The Journal of Experimental Biology 202, 2663–2677 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB2041

CONTRACTILE PROPERTIES OF ATRIAL AND VENTRICULAR MYOCARDIUM OF THE HEART OF RAINBOW TROUT *ONCORHYNCHUS MYKISS*: EFFECTS OF THERMAL ACCLIMATION

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Accepted 8 July; published on WWW 13 September 1999

Summary

Atrial and ventricular myocardium perform different tasks in the pumping work of the vertebrate heart, which are reflected in their contractile properties. Although atrial contraction is assumed to have an important role in the function of fish heart, the contractile properties of atrial and ventricular myocardium have not been directly compared in any fish species. The objective of this study was to clarify any contractile differences in the heart of teleost fish and, in particular, to elucidate the contribution of myofibrillar ATPase and intracellular Ca^{2+} stores to the characteristics of atrial and ventricular contraction. Experiments were conducted on thermally acclimated rainbow trout *Oncorhynchus mykiss* to determine whether the effects of temperature adaptation are the same in atrial and ventricular tissue. It was shown that the rate of isometric contraction is much faster in atrial than in ventricular tissue of the fish heart and that acclimation to cold increases the rate of contraction in both cardiac compartments. The rapid contraction kinetics of the atrial tissue were associated with higher myofibrillar ATPase activity and faster Ca^{2+} uptake rate of the sarcoplasmic reticulum (SR) compared with ventricular tissue. Similarly, the faster kinetics of contraction following cold acclimation could be attributed to enhancement of the myofibrillar and/or SR function. The atrio-ventricular and temperature-induced differences were also expressed in the recovery of force from inactivation, i.e. in the mechanical restitution. The refractory period and the rate constant of force restitution were shorter in atrial than in ventricular muscle tissue. Similar differences also existed between the tissues of cold-acclimated (CA, 4 °C) and warm-acclimated (WA, 17 °C) fish. The fast recovery of force from inactivation in the heart of the CA trout was, at least in part, due to more active SR. Furthermore, it was shown that the force of atrial contraction in the CA trout is sensitive to ryanodine (10 μmol l^{-1}), a Ca^{2+}-release channel blocker of SR, at physiological body temperature (4 °C) and at a physiological pacing rate (0.6 Hz). This finding indicates that the Ca^{2+} stores of SR contribute to activation of cardiac contraction in the fish heart, and that the SR of fish heart is able to retain its Ca^{2+} load at low body temperatures, i.e. the Ca^{2+} release channels of SR are not leaky in the cold. The present data show that in the atrial tissue of CA trout, the SR directly contributes to the cytosolic Ca^{2+} and that in the atrium and ventricle of CA trout, the SR significantly accelerates the recovery of contractility from inactivation. The fast recovery from inactivation allows relatively high heart rates and therefore adequate cardiac outputs at low environmental temperatures for the cold-active rainbow trout.

Key words: fish, heart, ventricle, atrium, sarcoplasmic reticulum, ryanodine, force restitution, rest-potentiation, excitation–contraction coupling, rainbow trout, *Oncorhynchus mykiss*

Introduction

In mammals, atrial systole occurs immediately before ventricular systole and thus allows atrial contraction to augment the volume of blood in the ventricles at end-diastole when ventricular volume determines the strength of the ventricular contraction. The important timing of atrial systole is made possible by the delay of impulse conduction in the atrioventricular node (Katz, 1992) and by rapid contraction kinetics of the atrial muscle. Although atrial contraction exerts significant regulatory function in ventricular contractility through the Frank–Starling mechanism, its role in ventricular filling is relatively small; most of the blood flow into the ventricles of mammalian heart occurs directly from the veins, and atrial contraction provides only approximately 30 % of the end-diastolic blood volume (Guyton, 1981). The relative significance of atrial contraction for ventricular filling and the performance of the cardiac pump is generally assumed to be much larger in the fish heart than in the mammalian heart. In fact, atrial systole is considered to be the sole determinant of ventricular filling in fish heart, whereby the preceding atrial contraction would be a necessary prerequisite for the

subsequent ventricular contraction (see Johansen and Burggren, 1980; Farrell and Jones, 1992). According to this concept, atrial contractility would determine the extent of ventricle filling, the strength of ventricular contraction and hence the cardiac stroke volume in fish hearts. More recent findings suggest, however, that the contribution of the atrial systole to ventricular filling is relatively small in fish heart and that the ventricular filling mechanism is basically similar in all vertebrates (Lai et al., 1998).

In mammals, the atrial contraction is much faster than the ventricular contraction (Urthaler et al., 1975; Asgrimsson et al., 1995; Bottinelli et al., 1995). The cellular and molecular basis for this difference is complicated, involving action potential duration, kinetics of intracellular Ca^{2+} transients and properties of myofibrillar proteins (for references, see Minajeva et al., 1997). Although the importance of atrial contraction for the ventricular function is assumed to be larger in fish hearts than in other vertebrates, the contractile properties of the fish atrium are poorly known. The objective of the present study was to compare the basic characteristics of atrial and ventricular contraction in the heart of teleost fish. First, we wanted to determine whether the difference in contraction kinetics between the atrium and ventricle that is typical of the mammalian heart is similar in fish heart. Second, we tried to clarify the importance of myofibrils and sarcoplasmic reticulum (SR) in possible differences between the kinetics of atrial and ventricular contraction. Third, the effects of thermal acclimation on the contractile properties of atrial and ventricular muscle were compared. Experiments were conducted on a fish species (rainbow trout, *Oncorhynchus mykiss*) that remains active at low temperatures and is likely to show compensatory changes in cardiac function after acclimation to cold (Driedzic et al., 1996; Aho and Vornanen, 1998).

Materials and methods

Fish

Rainbow trout *Oncorhynchus mykiss* (Walbaum) (N=61) were obtained from a local fish farm, randomly divided into two groups and acclimated either to 4 °C (CA) or to 17 °C (WA) for more than 4 weeks. The fish were of three size classes: the smallest animals (WA fish, 53.4±3.9 g, N=16; CA fish, 39.3±2.0 g, N=17; values are means ± S.E.M.) were used in contractile measurements, the mid-sized fish (WA fish, 247.2±19.0 g, N=8; CA fish 216.9±33.6 g, N=8) for Ca^{2+} uptake measurements and the biggest fish (WA fish, 566.4±62.2 g, N=6; CA fish, 599.7±65.7 g, N=6) in myofibrillar Ca^{2+}/Mg^{2+}-ATPase determinations. These size groups were selected to fulfil special experimental requirements and were necessary because of the large difference in size between atrial and ventricular myocardium. Small fish were used in contractile measurements to achieve dimensionally appropriate preparations for force recording, whereas bigger fish were necessary for Ca^{2+} uptake and myofibrillar ATPase measurements in order to obtain a sufficient amount of muscle tissue without pooling from a large number of animals. During acclimation, the fish were held in 500 l stainless steel tanks with a continuous circulation of aerated tap water. The fish were fed five times a week with a commercial fish food (Ewos, Turku, Finland). The photoperiod was a constant 12 h:12 h L:D cycle. Fish were stunned by a sharp blow to the head and killed by cutting the spine.

Characterization of isometric contractions

Isometric contractile properties of excised atrial and ventricular tissues were determined at the acclimation temperatures of the animals as well as at the intermediate temperature of 11 °C. Whole atria (6.88±0.6 mg, N=23) or ventricular strips (32.5±1.03 mg, N=23, approximately 1 mm thick) were mounted on the bottom of a chamber by means of a stimulating platinum electrode while the other end of the muscle was connected to the lever arm of a force transducer (Grass FT03) by a short braided-silk suture (size USP 6–0; Davis and Geck, Great Britain). The muscles were stretched stepwise to the length where the developed force was maximal when stimulated and were then allowed to stabilize at this length for about 1 h before the experiments were started. The voltage of the 8 ms long square-wave pulse was twice the threshold value (1–5 V). The solution in the chamber was oxygenated (100 % O_2) continuously and consisted of (in mmol l^{-1}): NaCl, 140; KCl, 2.8; $CaCl_2$, 1.8; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; Hepes, 10; glucose, 10; pH 7.7, at 22 °C. The temperature of the saline was maintained at 4, 11 or 17 °C with the aid of a recirculating water bath. Preparations were stimulated to contract at physiological heart rates (Graham and Farrell, 1990; Farrell et al., 1988, 1996) of 0.6 Hz (36 beats min^{-1}), 0.8 (48 beats min^{-1}) and 1.2 Hz (72 beats min^{-1}) at 4, 11 and 17 °C, respectively. The time course of isometric twitches was monitored and recorded with a computer-based system. Analog signals from the chart recorder (Grass 7D) were digitised by an AD-converter (Digidata 1200, Axon instruments) and the recordings were stored on the hard disk of the computer by data acquisition software (PClamp 6.03 or AxoScope 1.1, Axon Instruments). The maximal developed force F_{max}, time-to-peak force T_{PF} (the time from the stimulus pulse to the peak force), time to half-relaxation $T_{0.5R}$ (the time from F_{max} to 0.5 F_{max}) and contraction duration T_{DC} (T_{PF}+$T_{0.5R}$) were determined off-line using the Clampfit-program of the PClamp 6.0.3 software package. The first derivative of the developed force (dF/dt_{max}) was sometimes calculated and is shown in some of the figures. We also studied the effects of verapamil, a sarcolemmal Ca^{2+} channel blocker, and ryanodine, an SR Ca^{2+}-release channel blocker, on isometric contractile characteristics. In each case the agent was added to the bathing solution to give a final concentration of 10 μmol l^{-1} after a 1 h equilibration period. During this initial period, there was a slight decline in F_{max} (see Fig. 2A). In order to allow a 'control' F_{max} to be predicted after addition of the drug, a double exponential equation, $y=ae^{-bt}+ce^{-dt}$ was fitted to the force during the equilibration period, where t is

time, *a* and *c* are the magnitudes of the two exponential components, and b and d the reciprocals of their time constants (Vornanen, 1996). By extrapolation, this function was used to predict the force at selected points during later phases of the experiment. The drug effect was obtained as the difference between the measured and the predicted value (Fig. 2A). By this means each muscle could be used as its own control. The effect of ryanodine was recorded 1 h after its addition. Due to its use-dependent properties, the effect of verapamil was recorded after a constant number of contractions, i.e. after 30 and 60 min of its application in WA and CA fish, respectively.

Rate dependence of isometric contractile force

Mechanical restitution and rest-potentiation were determined to characterize the force–frequency relationship of trout atrial and ventricular muscle. Muscle preparations were stimulated at physiological heart rates at the acclimation temperatures of the animals or at the mean experimental temperature of 11 °C (0.6 Hz at 4 °C, 0.8 Hz at 11 °C and 1.2 Hz at 17 °C). To record mechanical restitution, the regular pacing was interrupted by a single test pulse of variable interval, beginning from an interval just shorter than the regular pacing interval and proceeding to increasingly shorter intervals, until the preparation failed to elicit any contractile response. The force of the test contraction (F_T) was normalized to the force of the preceding control contraction (F_C) and was plotted as a function of the test interval (Δt) to produce a restitution curve. The force of the post-test contraction (F_{PT}), elicited at a constant delay after the extrasystolic test contraction, was also recorded. Two variables were used to characterize the mechanical restitution. First, the mechanical refractory period (MRP) is the shortest stimulus interval in which the muscle was able to produce a measurable contraction after the control contraction was measured. Second, the time course of mechanical restitution was determined by the time constant (τ) of the single-exponential equation, $y=a(1-e^{-b/t})+c$, where t is time, a is the magnitude of the exponential component, b is the reciprocal of the time constant and c is the part of force recovery that is not explained by the exponential component. This equation was used to fit the mechanical restitution curve. The time constant gives the time needed for F_{max} to recover to 63 % of its maximum value. To keep the cellular Ca^{2+} load constant, each restitution pulse sequence was separated by six conditioning pulses delivered at the control cycle length.

Rest-potentiation describes the force production at diastolic intervals longer than that of steady-state beating. Steady-state stimulation (as given above) was interrupted for 10 different rest periods ranging from 5 s to 300 s, after which regular pacing was resumed. In atrial preparations, spontaneous beats often limited the pause durations to less than 120 s. The force (F_{max}) of the first post-rest contraction was normalized to the force of the preceding steady-state beat and is a measure of rest-potentiation. The maximum potentiation of each preparation, achieved at slightly variable diastolic intervals, was recorded. When the effects of ryanodine (10 μmol l^{-1}) on restitution or rest-potentiation were studied, the drug was allowed to incubate for 60 min before the response was measured.

Rate of Ca^{2+} uptake into the sarcoplasmic reticulum

The ATP-dependent Ca^{2+} uptake of SR was measured fluorometrically using a Ca^{2+}-selective fluorescent dye, Fura-2 (Hove-Madsen and Bers, 1993a; Kargacin and Kargacin, 1994), as described in detail elsewhere (Aho and Vornanen, 1998). Crude cardiac homogenates were prepared in an ice-cold medium containing (in mmol l^{-1}): Hepes, 20; KCl, 100; $MgCl_2$, 4; pH 7.0 at 22 °C, and were used within 60 min of their preparation. Ca^{2+} uptake was measured at room temperature (22±1 °C) in a buffer containing (in mmol l^{-1}): Hepes, 20; KCl, 100; $MgCl_2$, 4; oxalate, 10; Na_2ATP, 1.25; creatine phosphate, 1.25; pH 7.0 at 22 °C. pH 7.0 was shown to be optimal for the Ca^{2+}-ATPase of fish muscle SR (Vornanen et al., 1999). Creatine phosphokinase (0.4 i.u. ml^{-1}) was included in the medium to regenerate ATP. Fura-2 was added to a final concentration of 2 μmol l^{-1} and Ca^{2+} uptake was initiated with 5 μmol l^{-1} $CaCl_2$. This gave an initial $[Ca^{2+}_{free}]$ of 1 μmol l^{-1}, which is close to the optimal Ca^{2+} concentration for SR Ca^{2+} uptake rate (Kargacin and Kargacin, 1994). Ca^{2+} removal from the solution was followed for 5 min, and the maximal rate of Ca^{2+} uptake by the SR was obtained by differentiation of this recording. Ca^{2+} uptake rate was also measured at the K_m (0.4 μmol l^{-1} Ca^{2+}) of the pump, which gave qualitatively similar results as at optimal $[Ca^{2+}_{free}]$. The contribution of mitochondrial Ca^{2+} uptake to Fura-2 fluorescence is excluded, since the concentration of free Ca^{2+} in the uptake medium was less than the threshold concentration (3 μmol l^{-1}) for mitochondrial Ca^{2+} uptake (Hove-Madsen and Bers, 1993b). The mitochondrial blocker Ruthenium Red has an inhibitory effect on SR Ca^{2+} uptake (Aho and Vornanen, 1998; Kargacin and Kargacin, 1998) and was therefore not included. As is normally assumed for an SR-dependent process, fluorescence changes were completely prevented by pretreatment of cardiac homogenates with 2 μmol l^{-1} thapsigargin (Aho and Vornanen, 1998).

Ca^{2+}/Mg^{2}-ATPase activity of myofibrils

The activity of the myofibrillar Ca^{2+}/Mg^{2+}-ATPase of both atrial and ventricular muscle tissues was measured from purified cardiac preparations (Vornanen, 1996). Briefly, the samples were homogenised three times for 5 s in 10 volumes of ice-cold buffer 1 containing (in mmol l^{-1}): KCl, 100; Tris-HCl, 10; dithiothreitol, 1; pH 7.4 at room temperature, and centrifuged at 10 000 ***g*** for 10 min. Pellets were resuspended in homogenising buffer 2 (buffer 1+1 % Triton X-100) and centrifuged again at 10 000 ***g*** for 10 min. The recovered pellets were washed three times in buffer 1 and between washings were centrifuged at 600 ***g*** for 15 min. After the last centrifugation, the pellets were resuspended in 20 volumes of low-ionic-strength buffer containing (in mmol l^{-1}): imidazole, 45; KCl, 50; dithiothreitol, 1; pH 7.0 at 22 °C. Ca^{2+}/Mg^{2+}-ATPase activity of purified atrial and ventricular

preparations was measured at three different temperatures (5, 10 and 15 °C). The pH was allowed to freely change according to temperature and was 7.30, 7.25 and 7.11 at 5, 10 and 15 °C, respectively. These pH changes have minimal effects on myofibrillar ATPase activity of the trout heart (Churcott et al., 1994). Total ATPase activity was measured from the liberated inorganic phosphate (Atkinson et al., 1973) in a solution containing (in mmol l^{-1}): imidazole, 45 (pH 7.0 at 22 °C); KCl, 50; EGTA, 5; $MgCl_2$, 5; Na_2ATP, 3; $CaCl_2$, 5 (pCa≈4). The background Mg^{2+}-ATPase activity was determined in the same solution, but without $CaCl_2$ (pCa>9). The Ca^{2+}/Mg^{2+}-ATPase activity was obtained as a difference between the two activities. The concentration of protein was determined by the method of Lowry et al. (1951).

Temperature-dependence of the measured variables

The terminology of Bennett (1984) was used to describe temperature-dependence of physiological variables. The temperature-dependence of a rate process is presented as its temperature coefficient (Q_{10}):

$$Q_{10} = (R_1/R_2)^{[10/(T_2-T_1)]}, \quad (1)$$

where R_2 and R_1 are rate processes at temperatures T_2 and T_1, respectively. The thermal dependence of variables that are not rate processes is given by an analogous expression, thermal ratio (R_{10}), which is the quotient of a quantity measured at two temperatures and expressed over a 10 °C interval:

$$R_{10} = (S_1/S_2)^{[10/(T_2-T_1)]}, \quad (2)$$

where S_1 and S_2 are quantities measured at temperatures T_2 and T_1, respectively. Numerically Q_{10} and R_{10} are identical.

Statistics

All results are given as mean ± S.E.M. Differences between acclimation groups were compared by one-way analysis of variance (ANOVA). Statistical differences between treatments were evaluated using a paired *t*-test. All percentage values were compared after arcsine-transformation with Student's *t*-test or ANOVA. Kruskal–Wallis ANOVA on ranks was used for populations that did not have equal variance. *Post-hoc* comparison of two groups were always accomplished using a Student–Newmann–Keuls test. The differences were considered to be significant at $P<0.05$.

Results

Contractile properties

Developed force was larger in atrial muscle (1.3±0.2 mN mg^{-1}, N=4, and 1.4±0.2 mN mg^{-1}, N=4, for CA and WA trout, respectively) than in ventricular muscle (0.3±0.05 mN mg^{-1}, N=4, and 0.3±0.08 mN mg^{-1}, N=4, for CA and WA fish, respectively), but there were no significant differences between acclimation groups ($P>0.05$). In both

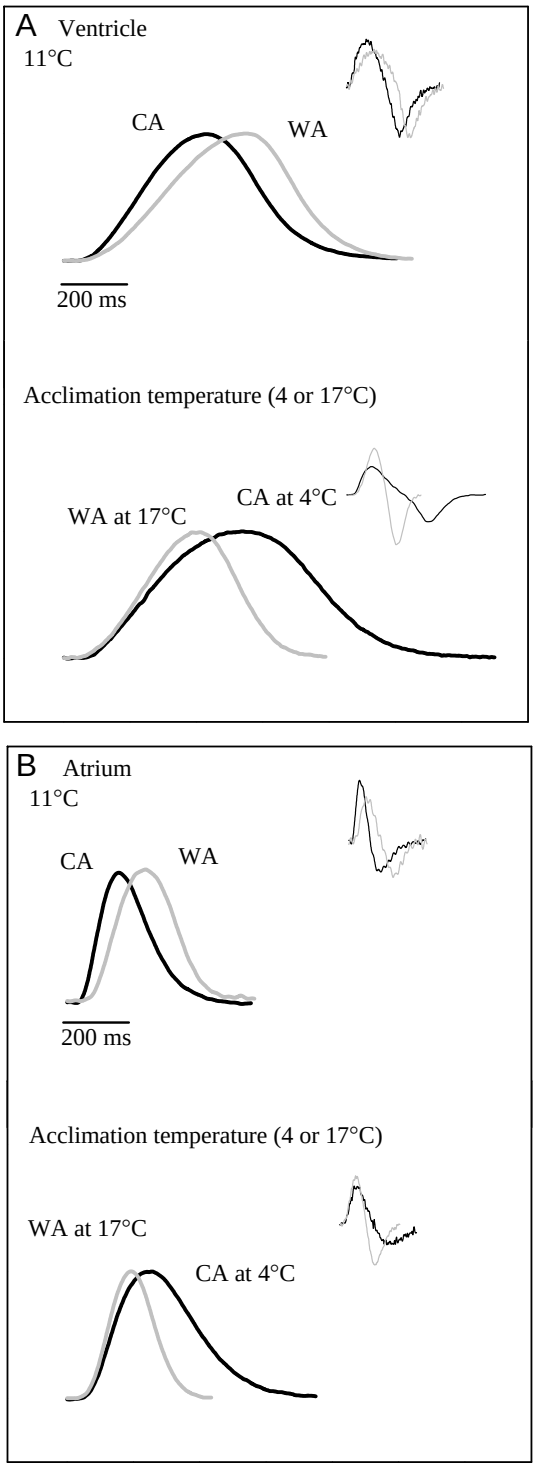


Fig. 1. Representative recordings of isometric contractions and their first derivatives from ventricular (A) and atrial (B) muscle of the rainbow trout heart. Superimposed recordings of force (F_{max}) normalized to the same height, and their first derivatives (dF/dt_{max}, where t is time) from warm-acclimated (WA) and cold-acclimated (CA) fish at the common experimental temperature of 11 °C are shown in the upper part of the figure. At this temperature it is evident that the contraction is faster in the heart of cold-acclimated than of warm-acclimated fish. Note also that thermal compensation is evident only in the rate of the contraction phase. The lower part of the figure shows recordings at the acclimation temperatures of the fish. The contraction is faster in warm-acclimated than in cold-acclimated fish, indicating that the compensation was only partial.

Table 1. *Kinetics of isometric contraction in atrial and ventricular muscle of warm- and cold-acclimated rainbow trout heart*

	CA atrium	CA ventricle	WA atrium	WA ventricle
Control				
T_{PF}	274±8[a,b,c] (5)	588±21[b] (5)	175±5[a] (7)	383±8 (9)
$T_{0.5R}$	171±6[a,b,c] (5)	277±8[b] (5)	78±5[a] (7)	122±4 (9)
T_{DC}	453±13[a,b]	865±28[b]	252±9[a]	504±10
Ryanodine				
T_{PF}	311±10[a,b] (4)	610±16[b] (4)	191±6[a] (4)	411±19 (4)
$T_{0.5R}$	151±4[a,b] (4)	272±9[b] (4)	87±5[a] (4)	122±9 (4)
T_{DC}	462±14[a,b]	882±25[b]	278±7[a]	543±16

Experiments were conducted at the acclimation temperatures of the animals; CA, cold-acclimated (4 °C) and WA, warm-acclimated (17 °C) in the absence (control) and presence of 10 µmol l^{-1} ryanodine.

Values are group means ± S.E.M. (*N*).

[a]Statistically significant differences (P<0.05) between atrium and ventricle within the acclimation group; [b]between the two acclimation groups; [c]between control and ryanodine treatment, determined using ANOVA (or Kruskal–Wallis ANOVA) and *post-hoc* Student–Newmann–Keuls test.

Time-to-peak force (T_{PF}); time to half-relaxation ($T_{0.5R}$); time of the contraction duration (T_{DC})

Table 2. *Kinetics of isometric contraction in atrial and ventricular muscle of warm- and cold-acclimated rainbow trout heart*

	CA atrium	CA ventricle	WA atrium	WA ventricle
Control				
T_{PF}	177±6[a,b]	394±14[b]	223±12[a,c]	517±22
$T_{0.5R}$	105±7[a]	173±8	107±4[a]	160±8
T_{DC}	282±8[a]	567±21[b]	330±10[a]	677±29
Ryanodine				
T_{PF}	222±15[a,b]	425±9[b]	288±13[a]	521±10
$T_{0.5R}$	115±15[a]	148±6	109±5[a]	142±7
T_{DC}	337±30[a,b]	573±9[b]	397±17[a]	663±15

Experiments were conducted at the common experimental temperature of 11 °C in the absence (control) and presence of 10 µmol l^{-1} ryanodine.

WA, warm-acclimated fish; CA, cold-acclimated fish.

Values are group means ± S.E.M. (*N*=4).

[a]Statistically significant difference (P<0.05) between atrium and ventricle within the acclimation group; [b]between the two acclimation groups; [c]between control and ryanodine treatment, determined using ANOVA (or Kruskal–Wallis ANOVA) and *post-hoc* Student–Newmann–Keuls test.

Time-to-peak force (T_{PF}); time to half-relaxation ($T_{0.5R}$); time of the contraction duration (T_{DC}).

acclimation groups, the rate of isometric contraction was much faster (P<0.05) in the atrial than in the ventricular preparation (Fig. 1, Table 1) and the rate of the contraction increased with temperature. Furthermore, temperature acclimation changed the contraction kinetics of the rainbow trout heart. When measured at 11 °C, the isometric contraction was significantly faster (P<0.05) in the heart of the CA fish than in the heart of the WA fish, indicating a positive thermal compensation in contraction kinetics (Fig. 1; Table 2). As indicated by the somewhat slower rate of contraction in the CA fish at 4 °C relative to the contraction of the WA fish heart at 17 °C, the compensation was only partial (Fig. 1). It is notable that the effect of thermal acclimation was mainly seen in the rate of contraction and less so in the rate of isometric relaxation. Ryanodine had no effect on the time course of ventricular contraction in either WA or CA trout. In the atrium of the CA fish, ryanodine prolonged T_{PF} and shortened $T_{0.5R}$ at 4 °C (Table 1). Significant prolongation of T_{PF} was also seen in the atrium of the WA fish at 11 °C (Table 2).

Verapamil (10 µmol l^{-1}), a blocker of L-type Ca^{2+} channels, strongly suppressed F_{max} in all preparations tested at the acclimation temperatures. In atrial and ventricular muscle of the WA fish, F_{max} dropped to 13±1 (*N*=4) and 19±5 % (*N*=4) of the control, respectively. In the ventricle of the CA fish, F_{max} decreased to 17±3 % (*N*=4) and in the atrium to 27±5 % (*N*=4) of the control value (Fig. 2B). The importance of Ca^{2+} entry through the channels may be due to its direct contribution to cytosolic Ca^{2+}, to the maintenance of the action potential plateau, allowing Ca^{2+} entry through the reverse Na^+/Ca^{2+} exchanger, or as a trigger for further Ca^{2+} release from the SR. The latter alternative was checked by the ryanodine sensitivity of contractility. When measured at physiological pacing frequencies and body temperatures, ryanodine had no effect on the F_{max} of atrial contraction in the WA fish or on the F_{max} of ventricular contraction in either WA or CA fish. In contrast, ryanodine had a clear (19±9 %, *N*=6) and consistent negative inotropic effect on the atrial contraction of the CA fish. This effect was seen in preparations paced to contract at the physiological heart rate (0.6 Hz) and acclimation temperature of the fish (4 °C) (Fig. 2B). This finding indicates that the Ca^{2+} stores of SR can contribute to the activation of contraction in the CA trout atrium under physiologically relevant conditions.

Frequency-dependent changes in the force of contraction

The recovery of contractility from inactivation was determined by restitution experiments as shown in Fig. 3. The mechanical refractory period and time course of restitution were determined from individual muscles. With the lengthening of the test interval, the force of contraction recovers approximately with a single-exponential time course, whereas the force of the post-extrasystolic contraction, delivered at constant delay,

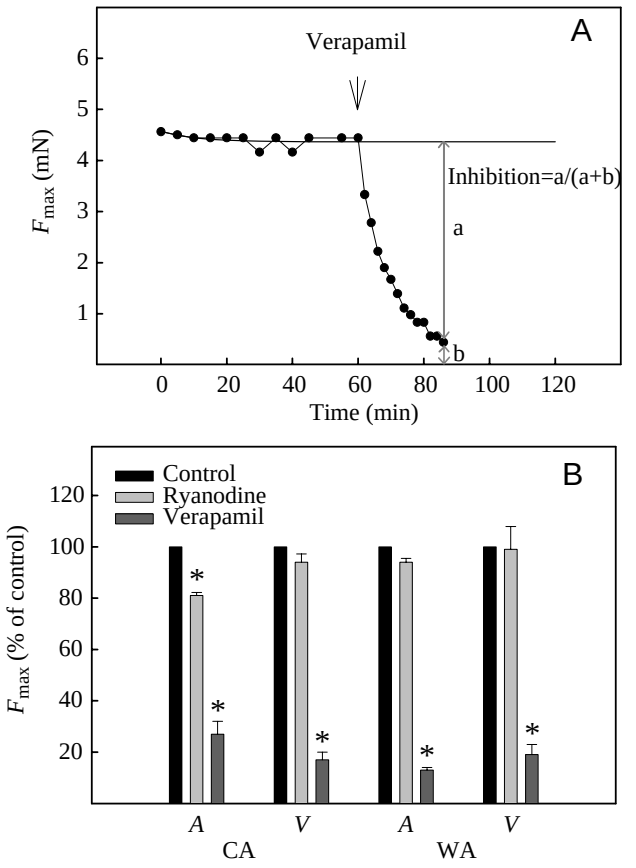


Fig. 2. Effects of the sarcolemmal Ca^{2+} channel blocker verapamil (10 μmol l^{-1}) and of the sarcoplasmic reticulum Ca^{2+} release channel blocker ryanodine (10 μmol l^{-1}) on the maximum force of contraction (F_{max}) in the atrial and ventricular muscle of the rainbow trout heart. (A) The time-dependent effects of the drug on force production was determined in each individual muscle. The change in force production during a 1 h equilibration period was fitted with double-exponential equation, and the fitted function was used to predict the control value at the moment of measurement in the presence of the blocker. The drug effect was obtained as the difference between the measured and predicted force. (B) The effects of verapamil and ryanodine in atrial (A) and ventricular (V) tissue of both acclimation groups. Verapamil caused a significant (P<0.05) decrease in force production in all preparations, whereas ryanodine had a negative inotropic effect only in the atrium of the cold-acclimated fish. The results are means ± S.E.M. of 4–6 animals. Experiments were conducted at the acclimation temperatures of the animals: 4 °C for cold-acclimated (CA) and 17 °C for warm-acclimated (WA) fish. *Statistically significant difference (P<0.05) relative to the control value.

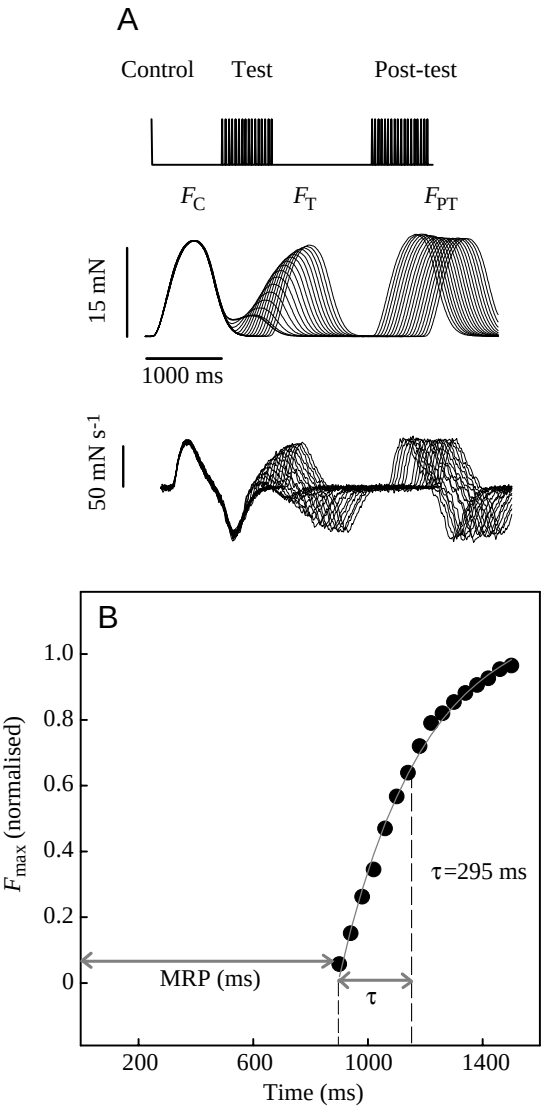


Fig. 3. Determination of restitution parameters, mechanical refractory period (MRP) and rate constant of restitution (τ) in the rainbow trout heart. (A) Superimposed force recordings from a complete restitution experiment in the ventricle of the cold-acclimated trout at 4 °C. The timing of the stimulus pulses is shown in the upper part of the figure, maximal force (F_{max}) in the middle part and its first derivative dF/dt_{max} in the lower part. With increasing diastolic interval, the force of the test contraction (F_T) increases, whereas the force of the first post-test contraction (F_{PT}), induced at constant delay of 1.4 s, remains practically unchanged. (B) The restitution curve from the above experiment and the two restitution variables. The line represents a single-exponential fit $y=23.46(1-e^{-0.0034t})-22.33$, to the data, where t=time; r^2=0.931; P<0.001).

remains virtually unaltered. The results of the restitution experiments are shown in Fig. 4. Mechanical restitution was much faster in atrial than in ventricular muscle. In both acclimation groups, the mechanical refractory period was much shorter and the rate constant of force restitution significantly smaller in atrial than in ventricular tissue (Table 3). Acclimation to cold shortened the mechanical refractory period, as was expected on the basis of temperature-induced changes in the duration of contraction. Furthermore, the rate constant of restitution was decreased in both atrial and ventricular tissue after acclimation to cold (Fig. 4). These findings indicate that partial thermal compensation occurs in the force restitution of the rainbow trout heart. Moreover, blockade of the SR Ca^{2+}-release channels with ryanodine caused a marked lengthening

Table 3. *Mechanical refractory period and the rate of force restitution of trout atrial and ventricular muscle at the acclimation temperatures of the fish and at the common experimental temperature of 11 °C in the absence and presence of ryanodine (10 µmol l^{-1})*

Time (ms)	CA atrium	CA ventricle	WA atrium	WA ventricle
MRP				
4/17 °C	480±16^{a,b,c,d} (4)	924±16^{b,c,d} (5)	230±6^{a,d} (6)	436±11^{d} (5)
4/17 °C+ ryanodine	520±23 (4)	976±13 (4)	225±7 (4)	444±7 (4)
11 °C	278±8^{a,b} (4)	358±8^{b} (4)	383±14^{a} (4)	505±5 (4)
τ				
4/17 °C	223±9^{a,b,c,d} (4)	328±30^{b,d} (4)	84±6^{a,d} (6)	112±3 (5)
4/17 °C+ ryanodine	332±12 (4)	339±54 (4)	87±3 (4)	102±7 (4)
11 °C	101±25^{a} (4)	160±17^{b} (4)	231±57 (4)	282±40 (4)

MRP, mechanical refractory period; τ, rate constant of force restitution.

Values are group means ± S.E.M. (*N*).

^{a}Statistically significant differences (P<0.05) between cardiac compartments within the acclimation group; ^{b}between two acclimation groups; ^{c}between control and ryanadine experiments; ^{d}between test temperatures (4 *versus* 11 °C and 17 *versus* 11 °C) within the acclimation group, determined using ANOVA (or Kruskal–Wallis ANOVA) and Student–Newmann–Keuls test.

Warm-acclimated (WA) fish acclimated at 17 °C; cold-acclimated (CA) fish acclimated at 4 °C.

of the refractory period and an increase in the rate constant of restitution τ in both the atrium and ventricle of the CA fish. This finding indicates that the Ca^{2+} handling of SR is involved in the restitution process of the fish heart (Fig. 4C). In the atrium and ventricle of the WA fish, ryanodine had no effect on the restitution process (Table 3). Furthermore, the mechanical refractory period and the time course of restitution were very sensitive to experimental temperature. The high R_{10} values (Table 4) suggest that the force restitution is not a passive diffusion-dependent process but more like an enzymatic reaction.

In mammalian hearts, the force of contraction of the post-extrasystolic beat is potentiated, which is considered to reflect the time-dependent redistribution of activator Ca^{2+} within the SR (Braveny and Kruta, 1958; Wohlfart and Noble, 1982). In the trout heart, the force of post-test contraction was not potentiated in any of the cardiac preparations. Even in the atrium of the CA trout, where ryanodine-sensitive Ca^{2+} stores contribute to steady-state force production, post-extrasystolic potentiation was absent. In this regard, fish and mammalian hearts are different.

Rest-potentiation describes the effect of long diastolic intervals on the force generation of the muscle. Prolonged diastole, measured at the acclimation temperatures of the fish, strengthened post-rest contraction in all cardiac preparations of the trout heart (Figs 5 and 6). Maximal rest-potentiation was achieved at rest durations of 40–60 s (being significantly shorter for atrial than for ventricular tissue), whereas at longer rests intervals it either remained relatively constant (atria) or gradually declined (ventricles). Rest-potentiation was significantly stronger (P<0.05) in ventricular (157±5, N=5, and 204±23 %, N=5, for WA and CA fish, respectively) than in atrial (129±1, N=5, and 148±9 %, N=5, for WA and CA fish, respectively) muscle (Fig. 7). When isometric force was used as a measure, the ventricular rest-potentiation was totally insensitive to ryanodine in both CA and WA fish, as was the atrial rest-potentiation of the WA fish (Figs 5 and 6). In contrast, in the atrium of the CA fish, rest-potentiation was completely abolished by ryanodine, as was expected on the basis of ryanodine sensitivity of the steady-state force production (Fig. 5). Again, this reinforces the importance of the SR as a source of cytosolic Ca^{2+} in the atrium of the CA trout. In contrast, if the rate of isometric force (dF/dt_{max}) was used as a measure, the depressive effect of ryanodine was also evident in the ventricle of the CA fish (Fig. 6).

Table 4. Q_{10} *or* R_{10} *values for various parameters in atrial and ventricular muscle of rainbow trout heart in the absence and presence of 10 µmol l^{-1} ryanodine*

	CA atrium	CA ventricle	WA atrium	WA ventricle
T_{PF}				
Control	2.3±0.12	2.1±0.09	2.1±0.07	2.2±0.09
Ryanodine	2.0±0.10^{b}	2.1±0.04	2.5±0.10^{a}	2.1±0.12
$T_{0.5R}$				
Control	2.5±0.10	2.5±0.20	2.5±0.10	2.3±0.10
Ryanodine	1.8±0.10^{a}	2.7±0.20^{b}	2.1±0.03	2.1±0.10
T_{DC}				
Control	2.3±0.10^{c}	2.2±0.10	2.1±0.10	2.3±0.03
Ryanodine	1.9±0.09^{b}	2.2±0.07	2.4±0.10^{a}	2.1±0.09
MRP				
Control	2.5±0.10^{a}	3.6±0.04^{b}	2.7±0.10^{a}	1.9±0.03
Ryanodine	2.6±0.04^{a}	3.8±0.07^{b}	2.9±0.20^{a}	1.9±0.05
τ_{fast}				
Control	3.4±0.7	3.4±0.3	4.4±0.7	3.7±0.20
Ryanodine	2.3±0.2	3.5±0.5	3.6±0.2	3.5±0.3

WA, warm-acclimated; CA, cold-acclimated fish.

TPF, time to peak force; $T_{0.5R}$, time to half relaxation; T_{DC}, time of contraction duration; MRP, refractory period; t, time constant of force restitution.

Values are group means ± S.E.M., N=4.

^{a}Statistically significant differences (P<0.05) between cardiac compartments within the acclimation group; ^{b}between the two acclimation groups; cbetween control and ryanodine treatment, determined with ANOVA (or Kruskal–Wallis ANOVA) and post-hoc Student–Newman–Keuls test.

At 11 °C, rest (60 s) increased the force to 145±7 (*N*=5) and 136±7 % (*N*=5) of the control contraction in the ventricles of WA and CA fish, respectively. Ryanodine had no effect on these responses. In the atrial tissue, rest (40 s) increased force production to 140±8 % (*N*=5) of the control value in both acclimation groups. Ryanodine had no effect on rest-potentiation in the atrium of the WA fish, whereas in the atrial tissue of the CA fish, rest-potentiation was depressed to 108±1 % (*N*=5) (*P*=0.025) after the ryanodine treatment. Regardless of the experimental temperature, the ventricular rest-potentiation was always associated with the prolongation of the twitch (*P*<0.001) (Fig. 6C). In contrast, rest had no significant effect on the time course of atrial contraction (*P*=0.094) (Fig. 5).

Briefly stated, rest-potentiation and its ryanodine sensitivity indicate that the prolonged diastole increases the SR Ca^{2+} stores in the atrium and possibly also in the ventricle of the CA trout, but not in the atrial or ventricular muscle of the WA trout.

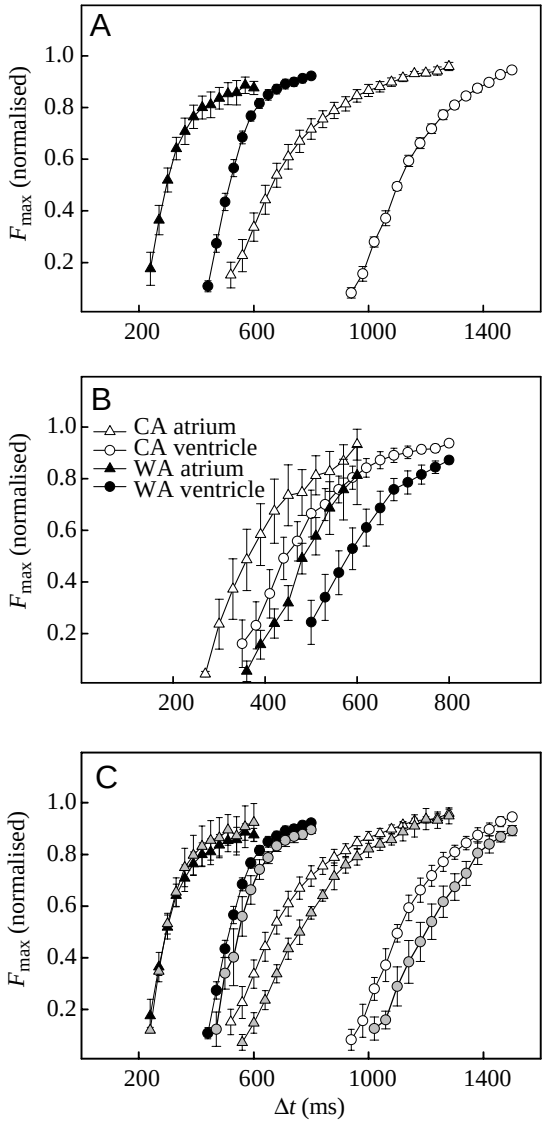


Fig. 4. Force restitution in atrial and ventricular tissue of the rainbow trout heart. (A,B) Restitution curves for atrial and ventricular muscle at the acclimation temperatures of the fish (A) and at the common experimental temperature of 11 °C (B). The force (F_{max}) of the test beat is plotted as a function of time between the control pulse and the test pulse (Δt). (C) The effect of 10 μmol l^{-1} ryanodine (grey symbols) on the force restitution at the acclimation temperatures of the fish. In the preparations of the cold-acclimated (CA) fish, but not in those of the warm-acclimated (WA) fish, ryanodine lengthens the refractory period and increases the time constant of restitution (τ). The results are means ± S.E.M. of 4–6 preparations.

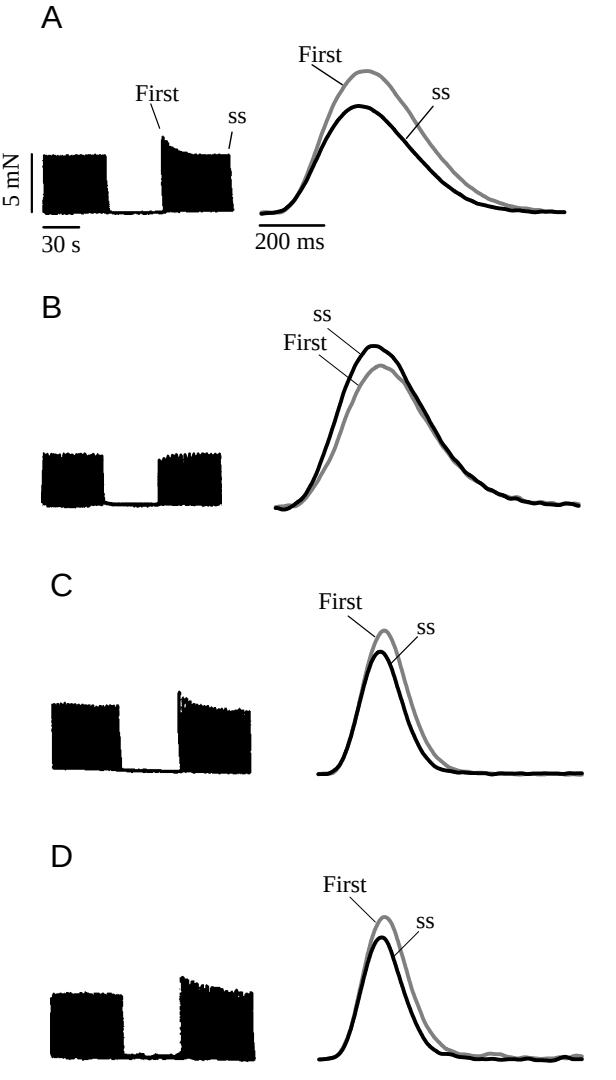
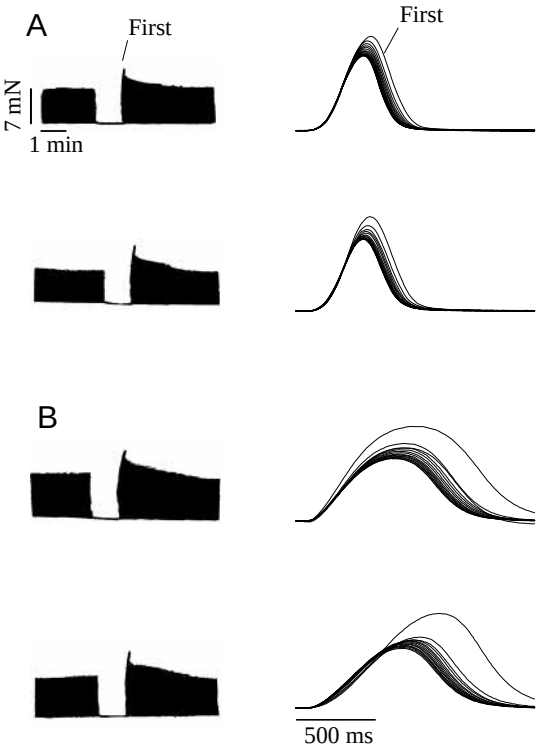


Fig. 5. Effect of ryanodine on rest-potentiation in atrial muscle of (A,B) the cold-acclimated (CA) and (C,D) the warm-acclimated (WA) rainbow trout heart. Slow time-base polygraph recordings for a 40 s pause, and fast superimposed computer prints for the first post-test contraction (First) and the steady-state (SS) contraction under control conditions (A,C) and in the presence of 10 μmol l^{-1} ryanodine (B,D) are shown. Note that, in the atrium of the CA trout, the first post-rest contraction is completely suppressed, whereas in the atrium of the WA trout, ryanodine has no effect on the force of contraction. Experiments were conducted at the acclimation temperatures of the animals (4 °C for CA fish and 17 °C for WA fish).



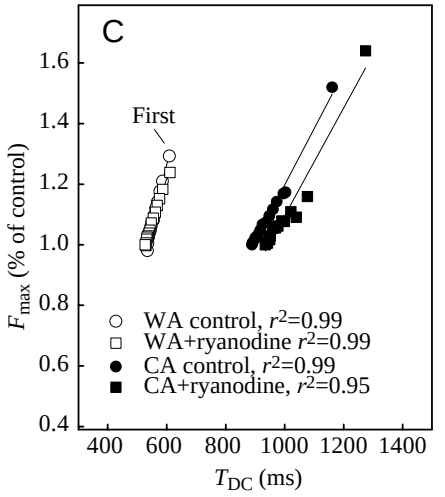


Fig. 6. Rest-potentiation in the ventricular muscle of (A) the warm-acclimated (WA) and (B) the cold-acclimated (CA) rainbow trout heart in the absence (upper trace) and presence (lower trace) of ryanodine. Slow time-base recordings for a pause of 60 s (left) and fast superimposed recordings of 12 post-rest contractions (right) from the same experiments are shown. First is the first post-rest contraction. Note that ryanodine has no effect on the force (F_{max}) of the post-rest contractions in the ventricle of either warm-acclimated (WA) or cold-acclimated (CA) trout. In the ventricle of the CA trout, however, ryanodine reduces the rate of the post-rest contractions. (C) Potentiation of the post-rest contraction (F_{max}) is linearly correlated (all P<0.001) with the duration of contraction (T_{DC}) for the preparations in A and B in the absence and in the presence of ryanodine. The experiments were conducted at the acclimation temperatures of the animals (4 °C for CA fish and 17 °C for WA fish).

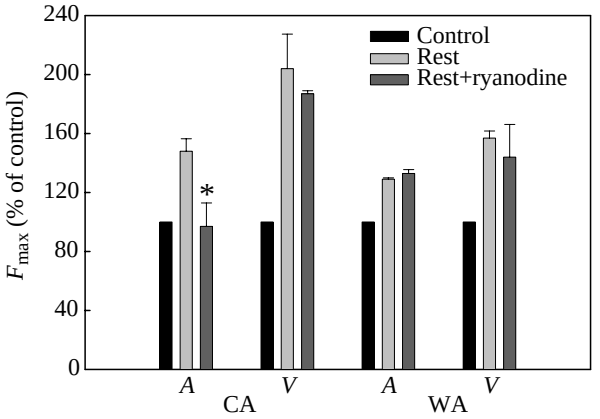


Fig. 7. The mean values (± S.E.M.) of rest-potentiation (maximal force=F_{max}) of cold-acclimated (CA) and warm-acclimated (WA) ventricular and atrial tissue of the rainbow trout heart in the absence (rest) and presence of 10 μmol l^{-1} ryanodine (rest+ryanodine) at the acclimation temperature of the fish. Rest-potentiation was taken at a pause of 40 s and 60 s for atrial (A) and ventricular (V) muscle, respectively. The force of the first post-rest contraction was normalized to the force of the last contraction before the pause. The results are means from 4–6 preparations. *A statistically significant difference (P<0.05) from the value in the absence of ryanodine. Note that only the rest-potentiation of the atrial tissue of the CA trout is ryanodine-sensitive.

Ca^{2+} uptake into the SR

ATP-dependent Ca^{2+} uptake of SR was measured with Fura-2 from crude atrial and ventricular homogenates. The rate of Ca^{2+} uptake of atrial preparations was significantly higher than that of ventricular preparations in both acclimation groups (Table 5). Furthermore, acclimation to cold caused thermal compensation in the rate of SR Ca^{2+} uptake, which was evident in both atrial and ventricular tissue (Table 5). When the measured Ca^{2+} uptake rates at 22 °C are transformed to the body temperatures of the fish, using the previously determined temperature coefficient (Q_{10}) of 1.64 (Aho and Vornanen, 1998), the SR Ca^{2+} uptake rate was highest in the atrium of the WA trout (100 %), and the relative activities of other preparations were 68, 66 and 52 % for CA atrium, WA ventricle and CA ventricle, respectively. These results indicate that thermal compensation of Ca^{2+} uptake rate in the atrial tissue was only partial, whereas compensation was more complete in the ventricular tissue.

Ca^{2+}/Mg^{2+}-ATPase activity of myofibrils

The activity of myofibrillar ATPase was determined at three different temperatures. For both acclimation groups, the myofibrillar ATPase activity was higher in atrial than in ventricular preparations (P<0.05). Furthermore, in both acclimation groups, the temperature-dependence of the ATPase activity (Q_{10}) was higher for atrial than for ventricular preparations (P<0.05) (Table 5). Acclimation to cold increased the activity of myofibrillar Ca^{2+}/Mg^{2+}-ATPase of the trout atrial tissue (P<0.05), whereas no temperature-induced

Table 5. *Sarcoplasmic reticulum Ca^{2+}-uptake rate of crude atrial and ventricular preparations and Ca^{2+}/Mg^{2+}-ATPase activity of purified myofibrils from rainbow trout*

Activity (μmol mg^{-1} protein min^{-1})	CA atrium	CA ventricle	WA atrium	WA ventricle
SR Ca^{2+} uptake	0.480±0.027[a,b]	0.354±0.035[b]	0.374±0.021[a]	0.246±0.036
	(7)	(6)	(7)	(6)
Myofibrillar Ca^{2+}/Mg^{2+}-ATPase				
at 15 °C	0.516±0.021[a,b]	0.162±0.029	0.390±0.045[a]	0.234±0.020
at 10 °C	0.326±0.031[a,b]	0.063±0.018	0.123±0.020	0.110±0.006
at 5 °C	0.083±0.014	0.047±0.012	0.032±0.003	0.045±0.007
Q_{10} (5–15 °C)	5.7±0.56[a,b]	3.4±0.23[b]	12.2±1.60[a]	5.2±0.36
	(5)	(5)	(6)	(6)

SR, sarcoplasmic reticulum; CA, cold-acclimated fish; WA, warm-acclimated fish.

Samples from the same myofibril preparations were used for Ca^{2+}/Mg^{2+}-ATPase determinations at the different temperatures.

Values are means ± S.E.M. (*N*).

[a]Statistically significant difference ($P<0.05$) between atrial and ventricular tissue within the acclimation group; [b]between the two acclimation groups, determined using ANOVA (or Kruskal–Wallis ANOVA) and *post-hoc* Student–Newmann–Keuls test.

changes were found in the activity of ventricular ATPase. The Q_{10} values of the CA fish were lower than those of the WA fish ($P<0.05$, Table 5), indicating temperature-induced changes in myofibrillar function.

Discussion

Contractile differences between atrium and ventricle

The current concept of fish heart function assumes that filling of the ventricle is primarily determined by atrial contraction (see Johansen and Burggren, 1980; Farrell and Jones, 1992), in contrast to the mammalian heart in which atrial systole has only a relatively small regulatory function. The fast contraction of the mammalian atrium (Urthaler et al., 1975; Asgrimsson et al., 1995; Bottinelli et al., 1995; Minajeva et al., 1997), together with the atrioventricular delay in impulse conduction, is considered to provide accurate timing for the atrial 'kick' to augment end-diastolic volume before ventricular systole (Katz, 1992). If atrial function in fish heart is decisively different from that of mammalian heart, it might be assumed that the contractile properties of the fish atrium would also differ from its mammalian counterpart. Specifically, it might be anticipated that contractile differences between atrial and ventricular muscle would be minor if the atrium and ventricle were working as two separate pumps in series. The present results indicate, however, that the rainbow trout heart atrial contraction is much faster than ventricular contraction, as has been observed in mammalian hearts. This suggests that the demands on atrial contractility are not that different in fishes and mammals. This agrees with the recent findings of Lai et al. (1998), indicating that a major part of ventricular filling in fish hearts, as in mammals and other vertebrates, occurs during ventricular relaxation and well before atrial contraction. In this scheme, the fast contraction of atrial cells generates a relatively short and accurately timed atrial systole, which adjusts the end-diastolic volume of ventricle according to the circulatory demands.

The rate of isometric contraction in atrial tissue of the trout heart was approximately double the contraction rate of ventricular tissue (Fig. 1; Table 1). Similar contraction (T_{PF}) and relaxation ($T_{0.5R}$) times to those presented here have also been reported by Ask et al. (1981) for rainbow trout atrium. The fast contraction kinetics of trout atrial tissue was associated with significantly higher myofibrillar ATPase activity relative to the ATPase activity of ventricular tissue, which can explain most of the atrioventricular differences in the rate of isometric contraction (Bárány, 1967). Our values for the specific activity of trout ventricular ATPase (Table 5) are similar to those reported by Churcott et al. (1994), and the difference in myofibrillar ATPase activity between the atrium and ventricle agrees with the findings of Deng and Gesser (1997). In addition to faster myofibrillar ATPase, atrial tissue also had a higher rate of Ca^{2+} uptake into the SR than ventricular tissue (Table 5). It could therefore be assumed that the greater participation of SR in the contractile activation might make the intracellular Ca^{2+} transient faster and thus contribute to faster contraction kinetics in the atrial cells. The slight lengthening of contraction duration in atrial tissue after ryanodine treatment supports this assumption (Table 1). In addition to the function of myofibrils and SR, differences in action potential duration could contribute to atrioventricular differences in the kinetics of contraction (Asgrimsson et al., 1995), although this aspect was not studied systematically in the present work.

Cellular processes that are responsible for the initiation of cardiac contraction need a finite time to recover from inactivation. Mechanical restitution describes this recovery process at short diastolic intervals and reflects the basic cellular mechanisms underlying excitation–contraction coupling (Wohlfart and Noble, 1982). The rate constant of restitution was much shorter in the atrial than in the ventricular muscle of the trout heart (Table 3), indicating that the processes that regulate the recovery of contraction from inactivation are faster in atrial cells than in ventricular myocytes. This kind of atrioventricular difference is also typical of the mammalian heart (Asgrimsson et al., 1995). In mammals, cardiac

contraction is initiated by sarcolemmal Ca^{2+} influx, mainly through L-type Ca^{2+} channels, which triggers a large Ca^{2+} release from the SR (Fabiato, 1983; Cheng et al., 1996). Accordingly, mechanical restitution might be explained in terms of either the trigger Ca^{2+} or the SR Ca^{2+} store. Sarcolemmal Ca^{2+} influx is the first step in excitation–contraction coupling, and the force restitution could therefore represent recovery of the Ca^{2+} channels from inactivation. In the function of the release store, at least two different mechanisms could contribute to mechanical restitution: slow replenishment of the releasable Ca^{2+} in the SR, or time-dependent recovery of the SR release channels from inactivation (for references, see Vornanen and Shepherd, 1997). Quantitative analysis of the sarcolemmal Ca^{2+} current has shown that, in trout ventricular myocytes, L-type Ca^{2+} channels can provide a significant proportion of the activator Ca^{2+} (Vornanen, 1998). Therefore, it might be assumed that the time course of restitution in the fish ventricle would, to a relatively large extent, reflect the time- and voltage-dependent recovery of sarcolemmal Ca^{2+} channels from inactivation. Our preliminary findings on isolated ventricular myocytes suggest that, at the physiological resting potential, the time-dependent recovery of Ca^{2+} current from inactivation is significantly faster than the rate of mechanical restitution in multicellular preparations. For example, in ventricular myocytes of the WA trout, the recovery of L-type Ca^{2+} current from inactivation occurred with a time constant of 52±7 ms (holding potential –70 mV, 19 °C, N=4), which is approximately twice as fast as the rate of force recovery (112±3 ms, N=5, at 17 °C) in the ventricular strips. Nor can the atrio-ventricular difference in the rate of force restitution be ascribed to the kinetic properties of the L-type Ca^{2+} current, because the recovery of Ca^{2+} current from inactivation in the atrial cells of the WA trout occurred with a time constant of 41.6±7 ms (N=3), which is not significantly different (P=0.37) from that of ventricular cells. The recovery of Ca^{2+} channels from inactivation is much slower at more depolarized membrane potentials, and it is therefore plausible that the time course of force restitution also partly reflects frequency-dependent changes in the shape of the action potential and hence in the voltage-dependent recovery of the L-type Ca^{2+} current from inactivation. In any case, the atrio-ventricular differences in the time course of mechanical restitution were not abolished by ryanodine, which excludes SR as a major determinant for frequency-dependent differences between the two cardiac compartments. Clearly, the connection between electrical and mechanical restitution in fish cardiac cells needs to be studied in detail. The shorter mechanical refractory period of atrial tissue is probably explained by the shorter contraction and the shorter action potential (Asgrimsson et al., 1995; M. Vornanen, unpublished observations) compared with ventricular muscle.

The force recovery from inactivation at long diastolic intervals is expressed in the potentiation of the post-rest contraction, which was present in both atrial and ventricular tissue of the rainbow trout heart (Figs 5, 6 and 7). The maximum potentiation was smaller and it was achieved at shorter rest intervals in atrial than in ventricular muscle. In this regard, fish and mammalian hearts are again quite similar (Minajeva et al., 1997). The smaller size of the rest-potentiation in atrial tissue suggests that, because of the faster recovery process, the inactivation of force development is reduced in atrial compared with ventricular tissue at physiological heart rates. This means that, with increasing heart rate, the atrial tissue will be able to maintain its force production better than the ventricular muscle. The fast recovery of atrial contraction from inactivation may be significant for cardiac function in fishes, in as much as the strong atrial contraction will maintain adequate ventricular filling and thereby efficient cardiac output at high heart rates.

Effects of thermal acclimation

The present results show that there is partial thermal compensation in the contractile properties of the rainbow trout cardiac muscle. In both cardiac compartments, the effects of thermal adaptation are seen in the kinetics of isometric contraction, in the time course of restitution, in the mechanical refractory period and in rest-potentiation (Fig. 1; Tables 1 and 2). In the subcellular systems, positive thermal compensation was expressed in the Ca^{2+} uptake rate of SR in both the atrial and the ventricular myocardium and in the myofibrillar ATPase activity of the atrial tissue. In fact, the only site where the compensation was clearly absent was the myofibrillar ATPase of the ventricular muscle (Table 5). These temperature-induced changes in contraction kinetics provide partial compensation for the effects of low ambient temperature in the cardiovascular function of the cold-active rainbow trout.

We have demonstrated that acclimation to cold increases the rate of isometric contraction in the rainbow trout heart. A decrease in relaxation time has been found in the heart of the yellow perch (*Perca flavescens*) (Bailey and Driedzic, 1990), suggesting that this kind of response might be common for teleost fish that remain active in the cold. The kinetics of isometric contraction are determined by the attachment and detachment rates of cross bridges (i.e. it is a function of myofibrillar ATPase activity), and by the rate of activation induced by Ca^{2+} and by Ca^{2+} removal (i.e. the rate of the Ca^{2+} transient) (Rossmanith et al., 1986; Hoh et al., 1988; Ruegg, 1990). The faster kinetics of atrial contraction after cold-acclimation can be explained, to large extent, by positive thermal compensation in the activity of myofibrillar ATPase. We do not know whether this change in myofibrillar function is associated with alterations in myosin heavy chain or in myosin light chain composition, both of which can affect myosin ATPase activity (Bottinelli et al., 1995). In another teleost fish, the crucian carp (*Carassius carassius* L.), temperature-induced changes in myofibrillar ATPase activity are associated with altered myosin heavy chain expression (Vornanen, 1994). It is notable that positive thermal compensation was absent in the ventricular myofibrils of the rainbow trout. Thus far, we have no explanation for this. The temperature-dependence of ATPase activity was, however, weaker in the CA trout, which suggests that the myofibrillar

function of the ventricular myocardium is also modified by the thermal history of the animal.

In part, the faster kinetics of contraction after cold acclimation could be ascribed to enhanced Ca^{2+} handling by the SR, which was evident in the Ca^{2+} uptake rate of SR in both atrial and ventricular muscle. The enhanced activity of the SR Ca^{2+} pump in the heart of the CA trout is in agreement with our previous findings on ventricular muscle of the rainbow trout (Aho and Vornanen, 1998) and extends it to the atrial tissue. A small SR Ca^{2+} leakage was noted when thapsigargin (TG) was applied to Ca-loaded vesicles. Although we have not determined it quantitatively, the leakage is apparently relatively constant in different cardiac preparations since the rate of TG-sensitive Ca^{2+} uptake and the activity of SR Ca-ATPase (not affected by Ca^{2+} leakage) are linearly correlated (Aho and Vornanen, 1998). Therefore, we assume that the temperature-induced changes in TG-sensitive Ca^{2+} uptake of cardiac homogenates reflect true enhancement of SR Ca^{2+} handling after acclimation to cold and not variable Ca^{2+} leakage of different preparations. The proliferation of cardiac SR as a consequence of cold-acclimation has been suggested to occur in the perch (*Perca fluviatilis*) heart (Bowler and Tirri, 1990), and an increase in the ryanodine sensitivity of contraction has been demonstrated in the ventricle of CA rainbow trout (Keen et al., 1994). Thus, it seems clear that the enhanced activity of SR is part of the thermal adaptation of cardiac function in cold-active fish species.

The ryanodine sensitivity of force (Fig. 2B) and the time course (Table 2) of the atrial contraction suggest that the SR is involved in the kinetics of atrial contraction. Although the acceleration of atrial contraction after cold acclimation can be explained largely by changes in myofibrillar ATPase activity and SR Ca^{2+} uptake rate, these mechanisms are not valid explanations for the kinetic changes in ventricular tissue where ryanodine had no effect on either force or time course of contraction. In trout ventricle, which on the basis of ryanodine sensitivity of force generation (Hove-Madsen and Gesser, 1989; Møller-Nielsen and Gesser, 1992; Shiels and Farrell, 1997; present study) is almost exclusively dependent on extracellular Ca^{2+}, cold adaptation may alter membrane excitation, which would lead to shorter action potential duration or faster recovery of Ca^{2+} channels from inactivation. In fact, we have shown that the inactivation kinetics of L-type Ca^{2+} channels is increased after acclimation to cold (Vornanen, 1998). Whether this also applies to recovery from inactivation has not yet been studied.

Adaptation to cold increased the rate of force restitution in the trout heart (Table 3). In both atrial and ventricular tissue the compensation was partial. Furthermore, in the heart of the CA trout, but not in the heart of the WA trout, the time constant of restitution was significantly suppressed by ryanodine, whereas ryanodine had no effect on the force of the post-extrasystolic beat or on the force of the steady-state twitch (ventricle). Thus, while SR may not affect the steady-state force of contraction, it appears to alter the frequency response of the heart in a manner that allows better force generation and greater pumping capacity at high contraction frequencies (Shiels and Farrell, 1997). A similar rightward shift of the restitution curve by ryanodine, as was noted here in the atrium and ventricle of the CA trout, has been found in the rainbow trout heart in response to adrenaline stimulation (Gesser, 1996). In contrast to the trout heart, ryanodine increases the rate of cardiac force restitution (leftward shift of the curve) in mammals, suggesting that some function in the SR forms the rate-limiting step for the restitution process of the mammalian heart (Cooper and Fry, 1990). On the basis of the opposite effects of ryanodine on the time course of restitution in fish and mammalian hearts, it might be anticipated that the effect of the SR on the restitution process of fish heart is indirect; the SR may not provide activator Ca^{2+} to the myofilaments, but it might modify subsarcolemmal Ca^{2+} concentration locally and, thereby, membrane excitation (e.g. by affecting K^+ and Ca^{2+} currents of the sarcolemma). The lengthening of the refractory period after ryanodine treatment in the atrium and ventricle of the CA trout is in agreement with this hypothesis. Alternatively, it is possible that, in effect, the SR makes a small contribution to cytosolic Ca^{2+} but that, after the blockade of the SR release channels, this is compensated for by enhanced sarcolemmal Ca^{2+} influx, which obscures the inhibitory effect of ryanodine on the steady-state twitch force. The latter hypothesis is based on mutual inhibition between SR and sarcolemmal (SL) Ca^{2+} channels (Adachi-Akahane et al., 1996) and could explain the ryanodine-induced slowing of the restitution rate which would occur if SL Ca^{2+} influx were unable to compensate for the missing Ca^{2+} release from SR due to the incomplete recovery of SL Ca^{2+} influx from inactivation. Regardless of the mechanism, the ryanodine sensitivity of the restitution rate in the CA trout indicates that temperature-induced enhancement of the SR function underlies the increased rate of recovery from inactivation. The positive thermal compensation in the recovery of contractility from inactivation would make room for positive compensation in heart rate, thereby counteracting the temperature-dependent reduction in cardiac output. Thus, temperature-induced changes in the kinetics of contraction will probably be very important for the function of the intact heart at low ambient temperatures.

Rest-potentiation has been suggested to result from the increase in Ca^{2+} release from the SR as a consequence of either the increase in SR Ca^{2+} content or the increase in fractional SR Ca^{2+} release (Bouchard and Bose, 1989; Bers et al., 1993; Bassani et al., 1995). One important finding in the present study is that rest-potentiation can, in different parts of the heart of the same species, be either ryanodine-resistant or ryanodine-sensitive (i.e. rest-potentiation is not necessarily an expression of the SR Ca^{2+} release in fish heart). Indeed, the present findings suggest that, in the majority of cases, rest-potentiation reflects the lengthening of contraction duration (active state), and only in the atrium of the CA trout can it be ascribed solely to the function of SR. It is likely that prolonged rest causes a redistribution of intracellular Ca^{2+}, so that sarcolemmal Ca^{2+} entry during the first, long action potential after a rest is enhanced, resulting in potentiated contraction. In addition to the atrium of the CA fish, in the ventricle of the CA trout SR

also seems to be affecting the post-rest contraction. However, in this case the force (F_{max}) of post-rest contraction is not affected by ryanodine, only the rate of force generation (dF/dt_{max}) is altered. This would again suggest that, under control conditions, a small part of the cytosolic Ca^{2+} is released from the SR and that in the presence of ryanodine this Ca^{2+} fraction is compensated for by a larger sarcolemmal Ca^{2+} influx. This could, in principle, also explain some of the earlier findings where the contraction of fish heart is apparently ryanodine-insensitive even though the SR is powerful. The effect of ryanodine would not be apparent if maximal force was measured but could be evident in its first derivative.

Our results on trout ventricle differ from earlier findings which suggested that rest-potentiation is not associated with lengthening of contraction duration and that the potentiation was completely abolished by ryanodine (Hove-Madsen, 1992). These discrepancies may involve differences in acclimation and experimental temperatures as well as in pacing protocols. In Hove-Madsen's (1992) experiments, pacing rate was low (0.2 Hz), which might have abolished the difference in time course between steady-state and post-rest contractions, elicited after a 5 min rest. However, the SR Ca^{2+} uptake in trout ventricular myocytes may be so slow that the effect of ryanodine on the force of post-rest contraction would appear only after several minutes rest (Møller-Nielsen and Gesser, 1992), and thus would not be evident in our experiments with a rest duration of 1 min. Contrary to this assumption, under our experimental conditions, ryanodine did not abolish rest-potentiation in trout ventricle even at a rest duration of 5 min.

Physiological importance of the sarcoplasmic reticulum in fish cardiac muscle

The present work shows that the SR is functionally important in the atrial tissue of rainbow trout, whereas its physiological role in ventricular contraction is less important and more concealed. We measured the function of the SR by its Ca^{2+} uptake rate and ryanodine sensitivity of contraction. When transformed to the physiological body temperatures of the fish, the rank order for the rate of SR Ca^{2+} uptake was WA atrium (100 %), CA atrium (68 %), WA ventricle (66 %) and CA ventricle (52 %). If this reflects the functional capacity of the SR, it would suggest that the inotropic effects of ryanodine would also follow the same order and would be more evident in the tissues of WA trout than CA trout. However, contrary to this assumption, the effectiveness of ryanodine was more evident in the tissues of the CA fish and weak or nonexistent in the heart of the WA trout. Several putative explanations exist for this apparent discrepancy. First, it is possible that the number of SR Ca^{2+} release channels is lower in WA than CA fish, resulting in less extensive Ca^{2+} release. Unfortunately, there are no data available about the effects of thermal acclimation on the number of ryanodine receptors in fish myocardium. Second, the trigger might be less effective in WA trout than in CA trout. The density of L-type Ca^{2+} current is, however, virtually the same in the ventricular myocytes of WA and CA trout, suggesting that there are no great differences in the trigger (Vornanen, 1998). Third, it is possible that the SR membrane is less leaky to Ca^{2+} in CA fish than in WA fish or at low rather than high experimental temperatures. Our data are consistent with the latter hypothesis, which can explain the finding that, in spite of the lower absolute activity of SR Ca^{2+} uptake in the cold, the ryanodine sensitivity of the force is higher in CA than in WA fish. Indeed, the ryanodine sensitivity of the atrial contraction of the CA fish indicates that the SR Ca^{2+} release channels of the trout heart are not very leaky at low ambient temperatures. This is in contrast to the general idea that, as a result of the Ca^{2+} leakiness of the SR membrane, the contraction of fish cardiac muscle is insensitive to ryanodine at cold temperatures and the contribution of SR Ca^{2+} stores to contractile force is seen only at high experimental temperatures. This assumption is based on observations on sheep cardiac SR, where cooling produces an increase in Ca^{2+} efflux from the SR owing to a cold-induced increase in the open probability of the release channel (Sitsapesan et al., 1991). Our findings, especially for the atrium of the CA trout, clearly demonstrate that the SR of the trout heart is not leaking very much Ca^{2+}, otherwise the Ca^{2+} pump of SR could not maintain the Ca^{2+} load that is expressed in the ryanodine sensitivity of the force of the steady-state contraction at physiological heart rates at 4 °C. Therefore, the present results indicate that the Ca^{2+} handling by the cardiac SR is physiologically important for the contractile function of the fish heart, and this is especially characteristic for fish that are physiologically adapted to low ambient temperature. The ability to retain adequate SR Ca^{2+} load in the cold is not limited to the fish heart, as shown by experiments on hibernating mammals (Zhou et al., 1991; Belke et al., 1991). It should be remembered, however, that even if the SR is functionally important in the trout heart, it is still a minor source of cytosolic Ca^{2+}, the major part of the Ca^{2+} coming from the extracellular space through the sarcolemma (Tibbits et al., 1990; Møller-Nielsen and Gesser, 1992; Vornanen, 1998).

In summary, the present data indicate that there are marked differences in the kinetics of isometric contraction between atrial and ventricular myocardium, as well as between the hearts of WA and CA rainbow trout. Moreover, it is shown that, in the atrial tissue of the CA trout, the SR contributes cytosolic Ca^{2+} directly, and in the atrium and ventricle of the CA trout the SR significantly increases the rate of force recovery from inactivation. The fast kinetics of contraction and the rapid recovery from inactivation allow relatively high heart rates and therefore adequate cardiac outputs at low environmental temperatures in the cold-active rainbow trout.

This work was supported by The Academy of Finland (project No. 7641). We would like to thank the Kontiolahti fish farm for supplying the trout.

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