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Regulation of cardiac contractility in a cold stenothermal fish, the burbot *Lota lota* L.

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Summary

In the present study, burbot (*Lota lota* L.) was used as a model to study the effects of acute temperature changes on cardiac contractility in a cold stenothermal fish. The burbot were captured in the breeding season (February) and were maintained for 4 weeks at 1–2 °C in the laboratory before the contractile properties of the heart were measured. Both isometric force and the pumping capacity of *in vitro* perfused hearts were maximum at the acclimation temperature (1 °C) and declined markedly when the temperature increased. At 1 °C heart rate was 25 beats min^{-1} and increased to a maximum of 72 beats min^{-1} at 18 °C, above which atrio–ventricular block was observed. Ryanodine (10 μmol l^{-1}), an inhibitor of sarcoplasmic reticulum (SR) Ca^{2+} release channels, reduced the maximum developed force of paced atrial and ventricular preparations at 1 °C by 32±8 % and 16±3 %, respectively. At 7 °C, ryanodine-induced inhibition of force increased to 52±3 % and 44±5 % in the atrium and ventricle, respectively. At 1 °C, ryanodine abolished rest-potentiation and turned it into rest-decay in both atrial and ventricular muscle. Ryanodine, however, had no effect on the mechanical refractory period or on the rate constants of mechanical and relaxation restitution in either preparation at 1 °C. The activity of myofibrillar $Ca^{2+}Mg^{2+}$-ATPase was higher in atrial than ventricular muscle and the temperature optimum of the ATPase *in vitro* was approximately 10 °C in both preparations. Our results indicate a significant dependence on SR Ca^{2+} stores for contractile activation in the burbot heart at temperatures that are known to inhibit SR function in mammalian heart. This suggests that the ryanodine receptors of the teleost heart, unlike those of the endotherms, are not leaky as temperatures approach 0 °C. Reliance on SR Ca^{2+} stores in both cold stenothermal burbot and cold-acclimated eurythermal teleosts suggests that enhanced SR Ca^{2+}-release is a common characteristic of cold-living fish and may improve cardiac contractility in the cold.

Key words: ryanodine sensitivity, restitution, rest-potentiation, myofibrillar ATPase, heart rate, burbot, *Lota lota*.

Introduction

Fish may adopt various strategies to overcome the effects of changes in water temperature on physiological processes (see, for example, Bailey and Driedzic, 1990; Matikainen and Vornanen, 1992). Some migrate with the temperature gradient in order to maintain a constant body temperature, others become dormant in the cold, and others remain active in the cold by making physiological adjustments. In eurythermal fish species, cardiac function is subject to large temperature changes and the effects of temperature acclimation on cardiac contractility vary according to the strategy of environmental adaptation, being opposite in cold-active and cold-dormant fish (Aho and Vornanen, 1999; Tiitu and Vornanen, 2001). In cold-active rainbow trout (*Oncorhynchus mykiss)*, heart rate and velocity of cardiac contraction are higher in animals that have been allowed to acclimate to cold than in animals reared at warm temperatures (Aho and Vornanen, 2001). The faster kinetics of contraction are associated with increased myofibrillar ATPase activity and faster SR Ca^{2+} uptake (Aho and Vornanen, 1999). A stronger inhibitory effect of ryanodine on developed force is often interpreted as improved sarcoplasmic reticulum (SR) Ca^{2+} management (Keen et al., 1994; Shiels and Farrell, 1997; Aho and Vornanen, 1998). In cold-dormant species, temperature acclimation acts on the same physiological functions and involves the same subcellular mechanisms as in the cold-active fish, but the physiological responses to temperature are the opposite, i.e. heart rate and velocity of contraction are depressed after cold acclimation, and these functional changes are associated with the depression of SR Ca^{2+} handling and lower activity of myofibrillar ATPase (Aho and Vornanen, 1998; Tiitu and Vornanen, 2001).

In contrast to eurythermal fish species, relatively little is known about cardiac contractile activity and Ca^{2+} management in cold stenothermal fish, such as the burbot. Indeed, we know of no studies that examine cardiac function in burbot. This is surprising as burbot are a commercially valuable fish species,

having the widest distribution of all freshwater fish, ranging from the British Isles across Europe and Asia to the Bering Strait and from Alaska across the North American continent to the Atlantic coast (McPhail, 1997). Burbot are benthic, omnivorous fish that prefer cold waters and are seldom found at temperatures above 13 °C (Edsall et al., 1993; Carl, 1995; Pääkkönen and Marjomäki, 2000). In summer, burbot occupy hypolimnion of the lakes and feed on invertebrates and fish. Indeed, burbot are more active and better able to catch prey in winter than in summer. Although burbot are piscivorous they are relatively poor swimmers and their metabolic rate is low (Pääkkönen and Lyytikäinen, 2000). Spawning and embryonic development of burbot occur from January to March under ice at water temperatures below 4 °C. Together, these features suggest that burbot is a cold stenothermal species and therefore a suitable model animal for our study.

In this paper we report our investigations into the temperature-dependence of cardiac function and Ca^{2+} regulation of contractility in burbot atrium and ventricle, to test whether temperature adaptation in a cold stenothermal teleost involves similar functional changes and the same subcellular mechanisms that are typical for cold-acclimated eurythermal fish species.

Materials and methods

Fish

Sexually mature burbot (N=30) of both sexes, were caught during spawning time in February 1999 from Lake Orivesi (62°30′ N) in Finland. In the laboratory, the fish were held in 500 l stainless steel tanks at 1–2 °C with continuous circulation (approximately 0.5 l min^{-1}) of aerated tap (ground) water. Fish were fed with dead vendace (*Coregonus albula*) three times a week. The light cycle was 15 h:9 h dark:light. Fish body mass was 278±39 g (mean ± S.E.M.).

Contractile properties

Experiments were conducted on perfused spontaneously beating whole hearts, and atrial and ventricular strips. Fish were stunned by a blow to the head, the spine was cut and the heart was carefully removed. Atrial (16.4±1.7 mg wet mass) and ventricular strips (27.2±1.7 mg wet mass) were mounted on the bottom of a tissue bath with small needle electrodes and connected with small stainless steel hooks and braided silk threads to the force transducers (Grass FT03). A stimulus electrode was positioned in the tissue bath near the preparations. The duration of the stimulus pulse was 10 ms and voltage 60 V. The tissue bath was filled with 10 ml of oxygenated (100 % O_2) physiological solution containing (in mmol l^{-1}): NaCl, 150; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 10; Hepes, 10; glucose 10; pH 7.64 at 1 °C. The temperature of the saline was regulated to 1±0.5 °C with the aid of a recirculating water bath. Some experiments were also done at 7±0.5 °C (pH 7.57).

At the beginning of the experiment, the muscles were stretched stepwise to the length at which the developed force was close to maximum, after which the preparations were allowed to stabilize for a minimum of 30 min at a stimulation frequency of 0.25 Hz (1 °C) or 0.65 Hz (7 °C). Force of contraction was recorded on paper using a chart recorder (Grass 7D) and analog signals were digitized by an AD-converter (Digidata 1200, Axon instruments) and stored on the computer hard disk for later analysis. The recordings were analysed off-line using the Clampfit analysis program (Axon Instruments). Maximum developed force (F_{max}), maximum rate of relaxation (dF/dt_{min}), time to peak force (T_{PF}), time of half relaxation ($T_{0.5R}$) and duration of contraction (T_{DC}=T_{PF}+$T_{0.5R}$) were measured.

For whole-heart (0.54±0.06 g wet mass, N=8) experiments *in vitro*, an input cannula was secured through the bulbus arteriosus into the ventricle and the heart retrogradely perfused with oxygenated physiological saline (containing, in mmol l^{-1}, NaCl, 150; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 10; Hepes, 10; glucose 10; pH 7.4 at room temperature) with a pressure head of 4 cm. The heart was connected to a force transducer from the apex of the ventricle by a hook and short braided silk thread and tensioned to an afterload of 0.5 g. The heart was immersed in a temperature-regulated tissue bath, and the incoming perfusion saline and saline in the bath were regulated to the same temperature. Heart function was allowed to stabilize at 1±0.5 °C before heart rate and force contraction were recorded. The temperature was then raised from 1 to 18 °C in 2 °C steps. Contractile parameters were recorded at each temperature after the stabilization.

Mechanical restitution, relaxation restitution and rest-potentiation

Atrial and ventricular preparations were paced with a frequency of 0.25 Hz at 1±0.5 °C and the force was recorded on a computer. To measure mechanical and relaxation restitution, the regular pacing was intervened by test protocols. Each protocol consisted of six contractions at the steady-state frequency (0.25 Hz) to keep a constant cellular Ca^{2+} load. With these six contractions, the steady-state force was reached. The steady-state contractions were followed by a single extra stimulus with a variable delay from the last steady-state pulse and a post-extrasystolic stimulus with a constant delay of 4 s from the extra stimulus. The extrasystolic intervals were generated in the computer (Clampfit, Axon Instruments) from where the trigger signals were delivered through a D/A board (DigiData 1200, Axon Instruments) and a custom-made signal conditioner to the stimulator (SD-7, Grass Instruments). The extrasystolic intervals covered a range from the absolute refractory period to the steady-state interval (4 s). The force of the extrasystolic contraction (F_{ES}) was normalized to the force of the preceding control contraction (F_C) and plotted as a function of a extrasystolic interval (t) to produce a mechanical restitution curve. For relaxation restitution, the first derivative of the force recording was generated in the computer and relaxation restitution curves were constructed by plotting the inverse of dF/dt_{min} for extrasystolic beat (normalized to that of the preceding control beat) against extrasystolic interval. Contraction and relaxation restitution curves were fitted

to single exponential equations to give rate constants of restitution (see Aho and Vornanen, 1999).

For the determination of rest-potentiation at 1 °C, steady-state stimulation was interrupted for 10, 30 or 60 s, after which the normal pacing was resumed. The force of the first post-rest contraction was normalized to the force of the previous steady-state beat to give rest-potentiation.

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

Atrium from six fish and one ventricle were needed for the purification of one sample of myofibrils (Aho and Vornanen, 1999). Tissues were minced with scissors and then homogenized 3 times for 10 s in 20 volumes of ice-cold buffer 1 (containing in mmol l^{-1}: KCl, 100; Tris-HCl, 10; dithiothreitol, 1; pH 7.4), and centrifuged at 10,000 ***g*** for 10 min. The pellets were resuspended in the homogenization buffer 2 (buffer 1 + 1 % Triton X-100) and were centrifuged again at 10,000 ***g*** for 10 min. The recovered myofibrils were washed three times in buffer 1 and centrifuged at 600 ***g*** for 15 min between washes. The pellets from the last centrifugation were suspended in 20 volumes of low-ionic-strength buffer containing (in mmol l^{-1}): imidazole, 45; KCl, 50; dithiothreitol, 1; pH 7.0. ATPase activities of the purified myofibrils were determined at four different temperatures (1, 5, 10 and 15 °C) by liberation of inorganic phosphate (Atkinson et al., 1973). The pH was allowed to change freely according to temperature and was 7.73, 7.69, 7.64 and 7.59 at 1, 5, 10 and 15 °C, respectively. Total ATPase activity was measured in a solution containing (in mmol l^{-1}): imidazole, 45; KCl, 50; EGTA, 5; $MgCl_2$, 5; Na_2ATP, 3; $CaCl_2$, 5; pH 7.0. Activity of the background Mg^{2+}-ATPase was determined in the same solution, but without $CaCl_2$. Activity of the myofibrillar Ca^{2+}/Mg^{2+}-ATPase was obtained as a difference between the total and the background activity. Protein concentration was determined by the method of Lowry et al. (1951).

Blood analysis

Fish were slightly anaesthetised with 0.1 % MS 222 and arterial blood was collected from the caudal vessel into heparinized syringes. Hematocrit (Hct) was determined in 75 µl blood samples after centrifugation at 7000 ***g*** for 5 min (Hermle Z 231 M). Haemoglobin (Hb) was determined with the cyan-methemoglobin method using a 20 µl sample of blood. Mean cellular Hb concentration (MCHC) was calculated by dividing the Hb concentration by the Hct value. Blood plasma was diluted 1:10 with 0.5 mol l^{-1} NaOH and the protein concentration was determined (Lowry et al., 1951) using bovine serum albumin as a standard.

Statistics

All results are given as mean ± S.E.M. Differences between atrium and ventricle were compared by one-way analysis of variance (ANOVA). Statistical differences between treatments were evaluated with a paired *t*-test. All percentage values were compared after arcsin-transformation with Student's *t*-test and ANOVA. The differences were considered to be significant at $P \leq 0.05$.

Results

Contractile properties of spontaneously beating heart

In spontaneously beating perfused burbot hearts, the heart rate was relatively high, 25±1 beats min^{-1} at 1 °C, and increased with increasing temperature up to a maximum of 72±3 beats min^{-1} at 18 °C (Fig. 1A). At temperatures above 18 °C, the rhythm of contractions became irregular due to atrio–ventricular block of impulse conduction. In the present experimental protocol, the developed force F_{max} of the burbot heart was maximum at the acclimation temperature (1 °C) and decreased dramatically when temperature increased from 1 °C to 18 °C (Fig. 1B). The decrease in force was associated with simultaneous decrease in the T_{PF} and $T_{0.5R}$ (Fig. 1C). The pumping capacity of the heart (calculated as the product of F_{max} and heart rate) was maximum at 1 °C and decreased strongly with increasing temperature (Fig. 1D).

Contractile properties of paced atrial and ventricular muscle

Stable force production, for up to 4 h, was characteristic of both atrial and ventricular preparations from burbot heart. Accordingly, no correction for time-dependent deterioration of contractile function was needed when the effects of the SR Ca^{2+} release channel inhibitor, ryanodine, were examined. At the imposed preload of 0.5 g, there was no difference in the absolute force between atrial (5.37±1.5 mN) and ventricular (5.94±1.2 mN) muscle at 1 °C ($P<0.05$; note, however, that the ventricular preparations were, on average, 65 % larger).

The duration of ventricular contraction was approximately double the duration of atrial contraction. T_{PF} values in atrial and ventricular tissue at 1 °C were 610±18 and 1403±61 ms ($P<0.05$), respectively. The corresponding values for $T_{0.5R}$ were 383±17 and 563±30 ms ($P<0.05$). The rate of contraction increased with Q_{10} values between 1.9 and 3.8 when the temperature rose from 1 ° to 7 °C. Ryanodine (10 µmol l^{-1}, 60 min), strongly suppressed F_{max} in both atrial and ventricular preparations. At 1 °C, F_{max} decreased by 16±3 ($N=5$) and 32±8 % ($N=6$) ($P<0.05$) of the control in ventricular and atrial preparations, respectively (Fig. 2). At 7 °C, the inhibitory effect of ryanodine was even stronger as F_{max} decreased 52±3 % ($N=6$) in atrial and 44±5 % ($N=6$) in ventricular preparations. Despite its strong inhibitory effect on F_{max}, ryanodine had only marginal effects on the time course of contraction.

Restitution

The fast component of mechanical restitution describes force production at diastolic intervals shorter than the regular pacing interval. As expected on the basis of twitch duration, mechanical refractory period (MRP; the shortest extrasystolic interval where force was generated) was much shorter in atrial (1108±130 ms) than ventricular muscle (1975±81 ms, $N=6$; $P<0.05$). Ryanodine had no effect on MRP in either atrial or ventricular preparations

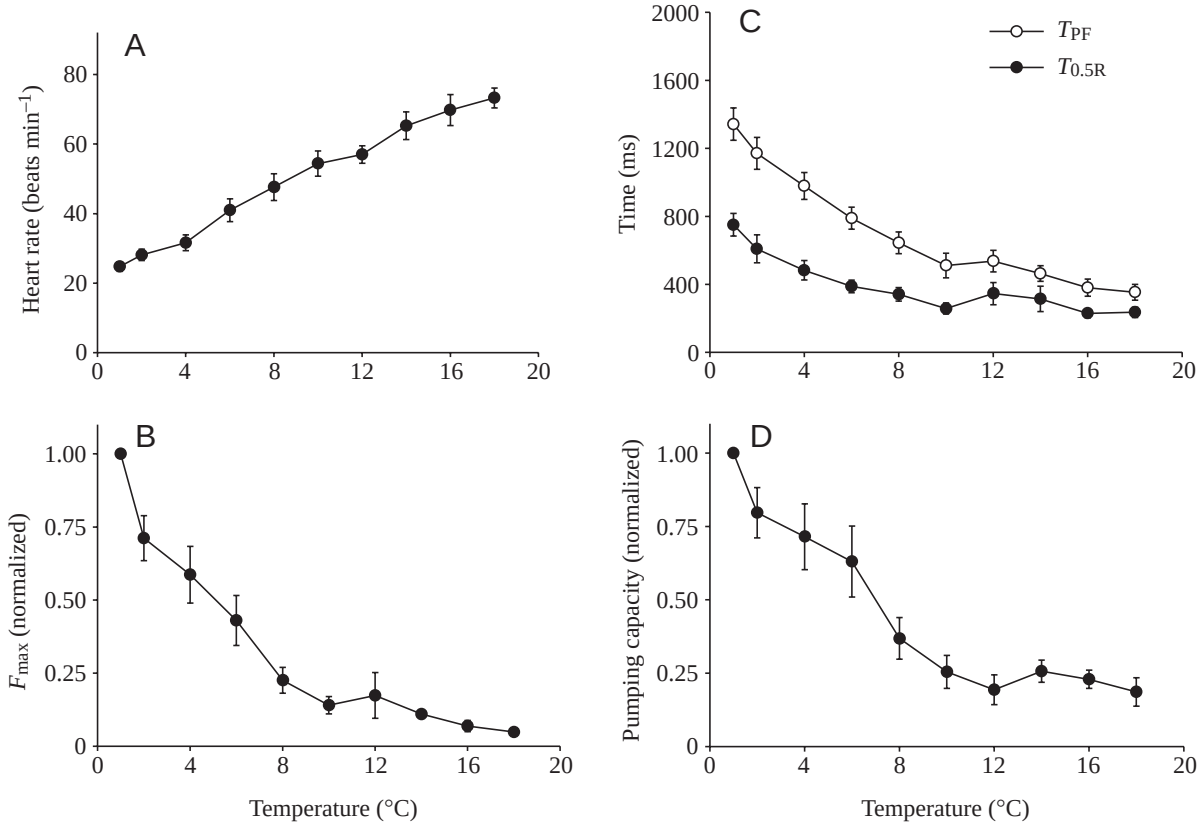

Fig. 1. Temperature-dependence of contractility in spontaneously beating perfused burbot hearts with acute temperature changes from 1±0.5 °C to 18±0.5 °C. (A) Heart rate, (B) maximum developed force F_{max}, (C) time course of contraction, (D) pumping capacity of the heart (F_{max}×heart rate).Values are means ± S.E.M. from eight hearts.

(Fig. 3A). Following MRP, the rate of force recovery (τ) was very similar in atrial (781±141 ms, *N*=6) and ventricular (628±65 ms, *N*=6; *P*>0.05) preparations and was not influenced by ryanodine (724±196 ms and 755±186 ms, for atrium and ventricle, respectively; *P*>0.05). The force of the post-extrasystolic contraction, following premature extrasystolic contractions, was not potentiated in the burbot heart (not shown).

Analogous to the recovery of contractile force, cardiac relaxation also follows a pattern of restitution, which can be determined from the time derivative of the force recording, dF/dt_{min} (Fig. 3B). Under control conditions, the time constants (τ) of relaxation restitution were 460±75 ms and 608±25 ms (*N*=6; *P*>0.05) for atrium and ventricle, respectively. Ryanodine had no effect on the time constants, which were 530±81 ms and 540±91 ms (*N*=6) (*P*>0.05) for atrium and ventricle, respectively.

Rest-potentiation represents the slow phase of mechanical restitution at diastolic intervals longer than the regular pacing interval. The first contraction after the prolonged diastolic interval (10–60 s) is bigger than the preceding control contraction and the potentiation dissipates gradually during the consecutive 15 beats (Fig. 4A). The maximum rest-potentiation was achieved at the diastolic interval of 60 s and was slightly stronger in atrial (154±8 %) than ventricular (122±2 %) preparations (*P*<0.05) (Fig. 4C). The rest-potentiation was completely abolished by

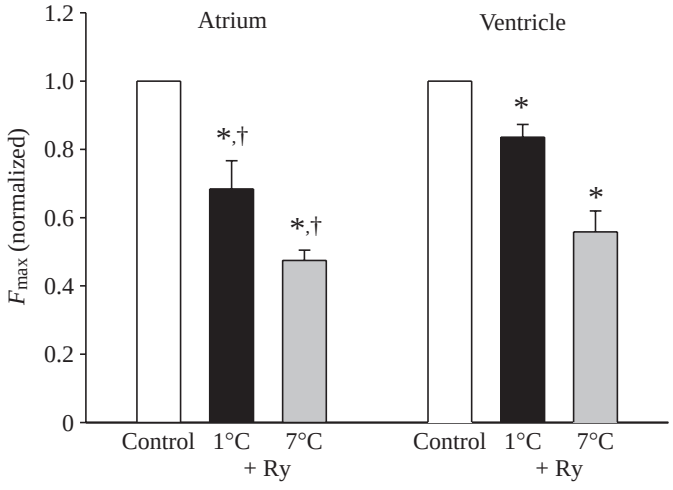

Fig. 2. Effect of ryanodine on the contractility (maximum developed force F_{max}) of burbot atrial and ventricular muscle at 1 ° and 7 °C, normalized against control value (at 1 or 7 °C). Values are means ± S.E.M. of 5 preparations for both atrial and ventricular tissue. *Statistically significant difference (*P*<0.05) between values of control at both 1 °C and 7 °C and after ryanodine treatment (+Ry); †, between atrial and ventricular muscle.

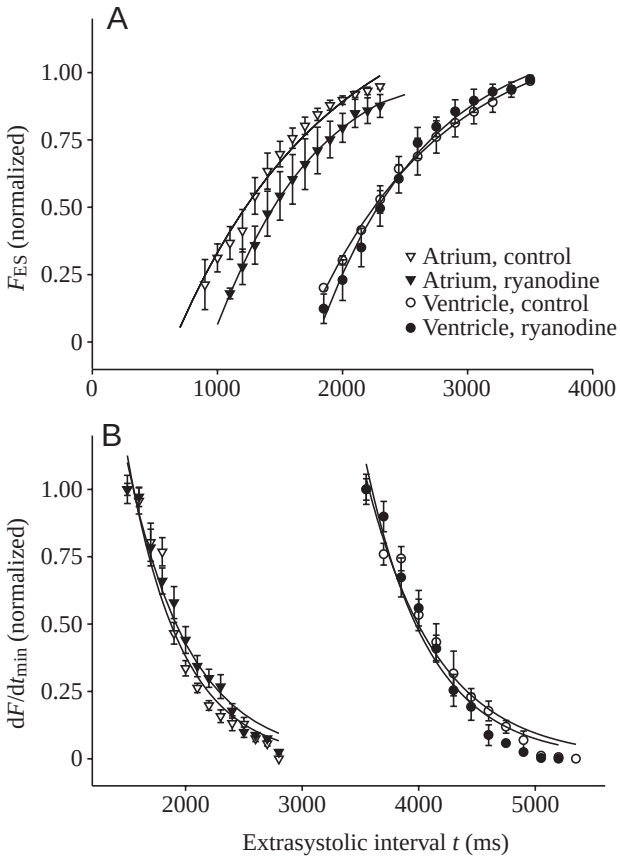

Fig. 3. Force and relaxation restitution of the burbot atrial and ventricular muscle in the absence (open symbols) and presence (filled symbols) of 10 μmol l^{-1} ryanodine. F_{ES} (A) and dF/dt_{min} (B) are plotted as a function of the extrasystolic interval (t) and the plots were fitted by single exponential equations. Values are means ± S.E.M. of 5 preparations. The experiments were conducted at 1±0.5 °C. See Materials and methods for details.

ryanodine and, in fact, turned it to a rest-decay; the first contraction after the rest was smaller than the control contraction and was followed by gradual increase of force to the steady-state level during the following 15 beats (Fig. 4C). At the rest-period of 60 s, the force of the first post-rest contraction in ryanodine-treated preparations was only 72±2 % in atrium and 63±9 % in ventricle from the force of the control (P<0.05) (Fig. 4A). In atrial muscle, the duration of the first post-rest contraction was the same as that of the preceding control contraction and ryanodine increased both. In ventricular preparations, the first post-rest contraction was longer in duration than the control contraction and it became shorter during the following 15 beats, irrespective whether the force declined (control) or increased (ryanodine). Thus, the duration of contraction was positively correlated with F_{max} in control preparations but negatively correlated in ryanodine-treated preparations (Fig. 4B).

Myofibrillar Ca^{2+}/Mg^{2+}-ATPase activity

The activity of myofibrillar ATPase was determined at four different temperatures. The Ca^{2+}/Mg^{2+}-ATPase activity was significantly higher in atrial than in ventricular preparations (P<0.05) and the temperature optimum of the ATPase was 10 °C in both tissues (Fig. 5). Furthermore, the temperature dependence of the ATPase activity was remarkably strong between 1 and 10 °C (Q_{10} as great as 15) and much less between 10 and 15 °C (Q_{10} approx. 0.5).

Blood composition

Blood composition was determined from nine burbot. Blood analysis showed a Hb value of 67.2±4.0 g l^{-1} and Hct was 31.6±1.63 %, yielding MCHC 212.7±7.4 g l^{-1}. The concentration of plasma protein was 9.4±0.7 g l^{-1}.

Discussion

Functional characteristics of the burbot heart

Low temperature reduces cardiac contractility and increases the viscosity of the blood. Without any compensatory changes in cardiac function, structure of microvasculature or flow properties of the blood, circulation would be severely compromised in the cold. The compensatory changes of the fish heart to the cold include an increase in heart size (Goolish, 1987; Driedzic et al., 1996; Graham and Farrell, 1989), higher frequency of beating (see Farrell, 1984) and faster rate of contraction (Driedzic, 1992; Aho and Vornanen, 1999). In Fig. 6, heart rate, duration of contraction of spontaneously beating heart and atrial ATPase activity of myofibrils are compared under the same experimental conditions in three different fish: cold-active and relatively eurythermal rainbow trout, cold-dormant and very eurythermal crucian carp, and cold-active and stenothermal burbot. It is clear that the burbot heart is more similar to the rainbow trout heart than to crucian carp heart with respect to both heart size and functional parameters. The large heart (Tiitu and Vornanen, 2002), high heart rate and relatively fast myosins in burbot (present study) are in apparent contradiction to the low standard metabolic rate of the burbot (Pääkkönen and Lyytikäinen, 2000). Therefore, these characteristics of the burbot heart are probably adaptations that maintain cardiac activity and adequate rate of circulation at temperatures approaching 0 °C.

Temperature tolerance of cardiac function is similar in burbot and cold-acclimated (4 °C) (Aho and Vornanen, 1999) rainbow trout, since arrhythmic contractions appear at approximately 18 °C in both species. More experiments are needed, however, to clarify whether acclimation to higher temperatures can increase thermal tolerance of the burbot heart, as happens in eurythermal rainbow trout and crucian carp (Matikainen and Vornanen, 1992; Aho and Vornanen, 2001). Such experiments would shed light on whether the contractile characteristics of the burbot heart also include a non-genetic component.

Pumping capacity, the product of heart rate and peak force, can be used as an index of power output for isolated muscle preparations since it integrates the effects of changes in tension and heart rate, and usually achieves the maximum value at the preferred temperature of the fish (Matikainen and Vornanen, 1992; Shiels and Farrell, 1997). In agreement with those

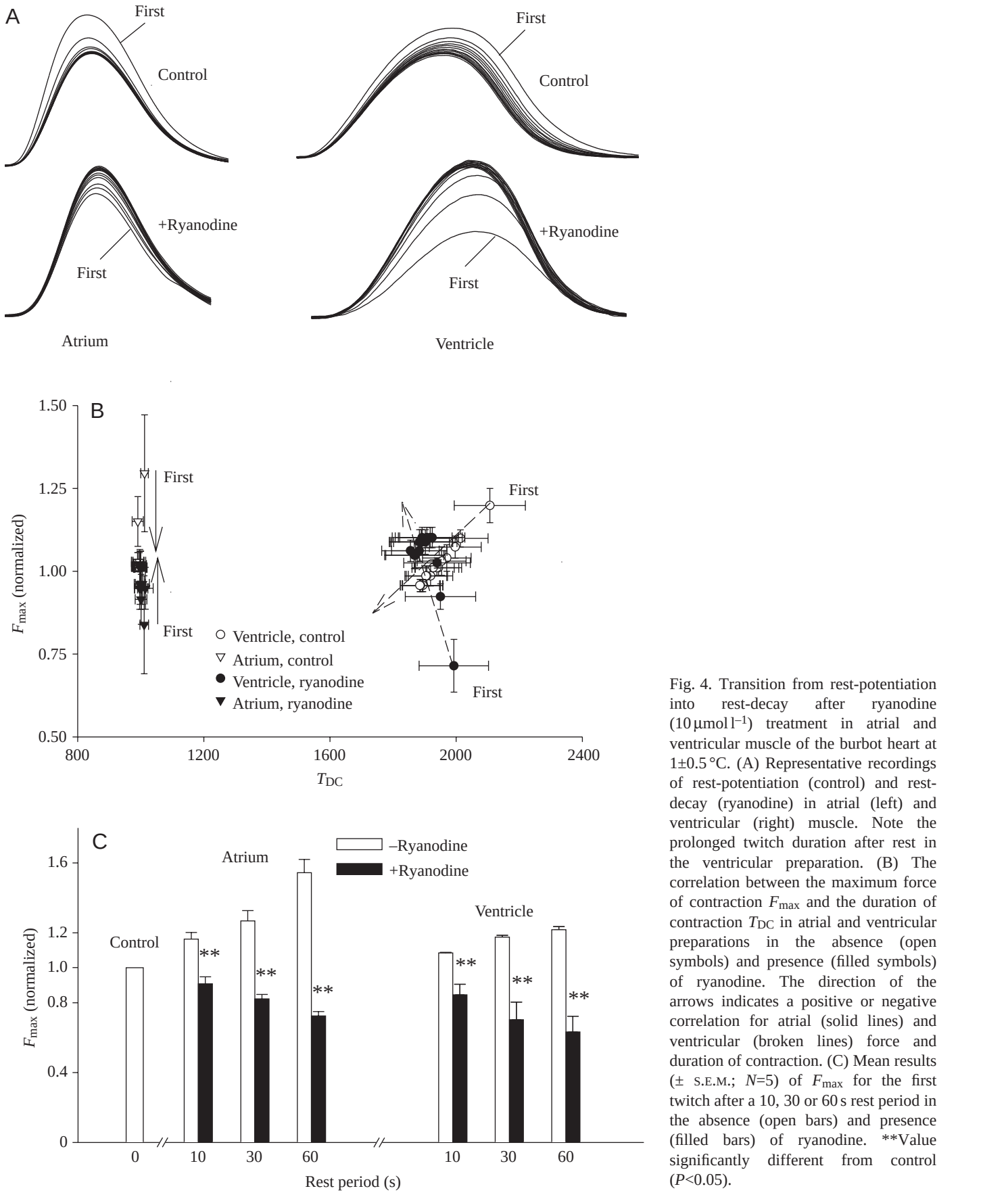

Fig. 4. Transition from rest-potentiation into rest-decay after ryanodine (10 μmol l^{-1}) treatment in atrial and ventricular muscle of the burbot heart at 1±0.5 °C. (A) Representative recordings of rest-potentiation (control) and rest-decay (ryanodine) in atrial (left) and ventricular (right) muscle. Note the prolonged twitch duration after rest in the ventricular preparation. (B) The correlation between the maximum force of contraction F_{max} and the duration of contraction T_{DC} in atrial and ventricular preparations in the absence (open symbols) and presence (filled symbols) of ryanodine. The direction of the arrows indicates a positive or negative correlation for atrial (solid lines) and ventricular (broken lines) force and duration of contraction. (C) Mean results (± S.E.M.; N=5) of F_{max} for the first twitch after a 10, 30 or 60 s rest period in the absence (open bars) and presence (filled bars) of ryanodine. **Value significantly different from control (P<0.05).

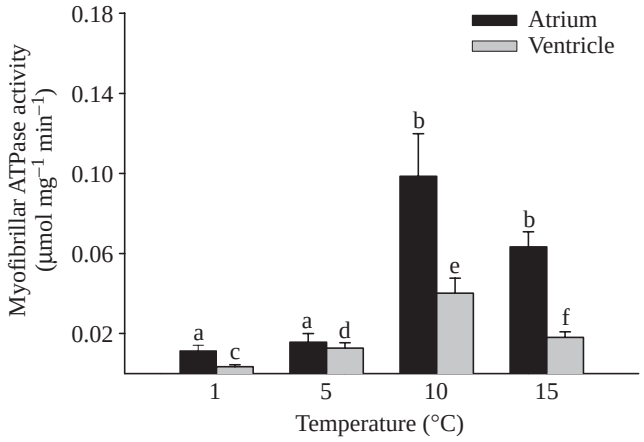

Fig. 5. Ca^{2+}/Mg^{2+}-ATPase activity of the purified atrial and ventricular myofibrils of the burbot heart. The experiments were conducted at four different temperatures (1, 5, 10 and 15 °C). Dissimilar letters indicate statistically significant differences between different temperatures. Values are means ± S.E.M. of 5–7 preparations.

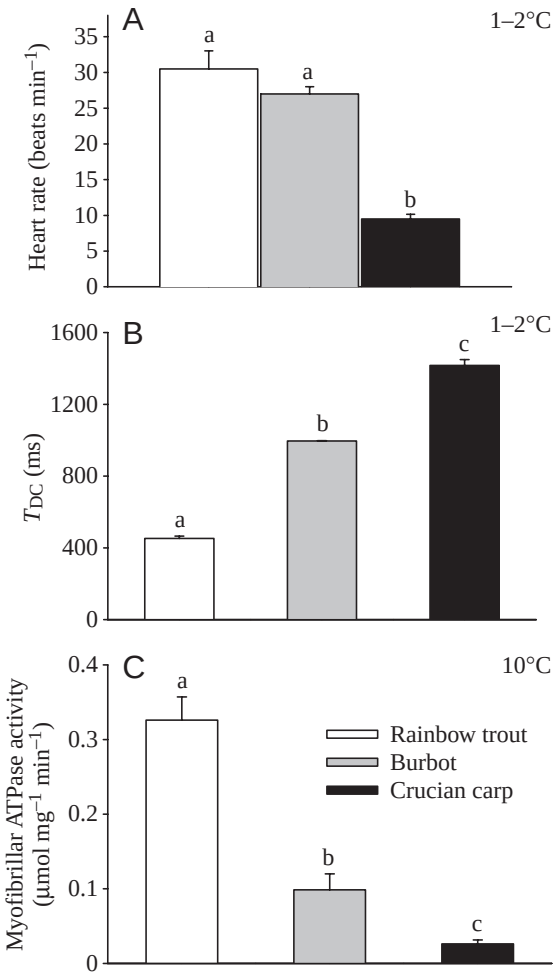

Fig. 6. Species-specific differences in heart rate (A), duration of contraction T_{DC} (B) and activity of myofibrillar ATPase of atrial muscle (C) between cold-acclimated (4 °C) rainbow trout, cold-acclimated (4 °C) crucian carp and cold-adapted (1 °C) burbot. Dissimilar letters indicate statistically significant differences (P<0.05) between species. Values are means ± S.E.M. of 5–7 preparations as indicated. The data for myofibrillar ATPase activity (C) of rainbow trout and crucian carp are from Aho and Vornanen (1999) and Tiitu and Vornanen (2001), respectively.

studies, pumping capacity of the burbot heart was maximum at the acclimation temperature (1 °C) and declined at higher temperatures. This strongly suggest that the burbot heart is best able to propel blood through the vasculature at near freezing temperatures.

The viscosity of blood is the major factor for the resistance to blood flow (Guyton and Richardson, 1961) and is largely determined by the number of red blood cells and concentration of proteins in the plasma. In most teleosts the Hct values are greater than 20 % and Hb concentration is between 4 and 15 g % (see Gallaugher and Farrell, 1998). The Hct (32 %) and Hb (6.7 g %) of the burbot blood seem to be slightly less than in many active teleosts, but not compared to the values of other sluggish fish (see Gallaugher and Farrell, 1998). Some caution is needed, however, when making interspecies comparisons, because handling stress and method of blood sampling can effect Hct values (Franklin et al., 1993). The total protein concentration in the burbot plasma (10.7 g l^{-1}) is remarkably low when compared to the concentration (20 to 80 g l^{-1}) of most other teleosts (see McDonald and Milligan, 1992). On the basis of Hct and protein values, it seems the burbot blood is slightly diluted, which could reduce its viscosity and the resistance to blood flow at the expense of oxygen-carrying capacity. In this respect the burbot heart has similarities to the ‘volume pumps’ of the haemoglobin-free Antarctic ice-fish, which circulate large volumes of dilute blood at sub-zero temperatures (Tota et al., 1998).

Subcellular mechanism of cold-adaptation

In addition to the characteristics of the volume pump, in many respects (heart rate, contraction velocity, ryanodine sensitivity) the burbot heart resembles the ‘power pump’ of the cold-acclimated rainbow trout heart and other cold-active fish. Some of the mechanisms that underlie the improved contractility in cold-acclimated fish heart include enhanced Ca^{2+} management by the SR (Bowler and Tirri, 1990; Keen et al., 1994; Aho and Vornanen, 1998, 1999). In cold-acclimated rainbow trout, the rate of Ca^{2+} uptake into the SR is enhanced (Aho and Vornanen, 1998), thereby increasing the relative importance of SR Ca^{2+} release in excitation–contraction coupling (Keen et al., 1994; Aho and Vornanen, 1999). SR Ca^{2+} uptake was not directly assessed in the burbot heart, but ryanodine inhibition of contraction force indicates that Ca^{2+} release from the SR contributes to the activation of contraction in this cold-active, stenothermal fish. Indeed, the ryanodine inhibition of force is somewhat larger in burbot (1 °C) than trout (4 °C) heart, in both atrial (32 % *versus* 20 %) and ventricular (17 % *versus* 6 %) muscle (present results; Aho and Vornanen, 1999). Thus, it is clear that in the cold-active fish,

cardiac excitation–contraction coupling is modified so that the myocyte relies more on the SR as a source of activator Ca^{2+}.

Since the effect of ryanodine is frequency-dependent and the burbot preparations were paced at a slightly slower rate (15 beats min^{-1}) than the real heart rate (25 beats min^{-1}), the extent of ryanodine inhibition under physiological conditions remains elusive. It appears, however, that the role of the SR in excitation–contraction coupling of the burbot heart is even more important at warmer (7 °C) temperatures, which agrees with findings from rainbow trout (Hove-Madsen, 1992; Keen et al., 1994; Shiels and Farrell, 1997) and mackerel (*Scomber japonicus*) heart (Shiels and Farrell, 2000). Indeed, at 7 °C (at physiological heart rate) ryanodine abolished about 50 % of the force production, which is similar to the values discovered in the hearts of highly active tunas (Keen et al., 1992; Shiels et al., 1999). Thus movement of the burbot across the thermocline from cold to warm water may change the functional characteristics of the heart from volume pump to those of power pump, which might be more suitable for circulating the less viscous blood.

Quantitative estimations of the relative significance of SR and sarcolemma (SL) Ca^{2+} management on the basis of ryanodine inhibition are not completely accurate and may underestimate the real SR Ca^{2+} release. This is because negative feedback of SR Ca^{2+} release on the SL Ca^{2+} influx is absent in the presence of ryanodine. Furthermore, other Ca^{2+} cycling pathways may compensate for the inhibition of SR in the presence of ryanodine. Direct measurements of intracellular Ca^{2+} on single cardiac cells are needed to resolve these issues. Nevertheless, the present experiments show that the SR Ca^{2+} release is physiologically important for excitation–contraction coupling in the cold-stenothermal burbot and is in agreement with electron-microscopic documentation of well-developed dyadic couplings in the burbot heart (Tiitu and Vornanen, 2002). Furthermore, the present findings indicate clearly that cardiac ryanodine receptors of the ectothermic fish are functional at near freezing temperatures (1 °C) and do not allow leakage of Ca^{2+} from the SR. This is a remarkable difference between fish and mammals. In mammalian cardiac myocytes, ryanodine receptors are locked in the open state at 1 °C and Ca^{2+} leaks out of the SR (Sitsapesan et al., 1991). The molecular basis of this interesting difference remains to be shown.

Activity of myofibrillar ATPase is a significant determinant of contraction rate (Barany, 1967). In accordance with previous findings from mammals (see Minajeva et al., 1997) and ectotherms (Deng and Gesser, 1997; Aho and Vornanen, 1999), the myofibrillar ATPase activity was higher in atrial than ventricular muscle and explains, to a large extent, the faster contraction of the atrial muscle. The temperature optimum of the burbot ATPase (10 °C) is much lower than those of cold-acclimated trout and cold-acclimated crucian carp (>15 °C) (Aho and Vornanen, 1999; Tiitu and Vornanen, 2001) and underscores the narrow thermal tolerance of the burbot heart. Furthermore, temperature dependence of the myofibrillar ATPase of the burbot heart was particularly marked below 10 °C. Also in the trout heart, temperature dependence of the myofibrillar ATPase is high at low temperatures (Aho and Vornanen, 1999). Although it is a general biological rule that Q_{10} values are high near zero temperatures (Bennett, 1984), it is unlikely that temperature-sensitivity of the ATPase activity would be as high *in vivo* as we observed *in vitro* since there were no dramatic and abrupt changes in the duration of contraction at temperatures below 10 °C. There may be some modulating factors absent from the purified myofibrils that might regulate ATPase activity *in vivo*. A similar discrepancy between the temperature dependence of myofibrillar ATPase activity and unloaded velocity of shortening was found in myotomal muscle of the bullrout (*Myoxocephalus scorpius* L.) (Johnston and Sidell, 1984). Myosin structure and function of the fish heart are poorly understood and require further research.

Restitution

During cardiac contraction, molecular mechanisms responsible for the initiation of contraction are inactivated. Recovery from inactivation occurs gradually with time and determines the force–interval relationship of cardiac muscle. Mechanical restitution represents the increase in force of contraction associated with progressively longer extrasystolic intervals and is linearly related to time-dependent increases in intracellular Ca^{2+} activator (Wier and Yue, 1986; Cooper and Fry, 1990). In mammalian heart, the activator Ca^{2+} comes primarily from the SR and therefore the time course of restitution is assumed to be due to time-dependent restoration of Ca^{2+} release from the SR. Ca^{2+} release from the SR is influenced by the rate of Ca^{2+}-uptake, extent of Ca^{2+} loading and availability of Ca^{2+} release channels (Fabiato, 1983) and, in principle, any factor involved in these processes could contribute to mechanical restitution. Thus, in mammals, when Ca^{2+} release from the SR is impaired by ryanodine, the rate of restitution increases. This is because SL Ca^{2+} influx through L-type Ca^{2+} channels, with faster recovery kinetics than SR processes, becomes the limiting step in restitution (Cooper and Fry, 1990; Prabhu, 1998).

Although ryanodine decreased the force of steady-state contraction in ventricular (16 %) and atrial (32 %) muscle, the rate of restitution in burbot heart was not influenced by ryanodine, which contrasts with previous findings on trout cardiac muscle. In the cold-acclimated rainbow trout, where the steady-state force of contraction was inhibited by 6 % and 17 % in ventricle and atrium, respectively, ryanodine clearly prolonged MRP and reduced the rate of restitution (Aho and Vornanen, 1999). The findings on trout cardiac preparations indicate that mechanical restitution is a relatively sensitive indicator of the contribution of SR Ca^{2+} to contraction and that in the trout heart the ryanodine-sensitive component of restitution is faster than the voltage-dependent component (i.e. the opposite to the situation in mammals). The present findings with burbot heart suggest that either the contribution of SR Ca^{2+} release to the rate of restitution is rather small and has therefore previously been unnoticed, or the recovery rates

of SR and SL mechanisms from inactivation are similar and not easily separated from each other. In this context it should be noted that rate constants of mechanical restitution are strongly temperature-dependent (Aho and Vornanen, 1999), and are much larger in the burbot (approximately 700 ms at 1 °C) than trout (200–300 ms at 4 °) heart. Furthermore, in the burbot heart, the rate of restitution was very similar in atrium and ventricle, whereas in trout atrial restitution was much faster than ventricular restitution (Aho and Vornanen, 1999).

Relaxation restitution is the increase in the rate of atrial and ventricular relaxation with progressively longer extrasystolic intervals. In mammalian heart, where the major part of activator Ca^{2+} is recycled through the SR, relaxation restitution is governed by SR Ca^{2+}-uptake. Accordingly, the rate of relaxation restitution is decreased by ryanodine and increased in transgenic mice lacking phospholamban, the inhibitory regulator of SR Ca^{2+}-ATPase (Prabhu, 1998; Hoit et al., 2000). The absence of any ryanodine effect on relaxation restitution in burbot suggests that SR Ca^{2+} uptake is not an important determinant for the recovery of relaxation from inactivation in this species. Although over 30 % of the activating Ca^{2+} in the atrial muscle recycles through the SR at the pacing rate of 0.25 Hz, no ryanodine-sensitive component of relaxation restitution was found. It is, however, possible that the ability of the SR to take up Ca^{2+} is impaired at short extrasystolic intervals and that the inhibitory effect of ryanodine appears only at the steady-state frequency of 0.25 Hz or lower. The SL Na^{+}-Ca^{2+} exchange and SR Ca^{2+}-pump are the major Ca^{2+} removal pathways in cardiac myocytes and therefore the ryanodine-resistant component of relaxation restitution in burbot is probably related to the operation of the Na^{+}–Ca^{2+} exchange, with little contribution by the SR Ca^{2+} pump. As the Na^{+}–Ca^{2+} exchange is voltage-dependent, the rate of relaxation restitution should describe the recovery of the action potential from inactivation. The similarity of time constants for mechanical and relaxation restitution suggests that both processes might be controlled by the same mechanisms, possibly the membrane potential of the SL. Clearly single-cell experiments are needed to clarify the excitation-contraction coupling of the burbot heart.

The slow phase of mechanical restitution appears as rest-potentiation. In mammals, increase in force at the post-rest contraction is associated with the larger release of Ca^{2+} from the SR (Lewartowski and Zdanowski, 1990; Bassani et al., 1995). The potentiation is not, however, associated with a higher SR Ca^{2+} load, but is due to a larger fractional release, i.e. the same Ca^{2+} trigger releases a larger proportion of the SR Ca^{2+} content (Bassani and Bers, 1994; Bouchard and Bose, 1989). The force of post-rest contraction decreases with the duration of rest period, which is known as rest-decay (Allen et al., 1976; Bers, 1985), and is related to the leak of SR Ca^{2+} into the cytoplasm, where it is extruded by the Na^{+}–Ca^{2+} exchange (Bers and Christensen, 1990). Furthermore, rest-potentiation and rest-decay are interconvertible by modulating the intracellular Na^{+} concentration, suggesting that SR Ca^{2+}-pump and the Na^{+}–Ca^{2+} exchange compete for the same Ca^{2+} and, depending on the relative competitiveness of the two systems, either rest-potentiation or rest-decay is expressed. Accordingly, ryanodine abolishes rest-potentiation in the mammalian cardiac muscle and reveals the underlying rest-decay (Bers, 1985). Like mammalian myocardium, the burbot atrium and ventricle show rest-potentiation, which is transformed into rest-decay in the presence of ryanodine. According to the mammalian restitution model, under normal control conditions the SR Ca^{2+}-pump is more powerful than Na^{+}–Ca^{2+} exchange in removing Ca^{2+} from the cytosol of the resting myocytes, but after ablation of the SR by ryanodine, Na^{+}–Ca^{2+} exchange remains the only relaxation mechanism and extrudes Ca^{2+} from the cell. In addition to the SR, other intracellular Ca^{2+} buffers are also partially depleted of Ca^{2+} during the rest, since the first post-rest contraction is weaker than the steady-state contraction. The depletion of intracellular Ca^{2+} buffers might explain the inability of SL mechanisms to activate full-strength twitch immediately after the rest and the negative correlation between force and duration of contraction. In brief, the strong effect of ryanodine on the force of the first post-rest contraction indicates the potential power of the SR in regulating myoplasmic Ca^{2+}, but at the same time reveals the effectiveness of SL mechanisms in regulating the Ca^{2+} management of the myocyte with only a small reduction in amplitude and without changes in time-course of contraction.

Conclusions

This study demonstrates that the burbot heart works best at its physiological body temperature (1 °C) and that the low temperature optimum of the heart is associated with significant dependence on SR Ca $^{2+}$ stores for contractile activation. This indicates that the ryanodine receptors of the burbot heart are operative at near freezing temperatures and thus functionally different from the ryanodine-receptors of mammalian heart, which become leaky at cold temperatures. In many respects (heart rate, contraction velocity, ryanodine sensitivity) the burbot heart resembles the ‘power pump’ of the cold-acclimated rainbow trout heart and other cold-active fish. It therefore seems that cardiac function at low temperatures is governed by similar subcellular mechanisms in both eurythermic and stenothermic teleosts.

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