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SHORT COMMUNICATION

Dynamic changes in scope for heart rate and cardiac autonomic control during warm acclimation in rainbow trout

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ABSTRACT

Time course studies are critical for understanding regulatory mechanisms and temporal constraints in ectothermic animals acclimating to warmer temperatures. Therefore, we investigated the dynamics of heart rate and its neuro-humoral control in rainbow trout (*Onchorhynchus mykiss* L.) acclimating to 16°C for 39 days after being acutely warmed from 9°C. Resting heart rate was 39 beats min^{-1} at 9°C, and increased significantly when fish were acutely warmed to 16°C (Q_{10}=1.9), but then declined during acclimation (Q_{10}=1.2 at day 39), mainly due to increased cholinergic inhibition while the intrinsic heart rate and adrenergic tone were little affected. Maximum heart rate also increased with warming, although a partial modest decrease occurred during the acclimation period. Consequently, heart rate scope exhibited a complex pattern with an initial increase with acute warming, followed by a steep decline and then a subsequent increase, which was primarily explained by cholinergic inhibition of resting heart rate.

KEY WORDS: Acclimation dynamics, Cholinergic tone, Heart rate scope, Intrinsic heart rate, Maximum heart rate, Time course

INTRODUCTION

Acute warming in fish typically results in an exponential rise in oxygen consumption rate and cardiac output, driven by increased resting heart rate ($f_{H,rest}$), with Q_{10} values of ~2–3 (Clark et al., 2008; Gollock et al., 2006). However, with more chronic seasonal temperature changes, compensatory physiological adjustments (i.e. thermal acclimation) can be initiated to counteract the thermal effects, hence reducing Q_{10} (see Seebacher et al., 2015). Moreover, thermal acclimation capacity may be crucial for the resilience of ectotherms to global warming (see Chevin et al., 2010; Seebacher et al., 2015). While most thermal acclimation studies characterize physiological traits after extended exposure to fixed temperature regimes, the time course and rate of the acclimation response have received much less attention although this information can be essential for revealing the underlying mechanisms and time constraints associated with thermal acclimation (Sandblom et al., 2014; Somero, 2015).

If the increase in $f_{H,rest}$ during acute warming is greater than the increase in maximum heart rate ($f_{H,max}$), the scope for heart rate ($f_{H,scope}$) decreases, which may constrain aerobic performance capacity (Farrell et al., 2009). Conversely, if thermal acclimation lowers $f_{H,rest}$, the scope may be restored (Franklin et al., 2007). Hence, the thermal plasticity of $f_{H,rest}$ and $f_{H,scope}$ is a key component in the overall compensatory acclimation response to elevated temperatures in ectotherms. Under steady-state conditions, $f_{H,rest}$ is determined by the tonic activity of extrinsic stimulatory adrenergic nerves and circulating catecholamines, inhibitory cholinergic ('vagal') nerves, as well as the intrinsic heart rate ($f_{H,intr}$) (Nilsson, 1983; Sandblom and Axelsson, 2011). Thermal acclimation of $f_{H,rest}$ in the intact animal probably involves changes to both $f_{H,intr}$ (Aho and Vornanen, 2001; Haverinen and Vornanen, 2007) and extrinsic control mechanisms (Graham and Farrell, 1989; Priede, 1974; Sureau et al., 1989; Wood et al., 1979). Yet, there is little information about the time course and dynamic interaction between intrinsic and extrinsic factors determining $f_{H,rest}$ and $f_{H,scope}$ during thermal acclimation.

We determined resting, intrinsic and maximum heart rates, as well as adrenergic and cholinergic tone in separate groups of rainbow trout, *Oncorhynchus mykiss*, acclimated to 9°C and when acutely transferred to 16°C. The subsequent dynamic warm acclimation response was then determined after 2, 4, 7, 11, 18 and 39 days at 16°C. This temperature span is well within the natural thermal tolerance range and has previously been used in thermal acclimation studies on rainbow trout (Gräns et al., 2009; Priede, 1974). We hypothesized that an initial increase in $f_{H,rest}$ with acute warming would be counteracted by increased cholinergic tone, reducing $f_{H,rest}$ (Ekström et al., 2014). However, as $f_{H,intr}$ was expected to gradually decline during the acclimation period, the importance of cholinergic inhibition of $f_{H,rest}$ was predicted to decrease as warm acclimation progressed. We further hypothesized that $f_{H,scope}$ would initially be lowered with the acute warming as a result of a greater increase in $f_{H,rest}$ than $f_{H,max}$, but would then recover because of gradually reduced $f_{H,rest}$.

MATERIALS AND METHODS

Animals

Rainbow trout (*O. mykiss*, N=115, mass 37.4±0.6 g) of mixed sexes were obtained from Antens Laxodling AB (Alingsås, Sweden) and kept in a 500 l holding tank supplied with aerated freshwater from a recirculating system. They were acclimated to 9°C for at least 8 weeks prior to experimentation under a daily 12 h:12 h light:dark cycle and fed commercial feed once weekly. Experimental procedures were covered by ethical permit 65-2012 from the ethical committee of Gothenburg.

Instrumentation

Fish were anaesthetized in freshwater containing MS-222 (tricaine methanesulphonate; 150 mg l^{-1}) buffered with $NaHCO_3$ (300 mg l^{-1}). The fish was placed ventral side up on wet foam on an operating table where anaesthesia was maintained by irrigating the gills with water containing MS-222 (75 mg l^{-1}) and $NaHCO_3$ (150 mg l^{-1}). For heart rate measurements, two ECG electrodes (AS 631-2, Cooner Wire, Chatsworth, CA, USA) were inserted between

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Received 6 November 2015; Accepted 4 February 2016

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 1106-1109 doi:10.1242/jeb.134312

the pectoral fins using a 23 gauge needle (Sterican, B. Braun Medical AB, Danderyd, Sweden). One electrode was directed caudally and one was directed cranially with the tip close to the heart. A polyethylene catheter (PE 50) for injection of pharmacological substances was then introduced intraperitoneally. The electrode wire and catheter were secured to the skin using 4.0 silk sutures. Following surgery, fish were placed in the experimental setup (see below) and allowed at least 24 h of post-surgery recovery.

Experimental setup and protocols

The experiments were conducted between February and June. The fish were placed in opaque holding tubes (length 250 mm, diameter 63 mm) in a 250 l tank, continuously supplied with flow-through freshwater from the recirculating system. A 9 kW heater (K060, Värmebaronen, Kristianstad, Sweden) was used for temperature control. Eight separate groups of fish were examined at the initial acclimation temperature of 9°C (day 0), immediately after warming to 16°C (day 1) or subsequently after 2, 4, 7, 11, 18 and 39 days of acclimation to 16°C.

On each measurement day, $f_{H,rest}$ was first recorded for several hours in all fish to confirm a stable baseline. Individual fish were then randomly allocated to one of two experimental protocols, either to determine $f_{H,max}$ using a chasing protocol (protocol 1) or to determine cardiac autonomic tone and $f_{H,intr}$ (protocol 2), as described below.

Protocol 1: the chasing experiments to determine $f_{H,max}$ were performed in a round tank (diameter 0.6 m, height 0.3 m, ∼85 l) supplied with flow-through aerated water from the recirculating system, maintaining the same temperature as the experimental tank. Fish were individually chased for 5 min until fatigue and quickly returned to the holding tube to record $f_{H,max}$.

Protocol 2: the pharmacological treatment group largely followed the protocol of Altimiras et al. (1997) and modified by Sandblom et al. (2010). Briefly, fish were intraperitoneally injected with atropine sulphate (1.2 mg kg^{-1}) to block muscarinic receptors and f_H was recorded for approximately 30 min, allowing the atropine to take full effect. Next, β-adrenergic receptors were blocked by injecting propranolol (3 mg kg^{-1}) and f_H was recorded for 1 h to allow the drug to have full effect. Pharmacological substances were dissolved in saline (0.9% NaCl) and injected as a 1 ml kg^{-1} bolus followed by 0.5 ml of saline to flush the catheter. All chemicals and pharmacological substances were purchased from Sigma-Aldrich (St Louis, MO, USA). Following the experiments of either protocol, fish were killed in water containing a high dose of MS-222 (500 mg l^{-1}).

Data acquisition and calculations

The ECG electrodes were connected to bioamplifiers (ML136, AD Instruments, Castle Hill, Australia) connected to a PowerLab (AD Instruments) and a computer running LabChart Pro software (v7.2.2, AD Instruments). Resting and maximum heart rates were determined from the rate of ventricular depolarizations (R peaks) using the ECG module in LabChart Pro. $f_{H,rest}$ was taken from periods when the heart rate was low and stable, and $f_{H,max}$ was taken at the maximum heart rate response following the chase protocol. Heart rate scope was calculated as:

$$f_{H,scope} = f_{H,max} - f_{H,rest}. \quad (1)$$

In the pharmacological experiments, $f_{H,intr}$ was obtained after complete autonomic blockade and cholinergic and adrenergic tone were calculated according to Altimiras et al. (1997).

Q_{10} values for the group means between 9°C and subsequent days at 16°C were calculated for the rate-dependent variables ($f_{H,rest}$ and $f_{H,intr}$) using the Van't Hoff equation (Seebacher et al., 2015).

Statistics

Values are means±s.e.m. unless otherwise stated. Experimental sample sizes were based on previous studies on these variables in rainbow trout. To evaluate the effects of temperature change, a comparison between the values at 9°C (day 0) and values at subsequent days at 16°C (days 1–39), was conducted using a general linear model with Dunnett's 2-tailed *post hoc* tests. To further investigate the acclimation response at 16°C, a general linear model including the seven groups measured at 16°C was used. Normality and homogeneity of variances were verified using Shapiro–Wilk and Levene tests, respectively. Maximum heart rate was power transformed to meet these assumptions. Statistical significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

The $f_{H,rest}$ at 9°C was 39±2 beats min^{-1}, which corresponds well with previous studies on rainbow trout (Ekström et al., 2014; Gamperl et al., 1995; Priede, 1974). Acute warming to 16°C elicited an expected increase in $f_{H,rest}$, which was highest in the group acclimated to 16°C for 2 days (60±3 beats min^{-1}, Q_{10}=1.9; Fig. 1A,B). However, the $f_{H,rest}$ in the groups acclimated to 16°C was significantly affected by acclimation time and decreased from the initial acute response to 45±3 beats min^{-1} in the group acclimated for 39 days (Q_{10}=1.2; Fig. 1A,B). This is close to full thermal compensation (i.e. Q_{10}=1) and the degree of thermal compensation of $f_{H,rest}$ in this study is indeed high when compared with other acclimation studies on trout (Q_{10}=1.5–2.6) (Gräns et al., 2009; Priede, 1974; Wood et al., 1979). These discrepancies are probably due to the shorter acclimation times used in these studies (2–4 weeks), resulting in a lower degree of compensation. Nonetheless, the present study shows that following an acute increase from 9 to 16°C, $f_{H,rest}$ remains significantly elevated for the first week and it takes somewhere in the range of 18–39 days for the compensatory acclimation response to level out (Fig. 1A,B). However, we cannot exclude the possibility that further thermal compensation (e.g. complete) would have occurred had we extended the acclimation time further.

One of the few other investigations of dynamic changes in cardiovascular variables during thermal acclimation in fish is a recent study on $f_{H,rest}$ in the estuarine longjaw mudsucker, *Gillichthys mirabilis* (Jayasundara and Somero, 2013). In contrast to the present study, Jayasundara and Somero (2013) found little thermal compensation of $f_{H,rest}$ and suggested that this may reflect a strategy minimizing the energetic costs associated with acclimation in a highly thermally variable environment. In fact, the pronounced heart rate acclimation capacity observed in rainbow trout may reflect a physiological phenotype adapted to accommodate predictable seasonal temperature changes typical of temperate regions (see Klaiman et al., 2011).

The cholinergic tone at 9°C (36±16%) did not differ significantly from the group acutely warmed to 16°C (46±15%), but increased significantly with further warm acclimation to reach a maximum of 69±18% at day 18 (Fig. 1C). The adrenergic tone was 28±10% at 9°C and there was no significant change with acclimation to 16°C (Fig. 1C). The $f_{H,intr}$ was 41±3 beats min^{-1} at 9°C and peaked at 68±3 beats min^{-1} on day 2 at 16°C (Q_{10}=2.1; Fig. 1A,B). While this acute effect resembles the acute effect observed for $f_{H,rest}$, no significant acclimation effect was evident

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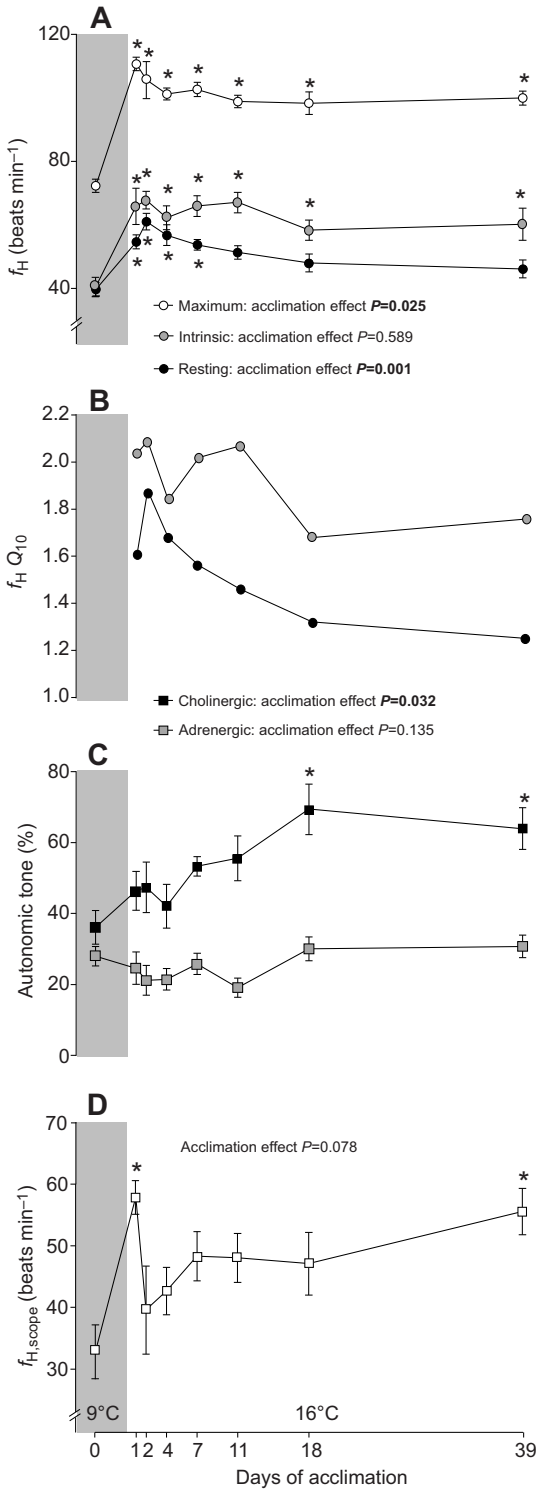

Fig. 1. Effects of warm acclimation on heart rate and cardio-regulatory mechanisms in rainbow trout (*Onchorhynchus mykiss*). (A) Resting, intrinsic and maximum heart rate (f_H); (B) Q_{10} for mean resting and intrinsic heart rates; (C) cholinergic and adrenergic tone; and (D) scope for heart rate ($f_{H,scope}$). Values were recorded in separate groups of fish (N=6–18) at the initial acclimation temperature of 9°C (day 0; shaded area), after acute transfer to 16°C (day 1) and subsequently at regular intervals during acclimation to 16°C (days 2–39). Sample sizes for each treatment group and acclimation day are specified in Table S1. *Statistically significant difference ($P \leq 0.05$) from the group acclimated to 9°C. P-values refer to the results from the general linear model determining the general acclimation effect in the seven groups acclimated to 16°C.

for $f_{H,intr}$ in the groups acclimated to 16°C and the Q_{10} was still 1.8 for this variable after 39 days at 16°C (Fig. 1A,B). Consequently, the decrease in $f_{H,rest}$ during warm acclimation was mainly due to an increase in cholinergic tone, which was significant from day 18 (Fig. 1A,C). This is consistent with a comparison of *in vivo* data with studies on *in situ* perfused hearts from thermally acclimated trout. In the perfused heart preparation, all extrinsic cardiac control is absent and the thermal compensation of heart rate is often relatively low (Q_{10}=2.0–2.2; Graham and Farrell, 1985; Graham and Farrell, 1989), whereas the thermal compensation of $f_{H,rest}$ *in vivo* can be considerably greater (Q_{10}=1.2–1.5, present study and Priede, 1974). Again, this highlights the importance of changes in autonomic tone for the thermal resetting of $f_{H,rest}$ in the intact animal.

$f_{H,max}$ increased from 72±2 beats min^{-1} in the 9°C acclimated group to a peak value of 111±2 beats min^{-1} in the group acutely warmed to 16°C (day 1), and then remained significantly elevated relative to the 9°C group throughout the warm acclimation period (Fig. 1A). These patterns are qualitatively consistent with previous studies on heart rate acclimation in various Arctic sculpin species, albeit at lower temperatures (Franklin et al., 2007, 2013). Even so, a significant general acclimation effect on $f_{H,max}$ was found in trout, suggesting a somewhat decreased $f_{H,max}$ with warm acclimation (Fig. 1A).

As a result of the concurrent changes in $f_{H,rest}$ and $f_{H,max}$, $f_{H,scope}$ initially increased significantly from 33±4 beats min^{-1} at 9°C to 58±3 beats min^{-1} in the group acutely warmed to 16°C, but then drastically declined to 40±7 beats min^{-1} after 2 days acclimation to 16°C, primarily due to the elevated $f_{H,rest}$ in this group (Fig. 1A,D). However, $f_{H,scope}$ subsequently increased and was again significantly elevated relative to the 9°C group after 39 days of acclimation to 16°C (Fig. 1D). Thus, while the increased $f_{H,max}$ with acute warming to 16°C explained the initial increase in $f_{H,scope}$, our data reveal that increased cholinergic inhibition of $f_{H,rest}$ was crucial for the subsequent increase in $f_{H,scope}$ during warm acclimation because $f_{H,max}$ did not increase further with acclimation. Interestingly, the current findings on the thermal acclimation of heart rate in trout are qualitatively consistent with a similar study on metabolic acclimation in the shorthorn sculpin, *Myoxocephalus scorpius*, where the recovery and increase in aerobic scope with warm acclimation were mainly the result of reduced standard metabolic rate, whereas maximum metabolic rate was little affected (Sandblom et al., 2014). Thus, the elevated cholinergic tone on the heart with warm acclimation may reflect an adaptive mechanism allowing warm-acclimated fish to quickly modulate heart rate and cardiac output through vagal release during transient periods of additionally elevated oxygen demand (e.g. exercise and digestion), and may therefore be fundamental to maintain a high metabolic scope following warm acclimation, as observed previously in various temperate and Arctic fish species (Gräns et al., 2014; Sandblom et al., 2014; Seth et al., 2013).

The mechanistic underpinnings of the increase in cholinergic tone with warm acclimation are presently not clear, but may reflect a greater expression of vagal activity as the metabolic oxygen demand is gradually reduced during warm acclimation. Another possible mechanism is an up-regulation of cardiac muscarinic receptor density, which would increase the cardiac sensitivity to cholinergic stimulation. While an up-regulation of cardiac β-adrenergic receptors has been demonstrated with cold acclimation in rainbow trout (Keen et al., 1993), the chronic temperature effects on cardiac muscarinic receptor density have to our knowledge not been examined.

The current study on rainbow trout provided several novel insights into the dynamic changes in cardiovascular function and its neuro-hormonal control during warm acclimation. The changes in $f_{H,scope}$ with warming exhibited a complex pattern with an initial increase due to a greater increase in $f_{H,max}$ than $f_{H,rest}$, followed by a decline due to a further increase in $f_{H,rest}$ and then a subsequent increase due to elevated cholinergic tone suppressing $f_{H,rest}$ during warm acclimation. Ultimately, increased knowledge about the dynamics and the temporal and physiological constraints to thermal acclimation will advance our understanding of how species biogeography and ecological interactions change seasonally, as well as how organisms may be affected by global change altering thermal conditions.

Acknowledgements
The authors thank Michael Axelsson, Catharina Olsson, Fredrik Jutfelt and Jeroen Brijs for valuable input during this study.

Competing interests
The authors declare no competing or financial interests.

Author contributions
E.S. and A.E. conceived and designed the experiments. K.H. and A.E. performed the experiments and analyzed the data. N.P. and A.G. contributed to the statistical analysis. A.E. wrote the manuscript and all authors provided feedback and contributed to its completion.

Funding
This research was funded by the Swedish Research Council (VR), the Swedish research council for environment, agricultural sciences and spatial planning (FORMAS), the Helge Axelsson Johnson foundation and Wilhelm and Martina Lundgren's research foundation.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.134312/-/DC1

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