The Journal of Experimental Biology 205, 3631–3639 (2002)
Printed in Great Britain © The Company of Biologists Limited
JEB4377

Temperature dependence of cardiac sarcoplasmic reticulum function in rainbow trout myocytes

Holly A. Shiels[1,*], Matti Vornanen[2] and Anthony P. Farrell[1]

[1]*Simon Fraser University, Biological Sciences, Burnaby, British Columbia, V5A 1S6, Canada* and [2]*University of Joensuu, Department of Biology, PO Box 111, 80101 Joensuu, Finland*

*Author for correspondence at present address: School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK (e-mail: hollys@sfu.ca)

Accepted 21 August 2002

Summary

To explore how the cardiac sarcoplasmic reticulum (SR) functions over a range of temperatures, we used whole-cell voltage clamp combined with rapid caffeine application to study SR Ca^{2+} accumulation, release and steady-state content in atrial myocytes from rainbow trout. Myocytes were isolated from rainbow trout acclimated to 14°C, and the effect of varying stimulation pulse number, frequency and experimental temperature (7°C, 14°C and 21°C) on SR function was studied. To add physiological relevance, in addition to 200 ms square (SQ) voltage pulses, myocytes were stimulated with temperature-specific action potentials (AP) applied at relevant frequencies for each test temperature. We found that the SR accumulated Ca^{2+} more rapidly and to a greater concentration (1043±189 μmol l^{-1} Ca^{2+}, 1138±173 μmol l^{-1} Ca^{2+}, and 1095±142 μmol l^{-1} Ca^{2+} at 7°C, 14°C and 21°C, respectively) when stimulated with physiological AP waveforms at physiological frequencies compared with 200 ms SQ pulses at the same frequencies (664±180 μmol l^{-1} Ca^{2+}, 474±75 μmol l^{-1} Ca^{2+} and 367±42 μmol l^{-1} Ca^{2+} at 7°C, 14°C and 21°C, respectively). Also, and in contrast to 200 ms SQ pulse stimulation, temperature had little effect on steady-state SR Ca^{2+} accumulation during AP stimulation. Furthermore, we observed SR-Ca^{2+}-dependent inactivation of the L-type Ca^{2+} channel current (I_{Ca}) at 7°C, 14°C and 21°C, providing additional evidence of maintained SR function in fish hearts over an acute range of temperatures. We conclude that the waveform of the AP may be critical in ensuring adequate SR Ca^{2+} cycling during temperature change in rainbow trout *in vivo*.

Key words: L-type Ca^{2+} current (I_{Ca}), I_{Ca} inactivation, sarcoplasmic reticulum, Ca^{2+} load, excitation–contraction coupling, heart rate, fish, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Eurythermal animals maintain aerobic performance throughout a range of acute temperature fluctuations. Rainbow trout (*Oncorhynchus mykiss*), for example, maintain swimming performance over a broad ambient temperature range (Randall and Brauner, 1991; Matthews and Berg, 1997; Reid et al., 1997), which implies that physiological systems that support exercise, such as the heart, also function well over a broad temperature range. The focus of the present study was to investigate how temperature influences cardiac function in trout heart and, in particular, how temperature affects Ca^{2+} flux through the sarcoplasmic reticulum (SR) in trout atrial myocytes.

In rainbow trout heart, Ca^{2+} delivery to the myofilaments during excitation–contraction (e–c) coupling primarily involves the influx of extracellular Ca^{2+} across the sarcolemmal membrane (SL) and secondarily involves the mobilization of the intracellular Ca^{2+} stores of the SR (Keen et al., 1994; Aho and Vornanen, 1999; Hove-Madsen et al., 1999, 2001; Harwood et al., 2000; Shiels et al., 2002a). The relative importance of the SR Ca^{2+} flux pathway in trout hearts is generally considered to be minor but can vary depending on a number of physiological parameters. For example, SR Ca^{2+} cycling appears to play a greater role in e–c coupling in (1) atrial tissue compared with ventricular tissue, (2) at low (<0.6 Hz) compared with high (>0.6 Hz) contraction frequencies, (3) after acclimation to cold temperatures (<12°C) and (4) after an acute change to warm temperatures (>18°C) (Hove-Madsen, 1992; Keen et al., 1994; Shiels and Farrell, 1997; Aho and Vornanen, 1999; Shiels et al., 2002a).

In most adult mammalian hearts, the SR is the major source of activator Ca^{2+} during e–c coupling, with SL Ca^{2+} influx serving primarily as the trigger for SR Ca^{2+} release (Bers, 2001). The SR Ca^{2+} flux pathways are known to be temperature-sensitive in many mammals, and the inhibition of SR function at cold temperatures is a major cause of cold-induced contractile dysfunction (Wang et al., 1997, 2000). Cold temperatures increase the open probability of the mammalian SR Ca^{2+} release channels (ryanodine receptors) causing the leak of available SR Ca^{2+} into the cytoplasm (Sitsapesan et al., 1991). Cold-induced slowing of ion transport

mechanisms prevents this Ca^{2+} from being easily removed from the cytoplasm, contributing to a loss of Ca^{2+} homeostasis (Bers, 1987; Sitsapesan et al., 1991).

Despite the fact that the fish heart continues to function at cold temperatures, early research on whole-muscle contractility in trout ventricle suggested that the teleost SR had a temperature dependency similar to that of the mammalian SR. This is because contractile force was found to be sensitive to ryanodine (an inhibitor of the SR Ca^{2+} release channel; Rousseau et al., 1987) at warm temperatures (18–25°C) but insensitive to ryanodine at cold temperatures (<15°C) (Hove-Madsen, 1992; Driedzic and Gesser, 1994; Keen et al., 1994). However, a study with isolated trout atrial tissue showed a 20% reduction in developed force after ryanodine treatment at 4°C (Aho and Vornanen, 1999), indicating maintained SR function in the cold and suggesting that, similar to mammals (Bers, 2001), SR involvement may be greater in fish atrium than in fish ventricle. These findings were supported in a recent cellular study that demonstrated maintained SR function at cold temperatures in rainbow trout atrial myocytes (Hove-Madsen et al., 2001). However, the experimental conditions in this cellular study favoured a large SR Ca^{2+} content, which could result in an overestimation of SR capabilities in the cold. High intracellular Na^+ concentrations (approximately 17 mmol l^{-1}) in conjunction with long depolarizing pulses (>30 s) to positive membrane potentials were used in this study to Ca^{2+}-load the SR *via* the reverse mode (Ca^{2+} in, Na^+ out) of the sarcolemmal Na^+/Ca^{2+} exchanger (NCX; Hove-Madsen et al., 1998, 1999, 2001). Indeed, mammalian and teleost studies have shown that experimentally elevating intracellular Na^+ concentrations to a range of 15–20 mmol l^{-1} (concentrations *in vivo* are approximately 7 mmol l^{-1}) induces supra-physiological Ca^{2+} influx *via* the NCX (Hove-Madsen et al., 2000; Bers, 2001). Thus, it is possible that previous studies with fish have overestimated the physiological SR Ca^{2+} loading and release capabilities at cold temperatures. In fact, cellular studies with permeabilized trout ventricular myocytes indicated that the trout SR Ca^{2+}-ATPase, which pumps cytosolic Ca^{2+} back into the SR during relaxation, is temperature-dependent, with an average Q_{10} of approximately 1.6 over a temperature range of 5–20°C (Aho and Vornanen, 1998; Hove-Madsen et al., 1998). As trout can experience acute temperature fluctuations of as much as ±10°C either while transversing thermoclines or as a result of diurnal changes in shallow streams (Matthews and Berg, 1997; Reid et al., 1997), the temperature dependency of the SR Ca^{2+}-ATPase, combined with temperature-sensitive SR Ca^{2+} release channels, could limit the utility of the SR as a source of activator Ca^{2+} during e–c coupling in trout heart.

In the present study, we were interested in assessing the physiological efficacy of the trout atrial SR at different temperatures. We acknowledge that it is difficult to be truly physiological using isolated voltage-clamped myocytes. However, to better approach the physiological situation, we included 10 mmol l^{-1} Na^+ in our pipette solutions and, in addition to stimulating cells with conventional square (SQ) voltage-clamp pulses of constant dimensions, we applied physiological action potentials (APs) whose shape and rate of firing varied dynamically with changes in temperature. We assessed SR Ca^{2+} accumulation using rapid caffeine application, and SR Ca^{2+} release, by examining the effect of SR Ca^{2+} content on the inactivation kinetics of I_{Ca} (L-type Ca^{2+} channel current). We find that the shape of the AP and the rate of AP firing may be critical for ensuring adequate atrial SR Ca^{2+} cycling during temperature change in rainbow trout *in vivo*.

Materials and methods

Fish origin and care

Rainbow trout *Oncorhynchus mykiss* Walbaum (female, mean mass 263.6±25.5 g, *N*=22) were obtained from a local fish farm (Kontiolahti, Finland) and held in the laboratory in a 500 l tank receiving aerated tapwater. The tank temperature was 14±1°C. Fish were held for a minimum of four weeks prior to experimentation and were fed daily on commercial trout pellets (Biomar, Brande, Denmark). The photoperiod was a 15 h:9 h L:D cycle. All procedures were in accordance with local animal handling protocols.

Isolated myocyte preparation

A detailed description of the myocyte preparation has been previously published (Shiels et al., 2000; Vornanen, 1998). Briefly, fish were stunned with a blow to the head, the spine was cut just behind the brain and the heart was excised. The heart was then perfused first with the isolating solution for 8–10 min, and then with the proteolytic enzyme solution for 20 min. After enzymatic treatment, the atrium was removed from the ventricle and placed in a small dish containing isolating solution. The atrium was cut into small pieces with scissors and then triturated through the opening of a Pasteur pipette. The isolated myocytes were stored in fresh isolating solution at 14°C.

Solutions

The isolating solution contained: NaCl, 100 mmol l^{-1}; KCl, 10 mmol l^{-1}; KH_2PO_4, 1.2 mmol l^{-1}; $MgSO_4$, 4 mmol l^{-1}; taurine, 50 mmol l^{-1}; glucose, 20 mmol l^{-1}; and Hepes, 10 mmol l^{-1}; adjusted to pH 6.9 with KOH. For enzymatic digestion, collagenase (Type IA), trypsin (Type IX) and fatty-acid-free bovine serum albumin (BSA) were added to this solution. The physiological saline used as the extracellular solution for recording Ca^{2+} currents (I_{Ca}) and NCX currents (I_{NCX}) contained: NaCl, 150 mmol l^{-1}; CsCl, 5.4 mmol l^{-1}; $MgSO_4$, 1.2 mmol l^{-1}; NaH_2PO_4, 0.4 mmol l^{-1}; $CaCl_2$, 1.8 mmol l^{-1}; glucose, 10 mmol l^{-1}; and Hepes, 10 mmol l^{-1}; adjusted to pH 7.7 with CsOH. Additionally, 1 µmol l^{-1} tetrodotoxin (TTX) was added to the perfusate to block fast Na^+ channels. Caffeine (10 mmol l^{-1}) was added to the extracellular solution to release the SR Ca^{2+} stores. For AP recordings, the extracellular solution was modified by replacing the CsCl with equimolar KCl, pH balancing with

KOH and by omitting the TTX. The pipette solution for I_{Ca} and I_{NCX} recordings contained: CsCl, 130 mmol l^{-1}; MgATP, 5 mmol l^{-1}; tetraethylammonium chloride (TEA), 15 mmol l^{-1}; $MgCl_2$, 1 mmol l^{-1}; oxaloacetate, 5 mmol l^{-1}; Na_2-phosphocreatine, 5 mmol l^{-1}; Hepes, 10 mmol l^{-1}; EGTA, 0.025 mmol l^{-1}; and Na_2GTP, 0.03 mmol l^{-1}; pH was adjusted to 7.2 with CsOH. For action potential recordings, the pipette solution contained: K-aspartate, 125 mmol l^{-1}; KCl, 15 mmol l^{-1}; $MgCl_2$, 1 mmol l^{-1}; MgATP, 5 mmol l^{-1}; EGTA, 0.05 mmol l^{-1}; Na_2-phosphocreatine, 5 mmol l^{-1}; and Hepes, 10 mmol l^{-1}; adjusted to pH 7.2 with KOH. All drugs, with the exception of TTX (Tocris, Bristol, UK), were purchased from Sigma (St Louis, MO, USA).

Experimental procedures

Myocytes (average cell capacitance 36.2±0.92 pF, *N*=92) were superfused at a rate of 2 ml min^{-1} with extracellular solution at 7°C, 14°C or 21°C. Each cell was tested at one experimental temperature only. The extracellular solution and the solution flowing from a rapid solution changing device (RS200, Biologic, Claix, France) was heated or chilled by water bath circuits before emptying into the recording chamber (RC-26, Warner Instrument Corp, Brunswick, USA; volume 150 µl). A thermocouple was placed inside the recording chamber and positioned no less than 5 mm from the cell to ensure it was experiencing the desired temperature. Rapid application (approximately 50 ms) of caffeine was achieved by switching between two barrels of the rapid solution changer; one containing control extracellular solution and the other containing the same solution plus caffeine, both flowing at approximately 0.5 ml min^{-1}.

Whole-cell voltage-clamp experiments were performed using an Axopatch 1D amplifier with a CV-4 1/100 headstage (Axon Instruments, Foster City, CA, USA). Pipettes had a resistance of 2.4±0.11 MΩ when filled with pipette solution. Junction potentials were zeroed prior to seal formation. Pipette capacitance (9.7±0.5 pF) was compensated after formation of a GΩ seal. Mean series resistance was 6.1±0.2 MΩ. Membrane capacitance was measured using the calibrated capacity compensation circuit of the Axopatch amplifier. Signals were low-pass filtered using the 4-pole lowpass Bessel filter on the Axopatch-1D amplifier at a frequency of 2 kHz for I_{Ca} and 10 kHz for action potentials, and were then analyzed off-line using pClamp 6.0 software (Axon Instruments).

Current-clamp and AP recording

Rainbow trout atrial myocytes were stimulated to elicit APs at 7°C, 14°C or 21°C at a frequency that corresponded to the resting heart rate of rainbow trout *in vivo* at each of these temperatures. The temperature/frequency pairs were as follows: 0.6 Hz at 7°C, 1.0 Hz at 14°C and 1.4 Hz at 21°C (Aho and Vornanen, 2001; Tuurala et al., 1982; Farrell et al., 1996). APs were elicited by the minimum voltage pulse able to trigger a self-sustained AP (1 ms, 0.8 nA). The resultant AP waveforms (Fig. 1) were subsequently used to provide a more physiologically relevant indication of how temperature and frequency affected SR Ca^{2+} accumulation and release in rainbow trout atrial myocytes. In all experiments, the capacitive transients resulting from the AP waveform were compensated for by the amplifier.

Assessing SR Ca^{2+} content

We used either SQ pulses (–80 mV to +10 mV, 200 ms, inter-pulse holding potential of –80 mV) or AP pulses (see Fig. 1) and varied both temperature and pulse frequency to examine their effect on SR Ca^{2+} accumulation. SR Ca^{2+} accumulation was assessed by the application of caffeine, which induces the release of Ca^{2+} from the SR. This Ca^{2+} is then extruded from the cell *via* the NCX generating an inward current (Fig. 2) that is directly proportional to the Ca^{2+} released from the SR (Varro et al., 1993). The time integral of the caffeine-induced I_{NCX} current was used to calculate the SR Ca^{2+} content (in pC) at the time of caffeine application. This value was expressed per unit capacitance (pC pF^{-1}). The SR Ca^{2+} content was also expressed in µmol l^{-1} and was calculated from the integral of I_{NCX} and the cell volume. Cell volume was calculated from cell surface area [obtained by measurements of cell capacitance (pF) and assuming a specific membrane capacitance of 1.59 µF cm^{-2}] and the surface:volume ratio of 1.15 [determined experimentally in previous studies; see Vornanen (1997)]. Finally, SR Ca^{2+} content was expressed as a function of myofibrillar volume (40%), as determined previously (Vornanen, 1998).

Interaction between the SL L-type Ca^{2+} channel and the SR Ca^{2+} release channel

Possible interaction between extracellular Ca^{2+} influx *via* the L-type Ca^{2+} channel (I_{Ca}) and intracellular Ca^{2+} release

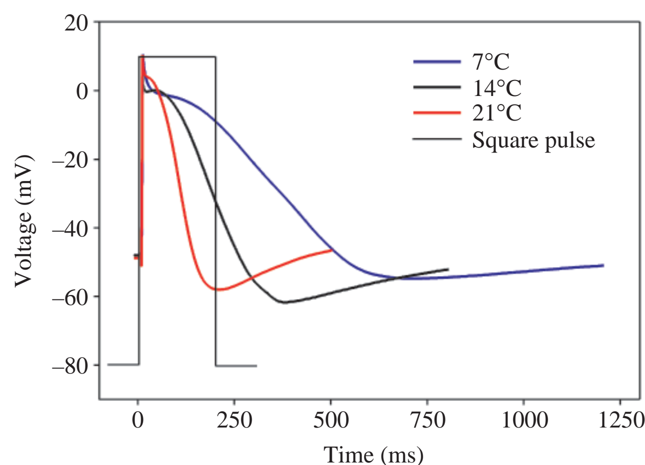

Fig. 1. The atrial action potential (AP) from isolated rainbow trout myocytes. AP waveforms were elicited by current-clamp at a rate that corresponded to the heart rate for rainbow trout *in vivo* at each temperature (0.6 Hz at 7°C, 1.0 Hz at 14°C and 1.4 Hz at 21°C). The steady-state APs from 6–10 cells at each temperature were averaged, and the resultant waveforms were used to stimulate L-type Ca^{2+} channel current (I_{Ca}) and sarcoplasmic reticulum (SR) Ca^{2+} accumulation in subsequent experiments where the inter-pulse holding level was –80 mV.

through the SR Ca^{2+} release channel was examined by investigating the effect of SR Ca^{2+} content on the inactivation of I_{Ca}. Previous work has shown that if there is interaction between these Ca^{2+} fluxes, I_{Ca} inactivation is faster due to SR Ca^{2+}-dependent inactivation (Lipp et al., 1992; Sham, 1997). To test whether this mechanism occurs in trout atrium, we fitted either a double (τ_f and τ_s; SQ) or single (τ; AP) exponential function to the inactivating portion of I_{Ca} immediately after the SR Ca^{2+} stores were depleted with caffeine (loading pulse 1) and then again after SR Ca^{2+} content had reached steady-state (after 25 pulses). In some cells, the effect of progressive SR Ca^{2+} accumulation on I_{Ca} with each pulse was examined between the first and 100th pulse. Single and double exponential fits were calculated using the standard exponential fitting procedure with the Chebyshev transformation with Clampfit 6.0 software (Axon Instruments).

Statistical Analysis

One-way repeated measures analysis of variance (RM ANOVA) was used to compare the effect of pulse number on SR Ca^{2+} content. One-way ANOVA was used to test the effects of temperature, frequency and shape of the stimulation pulse on SR Ca^{2+} accumulation. Significant differences ($P<0.05$) were assessed with Student–Newman–Keuls (SNK) *post-hoc* analysis. The effects of steady-state SR Ca^{2+} content on the inactivation kinetics of I_{Ca} were tested using paired Student's *t*-tests.

Results

Effect of temperature on steady-state SR Ca^{2+} accumulation

Our first experiments set out to investigate the effect of temperature on steady-state SR Ca^{2+} accumulation in rainbow trout atrial myocytes. At the onset of each experiment, caffeine was applied to the cell to release SR Ca^{2+} stores. The cell was then stimulated with 25 SQ-voltage pulses at 1.0 Hz and caffeine was re-applied. Application of caffeine caused the myocytes to contract strongly and induced a large inward current (I_{NCX}), as shown in Fig. 2. The time integral of this current was used to assess the SR Ca^{2+} content at the time of caffeine application (see inset in Fig. 2). Myocytes were then stimulated with 50 SQ-voltage pulses at 1.0 Hz and caffeine was again applied to assess SR Ca^{2+} accumulation. Finally, the cell was stimulated with 100 pulses at 1.0 Hz and SR Ca^{2+} accumulation was again assessed. The mean results for all cells at each temperature are given in Fig. 3A. At both 14°C and 7°C, the trout SR Ca^{2+} content had reached a steady-state of approximately 1 pC pF^{-1} by 25 pulses (equivalent to 474±75 μmol l^{-1} Ca^{2+} and 365±43 μmol l^{-1} Ca^{2+} at 14°C and 7°C, respectively, N=8–16). SR Ca^{2+} content did not increase significantly with additional pulses between 25 and 100 (Fig. 3, and also see Fig. 4).

At 21°C, SR Ca^{2+} accumulation was more variable, as only four out of eight cells demonstrated SR Ca^{2+} accumulation under these experimental conditions. Those cells that accumulated Ca^{2+} into the SR also reached a steady-state of approximately 1 pC pF^{-1} after 25 pulses (equivalent to 460±76 μmol l^{-1} Ca^{2+}) and did not change significantly with an additional number of pulses up to 100. Only the cells that were able to accumulate Ca^{2+} into the SR are shown in Fig. 3A (broken line).

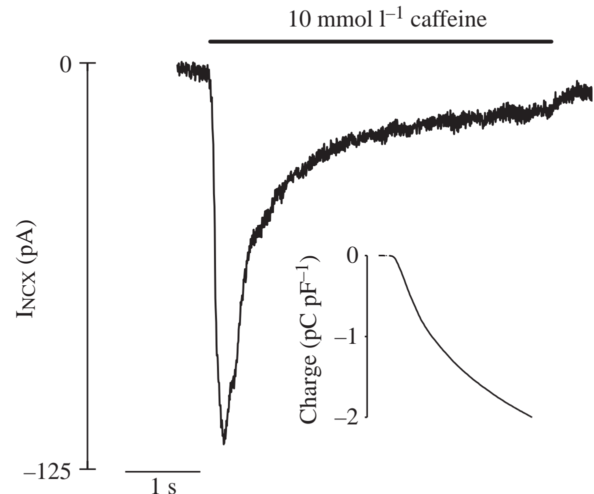

Fig. 2. A representative recording of the inward Na^+/Ca^{2+} exchanger (NCX) current (I_{NCX}) resulting from caffeine application in a trout atrial myocyte at 14°C. This current would be accompanied by a large contraction (not shown). The inset shows the time integral of the current that was used to calculate the Ca^{2+} content of the sarcoplasmic reticulum (SR) at the time that caffeine was applied.

Effect of frequency and temperature on steady-state SR Ca^{2+} accumulation

Because the frequency of cell depolarization should affect the Ca^{2+} available for uptake into the SR, our next experiment examined SR Ca^{2+} content at each temperature when the 200 ms SQ pulses were applied at a frequency that corresponds to the heart rate of the rainbow trout *in vivo* at each temperature. These results are given in Fig. 3B. In contrast to the results with 1.0 Hz stimulation, when frequency was increased to 1.4 Hz at 21°C, all cells displayed significant SR Ca^{2+} accumulation and reached a steady-state of 0.78±0.09 pC pF^{-1} (equivalent to 367±42 μmol l^{-1} Ca^{2+}) after 25 pulses (Fig. 3B). At 14°C, the physiologically relevant frequency was 1.0 Hz and the results were no different from those presented in Fig. 3A. At 7°C, a physiologically relevant stimulation frequency was 0.6 Hz, and the SR Ca^{2+} content after 25 pulses and 50 pulses was similar to that at 1.0 Hz but increased significantly ($P<0.05$, N=8) after 100 pulses (Fig. 3B). As a result, SR Ca^{2+} content was 2.1±0.3 pC pF^{-1} or 972±142 μmol l^{-1} Ca^{2+} after 100 pulses at 0.6 Hz, which was almost double the steady-state amount observed after stimulation with 100 SQ pulses at 1.0 Hz (550±90 μmol l^{-1} Ca^{2+}) (Fig. 3A).

Effect of AP shape on steady-state SR Ca^{2+} accumulation

The experiments with SQ-voltage-clamp pulses indicated that SR Ca^{2+} content can vary as a function of temperature and

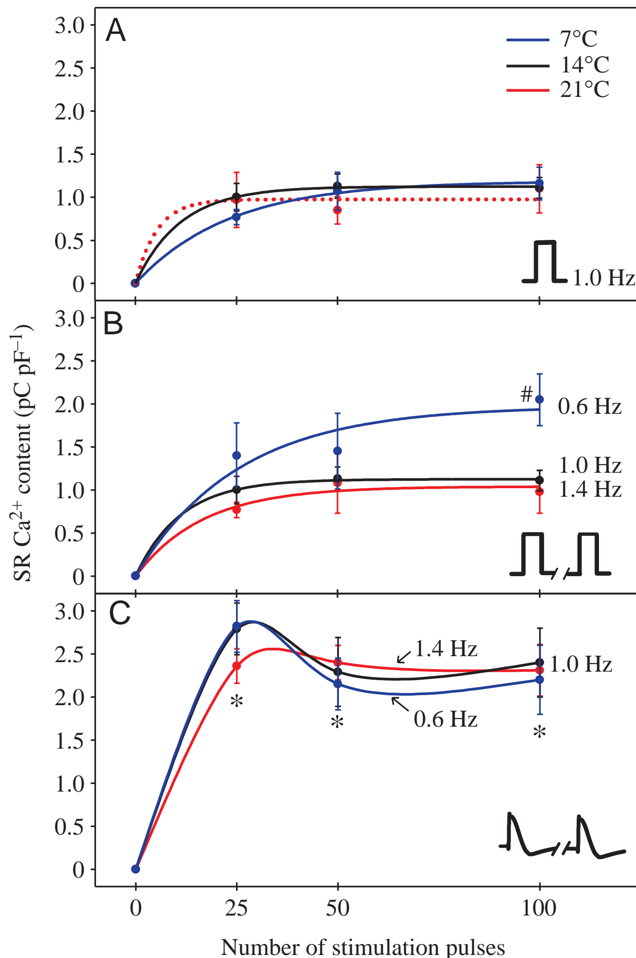

Fig. 3. The effect of pulse number, temperature, frequency and stimulus waveform on sarcoplasmic reticulum (SR) Ca^{2+} accumulation in rainbow trout atrial myocytes. At each temperature, myocytes were exposed to caffeine to release the SR Ca^{2+} stores (not shown) and then stimulated with an increasing number of stimulation pulses between 1 and 100. Stimulation pulse waveform and frequency are given in each panel. SR Ca^{2+} content is expressed in pC pF^{-1}, and the corresponding values for Ca^{2+} concentration (μmol l^{-1}) are given in the text. (A) The effect of temperature and pulse number on SR Ca^{2+} content when cells were stimulated with square (SQ) pulses applied at 1.0 Hz. The results at 21°C are given by a broken line, as only four out of eight cells achieved any SR Ca^{2+} content under these loading conditions (see text for details). (B) The effect of applying SQ pulses at a frequency that corresponds to the heart rate of rainbow trout *in vivo* at each test temperature on SR Ca^{2+} accumulation. # indicates an increase in SR Ca^{2+} content between 25 and 100 pulses at 7°C, and also a difference between 100 pulses at 0.6 Hz and 100 pulses at 1.0 Hz (part A) at 7°C. (C) The effect of temperature and frequency on SR Ca^{2+} content when action potentials (APs) were applied at a frequency that corresponds to the heart rate of rainbow trout *in vivo* at each test temperature. * indicates that the SR content after AP pulses is significantly greater than after SQ pulses. All values are means ± S.E.M. of 4–16 cells at each temperature. In (A) and (B), the lines were drawn by fitting the data to an asymptotic exponential function. In (C), the line is plotted through the data without a curve fit.

frequency. However, *in vivo*, extracellular Ca^{2+} entry into the myocyte does not occur during a SQ-voltage pulse but rather during an AP. As both the shape and rate of APs vary considerably with temperature (Coyne et al., 2000; Harwood et al., 2000; Shiels et al., 2000), studies with AP clamp may be essential for understanding the physiological capacity of the SR as a function of temperature in fish hearts. Indeed, we observed a pronounced effect of acute temperature change on AP duration, which is clearly illustrated in Fig. 1. AP duration at 50% repolarization varied by more than 50% among temperatures (increasing from approximately 90 ms at 21°C to 160 ms at 14°C and to 290 ms at 7°C; Fig. 1). Thus, our next experiments set out to examine the Ca^{2+}-accumulating abilities of the trout SR during AP stimulation.

When myocytes were stimulated at a physiologically relevant frequency, with the AP waveform specific for the test temperature, SR Ca^{2+} content reached a steady-state after 25 pulses at all three temperatures (2.8±0.3 pC pF^{-1}, 2.7±0.3 pC pF^{-1} and 2.3±0.2 pC pF^{-1} at 7°C, 14°C and 21°C, respectively; Fig. 3C) and the steady-state temperature and frequency-dependent changes in SR Ca^{2+} content observed with SQ pulses were no longer evident. The Ca^{2+} content of the SR was greater (1043±189 μmol l^{-1} Ca^{2+}, 1138±173 μmol l^{-1} Ca^{2+} and 1095±142 μmol l^{-1} Ca^{2+} at 7°C, 14°C and 21°C, respectively) when cells were stimulated with AP pulses compared with 200 ms SQ pulses (664±180 μmol l^{-1} Ca^{2+}, 474±75 μmol l^{-1} Ca^{2+} and 367±42 μmol l^{-1} Ca^{2+} at 7°C, 14°C and 21°C, respectively) applied at the same frequency (Fig. 3B).

Interaction between I_{Ca} and SR Ca^{2+} release

I_{Ca} records initiated immediately after depletion of SR Ca^{2+} by caffeine allowed us to monitor the effects of SR Ca^{2+} re-accumulation on I_{Ca}. Inactivation kinetics of I_{Ca} were significantly faster when the SR was loaded with Ca^{2+}, and this observation is consistent with a greater SR Ca^{2+} release resulting in greater SR-Ca^{2+}-dependent inactivation. Fig. 4 shows a representative recording of the progressive quickening of inactivation as SR Ca^{2+} content increases with each pulse between 1 and 25 at 21°C. After 25 pulses, inactivation kinetics did not change significantly with additional pulses, supporting the finding that SR Ca^{2+} content had reached a steady-state. Fig. 5 shows representative I_{Ca} currents with both SQ and AP pulses with the SR empty and at a steady-state at 14°C. The mean inactivation kinetics of I_{Ca} for each condition and temperature are given in Tables 1, 2. At 21°C, fast inactivation kinetics (τ_f) and slow inactivation kinetics (τ_s) were significantly faster than at 14°C and were significantly faster under the SR-loaded condition (Table 1). At 7°C, the inactivation of I_{Ca} was a better fit with a single exponential function. Inactivation of I_{Ca} at 7°C was faster as the SR refilled (Table 1). This suggests that, even with the reduced amplitude of I_{Ca} at 7°C (see Shiels et al., 2000), I_{Ca} elicited by a SQ stimulation pulse is still of sufficient magnitude to cause Ca^{2+}-dependent inactivation of SL Ca^{2+} influx.

The Ca^{2+} current is more complex during AP clamp

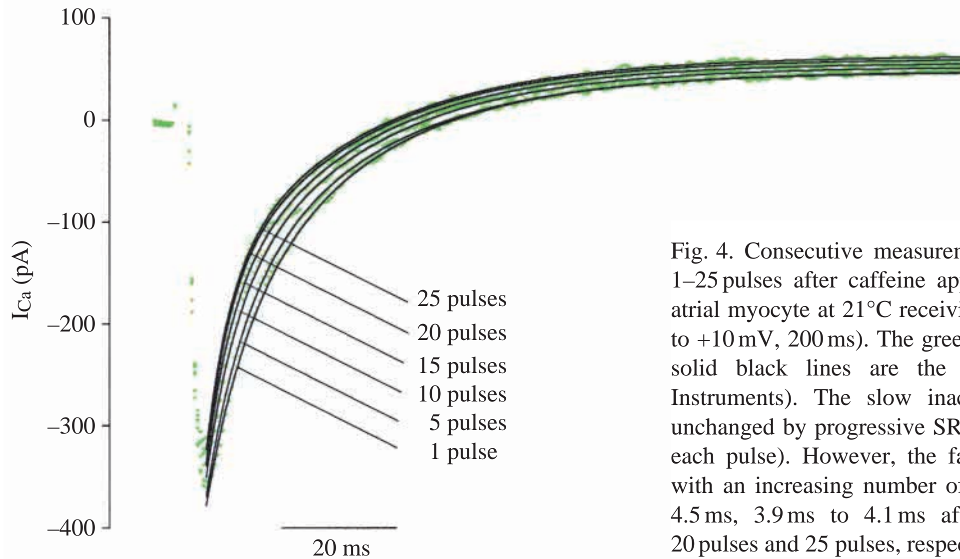

Fig. 4. Consecutive measurement of L-type Ca^{2+} channel current (I_{Ca}), 1–25 pulses after caffeine application in a representative rainbow trout atrial myocyte at 21°C receiving square (SQ) stimulation pulses (–80 mV to +10 mV, 200 ms). The green traces indicate the original curve, and the solid black lines are the double exponential fit (Clampfit, Axon Instruments). The slow inactivation kinetics (τ_s) for this cell were unchanged by progressive SR Ca^{2+} loading (τ_s = approximately 25 ms at each pulse). However, the fast inactivation kinetics (τ_f) became faster with an increasing number of pulses going from 7.1 ms, 6.7 ms, 5.2 ms, 4.5 ms, 3.9 ms to 4.1 ms after 1 pulse, 5 pulses, 10 pulses, 15 pulses, 20 pulses and 25 pulses, respectively.

(Fig. 5B). The effect of SR Ca^{2+} content on inactivation kinetics was examined by fitting a single exponential to the first part of the inactivation of I_{Ca} [first 200 ms after peak current at 14°C (see Fig. 5B) and first 100 ms after peak at 21°C (not shown)]. The AP waveform could not be consistently modelled at 7°C and so inactivation kinetics at 7°C are excluded from this part of the analysis. At both 14°C and 21°C, the inactivation of I_{Ca} elicited by AP pulses was faster with a steady-state Ca^{2+} SR content (Table 2).

Because the voltage waveform (either SQ or AP) was the same for each pulse and the amplitude of I_{Ca} was not significantly different, the change in inactivation kinetics observed in these experiments is unlikely to result from voltage-dependent inactivation or Ca^{2+}-dependent inactivation of Ca^{2+} entry through the channel. Thus, we conclude that there is SR Ca^{2+}-dependent inactivation of the SL I_{Ca} in rainbow trout atrial myocytes and that it is independent of temperature between 7°C and 21°C.

Table 1. *Effect of Ca^{2+} content on Ca^{2+}-dependent inactivation of I_{Ca} in rainbow trout atrial myocytes during stimulation with 200 ms SQ voltage pulses*

			τ_f		τ_s	
Temp.	*N*	Hz	SR empty	SR full	SR empty	SR full
21°C	5	1.4	11.47±2.4	8.33±1.8*	35.3±1.2	33.6±1.4
14°C	9	1.0	26.1±3.2	11.3±1.1*	161.7±26.1	96±5.9*

			τ	
Temp.	*N*	Hz	SR empty	SR full
7°C	8	0.6	154.3±17.8	119.2±0.60*

Inactivation of L-type Ca^{2+} channel (I_{Ca}) elicited by 200 ms square (SQ) pulses were fit with double exponential functions at 14°C and 21°C. τ_f is the time constant of fast inactivation and τ_s is the time constant of slow inactivation. At 7°C, inactivation was best fit with a single exponential (τ).

Values are means ± S.E.M. * indicates that the time constants with a steady-state sarcoplasmic reticulum (SR) Ca^{2+} content after 25 pulses are significantly faster than those after release of SR Ca^{2+} with caffeine (P<0.05, Student's paired *t*-test).

Discussion

The conventional SQ-voltage-clamp pulse helps characterize the kinetic behaviour of specific ion channels and separates the components of macroscopic current recordings but it does not emulate the change in membrane potential that occurs during an AP. This is a critical shortcoming in studies where the effects of temperature are being examined, as the shape of the cardiac AP (Coyne et al., 2000; Shiels et al., 2000) and the frequency of AP firing (Harwood et al., 2000) can both change as a function of temperature. The present study clearly shows that SR Ca^{2+} accumulation in rainbow trout atrial myocytes is neither temperature- nor frequency-dependent when cells are stimulated with AP pulses. This is a novel

Table 2. *Effect of Ca^{2+} content on Ca^{2+}-dependent inactivation of I_{Ca} in rainbow trout atrial myocytes during stimulation with APs*

			τ	
Temp.	*N*	Hz	SR empty	SR full
21°C	6	1.4	30.4±4.1	24.8±2.1*
14°C	9	1.0	54±5.1	42.0±3.2*

Inactivation of L-type Ca^{2+} channel (I_{Ca}) elicited by action potential (AP) pulses were fit with single exponential functions at 14°C and 21°C.

Values are means ± S.E.M. * indicates that the time constants with a steady-state sarcoplasmic reticulum (SR) Ca^{2+} content after 25 pulses are significantly faster than those after release of SR Ca^{2+} with caffeine (P<0.05, Student's paired *t*-test).

finding and is in contrast to the variability observed with 200 ms SQ stimulation pulses in the present study and with SQ pulses in other studies (Hove-Madsen et al., 1998, 1999). Our use of 200 ms SQ pulses and lower intracellular Na^+ concentrations resulted in a lower SR Ca^{2+} content than that reported in previous studies, which showed high and constant SR Ca^{2+} content at 7°C and 21°C in rainbow trout atrial myocytes [approximately 1568 μmol l^{-1} Ca^{2+} (Hove-Madsen et al., 2001) *versus* approximately 475 μmol l^{-1} Ca^{2+} (present study)]. However, when AP stimulation pulses were used in the present study, SR Ca^{2+} content more than doubled and there was no temperature dependence. These findings suggest that temperature- and frequency-dependent modulation of the AP may help to ensure a large and temperature-independent SR Ca^{2+} content in rainbow trout atrial myocytes *in vivo*.

The increase in AP duration at cold temperatures, and the increase in AP firing rate at warm temperatures, may underlie the temperature independence observed in the present study (see Fig. 1). Indeed, the duration of the AP plateau will profoundly affect SL Ca^{2+} influx and thus the Ca^{2+} available for uptake into the SR. Additionally, changes in stimulation frequency affect diastolic Ca^{2+} load in trout atrial myocytes (see Shiels et al., 2002b), which could also influence the activity of the SR Ca^{2+} ATPase. Thus, we suggest that temperature-dependent modulation of the AP shape and firing rate may help to offset the known temperature sensitivity of the SR Ca^{2+} ATPase (Q_{10} of approximately 1.6; Aho and Vornanen, 1998; Hove-Madsen et al., 1998).

Our results show greater SR Ca^{2+} accumulation with AP stimulation than with SQ pulses in trout atrial myocytes. This may be explained by the fact that I_{Ca} generated by an AP has a sustained phase during the AP plateau. The sustained phase is a result of an increased driving force for Ca^{2+} as a result of repolarization (to approximately 0 mV) after the peak and the reactivation of L-type Ca^{2+} channels during the AP plateau (window current) (Arreola et al., 1991). Indeed, the relatively depolarized resting membrane potential in the AP experiments (approximately –50 mV) may have increased Ca^{2+} influx *via* the window current under AP conditions. A –50 mV resting membrane potential is typical for isolated rainbow trout atrial myocytes (Shiels et al., 2000) and results from the loss of cholinergic tonus and also reflects the lower density of inward rectifier current (I_{K1}) in fish atrial cells compared with ventricular cells (Vornanen et al., 2002a). However, recent measurements *in vivo* suggest that the resting membrane potential in rainbow trout atrial cells is approximately –65 mV (Vornanen et al., 2002a). Therefore, future studies could consider adding tonic levels of acetylcholine to solutions when recording APs from isolated myocytes.

In previous studies (Shiels et al., 2000), we have measured the I_{Ca} window current in trout atrial cells at 7°C, 14°C and 21°C and found a peak Ca^{2+} contribution at –10 mV at all temperatures. However, we also found that the relative Ca^{2+} contribution from the window current was greater (0.08 relative units) and had a larger voltage window (–40 mV to +30 mV) at 7°C compared with at 14°C and 21°C (0.055 relative units, –30 mV to

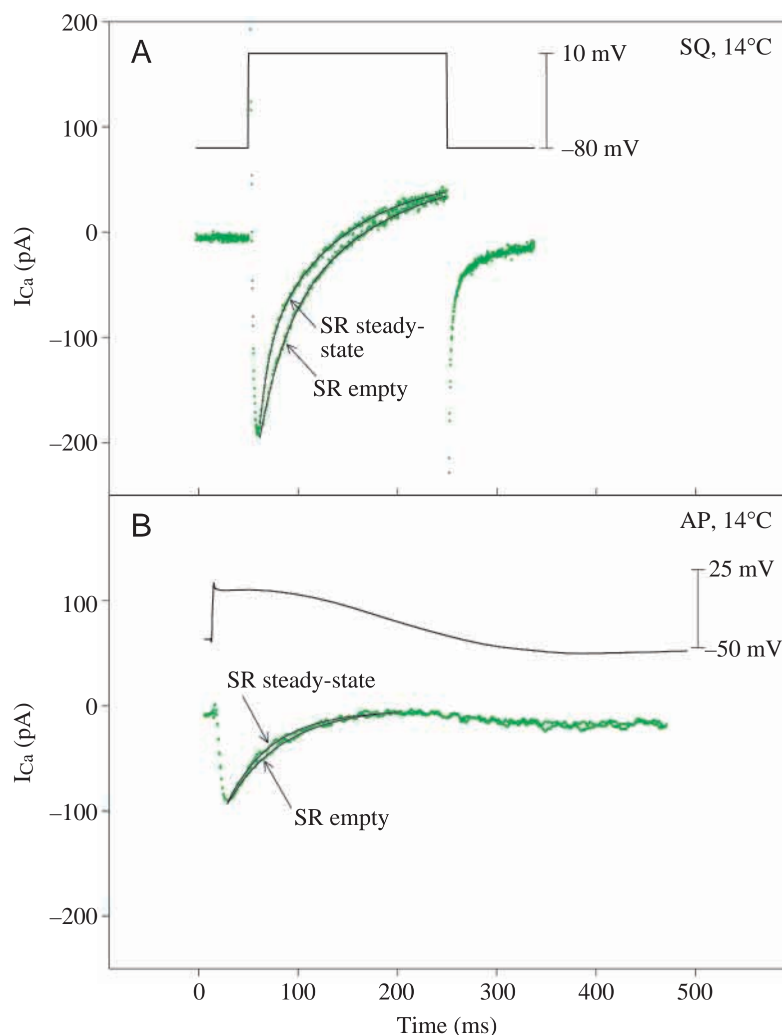

Fig. 5. Sarcoplasmic reticulum (SR) Ca^{2+}-dependent inactivation of L-type Ca^{2+} channel current (I_{Ca}) in representative rainbow trout atrial myocytes with square (SQ) and action potential (AP) pulses at 14°C. The green traces indicate the original curve, and the solid black lines are the double exponential fits (Clampfit 6.0, Axon Instruments). (A) I_{Ca} elicited by a SQ-voltage clamp pulse immediately after caffeine application [SR empty; fast inactivation kinetics (τ_f)=24.7 ms, and slow inactivation kinetics (τ_s)=113 ms] and after the SR Ca^{2+} content had reached a steady-state (SR steady-state; τ_f=10.7 ms and τ_s=80 ms). (B) The effect of steady-state SR Ca^{2+} content on I_{Ca} elicited by an AP pulse (green traces). The kinetics of inactivation were best fit with a single exponential (solid black line) over the first 200 ms after peak current. For this cell, τ decreased from 57 ms after caffeine application to 50 ms with a steady-state SR Ca^{2+} content. Means and statistics for SR-Ca^{2+}-dependent inactivation of I_{Ca} at 7°C, 14°C and 21°C are given in Tables 1, 2.

+20 mV). A larger window current at 7°C may explain, in part, the large SR Ca^{2+} content observed at 7°C with the SQ pulses at 0.6 Hz (Fig. 3B). Additionally, because kinetics are slower at 7°C (see Shiels et al., 2000), prolongation of the diastolic period at 0.6 Hz could allow for more complete recovery of I_{Ca}, thereby increasing Ca^{2+} influx and SR loading.

We report a high steady-state SR Ca^{2+} content during AP stimulation (approximately 1250 μmol l^{-1}). However, when trout atrial myocytes are depolarized to +50 mV for 32 s, the SR can accumulate even larger quantities of Ca^{2+} (approximately 2340 μmol l^{-1}; Hove-Madsen et al., 1998, 1999). In the adult mammalian heart, SR Ca^{2+} release predominates over SL Ca^{2+} delivery during e–c coupling and yet the steady-state SR Ca^{2+} content in mammalian myocytes (100–150 μmol l^{-1}; Bassani et al., 1995; Negretti et al., 1995; Díaz et al., 1997) is an order of magnitude less than in fish. An intriguing question for future study emerges: why does the SR of rainbow trout have such a large maximal and steady-state capacity to store Ca^{2+} when isometric muscle studies indicate that Ca^{2+} release from the SR contributes a rather small amount of the Ca^{2+} involved in contraction?

The Ca^{2+}-storing capacity of the SR is determined by the volume fraction of the SR in the myocyte, the concentration of low-affinity Ca^{2+} buffers inside the SR and the concentration gradient of free Ca^{2+} across the SR membrane (Shannon and Bers, 1997). It is unclear which of these factors contributes to the enhanced Ca^{2+}-storing capacity of the trout SR. It is possible that functional differences in the rainbow trout SR Ca^{2+} release channels, possibly at the luminal side of the channel, or differences in SR Ca^{2+} buffers may account for the difference in maximal SR Ca^{2+} content. However, to date there is no information available to accept or reject this idea. In mammals, it is difficult to increase steady-state SR Ca^{2+} above approximately 100 μmol l^{-1} without spontaneous release (Bassani et al., 1995), probably because of a direct effect of luminal Ca^{2+} on the SR Ca^{2+} release channel (Lipp et al., 1992; Sitsapesan and Williams, 1994; Bassani et al., 1995). One possibility is that the SR Ca^{2+} release channels in fish heart are approximately 10-fold less sensitive to luminal Ca^{2+} than those in mammals. Indeed, experiments with carp (*Cyprinus carpio*) suggest that their SR Ca^{2+} release channels may be an order of magnitude less sensitive to Ca^{2+} than mammals (Chugun et al., 1999). Furthermore, indirect studies of SR function do suggest striking differences between fish and mammalian SR Ca^{2+} release channels with respect to cold sensitivity. The results of the present study confirm earlier work (Bowler and Tirri, 1990; Hove-Madsen et al., 1998; Aho and Vornanen, 1999; Tiitu and Vornanen, 2002) indicating that the teleost SR Ca^{2+} release channels remain functional at cold temperatures whereas the mammalian SR Ca^{2+} release channels open such that Ca^{2+} leaks out of the SR (Bers, 1987; Sitsapesan et al., 1991).

One speculation as to why rainbow trout store large amounts of Ca^{2+} in their SR may be related to their burst mode of swimming. Burst swimming has been shown to decrease blood pH by as much as 0.5 pH units, and an extracellular pH change of such magnitude will significantly inhibit cardiac performance (Milligan and Farrell, 1986; Churcott et al., 1994). Although there is no information at present, a large SR Ca^{2+} store, if releasable, could serve as a safety factor during acidosis to override decreased myofilament Ca^{2+} sensitivity. Indeed, we know that adrenergic stimulation can protect force development in the acidotic rainbow trout myocardium (Farrell et al., 1985; Farrell and Milligan, 1986) although the mechanisms have not yet been defined. However, under normoxic conditions, a 30–60% release of the steady-state SR Ca^{2+} content in trout (as reported for mammals; Bassani et al., 1995) could cause severe contracture and could damage the contractile machinery. It may be that, regardless of SR Ca^{2+} content, there is an upper limit to the amount of Ca^{2+} that is releasable *via* Ca^{2+}-induced Ca^{2+} release, as the absolute amount of Ca^{2+} released in fish and mammalian cardiomyocytes at 21°C is comparable: 35–60 μmol l^{-1} Ca^{2+} (Bassani et al., 1995; Janczewski et al., 1995; Hove-Madsen et al., 1998).

In summary, the present study has demonstrated that the rainbow trout atrial SR Ca^{2+} content reaches a steady-state after approximately 25 stimulation pulses over a physiological range of temperatures and heart rates during AP stimulation. Furthermore, SR Ca^{2+} accumulation and release are not compromised by temperature change, indicating significant differences between rainbow trout and mammalian SR. Finally, because the temperature changes used in the present study represent a realistic physiological challenge for the rainbow trout heart, our results suggest that the plasticity of the rainbow trout contractile machinery (see Vornanen et al., 2002b for a recent review) may help to maintain SR Ca^{2+} content during temperature change *in vivo*.

We acknowledge Kontiolahti fish farm for donating the rainbow trout and Virpi Tiitu for her help in transporting them to the University of Joensuu. We thank Dr Leif Hove-Madsen for his critical reading of earlier versions of this paper. The study was supported by NSERC to A.P.F., Simon Fraser University Steele Memorial Fellowship to H.A.S., and the Academy of Finland to M.V.

References

Aho, E. and Vornanen, M. (1998). Ca^{2+}-ATPase activity and Ca^{2+}-uptake by sarcoplasmic reticulum in fish heart: effects of thermal acclimation. *J. Exp. Biol.* **201**, 525-532.

Aho, E. and Vornanen, M. (1999). Contractile properties of atrial and ventricular myocardium of the heart of rainbow trout (*Oncorhynchus mykiss*): Effects of thermal acclimation. *J. Exp. Biol.* **202**, 2663-2677.

Aho, E. and Vornanen, M. (2001). Cold acclimation increases basal heart rate but decreases its thermal tolerance in rainbow trout (Oncorhynchus mykiss). *J. Comp. Physiol. B* **171**, 173-179.

Arreola, J., Dirksen, R. T., Shieh, R.-C., Williford, D. J. and Sheu, S.-S. (1991). Ca^{2+} current and Ca^{2+} transients under action potential clamp in guinea pig ventricular myocytes. *Am. J. Physiol.* **261**, C393-C397.

Bassani, J. W., Yuan, W. and Bers, D. M. (1995). Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. *Am. J. Physiol.* **268**, C1313-C1319.

Bers, D. M. (1987). Ryanodine and the calcium content of cardiac SR assessed by caffeine and rapid cooling contractures. *Am. J. Physiol.* **253**, C408-C415.

Bers, D. M. (2001). *Excitation-Contraction Coupling and Cardiac Contractile Force*. Amsterdam: Kluwer Scientific Press.

Bowler, K. and Tirri, R. (1990). Temperature dependence of the heart isolated from the cold or warm acclimated perch (*Perca fluviatilis*). *Comp. Biochem. Phsyiol. A* **96**, 177-180.

Chugun, A., Oyamada, T., Temma, K., Hara, Y. and Kondo, H. (1999). Intracellular Ca^{2+} storage sites in the carp heart: comparison with the rat heart. *Comp. Biochem. Physiol. A* **123**, 61-67.

Churcott, C. S., Moyes, C. D., Bressler, B. H., Baldwin, K. M. and Tibbits, G. F. (1994). Temperature and pH effects on Ca^{2+} sensitivity of cardiac myofibrils: a comparison of trout with mammals. *Am. J. Physiol.* **267**, R62-R70.

Coyne, M. D., Kim, C. S., Cameron, J. S. and Gwathmey, J. K. (2000). Effects of temperature and calcium availability on ventricular myocardium from rainbow trout. *Am. J. Physiol.* **278**, R1535-R1544.

Díaz, M. E., Trafford, A. W., O'Neill, S. C. and Eisner, D. A. (1997). Measurement of sarcoplasmic reticulum Ca^{2+} content and sarcolemmal Ca^{2+} fluxes in isolated rat ventricular myocytes during spontaneous Ca2+ release. *J. Physiol.* **501**, 3-16.

Driedzic, W. R. and Gesser, H. (1994). Energy-metabolism and contractility in ectothermic vertebrate hearts: hypoxia; acidosis; and low-temperature. *Physiol. Rev.* **74**, 221-258.

Farrell, A. P., Gamperl, A. K., Hicks, J. M. T., Shiels, H. A. and Jain, K. E. (1996). Maximum cardiac performance of rainbow trout (*Oncorhynchus mykiss*) at temperatures approaching their upper lethal limit. *J. Exp. Biol.* **199**, 663-672.

Farrell, A. P. and Milligan, C. L. (1986). Myocardial intracellular pH in a perfused rainbow trout heart during extracellular acidosis in the presence and absence of adrenaline. *J. Exp. Biol.* **125**, 347-359.

Farrell, A. P., Wood, S., Hart, T. and Driedzic, W. R. (1985). Myocardial oxygen-consumption in the sea raven, *Hemitripterus americanus*: the effects of volume loading, pressure loading and progressive hypoxia. *J. Exp. Biol.* **117**, 237-250.

Harwood, C. L., Howarth, F. C., Altringham, J. D. and White, E. (2000). Rate-dependent changes in cell shortening; intracellular Ca^{2+} levels and membrane potential in single isolated rainbow trout (*Oncorhynchus mykiss*) ventricular myocytes. *J. Exp. Biol.* **203**, 493-504.

Hove-Madsen, L. (1992). The influence of temperature on ryanodine sensitivity and the force-frequency relationship in the myocardium of rainbow trout. *J. Exp. Biol.* **167**, 47-60.

Hove-Madsen, L., Llach, A. and Tort, L. (1998). Quantification of Ca^{2+} uptake in the sarcoplasmic reticulum of trout ventricular myocytes. *Am. J. Physiol.* **44**, R2070-R2080.

Hove-Madsen, L., Llach, A. and Tort, L. (1999). Quantification of calcium release from the sarcoplasmic reticulum in rainbow trout atrial myocytes. *Pflügers Arch.* **438**, 545-552.

Hove-Madsen, L., Llach, A. and Tort, L. (2000). Na/Ca-exchange activity regulates contraction and SR Ca(2+) content in rainbow trout atrial myocytes. *Am. J. Physiol.* **279**, 1856-1864.

Hove-Madsen, L., Llach, A. and Tort, L. (2001). The function of the sarcoplasmic reticulum is not inhibited by low temperatures in trout atrial myocytes. *Am. J. Physiol.* **281**, R1902-R1906.

Janczewski, A. M., Spurgeon, H. A., Stern, M. D. and Lakatta, E. G. (1995). Effects of sarcoplasmic reticulum Ca load on the gain function of Ca release by Ca current in cardiac cells. *Am. J. Physiol.* **268**, H916-H920.

Keen, J. E., Vianzon, D. M., Farrell, A. P. and Tibbits, G. F. (1994). Effect of temperature and temperature-acclimation on the ryanodine sensitivity of the trout myocardium. *J. Comp. Physiol. B* **164**, 438-443.

Lipp, P., Pott, L., Callewaert, G. and Carmeliet, E. (1992). Calcium transients caused by calcium entry are influenced by the sarcoplasmic reticulum in guinea-pig atrial myocytes. *J. Physiol.* **454**, 321-338.

Matthews, K. R. and Berg, N. H. (1997). Rainbow trout responses to water temperature and dissolved oxygen stress in two southern California streampools. *J. Fish Biol.* **50**, 50-67.

Milligan, C. L. and Farrell, A. P. (1986). Extra- and intracellular acid-base status following enforced activity in the sea raven (*Hemitripterus americanus*). *J. Comp. Physiol.* **156**, 583-590.

Negretti, N., Varro, A. and Eisner, D. A. (1995). Estimate of net calcium fluxes and sarcoplasmic reticulum calcium content during systole in rat ventricular myocytes. *J. Physiol.* **486**, 581-591.

Randall, D. and Brauner, C. (1991). Effects of environmental factors on exercise in fish. *J. Exp. Biol.* **160**, 113-126.

Reid, S. D., McDonald, D. G. and Wood, C. M. (1997). Interactive effects of temperature and pollutant stress. In *Global Warming: Implications for Freshwater and Marine Fish* (ed. C. M. Wood and D. G. McDonald), pp. 235-249. Cambridge, UK: Cambridge University Press.

Rousseau, E., Smith, J. S. and Meissner, G. (1987). Ryanodine modifies conductance and gating behavior of single Ca^{2+} release channel. *Am. J. Physiol* **253**, C364-C368.

Sham, J. S. (1997). Ca^{2+} release-induced inactivation of Ca^{2+} current in rat ventricular myocytes: evidence for local Ca^{2+} signaling. *J. Physiol.* **500**, 285-295.

Shannon, T. R. and Bers, D. M. (1997). Assessment of intra-SR free [Ca^{2+}] and buffering in rat heart. *Biophys. J.* **73**, 1524-1531.

Shiels, H. A. and Farrell, A. P. (1997). The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing Ca^{2+} to force development in isolated ventricular trabeculae from rainbow trout. *J. Exp. Biol.* **200**, 1607-1621.

Shiels, H. A., Vornanen, M. and Farrell, A. P. (2000). Temperature-dependence of L-type Ca^{2+} channel current in atrial myocytes from rainbow trout. *J. Exp. Biol.* **203**, 2771-2780.

Shiels, H. A., Vornanen, M. and Farrell, A. P. (2002a). The force-frequency response in fish hearts – a review. *Comp. Biochem. Physiol. A* **132**, 811-826.

Shiels, H. A., Vornanen, M. and Farrell, A. P. (2002b). The effects of temperature on $[Ca^{2+}]_i$ in rainbow trout atrial myocytes. *J. Exp. Biol.* **205**, 3641-3650.

Sitsapesan, R., Montgomery, R. A. P., Macleod, K. T. and Williams, A. J. (1991). Sheep cardiac sarcoplasmic-reticulum calcium-release channels: modification of conductance and gating by temperature. *J. Physiol.* **434**, 469-488.

Sitsapesan, R. and Williams, A. J. (1994). Regulation of the gating of the sheep cardiac sarcoplasmic reticulum Ca^{2+}-release channel by luminal Ca^{2+}. *J. Membr. Biol.* **137**, 215-226.

Tiitu, V. and Vornanen, M. (2002). Regulation of cardiac contractility in a stenothermal fish, the burbot (*Lota lota*). *J. Exp. Biol.* **205**, 1597-1606.

Tuurala, H., Soivio, A., and Nikinmaa, M. (1982). The effects of adrenaline on heart rate and blood pressure in *Salmo Gairdneri* at two temperatures. *Ann. Zool. Fennici* **19**, 47-51.

Varro, A., Negretti, N., Hester, S. B. and Eisner, D. A. (1993). An estimate of the calcium content of the sarcoplasmic reticulum in rat ventricular myocytes. *Pflügers Arch.* **423**, 158-160.

Vornanen, M. (1997). Sarcolemmal Ca influx through L-type Ca channels in ventricular myocytes of a teleost fish. *Am. J. Physiol.* **41**, R1432-R1440.

Vornanen, M. (1998). L-type Ca^{2+} current in fish cardiac myocytes: Effects of thermal acclimation and beta-adrenergic stimulation. *J. Exp. Biol.* **201**, 533-547.

Vornanen, M., Ryökkynen, A. and Nurmi, A. (2002a). Temperature-dependent expression of sarcolemmal K^+ currents in rainbow trout atrial and ventricular myocytes. *Am. J. Physiol.* **282**, R1101-R1199.

Vornanen, M., Shiels, H. A. and Farrell, A. P. (2002b). Plasticity of excitation–contraction coupling in fish cardiac myocytes. *Comp. Biochem. Physiol. A* **132**, 827-846.

Wang, S., Zhou, Z. and Qian, H. (2000). Recording of calcium transient and analysis of calcium removal mechanisms in cardiac myocytes from rats and ground squirrels. *Science In China (series C)* **43**, 191-199.

Wang, S. Q., Huang, Y. H., Liu, K. S. and Zhou, Z. Q. (1997). Dependence of myocardial hypothermia tolerance on sources of activator calcium. C*ryobiology* **35**, 193-200.