Comparative Biochemistry and Physiology, Part A 163 (2012) 66–73

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

The temperature challenges on cardiac performance in winter-quiescent and migration-stage eels *Anguilla anguilla*

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ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 8 May 2012
Accepted 8 May 2012
Available online 12 May 2012

Keywords:
A. anguilla
Adrenaline
Contractility
Relative ventricular mass
Temperature
Spawning migration
Winter-quiescence

ABSTRACT

The present study was undertaken to examine cardiac responses to some of the temperature challenges that eels encounter in their natural environment. The contractile properties of ventricular muscle was studied on electrically paced tissue strips after long term acclimation at 0 °C, 10 °C, or 20 °C, and following acute ± 10 °C temperature changes. The time-course of contraction, and thus maximal attainable heart rates, was greatly influenced by working temperature, but was independent of acclimation history. The absolute force of contraction and power production (i.e. the product of force and stimulation frequency) was significantly influenced by acute temperature decrease from 20 °C to 10 °C. The role of adrenaline as a modulator of contraction force, power production, rates of contraction and relaxation, and minimum time in contraction was assessed. Increased adrenergic tonus elicited a positive inotropic, temperature-dependent response, but did not influence twitch duration. This suggests that adrenaline acts as an agent in maintaining an adequate contractile force following temperature challenges. A significant increased relative ventricular mass was observed in 0 °C and 10 °C-acclimated eels compared to 20 °C-acclimated, which suggests that at low temperatures, eels secure cardiac output by heart enlargement. Inhibition of specific sarcolemmal Ca^{2+} channels by selective drug treatment revealed that, depending on temperature, L-type channels is the major entry site, but also that reverse-mode Na^{+}/Ca^{2+}-exchange and store operated calcium entry contribute to the pool of activator Ca^{2+}.

1. Introduction

The European eel (*Anguilla anguilla*) is an eurythermal fish which tolerates temperatures ranging from sub-zero to above 30 °C. For instance, during their spawning-migration to the Sargasso Sea, eels are faced with a gradual increase in temperature (McCleave, 2003; Aarestrup et al., 2009). Recently, it was reported that migrating eels perform diel vertical migration of up to 400 m, diving into colder waters (~6–8 °C) during the day and ascending to shallow warmer waters (12–14 °C) at night (Aarestrup et al., 2009). Meanwhile, non-migrating individuals are faced with decreased temperatures during fall and winter. When the temperature starts to decrease in the fall, eels cease activity, eventually become torpid and bury themselves in the sediment until spring where their activity increases along with the rise in temperature (Bertin, 1956; Nyman, 1972; Walsh et al., 1983). Under these conditions eels are able to survive to temperatures down to, or below 0 °C.

At all temperatures faced by the eel, a match must exist between activity levels and cardiac performance that may need to be modulated accordingly. A common, but not universal response to low seasonal temperatures is an increase in relative ventricular mass (RVM) or cardiac somatic index, but no clear correlation between cardiac enlargement and (in)activity during winter has been established (Driedzic et al., 1996). Thus cardiac growth has been observed in several species e.g. rainbow trout (Tsukuda et al., 1985; Graham and Farrell, 1989), goldfish (Tsukuda et al., 1985), common carp (Goolish, 1987), and sea raven (Graham and Farrell, 1985), while little or no change in RVM has been observed in other species like the crucian carp (Matikainen and Vornanen, 1992), white perch and yellow perch (Sephton and Driedzic, 1991). Intracellular responses to cold temperatures in fish include ultrastructural changes e.g. in mitochondrial volume (Rodnick and Sidell, 1997), expression of different myosin heavy chain isoforms (Vornanen, 1994), myofibrillar ATPase activity (Aho and Vornanen, 1999; Tiitu and Vornanen, 2001; Shiels et al., 2002), increased expression of β-adrenoreceptors (Keen et al., 1993) and increased SERCA2 activity and protein expression (Landeira-Fernandez et al., 2004). The effect of low temperature on excitation–contraction coupling in the fish myocardium includes prolonged action potential duration (APD) and slow deactivation of the SL L-type Ca^{2+} channels (Shiels et al., 2002). Adrenaline (AD) has long been recognized as a modulator of cardiac performance in fish (Nilsson, 1983; Farrell and Jones, 1992). In addition to regulating heart rate, cardiac contractility is also supported

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doi:10.1016/j.cbpa.2012.05.183

by AD, this by increasing the sarcolemmal (SL) Ca^{2+} influx through the L-type Ca^{2+} channels (Vornanen, 1998; Bers, 2002). In eels, AD has previously been demonstrated to have a positive inotropic effect on contractility under hypoxic and acidotic conditions (Gesser et al., 1982), but not during winter (Pennec and Peyraud, 1983). Contractility is ultimately determined by the flux of Ca^{2+} across the myocyte membrane and within the cell. Generally, the SL L-type channels are the major entry-ways for activator Ca^{2+}, although the Na^{+}/Ca^{2+}-exchanger (NCX), operating in reverse mode, has also been recognized as Ca^{2+} entry in teleosts (Shiels et al., 2002). In the crucian carp, as much as one third to one half of the SL Ca^{2+} influx can be ascribed to reverse mode NCX (Vornanen, 1999). In mammalian skeletal myocytes (Kurebayashi and Ogawa, 2001) and cardiocytes (Huang et al., 2006), another SL Ca^{2+} entry, Store Operated Calcium Entry (SOCE) has been described. SOCE increases the Ca^{2+} loading of the sarcoplasmatic reticulum (SR), and is suggested to play a role when the intracellular stores are low or depleted (Putney, 1986).

Cardiac function and performance at very low temperatures has only been studied in a limited number of species and it is unknown how eels, that are characterized as cold dormant, modulate cardiac function to meet the challenges imposed by low temperature. Moreover, because of the migrating life-stage, that imposes completely different challenges on cardiac performance, the European eel is an interesting species for studying cardiac function in fish.

With the following, we aimed partly to study the cardiac responses of winter-quiescent eels after long-term acclimation to 0 °C. We expected that at this very low temperature, some means of compensation might be necessary to ensure adequate cardiac output and hence survival. We investigated this by examining RVM and cardiac contractility — and how AD might support this. Secondly we aimed to study cardiac performance of migration-stage eels faced with acute temperatures changes, expecting that contractility would be modified as observed in other diving species (Shiels et al., 2002), and that AD would support contractility — especially during a temperature decrease. Finally, we wanted to investigate the individual contributions of three different SL Ca^{2+} entry-ways (L-type channels, NCX and SOCE) to force development, and how this might be affected by temperature.

2. Materials and methods

2.1. Fish origin and care

Silver stage female eels where caught in the Øresund by local fishermen and transported to the Marine Biological Laboratory in Helsingør, Denmark in the fall of 2007. Fish were kept in a large circular tank with a volume of 2500 l and supplied with 10 °C re-circulated 35‰ aerated seawater. The light–dark period was 16 h–8 h. The fish did not eat, although offered a variety of food items. Fish were acclimated to laboratory conditions for a minimum of 2 months prior to the experiments. During the acclimation period, the fish did not eat although a variety of food items was offered. The mass and length the experimental animals was 577.27 ± 151.58 g and 68.48 ± 6.26 cm respectively. For temperature acclimation to 0 and 20 °C, eels were transferred to separate insulated 450 l tanks supplied with 10 °C seawater at a reduced flow. Cooling was achieved by pumping water from the tank through the heat exchanger on a custom-built industrial-grade cooler. Flow of coolant through the heat exchanger was controlled by a motorized 3-way valve connected to a thermostat. Heating was achieved by submerging a set of 1000 W titanium aquarium heaters into the tank, and was regulated by a thermostat. Cooling/heating to desired acclimation temperature was done at a rate of 1 °C per day (Ta). Fish were kept at the final acclimation temperature for at least 4 weeks. The study was carried out in accordance with the Danish Animal Experimentation Act and the protocol was approved by the Danish Animal Experimentation Board (license number: 2004/561-894).

2.2. Tissue preparation

Eels were stunned by a blow to the head and killed by pithing of the spinal cord and brain. The heart was quickly and carefully excised and placed in cold ringer's solution. Four tissue strips (app. 1 mm thick) were cut longitudinally from the ventricle and the compact layer was trimmed. Strips were fixed to tissue holders in organ baths (Myobath, WPI) and connected to force transducers (Fort 10, WPI, Florida, USA). Signals were amplified using a Transbridge 4 M (TBM4M, WPI, USA) amplifier and recorded with the AcqKnowledge software (v.3.8.1 Biopac Systems Inc., California, USA) using a BioPac MP100 system. The bathing medium consisted of (in mM): 150 NaCl, 5.0 KCL, 1.5 $CaCl_2$, 0.17 $MgSO_4$, 0.17 $NaHPO_4$, 2.33 $Na_2H\ PO_4$, 11.0 $NaHCO_3$, 5.0 D-glucose, 10.0 HEPES. pH was adjusted to 7.80 at 10 °C and was allowed to change with the experimental temperature (T_t), (7.85 at 0 °C and 7.76 at 20 °C). The medium was continuously bubbled with pure oxygen throughout the whole of the experiment. A tonic level of adrenaline (1 nM) was added initially to the medium (standard condition). Experimental temperature (0 °C, 10 °C or 20 °C) was controlled by a set of coolers (CBN 8–30; Heto, Denmark), equipped with thermostated heaters (HMT 200; Heto) circulating a coolant. In each experiment, three of four preparations were working at the acclimation temperature while one was working at ±10 °C. The preparations were allowed to hang in the medium for 30 min before being stimulated with a square wave pulse (10 ms duration) at 0.1 Hz with a Grass SD9 stimulator (Grass Product Group, Rhode Island, USA). The stimulus voltage was slowly increased until contractions became apparent, after which the voltage was increased by 50%. To achieve maximum force of contraction, tension was increased by stretching the tissue strips and under these conditions they were allowed to stabilize for another 30 min.

2.3. Experimental protocol

Contraction of the ventricular muscle was studied at four different adrenaline concentrations [AD], (1 nM, 10 nM, 100 nM and 1 mM). Stimulation frequency was increased in steps until contractions became erratic. Data was collected for 5 min at each frequency. Stimulation frequency was then returned to 0.1 Hz and the bathing media was replaced with fresh ringer containing the next [AD]. Tissues were allowed to stabilize for 30 min and the above was repeated for all [AD]. Finally the stimulation frequency was returned to 0.1 Hz and the [AD] in the bathing media was returned to 1 nM.

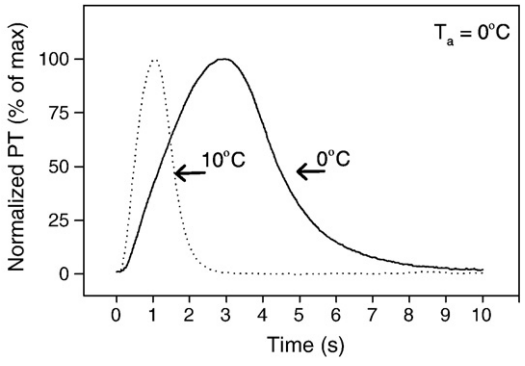

Fig. 1. Trace of isometric tension (PT) development in eel (*A. anguilla*) ventricle tissue strips paced at 0.1 Hz. The trace depicts a single contraction performed at either 0 °C or 10 °C with tissue taken from the same individual (acclimated to 0 °C). Values of peak tension normalized to percent of maximal tension (3.25 and 3.84 mN mm^{-1}, at 0 °C and 10 °C respectively) (see Materials and methods section).

Tissues were allowed 15 min to stabilize upon which, one of three different Ca^{2+} channel blocking agents was added to each separate preparation: Nifedipine, a drug that inhibits the SL L-type Ca^{2+} channels; KB-R7943, that inhibits the reverse mode NCX (both from Calbiochem); and SKF-96365, an inhibitor of SOCE (Cayman Chemical, MI, USA). All agents were added in concentrations of 10 μM according to the supplier's recommendations and previous studies (Vornanen, 1998; Woo and Morad, 2001; Huang et al., 2006). After 15 min 1 mM AD was added to each preparation to examine if this high [AD] could ameliorate the negative effect of the drug. As the blocking protocol was performed at the end of the experiment, some preparations performed poorly and were excluded from further analysis.

2.4. Data analysis

For each 5 min recording, only the last 10 consecutive peaks were used for further analysis. Peak tension (PT) (g), time to peak tension (TPT) (s) and time to half relaxation (THR) (s), was quantified using a custom made Matlab script (MathWorks, Inc., USA). Peak tension was standardized to mN mm^{-2}, where the mean cross-sectional area (A) of the tissues was calculated as $A = M/L \times 1.06$, where M is tissue mass in mg, L is the length of the strip in mm when positioned in the organ bath and 1.06 is the assumed density of muscle.

2.5. Calculations and statistics

Power production (PP) was calculated as the product of peak tension (mN mm^{-2}) and contractions per minute according to (Matikainen and Vornanen, 1992). Contraction rate (TR) and relaxation rate (RR) was calculated as PT/TPT and PT/THR respectively. The product of heart rate (min^{-1}) and the sum of TPT and THR was expressed as the variable minimum time in contraction (MTIC). The effects of temperature change and AD were assessed by a two-way repeated measures ANOVA, with pacing frequency as the second variable followed by the Holm–Sidak multi comparisons procedure (SigmaPlot v. 11, Systat systems inc. USA). For all statistics, a $p<0.05$ was considered significant. All data are presented as mean values ± SEM. Data points with $N<3$ were included in tables and graphs supplementary, but were not included in the statistical analysis.

3. Results

3.1. Acclimated to 0 °C

RVM was significantly higher (one-way ANOVA and Tukey post hoc test) in eels acclimated to 0 °C and 10 °C, being 0.073 ± 0.015 and 0.075 ± 0.016 respectively, compared to eels acclimated to 20 °C with a RVM of 0.049 ± 0.01. Twitch duration at 0 °C lasted approx. 8 s (Fig. 1), allowing a maximal stimulation frequency (F_{STIM}) of less than 0.2 Hz, as only few preparations could contract at 0.2 Hz (Table 1). Following an acute temperature increase to 10 °C, the twitch duration was significantly shortened and lasted ~3 s (Fig. 1). Rates of contraction and relaxation increased (Table 1), with Q_{10} values [$(R_1/R_2)^{(10/(T_1-T_2))}$] of 2.4 ± 0.2 and 2.0 ± 0.2 respectively (at 0.1 Hz), whereas peak tension and power production were not significantly affected by an increase in test temperature (Fig. 2A, B). AD elicited an inotropic response at 0 °C, with an increase in peak tension and power production of ~50% at the highest [AD] (Fig. 2C, D). Contraction and relaxation rates increased significantly at the high [AD] (Table 1) while MTIC was unaffected by adrenergic stimulation. A similar responsiveness to AD was not observed when tested at 10 °C. Blocking the SL Ca^{2+} influx via L-type channels with Nifedipine caused a significant reduction in peak tension, which was not abolished by addition of 1 mM AD (Fig. 3). Treatment with SKF-96365 a blocker of SOCE also caused a significant, albeit smaller reduction in peak tension, whereas blocking the reverse mode NCX Ca^{2+} entry with KB-R7943 had no effect on peak tension.

Table 1
Kinematic variables in eel (*A. anguilla*) ventricle tissue strips at 0 °C or 10 °C. Eels were acclimated to 0 °C and paced to contract at tonic (1 nM) or high AD concentration (1000 nM). Rates of contraction and relaxation expressed as mN mm^{-2} s^{-1}, and minimum time in contraction as s min^{-1}. An asterisk denotes significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). A dagger denotes a significant difference ($p<0.05$) between working temperatures. Values are mean ± s.e.m. (see Materials and methods section).

T_a/T_t (°C)	[AD] (nM)	F_{STIM} (Hz)	
		0.1	0.2
0/0	1 TR (mN mm^{-2} s^{-1})	2.28 ± 0.57	2.06 ± 0.87
	RR (mN mm^{-2} s^{-1})	5.40 ± 1.14	4.81 ± 1.71
	MTIC (s min^{-1})	22.99 ± 1.75	43.00 ± 0.36
	N	6	2
	1000 TR (mN mm^{-2} s^{-1})	3.14 ± 1.01*	2.45 ± 1.02
	RR (mN mm^{-2} s^{-1})	8.44 ± 2.21*	5.78 ± 2.40
	MTIC (s min^{-1})	25.28 ± 1.83	43.97 ± 1.59
	N	6	4
0/10	1 TR (mN mm^{-2} s^{-1})	5.86 ± 1.30†	6.91 ± 1.38†
	RR (mN mm^{-2} s^{-1})	11.85 ± 2.64†	13.82 ± 2.30†
	MTIC (s min^{-1})	10.89 ± 0.74†	19.51 ± 0.84†
	N	5	3
	1000 TR (mN mm^{-2} s^{-1})	6.45 ± 1.63	8.06 ± 1.71
	RR (mN mm^{-2} s^{-1})	13.20 ±	16.73 ± 3.49
	MTIC (s min^{-1})	11.17 ± 0.79	19.33 ± 0.67
	N	4	4

3.2. Acclimated to 10 °C or 20 °C

Twitch duration was affected by the acute change in temperature, i.e. prolonged after a decrease and shortened after an increase, but there were no differences in twitch duration between tissues working at the same temperature (Fig. 4). Preparations from 10 °C-acclimated

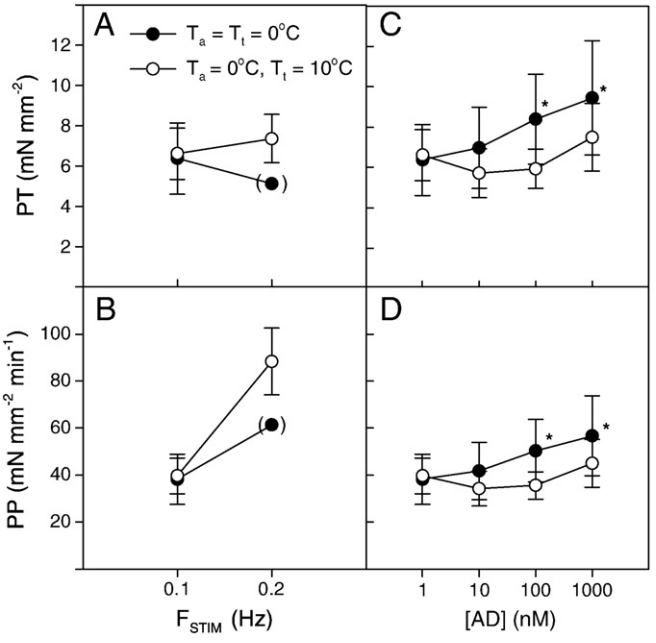

Fig. 2. Peak tension and power production in eel (*A. anguilla*) ventricle tissue strips at 0 °C or 10 °C. Eels were acclimated to 0 °C and the working temperature was either 0 °C or 10 °C. Left side: A) the force–frequency response and B) the power production as a function of pacing frequency. Right side: the effect of increased adrenergic tonus on C) peak tension and D) power production in strips paced at 0.1 Hz. Values presented are mean ± s.e.m. ($N = 2–6$). An asterisk signifies significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). Brackets denotes data points with $N<3$ (see Materials and methods section).

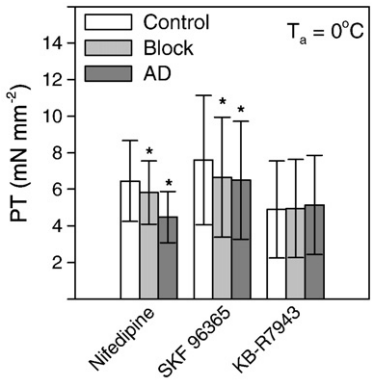

Fig. 3. Peak tension in eel (*A. anguilla*) ventricle tissue strips treated with Ca^{2+}-channel blocking agents. The acclimation and working temperature was 0 °C, and strips were paced at 0.1 Hz. Height of bars represents means and error bars s.e.m. (N=4–5). An asterisk signifies significant difference ($p<0.05$) from control conditions (see Materials and methods section).

animals did not display any changes in the force of contraction when working at 20 °C compared to 10 °C (Fig. 5A), consequently there was no difference in power production between the two working temperatures (Fig. 5B). The shortened twitch duration at the increased temperature significantly increased both contraction and relaxation rates with Q_{10} values of 2.2±0.2 and 1.8±0.2 (at 0.1 Hz) respectively (Table 2). A significant inotropic response to AD was observed at the high concentrations (100 and 1000 nM) only in tissues performing contractions at 10 °C (when compared at 0.1 Hz) (Fig. 5C, D). A decrease in temperature, from 20 °C to10 °C, increased contraction force with a resultant significant increase in power production (Fig. 6A, B) and decreased rates of contraction and relaxation with Q_{10} of 1.5±0.4 and 1.4±0.2 respectively at 0.1 Hz (Table 3). Similarly, a significant inotropic response to AD was observed at 100 and 1000 nM (Fig. 6C, D) in tissues performing contractions at 10 °C

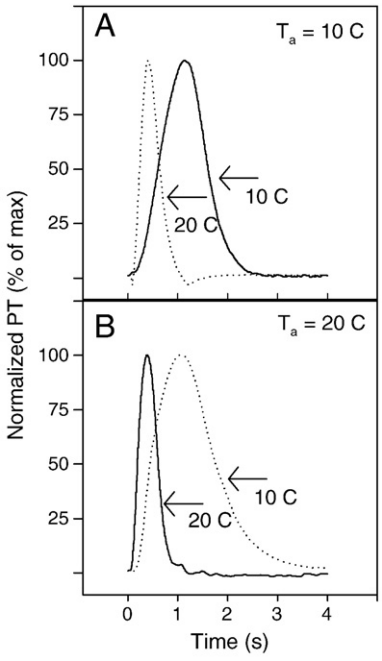

Fig. 4. Traces of isometric tension development in eel (*A. anguilla*) ventricle tissue strips paced at 0.1 Hz. The traces depict single contractions at either 10 °C or 20 °C by tissue taken from the same individual. A) Acclimated to 10 °C. B) Acclimated to 20 °C. Values of peak tension normalized to percent of maximal tension (in A, 7.53 and 4.4156 mN mm^{-1} at 10 °C and 20 °C respectively, and in B, 5.05 and 5.56 mN mm^{-1} at 10 °C and 20 °C respectively) (see Materials and methods section).

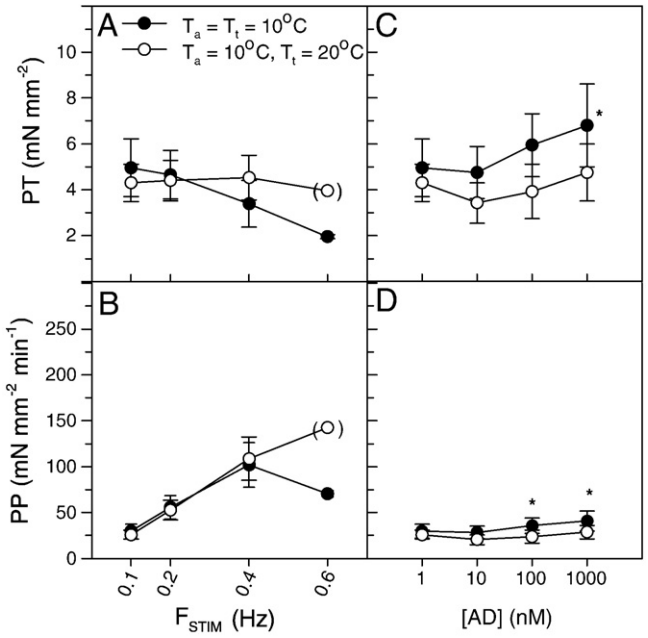

Fig. 5. Peak tension and power production in eel (*A. anguilla*) ventricle tissue strips at 10 °C or 20 °C. Eels were acclimated to 10 °C and the working temperature was either 10 °C or 20 °C. Left side: A) the force–frequency response and B) the power production as a function of pacing frequency. Right side: the effect of increased adrenergic tonus on C) peak tension and D) power production in strips paced at 0.1 Hz. Values presented are mean±s.e.m. (N=2–7). An asterisk signifies significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). Brackets denote data points with $N<3$ (see Materials and methods section).

only (paced at 0.1 Hz). At higher pacing frequencies, however, a significant inotropic response was also observed in tissues working at 20 °C (Fig. 7). Moreover, at both test temperatures, the magnitude of the inotropic response was positively correlated with pacing frequency. The time-course of contraction was unaffected by adrenergic stimulation so as a consequence of the increased peak tension, rates of contraction and relaxation were also increased (Table 3, 4). Treatment with Nifedipine (L-type channel blocker) caused a considerable and significant reduction in peak tension at both 10 °C and 20 °C, which was not abolished by the addition of AD. Inhibition of reverse mode NCX as well as SOCE caused a negative inotropic response at both temperatures. This effect, unlike that of Nifedipine, could be reversed by adrenergic stimulation (Fig. 8).

4. Discussion

4.1. Acclimated to 0 °C

Acclimation to cold temperatures (4 °C) reduces the heart rate, increases the duration of contraction and the refractoriness of the heart in the cold dormant crucian carp, but does not stimulate cardiac growth. These responses are considered an adaptive strategy to minimize energy expenditure during extreme winter conditions (Matikainen and Vornanen, 1992; Tiitu and Vornanen, 2001). The present observations on eels after cold acclimation partly resemble those of the crucian carp in that the time-course of contraction was noticeably prolonged thus allowing a maximal heart rate between 6 and 12 beats per minute. No in vivo heart rates, at 0 °C, have so far been reported for *A. anguilla*, however, Seibert (1979) recorded a resting heart rate of ~12 bpm at 5 °C in unrestrained eels. This value corresponds well with the present results, and with a study on the closely related American eel (*Anguilla rostrata*), where electrically paced ventricle strips and in situ perfused hearts could not be stimulated to contract above a frequency 18 bpm when tested at 5 °C (Bailey et al., 1991). A significantly increased RVM was presently

Table 2
Kinematic variables in eel (*A. anguilla*) ventricle tissue strips at 10 °C or 20 °C. Eels were acclimated to 10 °C and paced to contract at tonic (1 nM) or high AD concentration (1000 nM). Rates of contraction and relaxation expressed as mN mm^{-2} s^{-1}, and minimum time in contraction as s min^{-1}. An asterisk denotes significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). A dagger denotes a significant difference ($p<0.05$) between working temperatures. Values are mean ± s.e.m. (see Materials and methods section).

T_a/T_t (°C)	[AD] (nM)	F_{STIM} (Hz)			
		0.1	0.2	0.4	0.6
10/10	1 TR (mN mm^{-2} s^{-1})	3.67 ± 0.84	3.79 ± 0.78	3.37 ± 0.94	2.07 ± 0.10
	RR (mN mm^{-2} s^{-1})	9.16 ± 1.99	9.98 ± 2.21	8.67 ± 2.41	5.85 ± 0.12
	MTIC (s min^{-1})	11.04 ± 0.39	19.97 ± 0.55	34.84 ± 0.69	46.19 ± 1.08
	N	7	7	5	3
	1000 TR (mN mm^{-2} s^{-1})	5.10 ± 1.28	6.31 ± 1.61*	7.82 ± 1.86*	4.77 ± 0.31*
	RR (mN mm^{-2} s^{-1})	11.67 ± 2.56*	13.66 ± 2.87*	18.10 ± 3.91*	13.08 ± 0.95*
	MTIC (s min^{-1})	11.26 ± 0.33	20.28 ± 0.44	33.57 ± 0.57	43.16 ± 1.21
	N	7	7	7	3
10/20	1TR (mN mm^{-2} s^{-1})	8.90 ± 2.06†	9.32 ± 2.25†	9.75 ± 2.46†	8.39 ± 0.74†
	RR (mN mm^{-2} s^{-1})	18.89 ± 3.08†	19.92 ± 3.86†	21.25 ± 4.51†	19.02 ± 2.50†
	MTIC (s min^{-1})	4.34 ± 0.11†	8.48 ± 0.23†	16.580.39†	24.30 ± 1.78†
	N	6	6	6	2
	1000 TR (mN mm^{-2} s^{-1})	8.50 ± 2.18	10.41 ± 2.42	12.48 ± 2.71	15.48 ± 3.71
	RR (mN mm^{-2} s^{-1})	21.18 ± 503	24.06 ± 5.14	27.76 ± 5.32	35.56 ± 7.35
	MTIC (s min^{-1})	4.70 ± 4.70	9.21 ± 0.26	17.25 ± 0.41	24.45 ± 1.24
	N	6	6	6	4

observed after cold acclimation demonstrating that eels, unlike the crucian carp, compensate for the depressing effect of low seasonal temperature by increasing the size of the ventricle. Possibly other conditions, like anoxia, have necessitated a different survival strategy in the crucian carp. The specific temperature conditions (e.g. threshold value and/or rate of decrease), at which this response is initiated, is unknown, but evidently it occurs at 10 °C. At this temperature eels also reduce feed intake activity levels (Nyman, 1972; Walsh et al., 1983). The increase in RVM may be viewed as an early response in preparing for winter and possibly makes additional changes (increased contractility) redundant.

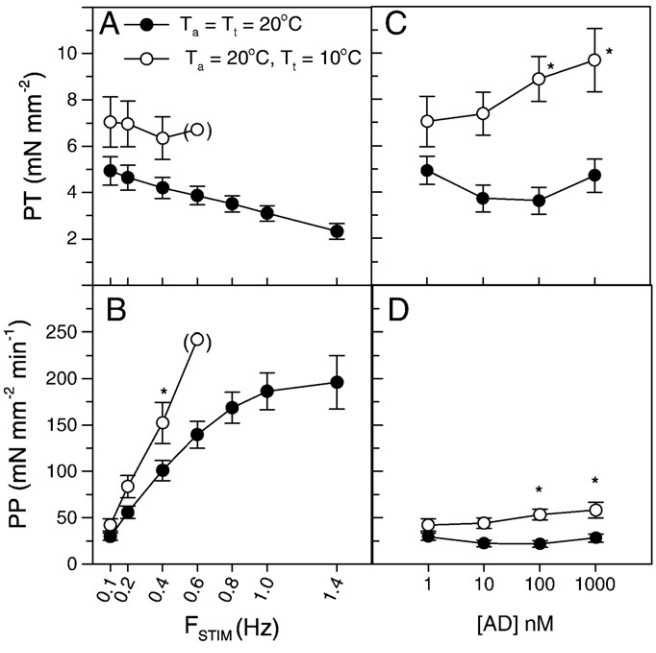

Fig. 6. Peak tension and power production in eel (*A. anguilla*) ventricle tissue strips at 20 °C or 10 °C. Eels were acclimated to 20 °C and the working temperature was either 20 °C or 10 °C Left side: A) the force–frequency response and B) the power production as a function of pacing frequency. An asterisk signifies significant difference ($p<0.05$) between test temperatures. Right side: the effect of increased adrenergic tonus on C) peak tension and D) power production in strips paced at 0.1 Hz. An asterisk signifies significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). Values presented are mean ± s.e.m. ($N=2$–7). Brackets denotes data points with $N<3$. (See materials and methods section).

Adrenergic stimulation did not affect the twitch duration, but it increased the force of contraction. This suggests that eels increase the adrenergic tone as a compensatory mechanism at low temperatures, and that cardiac performance is modulated via inotropic rather than chronotropic adjustments. A similar observation was recently made in two species of Antarctic fishes, *Chaenocephalus aceratus* and *Notothenia coriiceps*, where ventricle tissues working at 0 °C, responded to AD solely by increases in contraction force (Skov et al., 2009). Also, previous studies on *A. anguilla* reported that AD does not have a positive chronotropic effect, instead it prolongs the ventricular action potential duration (APD) and can even have a negative chronotropic effect (Peyraud-Waitzenegger et al., 1980; Pennec and Peyraud, 1983). Furthermore, the reactivity to catecholamines in eels was observed to be seasonally conditioned in that bradycardia and prolonged APD was mediated by a predominance of α-adrenoreceptors in winter-acclimated (8 °C) eels, while tachycardia, mediated by a predominance of β-adrenoreceptors, was observed in summer-acclimated (16 °C) eels (Peyraud-Waitzenegger et al., 1980; Pennec and Peyraud, 1983). More recently, it was observed that a β3-adrenoreceptor agonist caused a negative inotropic response in the eel myocardium (at 20 °C), higher temperatures (Imbrogno et al., 2006). Possibly, the expression of β3-ARs is also influenced by seasonal factors i.e. ambient temperature and the seasonal expression pattern of α- and β-ARs is an interesting subject for future studies.

4.2. Acclimated to 10 °C or 20 °C

In most species examined, an acute temperature decrease, increases the contractile force of the ventricular myocardium thus safeguarding cardiac output when e.g. diving to low ambient temperatures (Shiels et al., 2002). Conversely, increased temperatures have been demonstrated to decrease contractile force or cause a downward shift in the force–frequency curve in e.g. rainbow trout (Shiels and Farrell, 1997; Shiels et al., 2002). In accordance with this general observation, an increase in power production was also observed in eels when the working temperature was acutely decreased from 20 °C to 10 °C and it seems reasonable to interpret this as a mechanism to maintain cardiac output when eels make diurnal excursions to deeper cooler waters (Aarestrup et al., 2009). Unlike the response observed in rainbow trout, increasing the working temperature had no effect on isometric tension and did not cause a downward shift in the force–frequency response when working either at 10 °C or 20 °C (compared to 0 °C or 10 °C). From the present results, it appears that in eels, cardiac output is uncompromised during acute

Table 3
Kinematic variables in eel (*A. anguilla*) ventricle tissue strips at 20 °C or 10 °C. Eels were acclimated to 20 °C and paced to contract at tonic (1 nM) or high AD concentration (1000 nM). Rates of contraction and relaxation expressed as mN mm^{-2} s^{-1}, and minimum time in contraction as s min^{-1}. An asterisk denotes significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). A dagger denotes a significant difference ($p<0.05$) between working temperatures. Values are mean ± s.e.m. (see Materials and methods section).

T_a/T_t (°C)	[AD] (nM)	F_{STIM} (Hz)					
		0.1	0.2	0.4	0.6		1.4
20/20	1 TR (mN mm^{-2} s^{-1})	9.33 ± 1.34	8.92 ± 1.20	8.33 ± 0.97	8.09 ± 0.92	…	5.99 ± 0.90
	RR (mN mm^{-2} s^{-1})	20.34 ± 2.64	20.38 ± 2.49	20.41 ± 2.28	20.68 ± 2.28	…	17.05 ± 2.54
	MTIC (s min^{-1})	4.78 ± 0.25	9.26 ± 0.47	17.42 ± 0.85	24.54 ± 1.14	…	44.89 ± 1.36
	N	7	7	7	7	…	7
	1000 TR (mN mm^{-2} s^{-1})	7.44 ± 1.06	9.53 ± 1.27*	12.46 ± 1.69*	13.96 ± 1.88*	…	13.59 ± 1.84
	RR (mN mm^{-2} s^{-1})	23.43 ± 3.69	27.54 ± 4.34	33.75 ± 5.19	38.40 ± 6.05*	…	41.68 ± 6.24*
	MTIC (s min^{-1})	5.01 ± 0.13	9.45 ± 0.22	17.37 ± 0.42	24.20 ± 0.61	…	44.50 ± 0.65
	N	7	7	7	7	…	7
20/10	1TR (mN mm^{-2} s^{-1})	5.05 ± 0.67†	5.43 ± 0.61†	5.82 ± 0.71	6.95 ± 0.62		
	RR (mN mm^{-2} s^{-1})	12.86 ± 1.99	14.03 ± 2.02	15.49 ± 2.31	21.83 ± 0.95		
	MTIC (s min^{-1})	13.21 ± 1.64†	21.20 ± 0.62†	35.83 ± 0.90†	45.99 ± 0.36†		
	N	7	7	7	2		
	1000TR (mN mm^{-2} s^{-1})	7.37 ± 0.93*	8.69 ± 1.22*	10.07 ± 1.27*	9.63 ± 1.54		
	RR (mN mm^{-2} s^{-1})	17.13 ± 2.95*	18.65 ± 3.32*	23.73 ± 3.69*	25.00 ± 4.42		
	MTIC (s min^{-1})	11.38 ± 0.39	20.90 ± 0.74	35.24 ± 1.07	44.78 ± 0.83		
	N	6	6	7	4		

increases in temperature. This is in support of observations from *A. rostrata* where even an increase in isometric force was observed following an acute increase in temperature from 10 °C to 15 °C (Bailey et al., 1991).

Increasing the adrenergic tone as a regulatory mechanism is more efficient at low temperature in e.g. rainbow trout (Graham and Farrell, 1989; Keen et al., 1993; Farrell et al., 1996; Shiels and Farrell, 1997; Aho and Vornanen, 2001) and pacific mackerel (Shiels and Farrell, 2000). In rainbow trout the β-AR expression is increased at low ambient temperature (Keen et al., 1993) and the SL L-type Ca^{2+}-channel current, I_{Ca}, is more sensitive to AD after an acute temperature decrease (Shiels et al., 2003). On the other hand, the cardiac performance is less sensitive to adrenergic stimulation at warm temperatures (Graham and Farrell, 1989), and in trout atrial cells, I_{Ca} AD sensitivity is decreased when temperature is increased (Shiels et al., 2003). A similar temperature-dependence was observed in the present study, as isometric tension increased following the AD treatment when contracting at 10 °C, but not at 20 °C. However, this was also frequency-dependent as there was a positive inotropic response at both test temperatures when paced at higher frequencies. In perspective of diel vertical migrations, an increased adrenergic tone would act as compensation for the decrease in heart rate while descending. When ascending on the other hand, the increase in heart rate might not be sufficient to meet the concurrently increasing metabolic demands and during this period AD would then support cardiac performance. Besides AD, other factors have been shown to

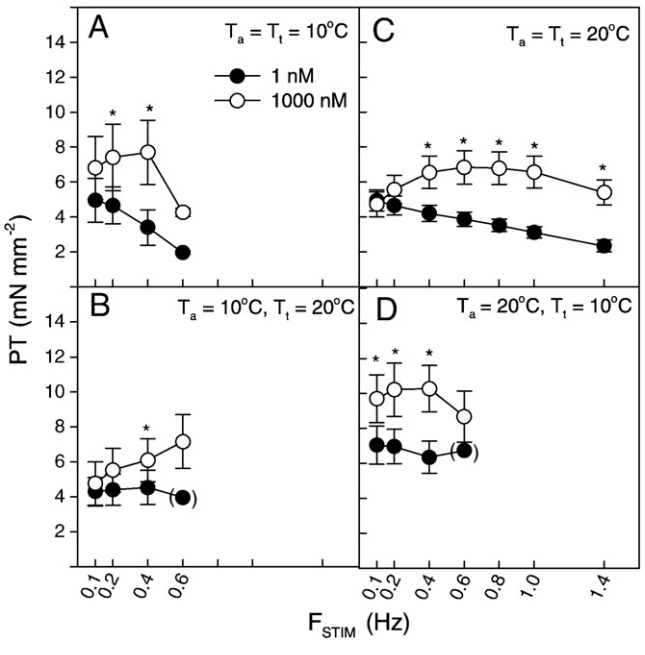

Fig. 7. Force–frequency response in eel (*A. anguilla*) ventricle tissue strips at tonic and high adrenaline concentrations. Eels were either acclimated to 10 °C or 20 °C and performed contractions either 10 °C (A, D) or 20 °C (B, C). An asterisk denotes a significant difference ($p<0.05$) from the lowest adrenaline concentration (1 nM). Values presented are mean ± s.e.m. ($N=2–7$). Brackets denotes data points with $N<3$. (See materials and methods section).

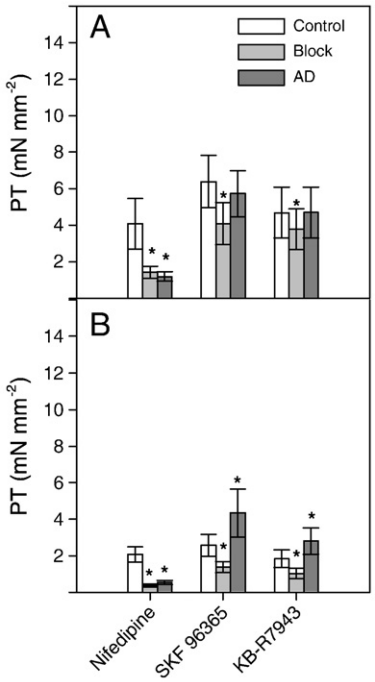

Fig. 8. Peak tension in eel (*A. anguilla*) ventricle tissue strips treated with Ca^{2+}-channel blocking agents. A) Acclimation and working temperature: 10 °C. B) Acclimation and working temperature: 20 °C. Strips were paced at 0.1 Hz. Height of bars represent means and error bars s.e.m. ($N=4–7$). An asterisk signifies significant difference ($p<0.05$) from control conditions (see Materials and methods section).

modulate cardiac performance in *A. anguilla*. Endogenous release of nitric oxide (NO) increases the sensitivity of the Frank–Starling response (Imbrogno et al., 2001) and NO can influence myocardial relaxation on a beat-to-beat basis by regulating the Ca^{2+} reuptake by the SR-Ca^{2+} ATPase (SERCA2a) (Garofalo et al., 2009).

Out of the 3 SL Ca^{2+}-blockers, Nifedipine caused the largest reduction in peak tension. This is in accordance with general observations on fish cardiocytes; that L-type Ca^{2+} channels serve as the main activator Ca^{2+} entry site (Driedzic and Gesser, 1988; Hovemadsen and Gesser, 1989; Vornanen, 1989). Also in line with previous studies using Nifedipine, the negative effect could not be abolished by AD as this agent binds irreversibly to the L-type Ca^{2+} channels. The reverse operating mode of NCX, i.e. bringing Ca^{2+} into the cell, is conditioned by the properties of the AP (Vornanen, 1999), and theoretically, low ambient temperature favors Ca^{2+}-influx via NCX due to a prolongation of the APD (Vornanen et al., 2002). But contrary to this assumption, the effect of blocking the reverse mode NCX was more pronounced at high temperature, while there was no effect at 0 °C. Possibly, the conditions favoring Ca^{2+}-influx via NCX are modified after thermal acclimation, but further insight must come from the study of AP characteristics at different temperatures.

As a novelty in fish cardiocytes, a decrease in isometric force could be attributed to the blockade of SOCE. The response was lower at 0 °C than at 10 °C and 20 °C, indicating also a temperature sensitivity of this Ca^{2+} entry. In mammalian myocytes, SOCE increases the Ca^{2+} loading of the SR, and is suggested to play a role when the intracellular stores are low or depleted (Putney, 1986). Assuming that the function of SOCE is homologous in mammals and teleosts, the current results suggest that the SR could be involved in force development in eel ventricular myocytes. To this end, it was demonstrated that treatment with ryanodine, a blocker of SR function, decreased isometric force at 20 °C and high Ca^{2+} loads in the American eel (*A. rostrata*) (Bailey et al., 2000). The contributing role of SR to force development is more significant in active species e.g. scombrids and in rainbow trout at high temperatures (Hovemadsen, 1992; Shiels and Farrell, 1997; Shiels et al., 2002), and possibly this could be true for migrating eels as well.

In summary we found that, in the European eel acclimatory responses to low temperature includes an increase in RVM that may compensate for the prolonged twitch duration and low heart rate. An acute temperature decrease, as experienced by migrating eels, increased the ventricular power production, which may serve as compensation for the upper limit placed on heart rate by acute cooling. Adrenergic stimulation generally improved contractility, suggesting a role for AD in supporting cardiac performance both during winter-quiescence and spawning migration. At all temperatures, contractions were mainly supported by L-type channel influx, but at higher temperatures also by reverse mode NCX and SOCE.

Acknowledgments

This study was funded by the Danish Agency for Science and Innovation, The Elisabeth and Knud Petersen Foundation and the Faculty of Science, University of Copenhagen.

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