Comparative Biochemistry and Physiology, Part A 205 (2017) 15–27

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effects of seasonal acclimatization on action potentials and sarcolemmal K^+ currents in roach (*Rutilus rutilus*) cardiac myocytes

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ARTICLE INFO

Article history:
Received 3 October 2016
Received in revised form 13 December 2016
Accepted 15 December 2016
Available online 19 December 2016

Keywords:
Thermal acclimatization
Fish heart
Electrical excitability
Temperature tolerance
Climate warming

ABSTRACT

Temperature sensitivity of electrical excitability is a potential limiting factor for high temperature tolerance of ectotherms. The present study examines whether heat resistance of electrical excitability of cardiac myocytes is modified by seasonal thermal acclimatization in roach (*Rutilus rutilus*), a eurythermal teleost species. To this end, temperature dependencies of ventricular action potentials (APs), and atrial and ventricular K^+ currents were measured from winter-acclimatized (WiR) and summer-acclimatized (SuR) roach. Under patch-clamp recording conditions, ventricular APs could be triggered over a wide range of temperatures (4–43 °C) with prominent changes in resting membrane potential (RMP), AP duration and amplitude. In general, APs of SuR were slightly more tolerant to high temperatures than those of WiR, e.g. the break point temperature (T_{BP}) of RMP was 37.6 ± 0.4 °C in WiR and 41 ± 1 °C in SuR ($p < 0.05$). Of the two major cardiac K^+ currents, the inward rectifier K^+ current (I_{K1}) was particularly heat resistant in both SuR (T_{BP} 39.4 ± 0.4 °C) and WiR (T_{BP} 40.0 ± 0.4 °C) ventricular myocytes. The delayed rectifier K^+ current (I_{Kr}) was not as heat resistant as I_{K1}. Surprisingly, I_{Kr} of WiR tolerated heat better (T_{BP} 31.9 ± 0.8 °C) than I_{Kr} of SuR (T_{BP} 24.1 ± 0.5 °C) ($p < 0.05$). I_{Kr} (Erg2) channel transcripts of both atrial and ventricular myocytes were up-regulated in WiR. I_{K1} (Kir2) channel transcripts were not affected by seasonal acclimatization, although ventricular I_{K1} current was up-regulated in summer. Collectively, these findings show that thermal tolerance limits of K^+ currents in isolated myocytes between seasonally acclimatized roach are much less pronounced than the heat sensitivity of ECG variables in intact fish.

1. Introduction

Temperature is the environmental master factor which substantially affects abundance and distribution of fishes due to its impact on physiology and physical performance of the ectothermic body (Hubbs, 1948; Ferguson, 1958; Brett, 1971; Sidell and Moerland, 1989; Pörtner, 2001). Increases and decreases of ambient temperature enhance and depress, respectively, fish cardiac function which is considered as a key physiological factor in environmental adaptation and acclimation of aquatic vertebrates due to its direct contribution to aerobic performance level (Brett, 1971; Cech et al., 1976; Driedzic and Gesser, 1994; Pörtner, 2001; Gamperl and Farrell, 2004; Gollock et al., 2006). Under acutely and seasonally changing thermal regimes of north-temperate latitudes, structural and functional plasticity of the fish myocardium is crucial for maintenance of proper excitability and contractility of the heart (Bowler and Tirri, 1990; Bailey and Driedzic, 1990; Keen et al., 1994; Aho and Vornanen, 1999; Aho and Vornanen, 2001; Vornanen et al., 2002b; Klaiman et al., 2011; Johnson et al., 2014; Vornanen, 2016; Keen et al., 2016). Electrical excitability of cardiac myocytes should be sensitive to acute temperature changes to produce temperature-dependent acceleration and deceleration of heart rate (f_H) and coordinated changes in conduction velocity of action potential (AP) through the heart, but at the same time stable enough to prevent arrhythmia that could compromise pump function of the heart (Hassinen et al., 2007; Haverinen and Vornanen, 2009; Vornanen et al., 2014; Badr et al., 2016; Vornanen, 2016).

Electrical excitation initiates each cardiac contraction, determines the rate and rhythm of the heartbeat and regulates contractility, and thereby cardiac output under constantly changing needs of the fish body.

Acute changes of temperature profoundly alter the shape of cardiac AP in atrial, ventricular and pacemaker myocytes of the fish heart by modulating the flow of inward and outward currents through the sarcolemma (Talo and Tirri, 1991; Harper et al., 1995; Vornanen et al., 2002a; Haverinen and Vornanen, 2007; Haverinen and Vornanen, 2009; Ballesta et al., 2012; Vornanen et al., 2014; Lin et al., 2014; Hassinen et al., 2014; Shiels et al., 2015). In several fish species, acute effects of temperature on cardiac excitability are counteracted under prolonged exposure to new thermal conditions by a physiological acclimation or acclimatization process, which modify f_H, AP duration (APD) and

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http://dx.doi.org/10.1016/j.cbpa.2016.12.017
1095-6433/

sarcolemmal ion currents by temperature-dependent changes in density and isoform profile of the cardiac ion channels. Indeed, shape and duration of fish cardiac APs and the underlying ion currents are highly sensitive to sustained temperature changes and important in thermal acclimation or acclimatization of both freshwater and marine teleosts to seasonal temperature regimes (Haverinen and Vornanen, 2009; Galli et al., 2009; Hassinen et al., 2014; Abramochkin and Vornanen, 2015). Although temperature is often a central factor in seasonal acclimatization of ectotherms at high latitudes, thermal acclimation and seasonal acclimatization are not equivalent (Kleckner and Sidell, 1985; Vézina and Guderley, 1991). While acclimation in the laboratory involves only changes in temperature, seasonal acclimation is associated, in addition to temperature, with changes in photoperiod, oxygen content of water, food availability and reproductive hormones. Therefore, studies on thermal acclimation and seasonal acclimation may provide complementary information about environmental adaptation of ectotherms.

Potassium ion currents of the fish heart seem to be particularly malleable entities under chronic temperature changes (Vornanen et al., 2002a; Haverinen and Vornanen, 2009). Fish cardiomyocytes have two major K^+ currents, the background inward rectifier current (I_{K1}) and the rapid component of the delayed rectifier current (I_{Kr}) (Vornanen et al., 2002a; Hassinen et al., 2007; Haverinen and Vornanen, 2009; Nemtsas et al., 2010). I_{K1} is responsible for maintaining the negative resting membrane potential (RMP) and setting the rate of the final phase-3 AP repolarization, while I_{Kr} strongly modulates APD (Vornanen et al., 2002a; Vornanen, 2016). I_{Kr} responds to sustained temperature changes in a way that the current is up-regulated in the cold and down-regulated in the warm in most fish species studies thus far (Vornanen et al., 2002a; Haverinen and Vornanen, 2009; Abramochkin and Vornanen, 2015). Thermal response of the I_{K1} is more variable. In some fish species, the density of I_{K1} is increased in the cold (*e.g.* crucian carp, *Carassius carassius*) while in others it is decreased or does not respond to temperature change (*e.g.* blue-fin tuna, *Thunnus thynnus*) (Vornanen et al., 2002a; Haverinen and Vornanen, 2009; Galli et al., 2009). These major repolarizing currents play a significant role in adjusting electrical excitability and stability of fish cardiac myocytes and are, therefore, potentially important mediators in adjusting season-specific thermal tolerance limits of the fish heart.

Heat tolerance of body functions is lowest in the intact animal and increases towards lower levels of biological organization (Lagerspetz, 1987). This rule also applies to fish cardiac function, since heat tolerance of cardiac excitability is lowest *in vivo*, but better in isolated tissues *in vitro* and in molecular level functions of enzymatically isolated cardiac myocytes. A recent study on brown trout (*Salmo trutta fario*), acclimated at 12 °C, showed that cardiac K^+ currents are markedly resilient to high temperatures (Vornanen et al., 2014). However, heat resistance of K^+ currents has not been measured from thermally acclimated/acclimatized fish. Since I_{K1} and I_{Kr} are important currents in regulation of AP shape and their densities are modified by thermal acclimation/acclimatization in fish, it is possible their heat resistance is also affected by sustained temperature changes, *e.g. via* changes in lipid membrane fluidity or isoform composition of channels. To this end, we examined the upper thermal tolerance of cardiac I_{K1} and I_{Kr} and their molecular background in winter- and summer-acclimatized roach (*Rutilus rutilus*). Roach have the ability to shift thermal tolerance window of cardiac excitability and to resist cardiac arrhythmia in season-specific manner (Badr et al., 2016). Therefore, it was hypothesized that those seasonally primed functional differences are partly dependent on thermal acclimatization of APs and K^+ currents.

2. Materials and methods

2.1. Animals

Roach (*Rutilus rutilus*) were caught from Lake Pyhäselkä in Central Finland (62°35′ N, 21°34′ E) and maintained in the aquarium facilities of University of Eastern Finland in temperature-controlled 500 l stainless steel tanks with a continuous supply of aerated groundwater and under a 12:12-h light-dark photoperiod. Winter acclimatized roach (WiR) (78.9 ± 4.5 g, n = 18) were caught at the end of February from ice-covered lake (water temperature 0–4 °C) and maintained for a minimum of 3 weeks at water temperature of 4 ± 1 °C until used in the experiments. Summer acclimatized roach (SuR) (56.6 ± 5.1 g, n = 17) were caught in June–September (water temperature 15–19 °C) and maintained in the laboratory at water temperature of 18 ± 1 °C for a minimum of 3 weeks prior to the experiments. SuR were slightly smaller than WiR ($p < 0.05$). Both groups were fed 5 times/week trout fodder (EWOS, Turku, Finland). All experiments were authorized by the national animal experimental board in Finland (permission ESAVI/2832/04.10.07/2015).

2.2. Isolation of cardiac myocytes

The roach were stunned by a blow to the head and killed by severance of the spine and destruction of the brain. The heart was quickly excised and rinsed in Ca^{2+}-free low Na^+ solution containing (in mmol l^{-1}): 100 NaCl, 10 KCl, 1.2 KH_2PO_4, 4 $MgSO_4$, 50 taurine, 20 glucose and 10 HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] with pH adjusted to 6.9 at 20 °C with KOH. Atrial and ventricular myocytes were isolated from the excised heart with enzymatic digestion as previously described (Vornanen, 1997). Briefly, after a 7-min perfusion with Ca^{2+}-free low-Na^+ saline the heart was digested by a 15-min perfusion with the same solution containing collagenase (Type IA; 0.75 mg ml^{-1}, Sigma, St Louis, MO, USA), trypsin (Type IX; 0.5 mg ml^{-1}, Sigma) and fatty acid-free bovine serum albumin (BSA; 0.75 mg ml^{-1}, Sigma). Solutions were continuously gassed with 100% O_2. Softened atrial and ventricular muscles were separately chopped in a small volume of low Na^+ solution and single myocytes were released by gentle agitation of muscle pieces through the opening of a Pasteur pipette. Isolated myocytes were stored at 5 °C in Ca^{2+}-free low-Na^+ solution and used within 8 h from isolation.

2.3. Whole-cell patch clamp

Ventricular APs and K^+ currents were recorded in current clamp and voltage clamp modes of the whole-cell patch-clamp, respectively, using an Axopatch 1D amplifier (Axon Instruments, Saratoga, CA, USA) equipped with a CV-4 1/100 head-stage (Axon Instruments). The digitized data were recorded using Clampex 9.2 software (Axon Instruments) and the recordings were analyzed using Clampex 10.4 software package. For the whole-cell patch-clamp a small aliquot of myocytes suspension was pipette into a 150 μl recording chamber (RC-26, Warner Instruments, Hamden, CT, USA) mounted on the stage of an inverted microscope (Nikon Eclipse TE200, Tokyo, Japan). Myocytes were allowed to adhere to the bottom of the chamber and then continuously superfused with the external saline solution (see below). The temperature of the external solution was regulated by using a Peltier device (HCC-100A, Dagan, MN, USA) and was continuously monitored with thermocouples positioned close to the myocytes and recorded on the same file with electrophysiological data.

Patch pipettes were pulled (PP-83-puller, Narishige, Tokyo, Japan) from borosilicate glass (King Precision, Claremont, CA) and filled with the same K^+-based electrode solution for both AP and K^+ current recordings (in mmol l^{-1}): 140 KCl, 4 MgATP, 1 $MgCl_2$, 5 EGTA (ethylene glycol tetra-acetic acid) and 10 HEPES at pH adjusted to 7.2 with KOH, giving a pipette resistance (means ± SEM) of 2.88 ± 0.08 MΩ (n = 204).The composition of the external solution for AP recordings was (in mmol l^{-1}): 150 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 1.8 $CaCl_2$, 10 glucose, and 10 HEPES at pH adjusted to 7.6 at 20 °Cwith NaOH. No ion channel blockers were included in the external solution. The external saline solution for K^+ current recordings contained (in mmol l^{-1}): 150 NaCl, 5.4 KCl, 1.5 $MgSO_4$, 0.4 NaH_2PO_4, 2 $CaCl_2$, 10 glucose, and 10 HEPES at