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RESEARCH ARTICLE

Effects of Na^+ channel isoforms and cellular environment on temperature tolerance of cardiac Na^+ current in zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Heat tolerance of heart rate in fish is suggested to be limited by impaired electrical excitation of the ventricle due to the antagonistic effects of high temperature on Na^+ (I_{Na}) and K^+ (I_{K1}) ion currents (I_{Na} is depressed at high temperatures while I_{K1} is resistant to them). To examine the role of Na^+ channel proteins in heat tolerance of I_{Na}, we compared temperature dependencies of zebrafish (*Danio rerio*, warm-dwelling subtropical species) and rainbow trout (*Oncorhynchus mykiss*, cold-active temperate species) ventricular I_{Na}, and I_{Na} generated by the cloned zebrafish and rainbow trout $Na_V1.4$ and $Na_V1.5$ Na^+ channels in human embryonic kidney (HEK) cells. Whole-cell patch-clamp recordings showed that zebrafish ventricular I_{Na} has better heat tolerance and slower inactivation kinetics than rainbow trout ventricular I_{Na}. In contrast, heat tolerance and inactivation kinetics of zebrafish and rainbow trout $Na_V1.4$ channels are similar when expressed in the identical cellular environment of HEK cells. The same applies to $Na_V1.5$ channels. These findings indicate that thermal adaptation of ventricular I_{Na} is largely achieved by differential expression of Na^+ channel alpha subunits: zebrafish that tolerate higher temperatures mainly express the slower $Na_V1.5$ isoform, while rainbow trout that prefer cold waters mainly express the faster $Na_V1.4$ isoform. Differences in elasticity (stiffness) of the lipid bilayer and/or accessory protein subunits of the channel assembly may also be involved in thermal adaptation of I_{Na}. The results are consistent with the hypothesis that slow Na^+ channel kinetics are associated with increased heat tolerance of cardiac excitation.

KEY WORDS: Atrioventricular block, Cardiac sodium channels, Electrical excitation, Heart rate, Thermal adaptation

INTRODUCTION

Temperature is a major environmental factor that has exerted a strong selective pressure on animal life forms. Temperature-driven evolution has led to significant variation in thermal tolerance of ectothermic vertebrates, including fishes (Beitinger, 2000; Johnston and Bennett, 2008). Some fish species are adapted to a relatively narrow range of temperatures such as the stenothermic teleosts of the Antarctic Ocean and the polar cod (*Boreogadus saida*) of the Arctic Ocean (Somero and DeVries, 1967; Beers and Sidell, 2011; Drost et al., 2016; Abramochkin et al., 2019). In temperate climates, fishes usually have a wider thermal tolerance range, e.g. rainbow trout (*Oncorhynchus mykiss*) are cold-water fish that survive temperatures between 0 and 28°C (Hokanson et al., 1977; Beitinger, 2000). The zebrafish (*Danio rerio*), a teleost fish of the lakes and rivers of South-eastern Asia, also tolerates a wide temperature range (7–41°C) but occupies warmer habitats than rainbow trout (Cortemeglia and Beitinger, 2005; López-Olmeda and Sánchez-Vázquez, 2011).

Despite considerable research efforts, the physiological basis for the high temperature tolerance of fishes and other ectotherms remains unresolved. Practically all major processes and functions of the animal body (e.g. circulation, sensory/motor functions, behaviour, metabolism, digestion, immune defence, reproduction) are affected by temperature and therefore more or less adapted to the habitat temperature of the animal (Angilletta et al., 2002; Gracey et al., 2004; Podrabsky and Somero, 2004; Vornanen et al., 2005; MacMillan, 2019). Therefore, it is likely that when approaching the upper temperature tolerance limit of an animal, several processes simultaneously weaken in the animal's body, but the severity of their effect at the organismal level may vary and manifest with varying delays (Vornanen, 2020). Alternatively, different processes/functions may have slightly different thermal optima or failure temperatures, depending for example on the level of biological organization where the process/function appears (Lagerspetz, 1987; Clark et al., 2013). A fundamental question arises: is there a single underlying process or interaction principle that drives the thermal collapse of multiple organ systems? Given that similar thermal limitations appear to apply to unicellular organisms and Metazoa, it has been suggested that the limiting processes of animal life are found at the molecular level (Tattersall et al., 2012).

Electrical excitability is a common process for most tissues of the animal body including nerves, skeletal muscles, heart and smooth muscles. These tissues are responsible for almost all vital functions of the animal body like sensation, learning, behaviour, locomotion, blood circulation, digestion and homeostasis of the body (Hille, 2001). Recently, we have gathered evidence showing that high temperatures can impair electrical excitability and therefore potentially limit the upper thermal tolerance of both ectothermic and endothermic animals (Vornanen, 2020). Electrical excitability or generation of propagating action potentials (APs) is the result of interaction between inward and outward directed flow of ions across the plasma membrane. In studies on thermal tolerance of electrical excitability we have used fish ventricular myocytes as a model system, as they are well suited for patch-clamp experiments. Atrial myocytes are a less suitable model as they fail to maintain stable resting potential in the current-clamp mode of patch-clamp due to

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the tiny background inward rectifier current (I_{K1}) and the absence of acetylcholine-dependent inward rectifier current (I_{KACh}) in isolated cells (Vornanen et al., 2002; Molina et al., 2007). In a quiescent ventricular myocyte, there is a negative resting membrane potential (V_{rest}), which is maintained by K^+ efflux via I_{K1}. For initiation and propagation of cardiac AP, V_{rest} must be depolarized to the threshold potential (V_{th}) of an AP. This is accomplished by Na^+ influx through the voltage-gated Na^+ channels, which generate the sodium current (I_{Na}). AP is initiated only when the charge transfer of the inward I_{Na} (the source current) exceeds the charge transfer of the outward I_{K1} (the sink current or resting membrane leak). At high temperatures, electrical excitability of fish ventricular myocytes may fail due to mismatch between the source current (I_{Na}) and the sink current (I_{K1}) (Vornanen et al., 2014). Acute warming reduces charge transfer via the I_{Na}, while K^+ leak via I_{K1} increases: I_{Na} fails to depolarize V_{rest} to the threshold potential of AP. At the level of a working heart this appears as atrioventricular block and depression of ventricular beating rate (Haverinen and Vornanen, 2020), which may eventually result in a collapse of cardiac output and thermal death of the fish.

The present study aimed to test the source–sink mismatch hypothesis with respect to the source current, I_{Na} (Vornanen, 2020). Given the differences in temperature tolerances between rainbow trout (a cold-active temperate species) and zebrafish (a warm-dwelling subtropical species), it was hypothesized that thermal tolerance of the zebrafish I_{Na} is higher than that of the rainbow trout I_{Na}. This assumption was tested by comparing I_{Na} of zebrafish and rainbow trout ventricular myocytes at different temperatures. Another prediction of the source–sink hypothesis is that the channels that generate currents with slow gating kinetics can withstand high temperatures better than channels that produce currents with fast kinetics (Touska et al., 2018; Vornanen, 2020). This is based on the assumption that stiffer molecules have higher activation energy and slower kinetics, i.e. there is a trade-off between flexibility and thermal stability of the proteins (Somero, 1995; Zavodszky et al., 1998; Fields, 2001). Studies on mammalian Na^+ channels have shown that the skeletal isoform, $Na_V1.4$, has faster inactivation kinetics than the cardiac isoform, $Na_V1.5$ (Wang et al., 1996). I_{Na} with slow inactivation kinetics is able to provide more depolarizing charge at high temperature than I_{Na}, which is rapidly inactivated. As zebrafish and trout ventricular I_{Na} are mainly generated by $Na_V1.5$ and $Na_V1.4$ channels, respectively (Haverinen et al., 2007; Haverinen et al., 2018), it was hypothesized that those channel isoforms are involved in adaptation of cardiac I_{Na} to high and low temperature, respectively. To this end, the inactivation kinetics and charge transfer of I_{Na} between rainbow trout and zebrafish ventricular myocytes were compared at different temperatures.

Finally, it was hypothesized that thermal stability and kinetics of fish I_{Na} depend in part on the cellular environment (e.g. biophysical properties of the lipid membrane and/or ancillary protein subunits) where they are expressed. To test this, the two main alpha subunits of zebrafish and rainbow trout Na^+ channels were cloned and expressed in a mammalian cell line, the human embryonic kidney (HEK) cell. This allowed comparison of inactivation kinetics and thermal resistance of the Na^+ channels in an identical cellular environment.

MATERIALS AND METHODS

Animals

The wild-type zebrafish, *Danio rerio* (F. Hamilton 1822) (*ab* strain, kindly donated by Dr Maxim Lovat, Lomonosov Moscow State University), were raised and maintained at the animal facilities of Lomonosov Moscow State University according to common practices (Westerfield, 2007). The rearing temperature of the fish was 28°C. Fish of either sex, about 1.5 years old, were used for electrophysiological experiments (N=8). Zebrafish were killed by immersion in an ice-water bath and cutting of the spine. Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), were obtained from a local fish farm (Kontiolahti, Finland). Fish weighing 118.7±20.6 g (N=11) of either sex, acclimated at 12°C for more than 3 weeks, were used in electrophysiological experiments and gene cloning. Trout were stunned by a quick blow to the head and killed by cutting of the spine immediately behind the head. The experiments conform to the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No. 123, Strasbourg 1985) and were authorized by the national animal experimental board in Finland (permission ESAVI/8877/2019).

Cloning of *SCN5LA* and *SCN4A* Na^+ channel genes of zebrafish and rainbow trout

Total cardiac RNA was extracted by TriReagent (Thermo Fisher Scientific, Vilnius, Lithuania) and the quality and quantity of RNA were determined by agarose gel electrophoresis and NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. RNA (2 µg) was treated with RNase free DNase (Thermo Fisher Scientific) and converted to cDNA using SuperScript IV reverse transcriptase (Invitrogen, Glasgow, UK) and oligo(dT) primers. cDNA (1 µl) was used as a template in 25 µl polymerase chain reaction (PCR) including a final concentration of 0.2 mmol l^{-1} dNTP mix, 0.2 mmol l^{-1} primers (synthesized by Invitrogen) as shown in Table 1, and 0.02 U $µl^{-1}$ Phusion High Fidelity DNA polymerase (Thermo Fisher Scientific). Cycling conditions for all PCR reactions were as follows: initial denaturation at 98°C for 1 min, 35 cycles at 98°C for 10 s, at 60°C for 30 s and at 72°C for 100–260 s (40 s per kb; for the length of the products see Table 1), followed by final extension at 72°C for 5 min. The PCR products were separated on a 0.8% agarose gel, and the nucleotide chains were extracted from the gel using a GeneJET Gel Extraction Kit (Thermo Fisher Scientific). Overhang adenines were added to the 3′-ends of PCR products using Dynazyme II DNA polymerase (Thermo Fisher Scientific) and products were ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA). The inserts were digested from the pGEM-T Easy vector and directionally cloned into the expression vector. If the coding sequence of the *SCN* gene was amplified by PCR in two parts, the pieces were ligated into the expression vector to form the entire coding sequence. Coding sequences of the *SCN5LA* genes of rainbow trout (*om* for *O. mykiss*) and zebrafish (*dr* for *D. rerio*) were cloned into the expression vector pcDNA3.1/Zeo(+) (Invitrogen). Several attempts to clone the full-length *om/drSCN4A* into the pcDNA3.1 failed. To overcome this problem, the high-copy number vector pcDNA3.1/Zeo(+) was converted to a low-copy number vector that was better tolerated by bacterial cells. To this end, the ColE1 origin was replaced by the pMB1 origin via digesting pcDNA3.1/Zeo(+) with BsmI and PvuI and replacing this fragment (bases 2768–4462) with the digested fragment from pBR322 (bases 1353–3733). The coding sequences of *dr/omSCN4A* were successfully cloned into this modified vector. Plasmids were isolated using PureLink HiPure Plasmid Midiprep Kit (Thermo Fisher Scientific) and sequenced by GATC Biotech AB (Köln, Germany). Nucleotide sequences were converted to protein sequences using EMBOSS Transeq software (https://www.ebi.ac.uk/Tools/st/emboss_transeq/), and the protein sequences of the species-specific isoforms were aligned with EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/). The fish *SCN4A* and *SCN5LA*

Table 1. Primers used in cloning of the *SCN* genes of rainbow trout and zebrafish

Target gene	Accession number	Primers in 5′–3′ orientation	Amplified region*	Product length (bp)
drSCN4Ab	NM_001045065	F: tgtcaagatggcgcgtct	−7 to 2562	2569
		R: gcgtcggccttctccatttac	2296 to +238	3489
		F: ggcatgtgcatcatcgtctt		
		R: tcatggcaggttctgagcat		
drSCN5LAb	NM_001045123	F: atggcagccatactgtttcc	1 to 3384	3384
		R: ctccgacgtgttgatgtcac	2770 to +9 bp	3105
		F: ctcttcttggctttgctgct		
		R: gtttttgcgtcacagaaaagt		
omSCN4Abb	XM_021624591	F: aggcacaaccgtagtgtgaa	−44 to 2848	2892
		R: atgggtacatccagggtcaa	140 to 5565	5426
		F: agcagaatgccaagatggtc		
		R: tcaaacatcggactctttcag		
omSCN5LAba	XM_021562569	F: ataagaagatggccaccctg	−8 to +632	6530
		R: tcccgggatatctttacgtg		

*Minus sign denotes bases of 5′ untranslated region upstream from the start codon; plus sign denotes bases of 3′ untranslated region downstream from the stop codon. F, forward; R, reverse; bp, base pair.

genes are orthologous to the mammalian *SCN4A* and *SCN5A* genes. For simplicity, the protein names ($Na_V1.4$ and $Na_V1.5$) of the orthologous genes are used throughout the text.

Heterologous expression of *SCN4A* and *SCN5LA* genes

Due to the whole genome duplications of the teleost lineage, the number of gene paralogues expressed in fish striated muscles is high (Alderman et al., 2012; Glasauer and Neuhauss, 2014). All eight teleost Na^+ channel genes are expressed in the heart of both zebrafish and rainbow trout (Haverinen et al., 2018; Hassinen et al., 2021). Because striated muscle isoforms, $Na_V1.4$ and $Na_V1.5$, make up the great majority (>99%) of all Na^+ channel transcripts in zebrafish and trout hearts, genes for heterologous expression were selected from these paralogues. For both $Na_V1.5$ and $Na_V1.4$, there are three paralogues in the trout genome and two paralogues in the zebrafish genome. For $Na_V1.5$ channels, *drSCN5LAb* and *omSCN5LAba*, the most abundant paralogues of ventricular myocytes, were expressed in HEK cells. For the expression of zebrafish $Na_V1.4$ channels, *drSCN4Ab*, the most abundantly expressed *SCNA* paralogue of ventricular myocytes, was inserted in HEK cells. In the case of trout $Na_V1.4$ channels, *omSCN4Abb*, the second most abundant *SCN4A* paralogue of ventricular myocytes (most abundant in atrial myocytes) was expressed in HEK cells. We failed to clone the most abundant paralogue of the ventricle, *omSCN4Aba*, and were therefore forced to use the major atrial isoform, *omSCN4Abb*. Human embryonic kidney (HEK293; ECACC) cells were grown at 37°C in DMEM (Biowest) supplemented with 10% fetal bovine serum (FBS, sterile-filtered and heat inactivated; Sigma-Aldrich) and 100 U ml^{-1} penicillin and streptomycin (Sigma-Aldrich) in a 5% CO_2 environment. HEK cells were transiently co-transfected with pEGFP-N1 (Clontech), and either *drSCN4Ab*, *drSCN5LAb*, *omSCN4Abb* or *omSCN5LAba* construct using TurboFect transfection reagent (Thermo Fisher Scientific). Cells were transfected for 16–18 h at 37°C; plate medium was then refreshed, and the cells were further incubated at 28°C in a 5% CO_2 environment for at least 24 h. Incubation at lower temperature increased the expression level of Na^+ channels. Whole-cell patch-clamp experiments were conducted 48–72 h after transfection.

Patch-clamp measurements of Na^+ current in fish cardiac myocytes and HEK cells

The procedure of isolating zebrafish cardiomyocytes was essentially similar to the original method of isolating crucian carp (*Carassius carassius*) cardiac cells (Vornanen, 1997), but scaled down to the size of small zebrafish hearts and using slightly higher enzyme concentrations (1 mg ml^{-1} collagenase Type IA and 0.67 mg ml^{-1} Trypsin IV; both from Sigma). A blunt-ended syringe needle (34 gauge, TE734025; Adhesive Dispensing Ltd, Milton Keynes, UK) cannula was inserted via the bulbus arteriosus into the ventricle and secured in place with a fine thread. The heart was perfused first with Ca^{2+}-free solution for 5 min and then with the same solution but with added hydrolytic enzymes together with fatty acid-free serum albumin (1 mg ml^{-1}; Sigma) for 25–30 min. Myocytes were stored at 5°C and used on the same day as they were isolated. The Ca^{2+}-free solution contained (mmol l^{-1}): 100 NaCl, 10 KCl, 1.2 KH_2PO_4, 4 $MgSO_4$, 50 taurine, 20 glucose and 10 HEPES at pH 6.9 (adjusted with KOH at 20°C). From rainbow trout heart, myocytes were obtained using essentially the same procedure, but with lower enzyme concentrations (0.75 mg ml^{-1} collagenase Type IA and 0.5 mg ml^{-1} Trypsin IV) and shorter digestion time (10–12 min).

The whole-cell voltage-clamp recording of I_{Na} was performed using an Axopatch 200B or an Axopatch 1-D amplifier (Molecular Devices, San Jose, CA, USA) as previously described in detail (Haverinen et al., 2018). Cardiac myocytes or coverslips containing cultured HEK cells were placed in a small chamber with a continuous flow of K^+-based external saline solution containing (mmol l^{-1}): 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose and 10 HEPES, with pH adjusted to 7.7 at 20°C with NaOH. The temperature of the saline solution was set using a Peltier device (CL-100, Warner Instruments, Hamden, CT, USA; or TC-10, Dagan, Minneapolis, MN, USA) and monitored continuously with thermistors placed close to the cells. Patch pipettes with a resistance of 1.5–2.5 MΩ were drawn from borosilicate glass (Hilgenberg GmbH, Malsfeld, Germany) and filled with Cs^+-based electrode solution containing (mmol l^{-1}): 5 NaCl, 130 CsCl, 1 $MgCl_2$, 5 EGTA, 5 Mg_2ATP and 5 HEPES, with pH adjusted to 7.2 with CsOH. Current amplitudes were normalized to the capacitive cell size to obtain current density (pA pF^{-1}).

During I_{Na} recording, myocytes were superfused with a Cs-based low-Na^+ external saline solution, which contained (mmol l^{-1}): 20 NaCl, 120 CsCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose and 10 HEPES at pH 7.7 (adjusted with CsOH at 20°C) (Haverinen and Vornanen, 2004). In experiments with cardiac myocytes, nifedipine (10 µmol l^{-1}; Sigma) was included to block I_{Ca}. The low concentration of Na^+ outside the cell reduced the driving force for Na^+ influx and made it possible to achieve stable and complete voltage control of I_{Na}. Approximately 80% of the series resistance was compensated for when recording fast and large I_{Na}. I_{Na} was leakage-corrected using the P/N procedure of Clampex 9.2 software. For determination of the current–voltage (I–V) relationship, I_{Na} was elicited from the holding potential of −120 mV with 60 ms depolarizing pulses (range −100 to +70 mV) at a frequency of 1 Hz (Fig. 1A). The voltage dependence of Na^+ channel conductance was calculated from the I–V recordings using the equation: $G_{Na}=I_{Na}/(V-V_{rev})$, where G_{Na} is the Na^+ conductance of the membrane, I_{Na} is the peak current at a given membrane potential (V) and V_{rev} is the reversal potential of I_{Na}. The steady-state (SS) voltage dependence of activation was obtained by plotting the normalized conductance (G/G_{max}) as a function of membrane potential and fitting it into the equation of Boltzmann distribution:

$$y = 1/\left(1+\frac{\exp(V-V_{0.5})}{S}\right), \quad (1)$$

where V is membrane potential, $V_{0.5}$ the midpoint potential and S is the slope of the curve. SS inactivation was determined using a two-step protocol where a 300 ms conditioning pulse to potentials between −110 and −20 mV was followed by a 60 ms test pulse to −20 mV. The normalized test pulse currents (I/I_{max}) were plotted as a function of membrane potential and fitted to the Boltzmann function with a negative slope ($-S$).

The time constant of I_{Na} inactivation at different membrane potentials (–30 to 0 mV) was derived by fitting the decay phase of I_{Na} using the double exponential function of the Chebyshev transformation procedure of the Clampfit 10.3 software package. The amplitude of the fast component (τ_f) was over 90% of the current. The slow component could only be reliably determined at the voltages at which I_{Na} was close to its peak amplitude. Therefore, only the results of τ_f are reported. Thermal coefficient (Q_{10}) values of I_{Na} inactivation rate were calculated using the equation: $Q_{10}=(R_2/R_1)^{10°C/(T_2-T_1)}$, where R_1 and R_2 are fast time constants of inactivation (τ_f) at temperatures T_1 and T_2.

All mentioned properties of I_{Na} were analysed in native trout or zebrafish myocytes and HEK cells expressing $Na_V1.4$ or $Na_V1.5$ channels at three different temperatures: 12, 20 and 28°C. However, additional experiments to estimate the dynamics of the temperature dependence of I_{Na} were done using the ‘heat ramp’ protocol. In these experiments I_{Na} was elicited by repetitive depolarizations to −20 mV and the temperature was steadily raised from 12 to 28°C (up to 39°C in the case of native zebrafish myocytes).

Statistics

Statistical analyses were performed using SPSS (IBM; version 25). One-way ANOVA (with Tukey’s or Dunnett’s T3 *post hoc* test) and unpaired *t*-test were used to compare normally distributed data with homogenous variances. If the assumptions of parametric tests were not met, non-parametric Kruskal–Wallis and *post hoc* Mann–Whitney *U*-tests were used. $P<0.05$ was considered to show a statistically significant difference between means.

RESULTS

Temperature dependence of I_{Na} density and charge transfer in zebrafish and rainbow trout ventricular myocytes

Temperature dependency of ventricular I_{Na} was markedly different between zebrafish and rainbow trout myocytes, as shown by the current–voltage (I–V) relationships (Fig. 1). In zebrafish myocytes, an acute rise of temperature from 12 to 20°C resulted in a significant increase in I_{Na} density at voltages between −50 and −30 mV (Fig. 1C). A similar difference was detected in I_{Na} density between 12 and 28°C ($P<0.05$). However, the current densities at 20 and 28°C were almost identical, suggesting plateauing of I_{Na} in this temperature range (Fig. 1C). In rainbow trout myocytes, warming from 12 to 20°C increased I_{Na} density at voltages from −30 to −10 mV, but further warming to 28°C caused a strong depression of I_{Na} (Fig. 1E). Although the density of the zebrafish I_{Na} was higher at 20 and 28°C than at 12°C, the charge transfer (integral of I_{Na}) was significantly smaller than at 12°C (Fig. 1D). In trout ventricular myocytes, the integral of I_{Na} decreased with increasing experimental temperature (Fig. 1F). Notably, charge transfer/I_{Na} density ratio, i.e. depolarizing power, was 4.5–22.2 times higher (at −20 mV) for zebrafish than rainbow trout I_{Na} at all experimental temperatures (0.6, 0.7 and 0.9 V A^{-1} for rainbow trout and 2.7, 4.5 and 20.0 V A^{-1} for zebrafish at 28, 20 and 12°C, respectively).

Temperature tolerance of peak density and charge transfer of I_{Na} (at −20 mV) were further studied using acute heat ramps. To this end, the cells were warmed from 12°C to their upper thermal tolerance limit while I_{Na} was elicited by depolarization from −100 to −20 mV for every second (Fig. 1G). Consistent with the I–V data, there was a dramatic interspecies difference in temperature dependence of the peak I_{Na} (Fig. 1G). Initially, when the temperature was raised above 12°C, the density of I_{Na} increased in both species. However, the breakpoint temperature (T_{BP}, the temperature above which peak I_{Na} started to decrease steadily) was much lower for rainbow trout I_{Na} (18.3±0.6°C, N=13) than for zebrafish I_{Na} (26.6±0.5°C, N=16) ($P<0.05$).

Taken together, these findings indicate that the ventricular I_{Na} of rainbow trout heart is much less tolerant of high temperatures than the zebrafish ventricular I_{Na}.

Inactivation kinetics of I_{Na} in zebrafish and rainbow trout ventricular myocytes

Temperature dependence of I_{Na} inactivation kinetics was measured in the voltage range −30 to 0 mV at 12, 20 and 28°C (Fig. 2). Kinetics of zebrafish or rainbow trout I_{Na} accelerated with increasing temperature and membrane depolarization. There was a striking difference in the rate of I_{Na} inactivation between trout and zebrafish ventricular myocytes (Fig. 2). At 12, 20 and 28°C, the time constant (τ_f) of I_{Na} inactivation (at 0 mV) was about 10.0, 6.3 and 4.0 times faster in rainbow trout ventricular myocytes in comparison with zebrafish ventricular myocytes ($P<0.05$).

Steady-state activation and inactivation of I_{Na} in zebrafish and rainbow trout ventricular myocytes

Voltage dependence of steady-state (SS) activation and inactivation of I_{Na} was studied at 12, 20 and 28°C (Fig. 3). In zebrafish ventricular myocytes, warming from 12 to 20°C and further to 28°C decreased the slope factor of the SS inactivation curve ($P<0.05$), while $V_{0.5}$ remained unaffected ($P>0.05$; Fig. 3C; Table 2). Voltage dependence of SS activation of I_{Na} in zebrafish myocytes was more temperature sensitive, as warming to either 20 or 28°C shifted $V_{0.5}$ to the left by almost 10 mV and decreased the slope factor ($P<0.05$) (Table 2; Fig. 3C). In trout ventricular myocytes, warming from 12 to 20°C and further to 28°C failed to shift $V_{0.5}$ or change slope factor of both curves (Table 2; Fig. 3D).

Temperature dependence of I_{Na} density and charge transfer generated by $Na_V1.4$ or $Na_V1.5$ channels in HEK cells

Zebrafish and rainbow trout $Na_V1.4$ and $Na_V1.5$ Na^+ channels were expressed in the same cellular environment for a direct comparison between temperature dependencies of the orthologous gene

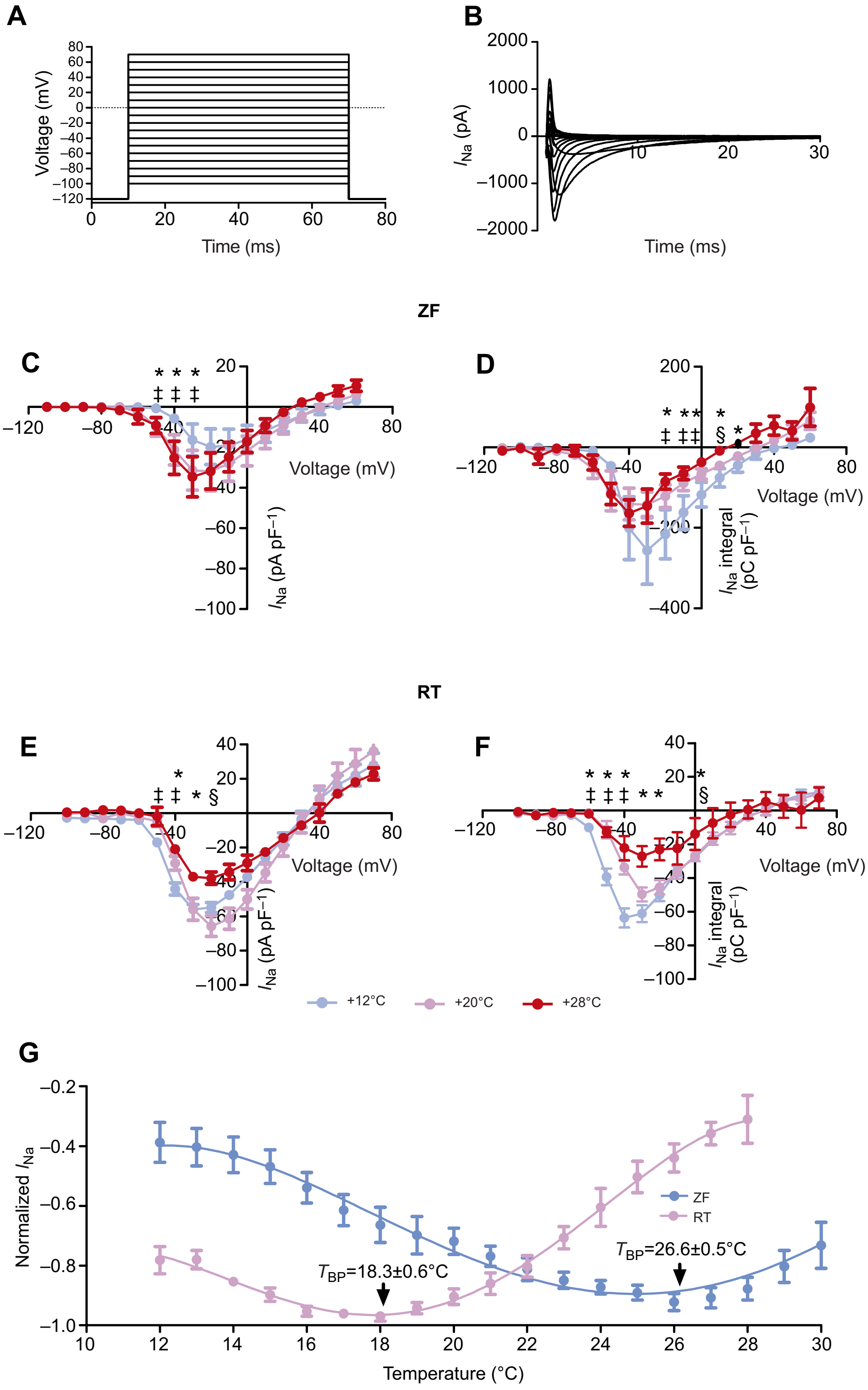

Fig. 1. Voltage and temperature dependence of current density and charge transfer of I_{Na} in zebrafish and rainbow trout ventricular myocytes. (A) The voltage protocol used to elicit I_{Na}. (B) A representative recording from a zebrafish ventricular myocyte indicating tracings of I_{Na} at different membrane voltages. (C–F) Mean results (±s.e.m.) for current–voltage relationship and charge transfer of I_{Na} at three different temperatures for zebrafish (ZF; *N*=47 myocytes from six fishes) (C,D) and rainbow trout (RT; *N*=33 myocytes from six fishes) (E,F) ventricle. (G) Current density of I_{Na} in heat ramp experiments with zebrafish (*N*=10 myocytes from two fishes) and rainbow trout (*N*=10 myocytes from four fishes) ventricle. Statistically significant differences (P<0.05) are shown as follows: *12 versus 28°C; ‡12 versus 20°C; §20 versus 28°C.

products (Fig. 4). Protein sequences of these Na^+ channels suggest a relatively high degree of structural similarity between the species. Trout and zebrafish $Na_V1.4$ shared 64.5% identity and 76.0% similarity, whereas identity and similarity for $Na_V1.5$ were 79.9 and 87.1%, respectively (Figs S1 and S2).

I_{Na} generated by the zebrafish $Na_V1.4$ channels responded to acute temperature increases in a similar manner to native ventricular I_{Na}: the current density increased, and the charge transfer decreased with increasing temperature (Fig. 4A,B). I_{Na} generated by the trout $Na_V1.4$ channels did not show any statistically significant

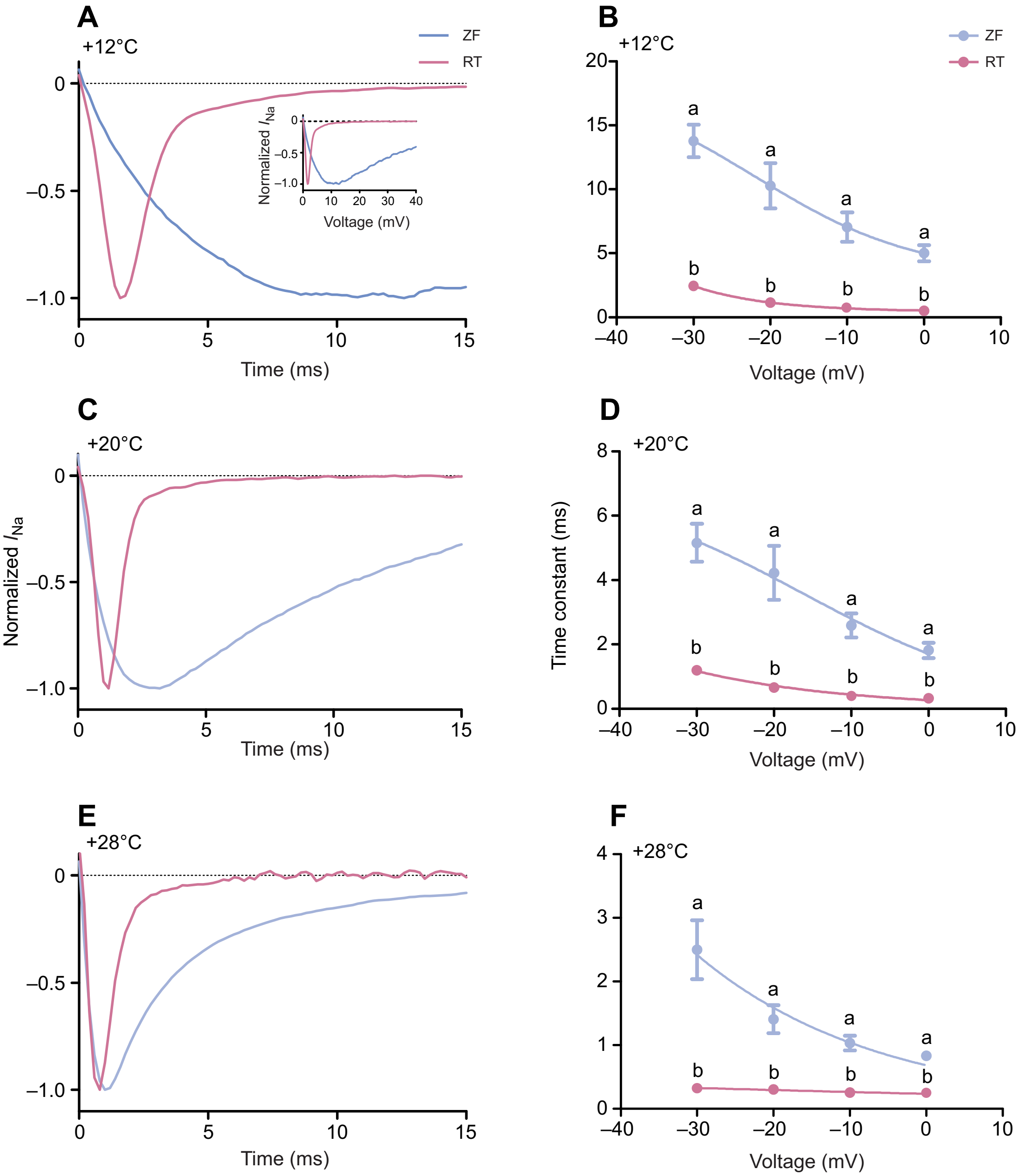

Fig. 2. Temperature dependence of inactivation kinetics of I_{Na} in zebrafish and rainbow trout ventricular myocytes. I_{Na} was elicited from a holding potential of −120 mV to −30 to 0 mV for 30 ms. (A,C,E) Representative tracings of I_{Na} at −20 mV at temperatures of 12°C (A), 20°C (C) and 28°C (E). (B,D,F) Means (±s.e.m.) of inactivation time constant (τ) at 12°C (B), 20°C (D) and 28°C (F). The results are from 31 and 30 myocytes from six zebrafish (ZF) and four rainbow trout (RT), respectively. Error bars of RT data are smaller than the size of the symbols. Statistically significant differences (P<0.05) between ZF and RT are shown by dissimilar letters (a and b).

differences between the three temperatures for either current density or charge transfer (Fig. 4C,D). (The expression level of trout $Na_V1.4$ channels in HEK cells was much lower than zebrafish $Na_V1.4$ channels, which may have reduced the resolution of the analysis.) The I_{Na} generated by trout $Na_V1.5$ channels was, however, much more heat tolerant than the native trout ventricular I_{Na}: current density and charge transfer strongly increased with increasing temperature in HEK cells (Fig. 4G,H), while in ventricular myocytes both variables were strongly reduced at 28°C (Fig. 1E,F).

I_{Na} density of the zebrafish $Na_V1.5$ channels in HEK cells increased with increasing temperature (P<0.05) (Fig. 4E). Differently from ventricular I_{Na}, the heterologously expressed $Na_V1.5$ channels positively responded to warming (at −50 mV) up to 28°C, i.e. I_{Na} indicated a better heat tolerance in HEK cell membranes than in ventricular myocytes. No statistically significant differences were found in charge transfer by the heterologously expressed $Na_V1.5$ channels (Fig. 4F). I_{Na} density of the rainbow trout $Na_V1.5$ channels in HEK cells increased with warming from 12 to 28°C (Fig. 4G). This is a striking deviation from the thermal response of the ventricular I_{Na}, which was strongly depressed at 28°C. Charge transfer by rainbow trout $Na_V1.5$ channels was significantly increased by acute warming in HEK cells, in contrast to the observations of ventricular I_{Na} (Fig. 4H).

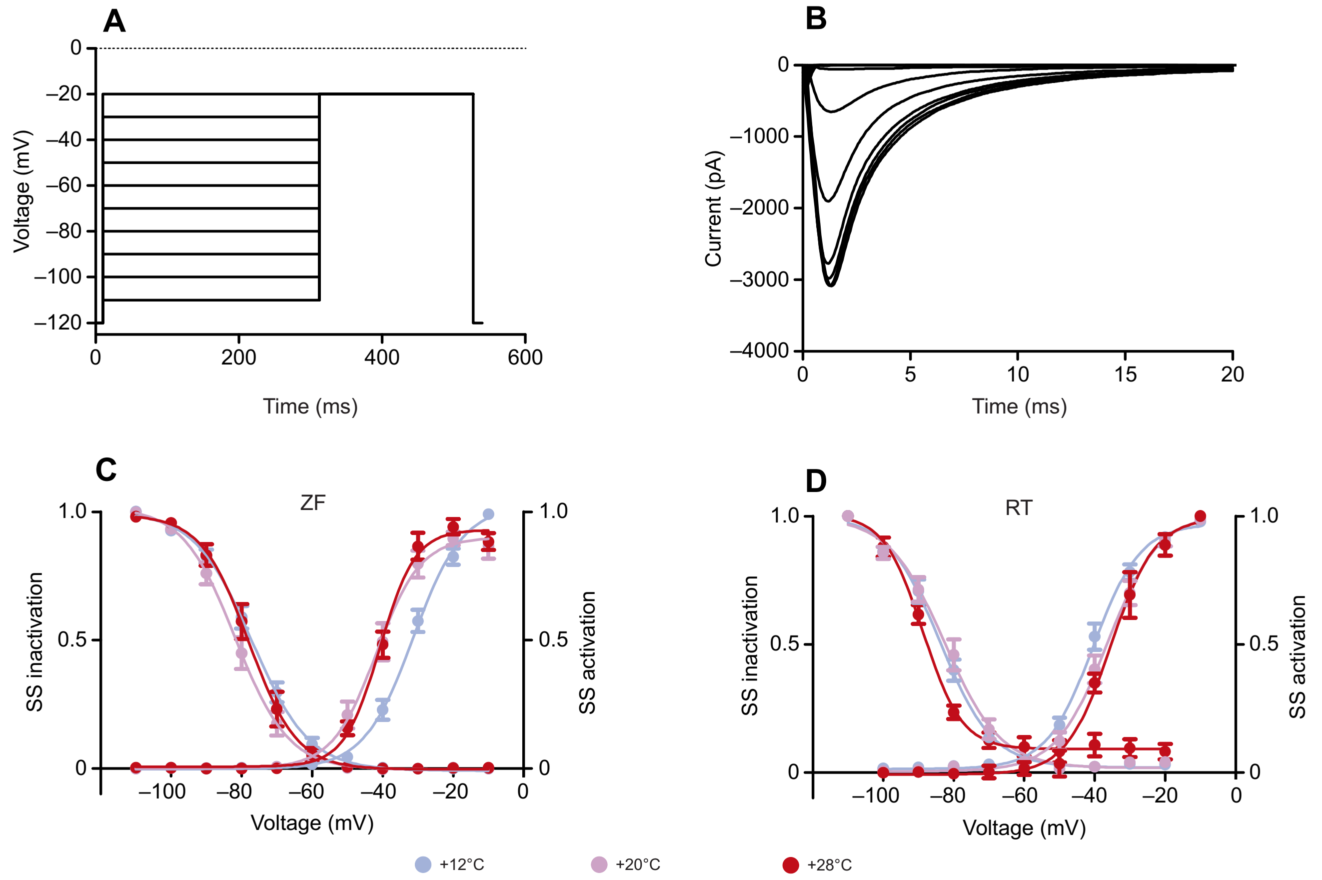

Fig. 3. Voltage and temperature dependence of steady-state activation and inactivation of I_{Na} in ventricular myocytes of zebrafish and rainbow trout. (A) The two-step voltage protocol used to elicit I_{Na}. (B) A representative recording from a zebrafish ventricular myocyte indicating tracings of I_{Na} at −20 mV following 1 s depolarizations from −120 mV to −110 to +30 mV. (C) Steady-state (SS) activation and inactivation of zebrafish (ZF) ventricular I_{Na} (N=87 myocytes from seven fishes). (D) Steady-state activation and inactivation of rainbow trout (RT) ventricular I_{Na} (N=58 myocytes from six fishes).

Inactivation kinetics of I_{Na} generated by Na$_{\mathrm{V}}$1.4 and Na$_{\mathrm{V}}$1.5 channels in HEK cells

The inactivation rate of I_{Na} increased with increasing temperature and with membrane depolarization. There were marked gene-specific differences in the rate of I_{Na} inactivation (Fig. 5). The rate of inactivation of I_{Na} produced by Na$_{\mathrm{V}}$1.5 channels was much slower than that produced by Na$_{\mathrm{V}}$1.4 channels for both zebrafish and trout genes (P<0.05). In contrast to gene-specific differences, interspecies differences in inactivation rate of I_{Na} were non-existent or small. The time constant of I_{Na} inactivation of Na$_{\mathrm{V}}$1.5 channels was almost identical for zebrafish and trout variants of the channel. In contrast, the I_{Na} generated by Na$_{\mathrm{V}}$1.4 channels was faster for zebrafish than the trout channel variant at more negative voltages (at −30 mV at 20°C, and at −30 and −20 mV at 28°C) (Fig. 5D,F). Temperature dependence (Q_{10} values) of I_{Na} inactivation varied between 1.8 and 6.0 with no differences between channel isoforms. However, the Q_{10} value of I_{Na} inactivation rate was higher in zebrafish ventricular myocytes than in trout ventricular myocytes at voltages of −10 and 0 mV (P<0.05) (Table S1).

Interestingly, the rate of I_{Na} inactivation of heterologously expressed zebrafish Na$_{\mathrm{V}}$1.4 and Na$_{\mathrm{V}}$1.5 Na$^+$ channels was much faster than the inactivation kinetics of the endogenous ventricular I_{Na}. Opposite to the findings in zebrafish, the I_{Na} inactivation rate of trout Na$_{\mathrm{V}}$1.5 channels was much slower than that of trout ventricular myocytes. In contrast, the inactivation rates of I_{Na} for heterologously expressed trout Na$_{\mathrm{V}}$1.4 channels and trout ventricular myocytes were similar.

Steady-state activation and inactivation of I_{Na} generated by Na$_{\mathrm{V}}$1.4 and Na$_{\mathrm{V}}$1.5 channels in HEK cells

Acute increases in temperature had only weak effects on the voltage dependence of SS activation and inactivation of I_{Na} generated by the heterologously expressed Na$^+$ channels (Table 2; Fig. 6). The only change for the zebrafish I_{Na} produced by Na$_{\mathrm{V}}$1.5 channels was the reduced slope of SS activation at 20 and 28°C relative to that at 12°C (P<0.05). Similar changes were observed for the rainbow trout I_{Na} generated by Na$_{\mathrm{V}}$1.5 channels (P<0.05) (Fig. 6). In addition, the rainbow trout Na$_{\mathrm{V}}$1.5 channels had a positive shift in the voltage dependence and a decrease in the slope factor of the I_{Na} SS inactivation (P<0.05) (Table 2; Fig. 6D). Elevated temperatures also reduced the slope factor of SS activation of the trout Na$_{\mathrm{V}}$1.4 channels (P<0.05) (Table 2; Fig. 6B).

DISCUSSION

The present results can be summarized in three major findings. (1) The properties of the endogenous I_{Na} of zebrafish and rainbow trout ventricular myocytes differ markedly in terms of heat tolerance and inactivation kinetics, with the zebrafish I_{Na} being more heat tolerant and more slowly inactivating. (2) The major Na$^+$ channel isoforms

Table 2. Midpoint membrane potential and slope factor of steady-state inactivation and activation for I_{Na} of zebrafish and rainbow trout ventricular myocytes and I_{Na} generated by their $Na_V1.4$ and $Na_V1.5$ channels in HEK cells

	SS inactivation		SS activation	
	$V_{0.5}$ (mV)	Slope	$V_{0.5}$ (mV)	Slope
Zebrafish myocytes				
12°C	−77.2±1.4^{a}	−7.3±0.5^{a}	−30.3±1.6^{a}	6.7±0.5^{a}
20°C	−80.6±1.8^{a}	−5.8±0.4^{b}	−40.2±2.3^{b}	4.9±0.3^{b}
28°C	−77.6±1.9^{a}	−5.3±0.6^{b}	−39.6±1.8^{b}	5.1±0.7^{b}
Zebrafish $Na_V1.4$ in HEK cells				
12°C	−83.5±2.1^{a}	−5.5±0.8^{a}	−35.9±3.2^{a}	8.0±0.8^{a}
20°C	−85.0±4.0^{a}	−6.5±0.6^{a}	−42.4±2.5^{a}	5.4±0.4^{b}
28°C	−76.8±2.3^{a}	−7.3±1.3^{a}	−39.0±3.6^{a}	6.6±0.7^{a,b}
Zebrafish $Na_V1.5$ in HEK cells				
12°C	−76.2±2.1^{a}	−7.8±1.5^{a}	−41.2±4.1^{a}	7.8±1.0^{a}
20°C	−78.3±2.4^{a}	−8.5±1.7^{a}	−33.4±1.7^{a}	7.0±0.8^{a}
28°C	−71.1±1.7^{a}	−5.5±0.5^{a}	−34.1±2.4^{a}	5.5±0.6^{a}
Rainbow trout myocytes				
12°C	−85.1±2.0^{a}	−7.7±0.7^{a}	−40.2±1.4^{a}	6.2±0.4^{a}
20°C	−83.1±2.4^{a}	−7.6±0.5^{a}	−36.9±1.7^{a}	6.5±0.3^{a}
28°C	−89.0±1.0^{a}	−8.7±0.3^{a}	−34.4±2.9^{a}	6.6±1.7^{a}
Rainbow trout $Na_V1.4$ in HEK cells				
12°C	−80.5±3.7^{a}	−10.1±1.9^{a}	−39.8±2.5^{a}	5.2±0.9^{a}
20°C	−81.0±3.9^{a}	−8.0±2.3^{a}	−29.2±4.2^{b}	9.0±2.7^{a}
28°C	−76.9±2.7^{a}	−8.0±1.5^{a}	−33.6±2.2^{a,b}	6.9±1.6^{a}
Rainbow trout $Na_V1.5$ in HEK cells				
12°C	−87.3±1.9^{a}	−8.5±0.5^{a}	−39.6±2.4^{a}	7.5±0.6^{a}
20°C	−77.9±1.9^{b}	−7.0±0.7^{a,b}	−39.1±1.6^{a}	5.4±0.4^{b}
28°C	−72.4±2.2^{b}	−5.7±0.4^{b}	−39.9±2.4^{a}	4.8±0.3^{b}

$V_{0.5}$, midpoint potential; SS, steady-state. Statistically significant differences ($P<0.05$) between temperatures (within species) are shown by different lower case letters.

of zebrafish and rainbow trout ventricles, $Na_V1.5$ and $Na_V1.4$, respectively, show only minor interspecies differences, when expressed in HEK cells, i.e. the orthologous Na^+ channel alpha subunits are functionally (heat tolerance, inactivation kinetics) similar in the same membrane matrix. (3) When expressed in HEK cells, I_{Na} generated by $Na_V1.4$ and $Na_V1.5$ isoforms of both species show large channel-specific differences in inactivation kinetics, with $Na_V1.4$ being fast and $Na_V1.5$ slow. Taken together, the species-specific properties of ventricular I_{Na} seem to be determined partly by the expressed Na^+ channel alpha subunit – $Na_V1.5$ in zebrafish and $Na_V1.4$ in rainbow trout – and partly by the biophysical properties of the lipid matrix/ancillary subunits of the channel assembly. Notably, the better heat tolerance of I_{Na} seems to be related to the slower inactivation kinetics of Na^+ channels.

The endogenous I_{Na} of zebrafish and rainbow trout ventricular myocytes have very different inactivation kinetics: the I_{Na} of zebrafish ventricular myocytes inactivates much more slowly than I_{Na} of rainbow trout ventricular myocytes. At 12°C, the inactivation time constant of the zebrafish I_{Na} was almost an order of magnitude bigger than that of the rainbow trout (5.0±0.7 versus 0.5±0.03 ms at −20 mV). This difference largely, but not completely, disappeared when the rate of I_{Na} inactivation was measured at the acclimation temperatures of the fish (0.8±0.09 ms for zebrafish at 28°C versus 0.5±0.03 ms for trout at 12°C). As thermal acclimation does not have any effect on the inactivation kinetics of the rainbow trout ventricular I_{Na} (Haverinen and Vornanen, 2004), the differences in the inactivation rate between the two species can be regarded as adaptations of I_{Na} to the respective habitat temperatures of the species. In general, electrical excitation in nerves and muscle tissues of ectothermic animals is adapted to work best at lower temperatures in comparison with the tissues of the endothermic animals. For instance, at the typical mammalian body temperatures (36–38°C), the AP conduction of ectothermic nerve fibres suffers from heat block (Hodgkin and Katz, 1949; Volgushev et al., 2000). The gating kinetics of the plasma membrane ion channels are probably responsible for the thermal adaptation of AP conduction, allowing APs to propagate at the proper rate and frequency at the typical habitat temperatures of the species. Because zebrafish live at warmer habitats than rainbow trout, the slow inactivation kinetics of its ventricular I_{Na} may provide better excitability at higher temperatures than the fast inactivating I_{Na} of the rainbow trout. However, when comparing the intrinsic heart rates of the two species at their acclimation temperatures (130 beats min^{-1} for zebrafish versus 60 beats min^{-1} for rainbow trout) (Aho and Vornanen, 2001; Vornanen and Hassinen, 2016), the rate of I_{Na} inactivation appears to be slow. Apparently, the rate of I_{Na} recovery from inactivation does not limit heart rate in zebrafish, although this was not experimentally confirmed.

The slow rate of I_{Na} inactivation in zebrafish ventricular myocytes may protect against heat-induced impairment of electrical excitability (Park et al., 2016; Touska et al., 2018). This is consistent with our hypothesis that Na^+ channels with slow gating kinetics better maintain electrical excitability at high temperatures (Vornanen, 2020). Indeed, findings from the pain receptors of human skin strongly suggest that the slow gating kinetics of Na^+ channels are needed for heat resistance of I_{Na} (Touska et al., 2018). At mammalian body temperatures, most Na^+ channel isoforms operate close to their optimum and a slight increase in temperature above the typical body temperature limits the density and charge transfer of I_{Na} (Touska et al., 2018). However, I_{Na} of the mammalian pain receptors works well at temperatures much above body temperature (43–50°C), largely owing to the thermal properties of the $Na_V1.9$ Na^+ channel isoform. Inactivation kinetics of this channel are more than an order of magnitude slower than those of $Na_V1.1$–$Na_V1.8$ isoforms (Balbi et al., 2017; Touska et al., 2018). Due to the slow inactivation of $Na_V1.9$, it provides enough charge for depolarization of the plasma membrane at high temperatures. Analogously, the slow inactivation rate of the zebrafish cardiac I_{Na} provides more inward charge for the same peak amplitude of I_{Na} or the same number of active Na^+ channels than the fast inactivating trout cardiac I_{Na} (Fig. 4). Indeed, the charge transfer/peak current ratio of the zebrafish I_{Na} is much higher at 28°C than that of the rainbow trout I_{Na} at 12°C.

The species-specific difference in the inactivation kinetics of the ventricular I_{Na} is partly explained by the difference in the alpha subunit composition of the Na^+ channels. In zebrafish ventricular myocytes, the main alpha subunit is $Na_V1.5$, which at the transcript level represents 83.1% of all ventricular Na^+ channels. Transcripts of the $Na_V1.4$ comprise only 16.2% of the zebrafish ventricular Na^+ channels (Haverinen et al., 2018). In the ventricle of the rainbow trout, the situation is opposite: $Na_V1.4$ channels form 80% of all Na^+ channel transcripts, while $Na_V1.5$ channels represent only 20% of the total channel population (Haverinen et al., 2007). Thus, in zebrafish ventricle Na^+ channels are mainly of the slow isoform, while in the ventricle of the rainbow trout they are mainly the fast isoform. Based on their tissue distribution in mammals, $Na_V1.5$ and $Na_V1.4$ are often called 'cardiac' and 'skeletal' isoforms, respectively (Zimmer et al., 2015). The mammalian $Na_V1.4$ is kinetically faster than $Na_V1.5$ and therefore functionally better at eliciting fast twitches at high frequencies in skeletal muscle fibres (Wang et al., 1996; Sheets and Hanck, 1999). Although the classification of $Na_V1.5$ and $Na_V1.4$ to cardiac and skeletal

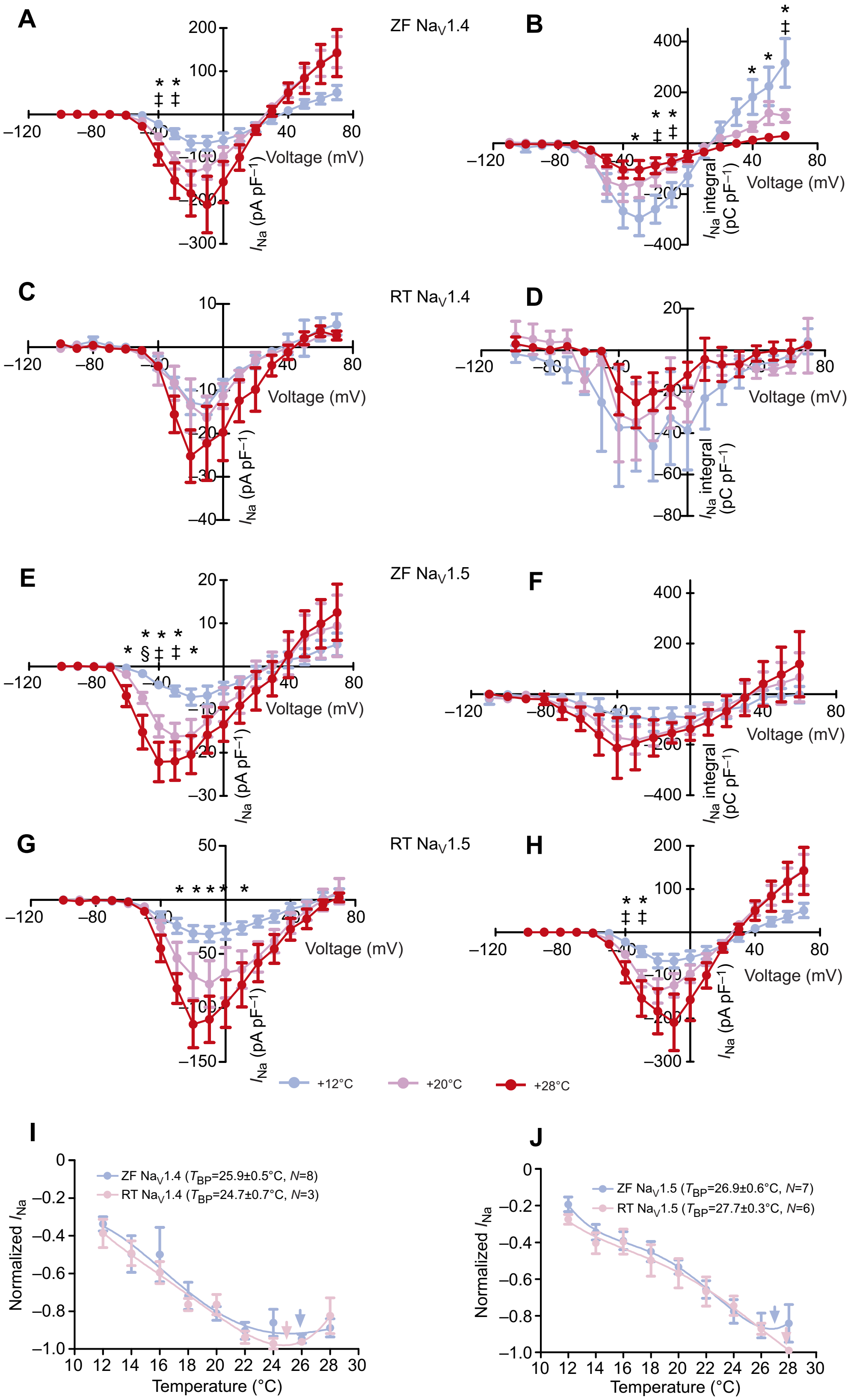

Fig. 4. Voltage and temperature dependence of current density and charge transfer of I_{Na} generated by zebrafish and rainbow trout Na$_V$1.4 and Na$_V$1.5 channels in HEK cells. (A–D) Mean results (±s.e.m.) for current–voltage relationship (A,C) and charge transfer (B,D) of I_{Na} at three different temperatures for zebrafish (ZF; *N*=49 cells) (A,B) and rainbow trout (RT; *N*=15 cells) (C,D) Na$_V$1.4 channels. (E–H) Mean results (±s.e.m.) for current–voltage relationship (E,G) and charge transfer (F,H) of I_{Na} at three different temperatures for ZF (*N*=30 cells) (E,F) and RT (*N*=27 cells) (G,H) Na$_V$1.5 channels. (I,J) Current density of I_{Na} in heat ramp experiments with ZF and RT Na$_V$1.4 (I) and Na$_V$1.5 (J) channels. Arrows (blue and red) mark the breakpoint temperature of each curve. Number of tested cells are shown in the figure. Statistically significant differences (*P*<0.05) are shown as follows: *12 versus 28°C; ‡12 versus 20°C; §20 versus 28°C.

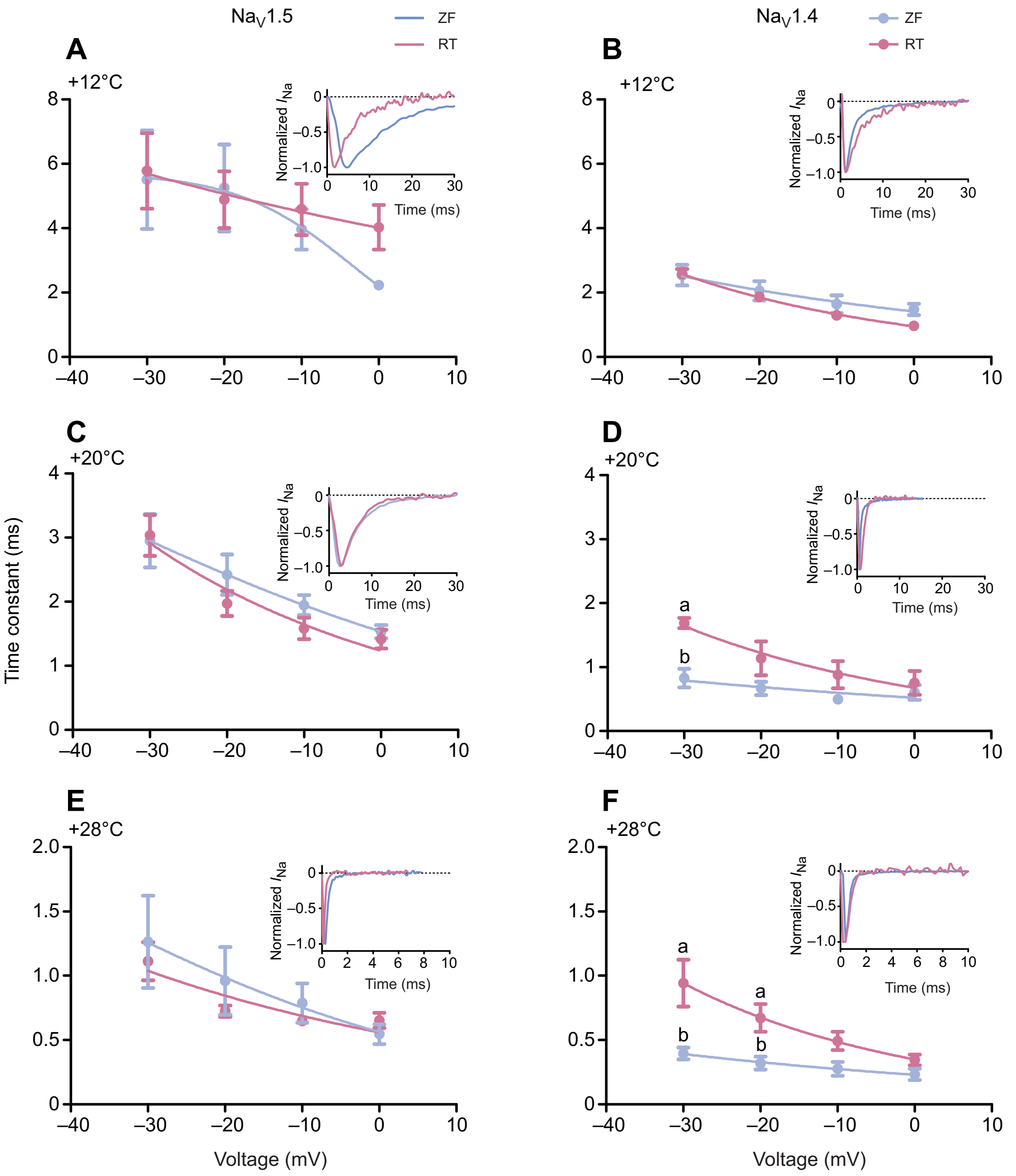

Fig. 5. Temperature dependence of inactivation kinetics of I_{Na} for zebrafish Na$_V$1.4 and Na$_V$1.5 channels in HEK cells. I_{Na} was elicited from the holding potential of −120 mV to −30 to 10 mV for 30 ms. (A–F) Means (±s.e.m.) of inactivation time constant (τ_f) for Na$_V$1.5 (A,C,E) and Na$_V$1.4 (B,D,F) at 12, 20 and 28°C as indicated. The results are from 39 and 42 cells from zebrafish (ZF) and rainbow trout (RT) channels, respectively. Representative tracings of I_{Na} at −30 mV are shown in the inset to each panel. Statistically significant differences ($P<0.05$) between ZF and RT are shown by dissimilar letters (a and b).

isoforms, respectively, is not valid for fish, the kinetic similarities between the orthologous mammalian and piscine Na$^+$ channels persist. The fast inactivation kinetics of the rainbow trout Na$_V$1.4 can be regarded adaptive for heart function of this cold-dwelling fish.

The dominance of Na$_V$1.5 alpha subunits in the zebrafish heart only partially explains the slow inactivation kinetics of the ventricular I_{Na}. Unlike the rainbow trout, where the inactivation rate of Na$_V$1.4 channels in HEK cells relatively closely matches the inactivation rate of the endogenous ventricular I_{Na}, in zebrafish there is a large difference in the inactivation kinetics of the heterologously expressed Na$_V$1.5 and the endogenous ventricular I_{Na}. As Na$_V$1.4 and Na$_V$1.5 Na$^+$ channel alpha subunits of zebrafish and rainbow trout share the inactivation kinetics in the heterologous environment of HEK cells, the slow inactivation rate of the zebrafish ventricular I_{Na} is probably partly due to the general biophysical properties of the bulk lipid bilayer of ventricular myocytes in the membrane domain where they are located (Lundbaek et al., 2004). Phospholipid bilayer composition of the plasma membrane affects function of the integral membrane proteins mainly in a non-specific manner by its biophysical properties (Lundbaek et al., 2004). Elasticity (stiffness) of the lipid bilayer, determined by the lipid composition, regulates the inactivation of the voltage-gated Na$^+$ channels. Decrease in membrane stiffness induced by amphiphiles like Triton-X or β-octyl-glucoside accelerate the rate of I_{Na} inactivation, while increase in membrane stiffness due to elevated

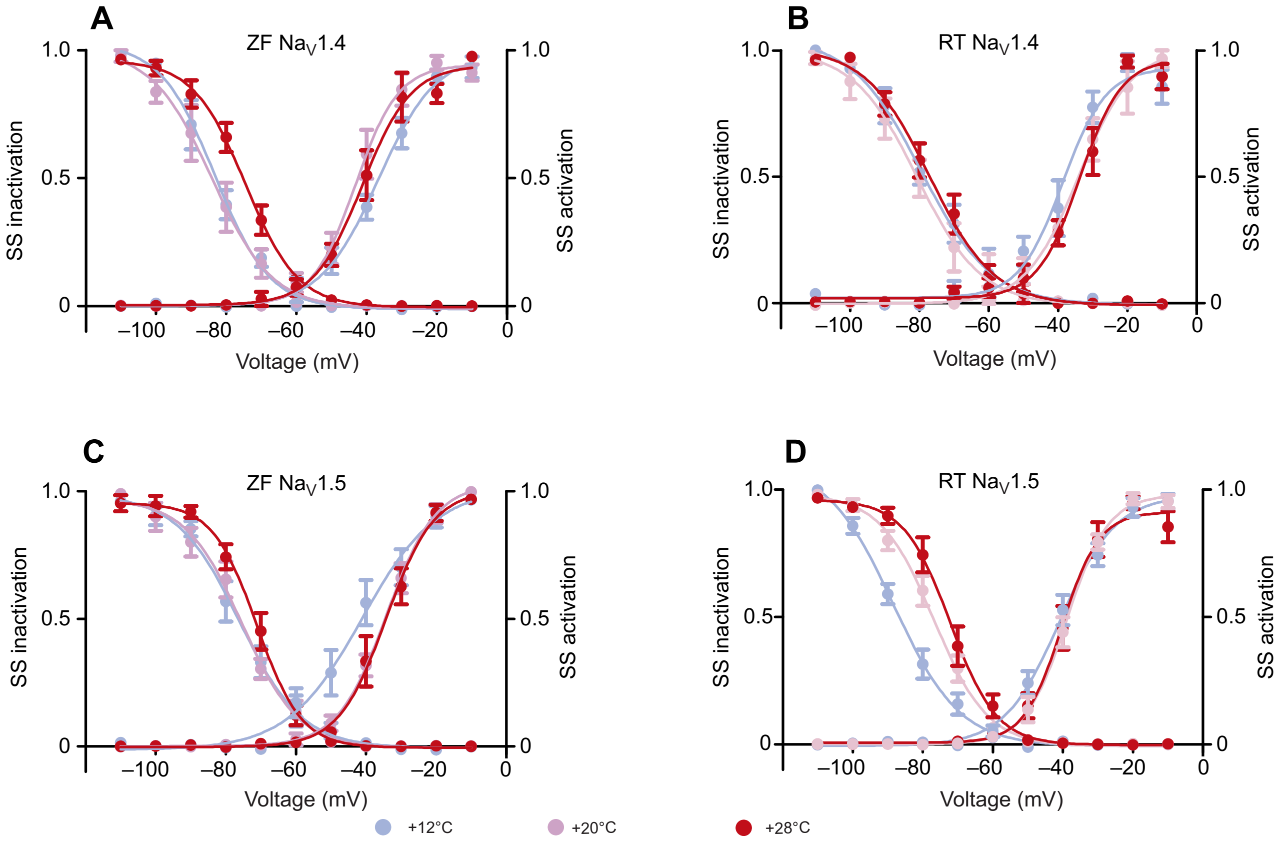

Fig. 6. Voltage and temperature dependence of steady-state activation and inactivation of I_{Na} for $Na_V1.4$ and $Na_V1.5$ channels in HEK cells. The voltage protocol was the same as in Fig. 2. (A,B) Steady-state activation and inactivation of zebrafish (ZF; *N*=49 cells) (A) and rainbow trout (RT; *N*=33 cells) (B) $Na_V1.4$ channels. (C,D) Steady-state (SS) activation and inactivation of zebrafish (*N*=49 cells) (C) and rainbow trout (*N*=50 cells) (D) $Na_V1.5$ channels.

cholesterol content decreases the rate of I_{Na} inactivation (Lundbaek et al., 2004). Therefore, it is possible that the lipid composition of the sarcolemma in zebrafish ventricular myocytes differs from that of HEK cells and rainbow trout myocytes, and results in increased stiffness, which slows the rate of I_{Na} inactivation.

Although we do not have a complete answer about the slow inactivation rate of the zebrafish ventricular I_{Na}, some possible explanations (in addition to membrane stiffness) can be mentioned to guide future studies. Na^+ channels are heteromultimers of large pore-forming alpha subunits and small accessory beta subunits. Beta subunits are needed for proper transportation of the alpha subunits into the plasma membrane and they may also affect kinetics of the I_{Na}. However, beta subunits are unlikely to cause the slow inactivation of the zebrafish I_{Na} as they tend to enhance the rate of Na^+ channel inactivation and recovery from inactivation (Chen and Cannon, 1995; Goldin, 2003). A more likely contributing factor is the fibroblast growth factor orthologous factor 2 (FGF2), which binds to the inactivation domain of the C-terminus in the Na^+ channel alpha subunit and strongly slows the rate of inactivation (Liu et al., 2003; Li et al., 2020). Interestingly, FGF2 knock-out mice are highly sensitive to temperature change and show more cardiac conduction defects when their core body temperature is elevated (Park et al., 2016). Future studies should examine the role of FGFs in temperature dependence of electrical excitability of the fish heart.

Acute heat challenge experiments indicated that the zebrafish ventricular I_{Na} is much more resistant to high temperatures than the rainbow trout I_{Na}. Thus, the heat tolerance of I_{Na} seems to positively correlate with the upper thermal tolerance of the fish and its heart rate, although in both species the optimum temperature of I_{Na} is lower than the critical thermal maximum temperature of the fish and the T_{BP} of the intrinsic heart rate (Beitinger, 2000; Aho and Vornanen, 2001; Cortemeglia and Beitinger, 2005; López-Olmeda and Sánchez-Vázquez, 2011; Vornanen and Hassinen, 2016). It is clear that I_{Na} of the trout ventricle would be almost non-functional at the acclimation temperature of the zebrafish, and it is likely that the kinetics of the zebrafish I_{Na} would be too slow for the trout heart at freezing temperatures.

Warming-induced decrease of heart rate is shown to be caused by atrioventricular block, probably due to the reduced excitability of the ventricle (Haverinen and Vornanen, 2020). Therefore, the thermal properties of I_{Na} are likely to affect the species-specific T_{BP} of heart rate. Warming-induced decrease of I_{Na} and simultaneous increase in membrane K^+ leak via I_{K1} results in source–sink mismatch, which may prevent AP generation (Vornanen, 2016; Haverinen and Vornanen, 2020; Vornanen, 2020). At low temperatures there is some excess of I_{Na} relative to the membrane leak via I_{K1} ($I_{Na}/I_{K1} \geq 1.0$) called safety factor. When temperature rises the charge transfer of I_{Na} starts to decline (at the species-specific T_{BP}) due to increased rate of inactivation: the safety factor is lost ($I_{Na}/I_{K1} < 1.0$) and excitation fails. In this respect, the difference in heat tolerance between the ventricular I_{Na} of rainbow trout and the I_{Na} generated by the heterologously expressed $Na_V1.4$ and $Na_V1.5$ channels of the trout may be important. Expression of trout I_{Na} in the HEK cell membrane makes it much more heat tolerant than it is in the native membrane surroundings. Notably, when zebrafish and trout $\mathrm{Na_V}$1.4 and $\mathrm{Na_V}$1.5 were expressed in HEK cells, inactivation rate and heat tolerance of I_{Na} were similar for the orthologous channels. It seems that the biophysical properties of the mammalian cell membrane shift the thermal tolerance window of the trout Na$^+$ channels to higher temperatures which would, however, be suboptimal at the habitat temperature of the cold-dwelling fish.

Summary and perspectives

Heat tolerance and inactivation kinetics of I_{Na} differ strongly between zebrafish and rainbow trout ventricular myocytes. In contrast, heat tolerance and inactivation kinetics of $\mathrm{Na_V}$1.4 and $\mathrm{Na_V}$1.5 channels of zebrafish and rainbow trout are similar when expressed in the same cellular environment of HEK cells. Species-specific thermal adaptation of the ventricular I_{Na} is largely achieved by expressing a specific alpha isoform subunit of Na$^+$ channel: the slowly inactivating $\mathrm{Na_V}$1.5 in zebrafish that tolerate higher temperatures, and the fast inactivating $\mathrm{Na_V}$1.4 in rainbow trout that favour cold waters. Differences in elasticity (stiffness) of the lipid bilayer and/or accessory protein components may also be involved in the thermal adaptation of I_{Na}. These findings are consistent with the hypothesis that slow Na$^+$ channel kinetics are associated with increased heat tolerance of cardiac excitation. Future studies should examine the extent to which these components are flexible under temperature acclimation and therefore able to accommodate the electrical excitability of the heart for seasonal temperature changes and peak summer temperatures. As electrical excitability is regulated in basically the same way in all excitable cells, studying the biophysical properties of neuronal and muscular I_{Na} could provide clues to its role in thermal homeostasis and death of ectotherms.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.V.; Methodology: J.H., I.D., M.H.; Investigation: J.H., I.D., D.V.A., M.H.; Writing - original draft: J.H., D.V.A., M.H.; Writing - review & editing: M.V.; Visualization: J.H., I.D.; Project administration: M.V.; Funding acquisition: M.V.

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Table S1. Q10-values for the rate of I_{Na} inactivation (τ_f) in zebrafish (ZF) and rainbow trout (RT) ventricular myocytes and I_{Na} generated by $Na_V1.4$ and $Na_V1.5$ channels in HEK cells.

	Q_{10}-value		**Membrane potential**
	12-20˚C	**20-28˚C**	
ZF myocytes	3.0±0.3	3.4±0.1	-30mV
	4.1±0.9	3.6±1.5	-20mV
	3.9±0.8[a]	2.8±0.8	-10mV
	4.0±0.7[a]	2.4±0.6	0mV
ZF $Na_V1.4$ in HEK cells	4.8±1.0	2.6±0.4	-30mV
	4.8±1.1	3.2±0.8	-20mV
	4.1±0.8	2.8±0.5	-10mV
	4.9±0.9	2.2±0.5	0mV
ZF $Na_V1.5$ in HEK cells	1.9±0.5	3.1±1.3	-30mV
	3.8±1.5	6.0±2.3	-20mV
	3.0±1.1	4.3±1.3	-10mV
	1.8±0.3	3.9±0.9	0mV
RT myocytes	2.8±0.4	3.1±0.1	-30mV
	2.1±0.2	2.9±0.4	-20mV
	1.9±0.1[b]	2.4±0.1	-10mV
	2.0±0.2[b]	2.0±0.3	0mV
RT $Na_V1.4$ in HEK cells	1.3±0.2	2.6±0.8	-30mV
	2.2±1.1	2.4±0.2	-20mV
	2.9±1.3	3.0±1.4	-10mV
	3.4±1.7	2.7±1.3	0mV
RT $Na_V1.5$ in HEK cells	3.3±1.0	4.5±1.1	-30mV
	3.9±1.1	3.7±0.6	-20mV
	4.7±1.2	3.2±0.5	-10mV
	4.0±0.8	2.0±0.3	0mV

Data are expressed as means ± S.E.M. Effects between species, channels and membrane voltages were assessed by unpaired *t*-test. [a, b] Temperature dependence of I_{Na} inactivation rate is higher in zebrafish ventricular myocytes than in trout ventricular myocytes at voltages of -10 and 0 mV ($p<0.05$).

Figure 1. Amino acid alignment of the cloned rainbow trout and zebrafish Nav1.4 proteins.

```
omSCN4Abb           1 MPTRKITLLEAWERNRTFRRKKAKDLKICPRLLPLLRDPGVVASLTQQNA     50
                                                                        
drSCN4Ab            1 --------------------------------------------------      0

omSCN4Abb          51 KMVTLLPPTGTEVFRHFTLESLVEIDRRMAEEAKEQERQKTLNIEVAEQD    100
                       |..|||||||:|||..|||||.||||||||||.||||.|..|::|||:|
drSCN4Ab            1 -MARLLPPTGTDVFRPLTLESLAEIDRRMAEEAAEQERMKEQNVKVAEED     49

omSCN4Abb         101 LPKPAVDLETGKILPFIYGDPPCELFNTPLEELDPFYKSHKTFIVITKRN    150
                      ||||..|||.||:|||||||||..|.|.|:|||||:||:.||||||.|:|
drSCN4Ab           50 LPKPTSDLEAGKVLPFIYGDPPPNLLNVPIEELDPYYKAQKTFIVIDKKN     99

omSCN4Abb         151 TIFRFNAEPACYILTPFSILRRGSIKILMHSLFSMFIMLTVVFNCAFMTI    200
                      ||:|||.|||||.|:||:.:||.:|:||:|||||:.||||::.||.||.:
drSCN4Ab          100 TIYRFNTEPACYCLSPFNPVRRAAIRILIHSLFSLVIMLTILTNCVFMAM    149

omSCN4Abb         201 SDPPASSKTVEYVFTGIYTFEATIKVLSSGFCVGDFTYFRDPWNWLDFIL    250
                      ||||..||.:||||||||||||.:||||.|||:||||:.|||||||||::
drSCN4Ab          150 SDPPGWSKILEYVFTGIYTFEAMVKVLSRGFCIGDFTFLRDPWNWLDFMV    199

omSCN4Abb         251 ISMTYLTEFVDLGNVSVFRMFRVLRALKIITVIPGLKTIVGTLIMSVKKL    300
                      |||.||||||||||:|..|.||||||||.||||||||||||.||.|||||
drSCN4Ab          200 ISMAYLTEFVDLGNISALRTFRVLRALKTITVIPGLKTIVGALIQSVKKL    249

omSCN4Abb         301 ADVMIITVFCLAVFAMIGLQMFMGNLKQKCVDWPPGEWHFNSTYMGNINM    350
                      |||||:|||||:|||:||||:|||||:||||.|||..|     |..|:.:
drSCN4Ab          250 ADVMILTVFCLSVFALIGLQLFMGNLRQKCVLWPPVGW-----YSDNLTV    294

omSCN4Abb         351 ANGFNDVMGYNDTNTGNSTWDLKAYINDKANHYFLPGMLDPLLCGNASDA    400
                      .:.:.|:   |...|.|||:|.:.|||.:.|:|::||.:|||:|||:|||
drSCN4Ab          295 LSNYTDI---NGNGTANSTFDYQKYINSEENYYYVPGQMDPLVCGNSSDA    341

omSCN4Abb         401 GICPEGYTCMKAGGNPNYGYTSYDNFGWAYLALIQLMTQDFWENLLQLTL    450
                      |:|||||.|:|||.|||||||||||||||:|||.:||||||||||.||||
drSCN4Ab          342 GLCPEGYICLKAGRNPNYGYTSYDNFGWAFLALFRLMTQDFWENLFQLTL    391

omSCN4Abb         451 RSAGKTYMIFFVVVIFLCSFYLINLILAVVAIAHAEQNEATRAEAKEKEA    500
                      |:|||||||||||:|||.|||||||||||||:|:|||||||.|||||||.
drSCN4Ab          392 RAAGKTYMIFFVVIIFLGSFYLINLILAVVAMAYAEQNEATAAEAKEKEE    441

omSCN4Abb         501 EYAMIMVQLKERREAEVK---------ERASRPKRSKDQ-----------    530
                      |||.||.|||  ::||.|         ..:|:.|...||
drSCN4Ab          442 EYAKIMEQLK--KQAEQKNGMVNGSKTSLSSKKKGDNDQMQSDYDGIALK    489

omSCN4Abb         531 --------------LEVAGLQKKTPSSPSVYSQ--DAMDDLEGELRPCPP    564
                                    |||...|.:.||..|....  ||.:|:|   |||||
drSCN4Ab          490 PLSKSNGSKGNINYLEVPDSQIRKPSVVSAVESALDAQEDIE---RPCPP    536

omSCN4Abb         565 CWYKCSDVFLKWNCCVPWVTFKKWVYFIVMDPFMDLVITICIVLNIMFLA    614
                      .|||.:|:||||:||:|||.||:.||..|||||:||.||:|||||.:|:|
drSCN4Ab          537 GWYKFADIFLKWDCCIPWVKFKRIVYLFVMDPFVDLGITLCIVLNTVFMA    586

omSCN4Abb         615 MEHYPMTIEFEEVLSVGNLVFIGIFTAEMVLKLIAMDPYYYFQVGGNIFD    664
                      ||||||::..||||::|||||.|||.|||||||||:|||||||||.||||
drSCN4Ab          587 MEHYPMSVHVEEVLAIGNLVFTGIFAAEMVLKLIALDPYYYFQVGWNIFD    636

omSCN4Abb         665 SIIVAISLMELWLADVEGLSVLRSFRLMRVFKQTKSWPTLNMTIKIIGNS    714
                      ||||.:||:||.||||||||||||||||||||..||||||||.|||||||
drSCN4Ab          637 SIIVTMSLVELMLADVEGLSVLRSFRLMRVFKLAKSWPTLNMLIKIIGNS    686

omSCN4Abb         715 VGTLVNITLVLAIIVFMFAAGGMHLFGKSYKECVCKISLDCELPRWHMEN    764
                      ||.|.|:|||||||||:||..||.||||||.:.|||||.|||||||||.:
drSCN4Ab          687 VGALGNLTLVLAIIVFIFAVVGMQLFGKSYTDSVCKISSDCELPRWHMAD    736

omSCN4Abb         765 FFHAFLTIFRVLCGEWIETMWECMEVAGQGICLVFFMMVMVIGKLVVLNL    814
                      ||||||.||||||||||||||:||||||||:|::.||||||||.||||||
drSCN4Ab          737 FFHAFLIIFRVLCGEWIETMWDCMEVAGQGMCIIVFMMVMVIGNLVVLNL    786

omSCN4Abb         815 FLTFLLSSFSRYNLAAPEEDGEMNNLQIATSRITRGMNWAKAYVIKQFCI    864
                      ||..||||||..||:|.::||| ||||||.||||||::|.||:|.|....
drSCN4Ab          787 FLALLLSSFSGDNLSASDDDGE-NNLQIAISRITRGIDWIKAFVNKHVRQ    835

omSCN4Abb         865 IIGNQTKEDGDGADGNGGSNGDVDGLPKDSLALKNINSSDNTKADLELKM    914
                      .:..:.||:|...:|.|.:..:         |:.|.:||
drSCN4Ab          836 CLNLKPKEEGAKVNGEGDAKMN---------AIMNSSSS-----------    865

omSCN4Abb         915 LDHVNPSGFLAHANLTLDVPIAKMESDDDEDD--SSEVKGEGRNEDAKRR    962
                                     .:.||||..|||||:.:  |.:...|||:.:.|::
drSCN4Ab          866 ---------------MVKVPIANGESDDDDGNGSSEDEDDEGRDINMKKK    900

omSCN4Abb         963 KPIAQDNASLICRT-----SVE--VEKEEANPTSPENCWTESCIGRCPCL   1005
                          ..:.|..|.|     .||  ||:||.:.||||:|:||:||.|||||
drSCN4Ab          901 ----NGDESSTCSTVDKPPEVEDLVEEEEEDLTSPEDCYTENCIRRCPCL    946

omSCN4Abb        1006 DCDITTGKGKTWWNFRKTCFLIVEHNYFQSFIIFMILMSSGALAFEDIHI   1055
                      |.|::.||||.|||||||||.||||:||::|||||||:||||||||||:|
drSCN4Ab          947 DLDVSQGKGKAWWNFRKTCFAIVEHSYFETFIIFMILLSSGALAFEDIYI    996

omSCN4Abb        1056 EQRRTIKVILEYADQVFTYVFIIEMLLKWVAYGFQAYFTNAWCWLDFLIV   1105
                      ||||.||:|||||||||||||::|||||||||||:.||||||||||||||
drSCN4Ab          997 EQRRMIKIILEYADQVFTYVFVVEMLLKWVAYGFKVYFTNAWCWLDFLIV   1046
```

```
omSCN4Abb       1106 NVSMISLTANILGYAELGSIKSLRKLRALRPLRALSRFEGMK-VVVNALV   1154
                     :||:||||||||||:|||:|||||.||||||||||||||||||: |||||||
drSCN4Ab        1047 DVSLISLTANILGYSELGAIKSLRTLRALRPLRALSRFEGMRVVVVNALV   1096

omSCN4Abb       1155 GAIPSIVNVLLVCVTFWLIFSIIGVNLFAGKYYYCFNTTSEEMFSADIVN   1204
                     ||||||.||||||:.|||||||:||||||||:|||||.||||:|..::||
drSCN4Ab        1097 GAIPSIFNVLLVCLIFWLIFSIMGVNLFAGKFYYCFNETSEEVFDHNVVN   1146

omSCN4Abb       1205 NMTECIQLTEGNADVRWMNSKINFDNVAMGYLSLLQVATFKGWLGIMYGA   1254
                     |.|:|.:|.|.:.:|||||.|||||||.||||:||||||||||:.|||.|
drSCN4Ab        1147 NKTDCYELMEFHPEVRWMNGKINFDNVGMGYLALLQVATFKGWMDIMYSA   1196

omSCN4Abb       1255 VDSRTVGEQPMYEDNVYMYIYFVMFIIFGTFVMLNLFIGVIIDNFNQQKK   1304
                     ||||.:..||:||.|:|||||||:|||||:|..||||||||||||||||.
drSCN4Ab        1197 VDSRAIESQPVYEANLYMYIYFVIFIIFGSFFTLNLFIGVIIDNFNQQKA   1246

omSCN4Abb       1305 KFGGKNIFMTEEQNKYYNAMKKLGSKKLQKPIPRPKNKFAGIVYDLITNQ   1354
                     |.||.:|||||||.|||||||||||||.|||||||.|...|:|:|.:|.|
drSCN4Ab        1247 KLGGTDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPTNCCQGLVFDFVTQQ   1296

omSCN4Abb       1355 FFEVFIIVLICLSMVTMMVDTDDQSEEKDSILFFINLVFIFIFVAECILK   1404
                     ||::||:|:|||:||||||:|||||.|.:.|||:||..||.:|..||:||
drSCN4Ab        1297 FFDIFIMVMICLNMVTMMVETDDQSAEIEEILFYINFAFIILFTGECVLK   1346

omSCN4Abb       1405 LIGLRQYYFTVGWNILDFVVVILSILGLLLADLIEKYFVSPTLFRVVRLA   1454
                     :..||.:||::||||.|||||||||||:.|||||||||||||||||:|||
drSCN4Ab        1347 ITALRYHYFSIGWNIFDFVVVILSILGIGLADLIEKYFVSPTLFRVIRLA   1396

omSCN4Abb       1455 RIGRVLRLIRGAKGIRTLLFSLRMSLPAIFNIGLLLLLIMFIFSIFGMSN   1504
                     ||||||||||||||||||||:|.|||||:|||||||.|||||||||||||
drSCN4Ab        1397 RIGRVLRLIRGAKGIRTLLFALMMSLPALFNIGLLLFLIMFIFSIFGMSN   1446

omSCN4Abb       1505 FAYVKKEVGIDDMINFETFGNSIICLFTITTLAGWDSVLSPMMSSTPPNC   1554
                     |||||||||||||:|||||||||||:|.|||.||||.:|:|:::| ||:|
drSCN4Ab        1447 FAYVKKEVGIDDMMNFETFGNSIICMFMITTSAGWDGLLAPILNS-PPDC   1495

omSCN4Abb       1555 DPYIENPGTDVRGNCSSLGWGILFICSYIIMCFFLVVNMYIAVILENFNV   1604
                     ||.::|||:..||||.:...||:|.||||:|.|.:|||||||:|||||||
drSCN4Ab        1496 DPDVDNPGSTTRGNCGNAAVGIVFFCSYIVMSFLVVVNMYIAIILENFNV   1545

omSCN4Abb       1605 VIEESGNPLCEEDFEMFYETWEKFDPDASQFVAYDILSEFCDTLKDPLRI   1654
                     ..|||.:||||:||||||||||||||.||||:.|:.||||||||||||||
drSCN4Ab        1546 ATEESSDPLCEDDFEMFYETWEKFDPTASQFIDYNRLSEFCDTLKDPLRI   1595

omSCN4Abb       1655 PKPNAIKLITMDLPIVPGDKIHCLDILLALTTEVLGESGEMDAMKESMEE   1704
                     ||||.:||||||:|:|.||||||||:|||||.||||.|.:||.||.:|||
drSCN4Ab        1596 PKPNTLKLITMDIPMVTGDKIHCLDLLLALTGEVLGGSDQMDGMKATMEE   1645

omSCN4Abb       1705 KFMAKNPFKVSFKPITTTLRRKQEEVAAVVIQRAYCKHLLRSSMKLASYK   1754
                     ||||.||.|.|::|||:||:||||||||..|||||..|:|:..:|.|||.
drSCN4Ab        1646 KFMANNPSKASYEPITSTLKRKQEEVAASTIQRAYRSHILKRCVKQASYM   1695

omSCN4Abb       1755 YHEKKELTKEDEAPPEQEGMIAIRMSKLYESQQSLGVDETVVDMD-----   1799
                     |.:|....|.....||:.||||..|..||..|        .|:.|
drSCN4Ab        1696 YRDKTGSKKPTGEAPEKVGMIAENMRSLYGDQ--------AVEDDHPVGC   1737

omSCN4Abb       1800 SYVKRERKELTATQLPVVTTETPPPVELQTEIISHSAPFVIP-ASTHNSQ   1848
                     |:.:..:.:..|.:         |||::|::::.|||||.:| :||....
drSCN4Ab        1738 SFSQHGKTQFGAKR---------PPVKVQSDVVLHSAPFPVPESSTAADN   1778

omSCN4Abb       1849 LKESHV   1854
                     |:||.|
drSCN4Ab        1779 LRESIV   1784
```

Figure S2. Amino acid alignment of the cloned rainbow trout and zebrafish Nav1.5 proteins.

```
omSCN5LAba         1 MATLLLPPGPDSLHRFTRESLAAVEQRIAEEEARRTKHYQEDLGDVELPR     50
                     ||.:|.||||||||||||||||.:||||||||||..|.||||.||||.|:
drSCN5LAb          1 MAAILFPPGPDSLHRFTRESLAGIEQRIAEEEARNAKRYQEDRGDVEPPK     50

omSCN5LAba        51 PRADLEAGKQLPRIFGDIPAGLVGVPLDDFDPFYFKNQRTFIVLNKGKAI    100
                     |||||||||||||||||||:.||||||:|.|||||:|:||||||||||||
drSCN5LAb         51 PRADLEAGKQLPRIFGDIPSALVGVPLEDIDPFYFQNRRTFIVLNKGKAI    100

omSCN5LAba       101 FRFSATSALYIFNPFHPVRRASIKVLVHSLFSLFIMCTILTNCCFMAMSE    150
                     ||||||||||||:||||:|||||::|||||||||||||||||||||||||
drSCN5LAb        101 FRFSATSALYIFSPFHPIRRASIRILVHSLFSLFIMCTILTNCCFMAMSE    150

omSCN5LAba       151 PAYWAKYVEYTFTGIYTFESLIKILARGFCVGPFTFLRDPWNWLDFSVIV    200
                     ||.|||||||||||||||||||||||||||:||||||||||||||||||:
drSCN5LAb        151 PAQWAKYVEYTFTGIYTFESLIKILARGFCIGPFTFLRDPWNWLDFSVIL    200

omSCN5LAba       201 MAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLAD    250
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
drSCN5LAb        201 MAYVTEFVDLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLAD    250

omSCN5LAba       251 VMILTVFCLSVFALIGLQLFMGNLRQKCVRSTAHCVNNTLNT--NTTFFC    298
                     |||||||||||||||||||||||||||||||.:.|:|.||.|  |:||.|
drSCN5LAb        251 VMILTVFCLSVFALIGLQLFMGNLRQKCVRSASQCLNTTLPTYNNSTFIC    300

omSCN5LAba       299 NNRTWPSLKDFIADEDNYYKVEGAKDALICGDGSDAGHCPDGFECLKAGR    348
                     |||:|.||::|..:|||::||||||||||||:.||||.|||||||||.||
drSCN5LAb        301 NNRSWASLEEFFNNEDNFFKVEGAKDALICGNASDAGKCPDGFECLKTGR    350

omSCN5LAba       349 NPNYGYTSFDSFGWAFLSLFRLMTQDYWENLYHQTLRSAGKTYMVFFVVV    398
                     ||||||||||:||||||||||||||||||||||.|||||||.||||||||
drSCN5LAb        351 NPNYGYTSFDTFGWAFLSLFRLMTQDYWENLYHHTLRSAGKAYMVFFVVV    400

omSCN5LAba       399 IFLGSFYLVNLILAVVAMAYEEQNQATIQEAWQKEKEFQLAMERLKRE--    446
                     ||||||||||||||||||||||||||||.||.|||:|||||||:||:|
drSCN5LAb        401 IFLGSFYLVNLILAVVAMAYEEQNQATIAEALQKEQEFQLAMEQLKKEQQ    450

omSCN5LAba       447 -AQKAQDTESLMSPELSPGLALPDHKEVQSRRRSLEELVEEVENGGDVEK    495
                      |||||:|||:::.::|| .:..|..::: ||:|...|.|........:.
drSCN5LAb        451 AAQKAQETESILTADVSP-FSTQDKAKLE-RRKSSRPLSEGTGEDKPAKL    498

omSCN5LAba       496 GYVTTLKPTHPLLARTISTRTRRGSNISIFSFRPRNKDSEGDFADDEFSI    545
                     ..:..:|.||.||.||:|.|.||.|.:|||:|||.|||||.||||||||.
drSCN5LAb        499 DPIEGIKQTHSLLVRTLSLRARRESAVSIFNFRPVNKDSELDFADDEFSN    548

omSCN5LAba       546 HGDNEGTHSRTGSLVIPWSTRRRPSTYSTGSRGSQVF---LNVNGKLFVA    592
                     |||::   ||.|:|.:||| |||.|..|:.|..||.|   .|:||||.||
drSCN5LAb        549 HGDSD---SRGGALALPWS-RRRTSAQSSCSHSSQFFFPSFNINGKLMVA    594

omSCN5LAba       593 MDQNGVTPQG----------LPACIMEKVKEESGPNSSTELSTMLLPR--    630
                     ::|||::.||          ||||.|||:|||||.|||.|||:||||:
drSCN5LAb        595 VEQNGISSQGPLTPMTPLTPLPACTMEKLKEESGQNSSNELSSMLLPQLP    644

omSCN5LAba       631 PVSREEHVQRDRALSGASYLTDALEELEESRQKCHPCWYEFAHKYLIWES    680
                     |..      ||||||..||:|||:|||||::||||||||.||||||:|..
drSCN5LAb        645 PEG------RDRALSATSYITDAMEELEEAQQKCHPCWYVFAHKYLVWTC    688

omSCN5LAba       681 SPRWLQLKALVKVMVMDPFLDLAITICIVLNTLFMAMEHYPMTDEFNGML    730
                     |||||::|..||:|||||||||||||||||||||||:||||||||||.||
drSCN5LAb        689 SPRWLKVKEWVKIMVMDPFLDLAITICIVLNTLFMALEHYPMTDEFNRML    738

omSCN5LAba       731 SIGNLVFSGIFTAEMVLKIIALDPYYYFQQGWNIFDSIIVCLSLMELGLS    780
                     |:|||||:|||||||||||||||||||||||||||||:||.|||||||||
drSCN5LAb        739 SVGNLVFTGIFTAEMVLKIIALDPYYYFQQGWNIFDSLIVSLSLMELGLS    788

omSCN5LAba       781 DVEGLSVLRSFRLLRVFKLAKSWPTLNTLIKIIGNSVGALGNLTLVLAII    830
                     :|||||||||||||||||||||||||||||||||||||||||||||||||
drSCN5LAb        789 NVEGLSVLRSFRLLRVFKLAKSWPTLNTLIKIIGNSVGALGNLTLVLAII    838

omSCN5LAba       831 VFIFAVVGMQLFGKNYQDCVCKISKDCTLPRWHMKDFFHSFLIVFRVLCG    880
                     ||||||||||||||||..||||||||||||||||||||||||||||||||
drSCN5LAb        839 VFIFAVVGMQLFGKNYDLCVCKISKDCTLPRWHMKDFFHSFLIVFRVLCG    888

omSCN5LAba       881 EWIETMWDCMEVAGQPLCLLVFMQVMVIGNLVVLNLFLALLLSSFSSDNL    930
                     ||||||||||||||||||:||||.||||||||||||||||||||||:|||
drSCN5LAb        889 EWIETMWDCMEVAGQPLCILVFMLVMVIGNLVVLNLFLALLLSSFSADNL    938

omSCN5LAba       931 SAPDDDGEMNNLQIAIGRIKSGMGWLRSQICDFFNGNFKRRQQKSKEAEA    980
                     ||||:|||||||||||.||:.||.|||..:|||||||||||:||:|||:|
drSCN5LAb        939 SAPDEDGEMNNLQIAIARIQRGMLWLRQALCDFFNGNFKRRRQKAKEAKA    988

omSCN5LAba       981 MLKLKRLSHSAPLGEVGNGT--VVAVAGGIGRHGEKIMVPEVDDSYMTNP   1028
                     ||||||||..|...| ||||  |:..:.|.| .||       ||||||||
drSCN5LAb        989 MLKLKRLSQQAHWAE-GNGTAGVIERSSGAG-SGE-------DDSYMTNP   1029

omSCN5LAba      1029 NLTISVPIAPGESDVEFPEDEDEEEEGESESSEKEEEEEESKQVKDDISL   1078
                     |||||||||||||||||||:|||||:.|.|:|...:||.|..:.:||.||
drSCN5LAb       1030 NLTISVPIAPGESDVEFPEEEDEEEDEEEEASASSDEEPEPCKPRDDTSL   1079

omSCN5LAba      1079 SEGSTVDLRKPGEEEDEYSEMAEETMDPDNCFPDVCVRHFQCCDIQTTEG   1128
                     |||||:||||||||||||||||||.|||:|||||||||||:||||.|:||
drSCN5LAb       1080 SEGSTIDLRKPGEEEDEYSEMAEEAMDPENCFPDVCVRHFKCCDINTSEG   1129
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omSCN5LAba      1129 LGQAWWRLRKTCFQIVEHSWFESFIIFMILLSSGALAFEDIYIEQRKVIK   1178
                     ||:|||||||||:|||||||||:||||||||||||||||||||:||||:|
drSCN5LAb       1130 LGRAWWRLRKTCYQIVEHSWFETFIIFMILLSSGALAFEDIYIDQRKVVK   1179

omSCN5LAba      1179 MVLEYADKIFTYIFILEMLLKWIAYGFKKYFTNYWCWLDFLIVDISVIGL   1228
                     ::||||||||||||||||.||||||||:||||||||||||||||
drSCN5LAb       1180 VILEYADKIFTYIFILEMSLKWIAYGFRKYFTNYWCWLDFLIVD------   1223

omSCN5LAba      1229 LASAVNIEQIGTMRVLRTLRALRPLRAVARFAGIRVSLVSLVANMLGYSD   1278
                                                        |||||||||.|||||
drSCN5LAb       1224 -----------------------------------VSLVSLVANTLGYSD   1238

omSCN5LAba      1279 FAAIKSLRTLRALRPLRALSRFEGMRVVVNALIGAIPSIMNVLLVCLIFW   1328
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
drSCN5LAb       1239 FAAIKSLRTLRALRPLRALSRFEGMRVVVNALIGAIPSIMNVLLVCLIFW   1288

omSCN5LAba      1329 LIFSIMGVNLFAGKYGRCVNRTGYIHNVSVVNNKTDCLAMNDTQFYWTKV   1378
                     ||||||||||||||:||||||||:|:|.|.:||:::||.||.||:|||||
drSCN5LAb       1289 LIFSIMGVNLFAGKFGRCVNRTGFIYNSSDINNRSECLEMNSTQYYWTKV   1338

omSCN5LAba      1379 KVNFDNVGAGYLALLQVATFKGWMDIMYAAVDSRAVEEQPIREVNMYMYL   1428
                     ||||||||||||||||||||||||:||||||||||||||||:|.::||||
drSCN5LAb       1339 KVNFDNVGAGYLALLQVATFKGWMEIMYAAVDSRAVEEQPIKENSLYMYL   1388

omSCN5LAba      1429 YFIIFIIFGSFFTLNLFIGVIIDNFNQQKRKLGGQDIFMTEEQKKYYNAM   1478
                     ||:|||||||||||||||||||||||||||||||||||||||||||||||
drSCN5LAb       1389 YFVIFIIFGSFFTLNLFIGVIIDNFNQQKRKLGGQDIFMTEEQKKYYNAM   1438

omSCN5LAba      1479 KKLGSKKPQKPIPRPVNQVQGFFFDLVGKQAFDIIIMVLILLNMITMMVE   1528
                     |||||||||||||||.|.|||||||||.||||||:||:||:|||:|||||
drSCN5LAb       1439 KKLGSKKPQKPIPRPPNPVQGFFFDLVSKQAFDILIMLLIILNMVTMMVE   1488

omSCN5LAba      1529 TDEQPARMEYILNKINLAFIIIFSCECLIKIVALRCYFFTIGWNIFDFVV   1578
                     ||||...:::||:.|||.||:||:.||::||:||||||||:.||||||||
drSCN5LAb       1489 TDEQSPSIKHILDCINLVFIVIFTSECILKIIALRCYFFTVSWNIFDFVV   1538

omSCN5LAba      1579 VILSIVGIVLADIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKGIRTLLF   1628
                     ||||||||||||||||||||||||||||||||||:|||||||||||||||
drSCN5LAb       1539 VILSIVGIVLADIIEKYFVSPTLFRVIRLARIGRILRLIRGAKGIRTLLF   1588

omSCN5LAba      1629 ALMMSLPALFNIGLLLFLVMFIYAIFGMANFAYVKKQAGIDDMFNFETFG   1678
                     |||||||||||||||||||||||||||||||||||||.||||||||||||
drSCN5LAb       1589 ALMMSLPALFNIGLLLFLVMFIYAIFGMANFAYVKKQGGIDDMFNFETFG   1638

omSCN5LAba      1679 NSMICLFQITTSAGWDGLLSPILNNSPEECNPNLIHTGTNARGNCGNPSV   1728
                     ||||||||||||||||.|||||||..||||:|.:.|||||||||||||||
drSCN5LAb       1639 NSMICLFQITTSAGWDNLLSPILNTPPEECDPEIPHTGTNARGNCGNPSV   1688

omSCN5LAba      1729 GITFFVTYIIISFLIVVNMYIAIILENFSVATEESTEPLSEDDFEMFYEV   1778
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
drSCN5LAb       1689 GITFFVTYIIISFLIVVNMYIAIILENFSVATEESTEPLSEDDFEMFYEV   1738

omSCN5LAba      1779 WEKFDPEATQFIEYAKLSDFADTLSEPLRIGKPNKIKLISMDLPMVSGDK   1828
                     ||||||||||||||.|||||||||||||||||||||||||||||||||||
drSCN5LAb       1739 WEKFDPEATQFIEYLKLSDFADTLSEPLRIGKPNKIKLISMDLPMVSGDK   1788

omSCN5LAba      1829 IHCLDILFAFTKRVLGESGEMDALKQQMEEKFMMANPSKISYEPITTTLR   1878
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
drSCN5LAb       1789 IHCLDILFAFTKRVLGESGEMDALKQQMEEKFMMANPSKISYEPITTTLR   1838

omSCN5LAba      1879 RKQEDVSAAVIQRCYRRHLVRRQMKQASFLYRSMQISLTQSPTGE--GGT   1926
                     ||||:|||.:|||.|||||:|||:||||.|||.|.:     |..:  |.:
drSCN5LAb       1839 RKQEEVSAIMIQRSYRRHLIRRQLKQASLLYRQMTM-----PDADKAGDS   1883

omSCN5LAba      1927 APEKEGLIASMIKEHYGGPEMELMETLSSTSSPPSYDSVTRATSELFQVL   1976
                     :||.:|||.|||.|:| ..|.|:  |:|:||||||||||||||||:|..|
drSCN5LAb       1884 SPESQGLIVSMIMENY-ATEAEI--TISATSSPPSYDSVTRATSEIFHAL   1930

omSCN5LAba      1977 ISEQSRNSDLREHCPAPDRVPDRETFL   2003
                     |.|:: |..|.||  ..|...:.||||
drSCN5LAb       1931 IPEET-NEILSEH--LADAEKESETFL   1954
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