na} exceeds the density of the outward I_{K1}. The TPs for atrial and ventricular myocytes of the cold-acclimated rainbow trout at +4°C are −49 and −46 mV, respectively (Haverinen and Vornanen, 2006). Considering an RMP of −75 mV, the critical depolarization (CD; see Glossary) needed to trigger an

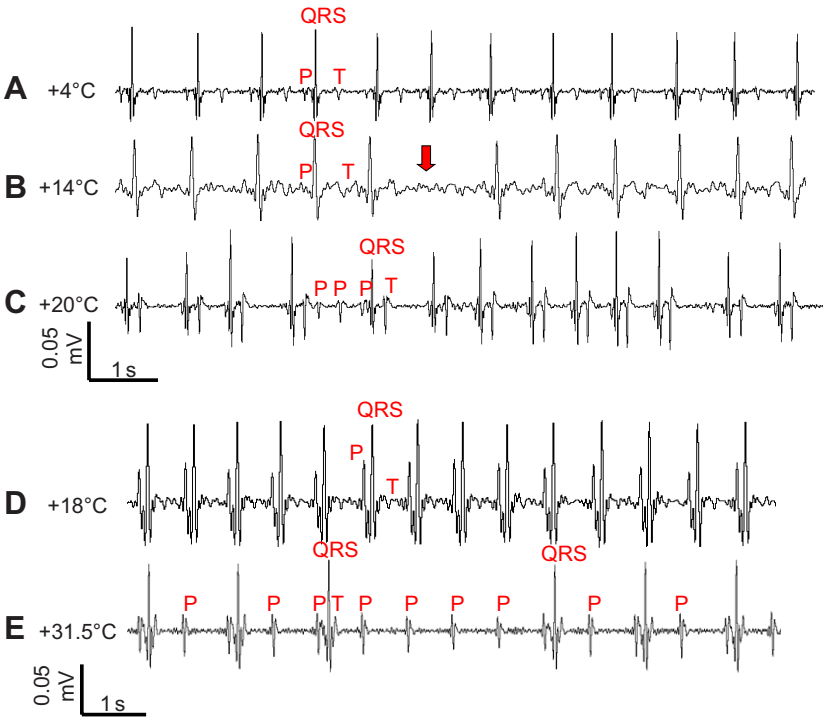

Fig. 6. Temperature-induced cardiac arrhythmia in roach (*Rutilus rutilus*). (A–C) ECG data from a cold-acclimated roach at +4°C, +14°C and +20°C, respectively. (A) Normal rhythmic cardiac activity at the acclimation temperature (+4°C). (B) The thick red arrow indicates a single missing beat at +14°C. (C) A short period of atrial tachycardia; three successive P waves, two of them without ventricular beats (no QRS complex). (D,E) ECG data from a warm-acclimated roach at +18°C (D) and +31.5°C (E). (D) Normal rhythmic cardiac activity at the acclimation temperature (+18°C). (E) A number of missing ventricular beats (no QRS complex), causing ventricular bradycardia. The figure is modified from Badr et al. (2016). The P wave (atrial depolarization), QRS complex (ventricular depolarization) and T wave (ventricular repolarization) are marked in the figure.

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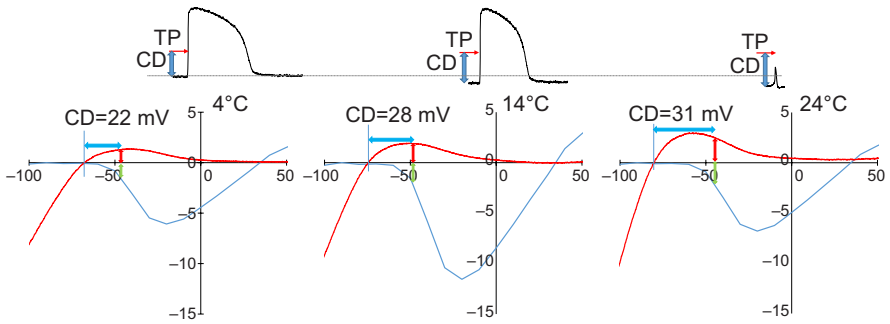

Fig. 7. Temperature-dependent depression of electrical excitation. A schematic representation of the current–voltage relationships of I_{K1} and I_{Na} under acutely rising temperature. The upper panels show ventricular action potentials and the lower panels show current–voltage relationships of I_{K1} and I_{Na} at different temperatures. Voltage is shown on the *x*-axis and current is shown on the *y*-axis. The magnitude of the outward I_{K1} (red line) increases across the whole temperature range from +4°C to +24°C, whereas that of the I_{Na} (blue line) first increases from +4°C to +14°C and then decreases between +14°C and +24°C. Because of the mismatched change in I_{K1} and I_{Na} as the temperature increases, the excitation threshold for AP firing can become too high, and APs fail at high temperatures. Red horizontal arrows indicate the threshold potential (TP), where the outward I_{K1} (red double-headed arrows) and the inward I_{Na} (green double-headed arrows) are equal in magnitude (but opposite in sign), and an all-or-none AP would be elicited if the membrane were depolarized to that level. The value of critical depolarization (CD, blue double-headed arrows) needed to trigger an AP is determined by the resting membrane potential (RMP) and the threshold potential: CD=TP–RMP. As a result of the leftward shift in the reversal potential of I_{K1} as temperature increases, the RMP becomes more negative, and because of the decreasing I_{Na} at high temperatures, the TP becomes more positive. Together, these two factors make the CD progressively larger as temperature increases. APs arriving from nodal tissues to the atrium or ventricle may thus be unable to generate the required critical depolarization of the myocyte membrane, and myocyte APs will fail at 24°C. This schematic and the current–voltage relationships of I_{K1} and I_{Na} are based on experiments performed on brown trout ventricular myocytes.

AP would be 26 and 29 mV for atrial and ventricular myocytes, respectively.

Under acute increases in temperature, cardiac excitability is affected by temperature-dependent changes in I_{Na} and I_{K1} (Fig. 7). Initially, with moderate increases in temperature, the increasing I_{Na} (Q_{10}~2) provides more depolarizing current, with a positive effect on excitability, whereas the simultaneous increase in the density of I_{K1} (Q_{10}=1.3–1.5) decreases membrane resistance and makes RMP more negative, thereby tending to decrease excitability. As long as the densities of I_{Na} and I_{K1} increase in a coordinated way, the balance between inward and outward currents is retained and the membrane remains excitable. However, experiments on brown trout cardiac myocytes show that an imbalance between I_{Na} and I_{K1} develops under acute heat ramps (Vornanen et al., 2014). Because of the heat sensitivity of Na^+ channels, I_{Na} starts to decline at +20.9°C (Q_{10} becomes negative), whereas the heat-resistant I_{K1} continues to increase. The larger I_{K1} makes the RMP more negative and decreases membrane resistance, such that stronger depolarization ($\Delta V=R\cdot I$, where V is voltage, R is resistance and I is current) is needed to bring the membrane potential to the threshold. The CD increases because of the more negative RMP and the more positive TP (CD=TP–RMP). At some point, the imbalance between I_{K1} and I_{Na} becomes so large that APs arriving from the nodal tissue or from the neighboring myocyte are insufficient to depolarize the membrane to the TP and, consequently, atrial or ventricular APs fail. This failure to elicit a myocyte AP appears sporadically as missing beats (sinoatrial block or atrioventricular block) and more regularly as ventricular bradycardia (Fig. 6). Finally, the imbalance between I_{K1} and I_{Na} can become so large that the nodal APs are unable to elicit APs in atrial or ventricular myocytes, resulting in complete cessation of the heartbeat. A similar sequence of events could occur at extremes of low temperature, because I_{Na} decreases faster with decreasing temperature than I_{K1}.

In reality, the loss of atrial and ventricular excitation could be partly due to the diminished amplitude of nodal APs, and not exclusively caused by the elevated TP. Heat-induced depression of I_{Na} diminishes the amplitude of ventricular APs (Vornanen et al., 2014), and therefore a smaller stimulus is available to depolarize the neighboring cell to the TP. If the AP amplitude of the sinoatrial pacemaker or the atrioventricular canal were reduced by high temperature in a manner similar to the ventricular AP, this would also appear as a blunted response of f_H to high temperatures; the pacemaker rate might well increase with temperature, but the diminished amplitude of the nodal APs could prevent excitation of atrial and ventricular myocytes. The heat-induced deterioration of excitability is expected to be more severe at the ventricle than at the atrium, because the density of ventricular I_{K1} is much larger than the density of atrial I_{K1}, whereas the density of I_{Na} is more similar in the two cardiac chambers (Vornanen et al., 2002a,b; Haverinen and Vornanen, 2006).

The experimental evidence for the TDEE hypothesis is mainly based on the study of the brown trout heart (Vornanen et al., 2014). However, antagonism between Na^+ and K^+ currents is not restricted to cardiac myocytes, but is present in all excitable tissues. It is likely that heat sensitivity of electrical excitation, similar to that shown for brown trout cardiac myocytes, could impair excitability in neurons, skeletal muscle cells, smooth muscle cells and excitable glandular cells. Indeed, a temperature-related mismatch between the rate of rise (depolarization) and fall (repolarization) of APs and the consequent (and reversible) heat block of neuronal conduction was noticed by the pioneers of electrophysiology in the squid giant axon (Hodgkin and Katz, 1949; Huxley, 1959; Guttman, 1962), and the findings have been confirmed more recently (Rosenthal and Bezanilla, 2002). A similar mechanism is suggested to cause cold-block in the central nervous system of fishes (Roots and Prosser, 1962). The nervous system is often considered to be the most vulnerable tissue to variations in temperature; it is therefore thought to be the primary determinant of animal death at low and high temperatures, because the homeostatic control of body functions and adaptive responses to stress are lost (Robertson and Money, 2012). The temperature-dependent mismatch between repolarizing K^+ currents and the depolarizing I_{Na} could have detrimental effects on cardiac function, sensory transduction, locomotion, hormonal secretion and behavior of ectothermic animals.

The TDEE hypothesis should be testable by a variety of electrophysiological experiments. The temperature dependence of the excitation threshold in cardiac myocytes (and other excitable

tissues) can be studied by conventional impulse strength/duration protocols, and the significance of Na^+ and K^+ currents for these responses could be assessed by pharmacological and ionic manipulation of their amplitudes (Golod et al., 1998). Thermal modulation of excitation may involve the accumulation of K^+ ions in the intercellular space and frequency-dependent changes in the amplitude of ion currents (Kline and Morad, 1978), which should be taken into account in experimental procedures. Comparison of results from isolated cells, spontaneously active multicellular preparations (sinoatrial tissues, working whole hearts) and *in vivo* ECG recordings would help to elucidate the significance of molecular and cellular mechanisms in the thermal response of the whole heart (Vornanen et al., 2014; Badr et al., 2016).

It is becoming increasingly obvious that the heat tolerance of ectotherms is a complex multifactorial and life history stage-dependent characteristic involving diverse physiological functions and various cellular and molecular mechanisms and targets, including cellular membranes, mitochondria, heat shock response and redox balance (Cossins and Prosser, 1978; Dilly et al., 2012; Clark et al., 2013; Gräns et al., 2014; Iftikar et al., 2014; Ekström et al., 2014; Brijs et al., 2015; Ern et al., 2015). An important focus of future research is to investigate what role, if any, membrane excitability plays in the thermal tolerance of ectotherms. The excitability of the heart, in several respects, is a good model system for testing the TDEE hypothesis.

Conclusions and perspective

The present state of knowledge indicates that the electrical excitability of the fish heart is thermally plastic, enabling acclimation of cardiac function to seasonally changing temperatures. K^+ and Na^+ currents are especially malleable entities that adapt to chronic temperature changes. Evolutionary adaptation to low temperature involves changes in the expression level of ion channels (I_{Kr}) and changes in ion channel kinetics (I_{Ks}); the latter are achieved by modification of ion channel assemblies (I_{Ks}) and specialization of channel isoforms (I_{K1}) for function under particular thermal regimes. Adaptation and acclimation mechanisms of electrical excitability provide the means by which the fish heart can respond to acute temperature changes. The balance between I_{Na} and I_{K1} seems to be crucial for the sensitivity of excitation under acute temperature changes. At high temperatures, this balance could be distorted by divergent temperature dependencies of I_{Na} and I_{K1}, as the density of I_{K1} overwhelms that of I_{Na} and makes the fish heart unexcitable. The temperature-dependent deterioration of electrical excitability that is due to I_{Na}–I_K antagonism may not be limited to cardiac myocytes, but could be common to all excitable cells. Further research in this area will allow us to determine the cells and tissues, life history stages and ectothermic species in which temperature-dependent failure of electrical excitation might limit sensory, locomotor, behavioral and circulatory functions – information that will be increasingly valuable given predicted changes in global temperatures.

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Competing interests

The author declares no competing or financial interests.

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