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ORIGINAL PAPER

The role of adrenaline as a modulator of cardiac performance in two Antarctic fishes

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Abstract The present work was performed to test the hypothesis that Antarctic teleosts rely mostly on cholinergic inhibition for autonomic modulation of the heart. The effects of adrenaline on the inotropic properties on paced, isometrically contracting muscle strips were examined in two distinct Antarctic teleosts, the haemoglobinless icefish *Chaenocephalus aceratus* and the red-blooded *Notothenia coriiceps*. All tissues examined revealed a negative force-frequency relationship. Under baseline conditions *C. aceratus* contracted with a force twice as great as that of *N. coriiceps*. While the degree to which ventricular tissues responded to adrenaline varied between species, adrenergic stimulation significantly increases myocyte contraction force in this group of fishes. Contraction and relaxation times were not significantly affected by adrenaline concentration while absolute rates of contraction were. Adrenergic stimulation does not enable tissues to achieve higher contraction frequencies, but is shown to be a potent modulator of contraction force.

Keywords Adrenaline · Acetylcholine · Ventricle · Atrium · Icefish · Isometric contraction · Peak tension · Chaenocephalus · Notothenia

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Introduction

Decreasing temperatures have a large effect on cardiac function, through impacts on biochemical pathways and biological structures (Axelsson 2005). At 0°C, fluid viscosity is 30–40% greater than at 10°C (Riley and Skirrow 1975) and a greater amount of energy must be expended to respire water and circulate blood. Antarctic fishes which have evolved under these cold and stable conditions have a number of remarkable adaptations to low temperatures. These include enlarged hearts, larger and more numerous blood vessels to reduce vascular resistance, larger blood volumes, decreased blood viscosity (either by a complete loss of haemoglobin or a reduction in haematocrit) and changes in cell membrane lipid composition (see e.g. Axelsson 2005; Kock 2005).

The hypertrophied ventricle appears to be a defining characteristic for most of the Antarctic fishes. This is most extreme in the haemoglobinless species, where the relative ventricular mass (RVM) range from 0.32 to 0.40% of body mass (Johnston et al. 1983; Johnston and Harrison 1987). In several red-blooded Notothenioids (*Notothenia neglecta*; *N. rossi* and *N. gibberifrons*) RVM is also high, in the range of 0.17 to 0.21% (Johnston et al. 1983; Harrison et al. 1991) compared to temperate active species such as rainbow trout (*Oncorhynchus mykiss*) where RVMs range from 0.10 to 0.11% (Clark and Rodnick; 1999). Temperate species with lifestyles comparable to the Antarctic notothenioids (i.e. sedentary) typically have even smaller ventricles, with RVMs of 0.05–0.08% (*Micropterus salmoides*, Cooke et al. 2003; *Pleuronectes americanus*, Joaquim et al. 2004).

It has been argued that the reduction in viscosity of haemoglobinless blood would result in energetic savings for the heart. Sidell and O'Brien (2006), however, used

data from isolated perfused heart preparations (Axelsson et al. 1998) to estimate the body-mass specific cardiac power development required for haemoglobinless fishes, and found that it remains twice as high as for their red-blooded relatives. In the haemoglobinless icefish, it has been estimated that 22% of resting metabolism goes towards operating the cardiac pump (Hemmingsen and Douglas 1972). In contrast, cardiac work expenditures in most red-blooded species are estimated to range between 0.5 and 5% of resting metabolism (Farrell and Jones 1992).

Red blooded Antarctic teleost fishes generally have a similar or higher cardiac output than their temperate relatives, while the icefish group, lacking respiratory pigment, have a much greater cardiac output (Axelsson 2005). The relative mass of their ventricles suggests that the Antarctic fishes have a morphological predisposition towards utilizing large stroke volumes rather than high heart rates to meet cardiac demand. Indeed, Antarctic teleosts in general have heart rates that are considerably lower than their temperate relatives, with average resting heart rates in the range of 10–20 bpm (Axelsson et al. 2000; Bastos-Ramos et al. 1998; Egginton et al. 2006; Hemmingsen and Douglas 1972; Hemmingsen et al. 1972; Holeton 1970).

Heart rate in fishes is set by the intrinsic rhythm of the pacemaker cells in the sino-atrial region which is altered by chronotropic modulators. Adrenergic stimulation by neurons of the sympathetic system have a positive chronotropic effect (increases heart rate) while parasympathetic cholinergic neuron activity reduces heart rate (negative chronotropic effect). Although there are some interspecific differences in the innervation of the teleost heart, all species are thought to possess parasympathetic cardiac branches from cranial nerve X (Santer 1985). In the majority of species, sympathetic axons are also present in this nerve (Santer 1985), and in the Atlantic cod a sympathetic supply also originates from the first two spinal nerves (Holmgren 1977). The use of specific antagonists shows that the extent to which the intrinsic heart rate is modulated by a cholinergic and adrenergic tonus is highly species specific (see Axelsson 2005 for review).

In some Antarctic fishes (e.g. *Pagothenia borchgrevinki* and *Trematomus bernacchii)*, intrinsic heart rates are high, but a large cholinergic tonus results in greatly reduced resting heart rates, while the adrenergic tonus is small (Axelsson et al. 1992). While this is by no means a common feature of the Antarctic fish fauna, it has led to the suggestion that sympathetic innervation of the heart is absent (Egginton et al. 2006), as seen in the European flounder (Santer 1985). Increasing heart rate could then only be achieved by a release in cholinergic tone, a mechanism considered the fastest ways to change heart rate in Antarctic fishes (Axelsson 2005), or by the release of catecholamines into the bloodstream.

Circulating catecholamines efficiently modulate contractility of cardiac myocytes in many fish species (Farrell 1984). However, the ability of Antarctic fishes to synthesise adrenaline is not clear. Initial investigations showed that stress induced by capture and surgery (Egginton 1994), or forced exercise (Egginton 1997) did not increase levels of circulating adrenaline (<1 nmol l^{-1}) in *Notothenia coriiceps* and *Chaenocephalus aceratus*. Similar results were obtained from *T. bernacchii* subjected to handling stress (Davison et al. 1995). More recently, Whiteley and Egginton (1999) suggested that previous findings may have been the result of depleted catecholamine stores due to events prior to measurement. This work reported plasma catecholamine levels ranging from 700 to 1,000 nmol l^{-1} following trawl capture and in a single instance 2,500 nmol l^{-1}. A possible explanation for this phenomenon is that while Antarctic fishes may possess catecholamine stores available for release, rates of synthesis are extremely slow and these stores are only slowly replenished (Whiteley and Egginton 1999).

It is presently accepted that Antarctic fishes lack vagal adrenergic control of the heart. As such, they merely "take the foot off the cholinergic brake" during increased demands for cardiac work (Axelsson 2005) resulting in an increase in heart rate rather than stroke volume (Axelsson et al. 1992). Therefore, the aim of the present study was to investigate the potential role of catecholamines as an inotropic modulator of cardiac function in Antarctic fish by examining the effect adrenaline has on isolated atrial and ventricular muscle preparations from two species of Antarctic teleost fishes from the suborder Notothenioidei. The species chosen for the study were the haemoglobinless icefish *Chaenocephalus aceratus*, a species which displays cardiomegaly (enlargement of the heart) and whose heart operates as a high-volume low-pressure pump, and the red-blooded *Notothenia coriiceps* which has no remarkable morphological traits compared to temperate species.

Materials and methods

This research was performed on board HDMS VAEDDEREN on the Antarctic leg of the Danish Galathea 3 expedition during January and February 2007.

Animals

Chaenocephalus aceratus (Lönnberg, 1906) (N = 13) were caught by benthic trawl or hand-line at a depth of 80–100 m in the vicinity of the US Palmer Station Antarctica (64°70′S, 64°00′W), and ranged in mass between 520 and 2,830 g (mean mass 1,295 ± 261) and in length from 425 to 620 mm (491 ± 21). *Notothenia coriiceps* Richardson,

1844 ($N = 7$) were caught by hand-line from Palmer Station (64°70′S, 64°00′W), and ranged in mass from 510 to 1,050 g (678 ± 79) and length from 290 to 380 mm (321 ± 11). Water temperature in this area was ~0°C. Fish were maintained on board ship in 1,000 l aerated plastic tubs below deck supplied with continuously running seawater. All fish were used within 1 week of capture, and were not fed while in captivity.

Experimental procedure

Fish were netted from the holding tank and euthanised by a blow to the head. The heart was exposed by a ventral midline incision, quickly excised and transferred to a beaker of ice-chilled oxygenated ringer. The remains of the sacrificed fishes were used by other projects on board the ship. For *Notothenia coriiceps* the ringer consisted of NaCl 160 mM; $MgCl_2$ 5 mM; KCl 5 mM; Na_2HPO_4 1 mM; $CaCl_2$ 3 mM; HEPES 10 mM, glucose 20 mM and adjusted to a pH of 7.8 at 5°C (Holeton 1970). Ringer for *Chaenocephalus aceratus* was prepared in the same manner except that the NaCl was increased to 260 mM (Holeton 1970).

All experiments were conducted in a Myobath II system equipped with Fort 10 transducers and a Transbridge 4M amplifier (World Precision Instruments, FL, USA). Data was recorded at a sampling frequency of 200 Hz using a BioPac MP100 system driven by AcqKnowledge software (v. 3.8.1.Biopac Systems Inc., CA, USA) which was also used for data analysis.

Strips of ventricular and atrial tissues with a diameter no greater than 2 mm were dissected out and hung between a force transducer and a fixed post in organ baths filled with oxygenated Ringers with a tonic level of adrenaline (1 nM). Organ baths were bubbled with pure oxygen throughout the course of the experiment, and maintained at 0°C for the duration of the experiments. The experimental protocol followed that described by Shiels and Farrell (2000). Briefly, once mounted between the clamps, the tissues were gently tensioned to remove the slack, and allowed to rest for 30 min before being stimulated at 0.1 or 0.2 Hz (baseline frequency), 14 ms duration and 10 V using a Grass SD9 stimulator (Grass Product Group, RI, USA) controlled via the Acknowledge software. Stimulating voltage and tissue tension were slowly increased until maximum contractions were obtained, after which voltage was increased by an additional 50%. Tissues were then allowed to stabilise at these settings for 30 min, before an experiment was begun.

The experimental protocol consisted of exposing the tissues to stepwise increases in pacing frequency lasting 5 min per step. The stimulating frequency was increased in 0.1 or 0.2 Hz increments until the rate of tissue contraction no longer tracked with the stimulating pulse, at which point the pacing frequency was returned to the baseline level. Organ baths were drained and fresh Ringer containing adrenaline (AD) stock solution to achieve a final concentration of 10, 100 or 1,000 nM AD. The tissues were allowed to rest for 30 min at the baseline pacing frequency before a new force-frequency trial was begun at the next AD concentration. As with pacing frequencies, [AD] levels were presented in step-wise increases from lowest to highest.

Chemicals, calculations and statistics

All chemicals were purchased from Sigma-Aldrich (Auckland, New Zealand). AD stock solution was made fresh daily. In order to compensate for the movement and vibrations of the ship, one strip was selected to monitor background noise. This preparation was disconnected from the stimulating system, but otherwise subjected to the same protocol. The change in baseline force recorded by this channel by the movement of the ship was mathematically subtracted from the other three recorded traces before wave analysis was begun (Fig. 1). Ventricular and atrial twitch responses were summarised by measuring the characteristics of five twitches recorded at 1, 2.5 and 4 min of each step. Data from the 15 twitches were then averaged to calculate a grand mean for each step. Characteristics that were quantified for each twitch were peak tension (PT), time to peak tension (TPT) and time to half-relaxation (THR) from which rates of force increase and decrease, normalised peak tension and power production were calculated.

At the conclusion of the experiment the length of the tissue between the clamps was measured, removed and frozen at −80°C for mass determination upon the ships return to Denmark. Mean cross-sectional area (A) was calculated from

$$A = M/L \times 1.06,$$

where M is the tissue mass in milligram (determined to the nearest 0.1 mg), L is length of the tissue in millimeter (determined to the nearest 0.1 mm) and 1.06 is the density of muscle (Layland et al. 1995). Power production (PP) was calculated according to Matikainen and Vornanen (1992) as the product of PT (mN mm^{-2}) and heart rate (min^{-1}). Relative changes were calculated by dividing by measurements recorded at the baseline pacing frequency and lowest adrenaline concentration (1 nM). Data are presented as mean values ± S.E. Significant effects of adrenaline and stimulation frequency were assessed using one-way ANOVA, while comparisons between species or tissues were made using a t-test (SigmaStat, Jandel Scientific). For all statistics, a $P \leq 0.05$ was considered significant.

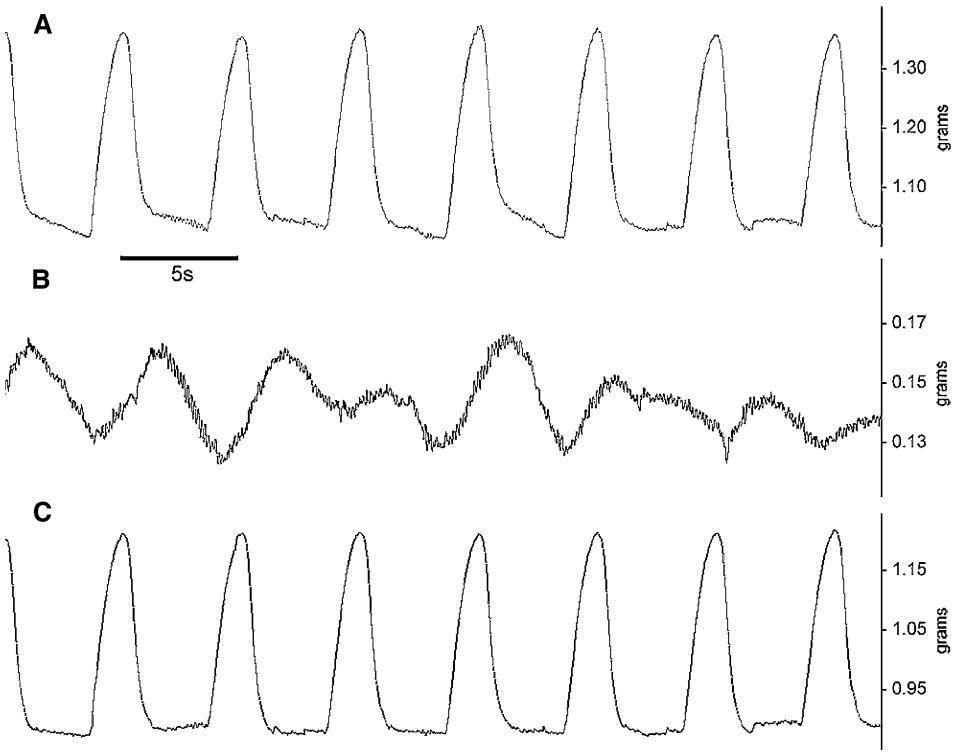

Fig. 1 Trace from *Notothenia coriiceps* ventricular tissue showing force of contraction in grams. **a** The raw signal from a working muscle preparation, **b** the background noise picked up from an unstimulated muscle preparation and **c** the corrected signal used for data analysis when the background noise was subtracted from the raw signal

Results

The number of species and number of individuals of each species we were able to collect was constrained by the cruise schedule, fishing success and holding facilities on board ship. While we successfully measured contraction properties of ventricular strips in both species examined, we were unable to gather sufficient data for contractions in atrial strips of *N. coriiceps* as several of the atrial preparations were unresponsive and would not contract when stimulated. Increasing stimulation frequency or [AD] had no significant effect on tension developed by atrial tissues from *C. aceratus*, nor did these variables affect contraction kinetics, therefore this data is not included.

Force of contraction

At the tonic adrenaline concentration [AD] (1 nM) and pacing frequency (0.1 Hz), ventricular tissue from *C. aceratus* generated peak tensions of 2.59 ± 0.37 mN mm^{-2} which was similar to that generated by the atrial tissues (3.73 ± 1.12 mN mm^{-2}). Ventricular tissue from *N. coriiceps* produced a PT of 1.14 ± 0.14 mN mm^{-2} (at 0.2 Hz), significantly less than that of *C. aceratus*. Changes in PT resulting from alterations in [AD] and pacing frequency are normalised to these values.

Increasing [AD] at baseline pacing frequencies caused a significant increase in ventricular PT in both *C. aceratus* (166%) and *N. coriiceps* (411%) (Figs. 2a, 3a).

Force–frequency response

Increasing the stimulation frequency reduced tension generation in all ventricular tissues (Figs. 2a, 3a). There was a marked difference, however, in the highest contraction rate they could achieve. In *C. aceratus* for instance, less than half of the ventricular samples were able to contract at 0.4 Hz (Table 1). The others tended to become unstable and only contracted in response to every other stimulus pulse. At tonic [AD] *N. coriiceps* ventricular tissue remained stable at pacing frequencies up to 0.5 Hz. With increasing adrenaline concentrations preparations became unstable at increasingly lower pacing frequencies (Table 2).

Increasing [AD] not only shifted the force frequency curves up (increased peak tension) in both species (Figs. 2a, 3a), but also severely compromised the ability of *N. coriiceps* to contract at the higher frequencies. At 1,000 nM [AD] maximum pacing frequency was reduced from 0.5 to 0.2 Hz (Fig. 3a). In no case did increasing [AD] alleviate the negative force–frequency relationship for any tissues.

Power production

Power production in *C. aceratus* under baseline conditions (1 nM [AD], 0.1 Hz) was 15.6 ± 2.2 mN mm^{-2} min^{-1} for ventricular tissue and 22.4 ± 6.7 mN mm^{-2} min^{-1} for atrial tissue. Increasing either pacing frequency or [AD]

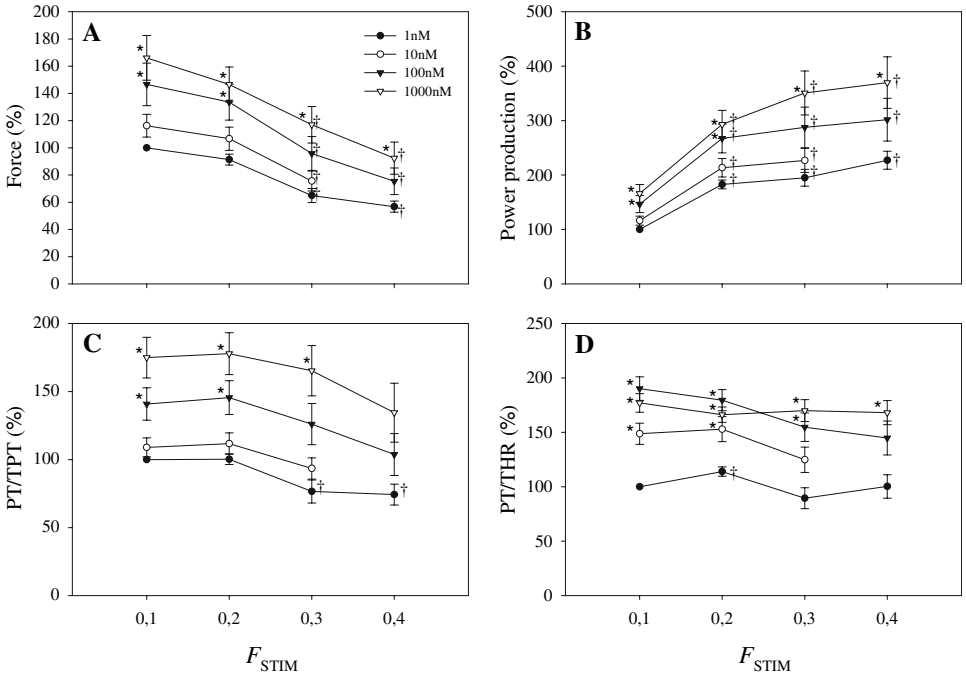

Fig. 2 The effects of stimulation frequency (F_{STIM}) and adrenaline concentration on **a** peak tension, **b** power production, **c** rates of contraction and **d** rates of relaxation in *Chaenocephalus aceratus* ventricular muscle preparations. Mean values ± S.E. An *asterisk* denotes significant difference from the lowest [AD] while a *dagger* denotes significant difference from the lowest pacing frequency. See text for details

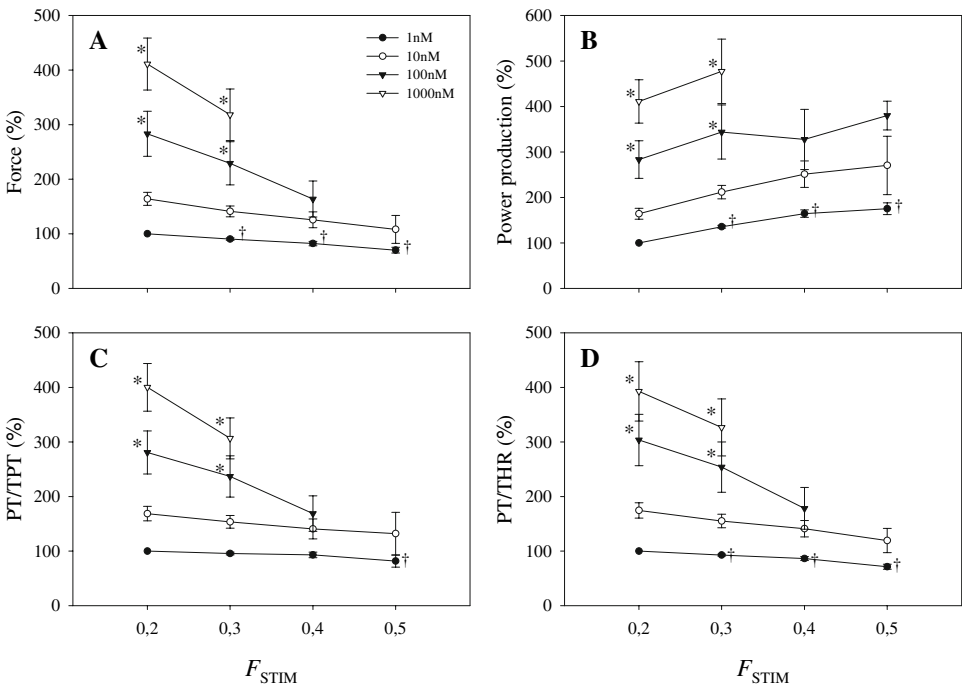

Fig. 3 The effects of stimulation frequency (F_{STIM}) and adrenaline concentration on **a** peak tension, **b** power production, **c** rates of contraction and **d** rates of relaxation in *Notothenia coriiceps* ventricular muscle preparations. Mean values ± S.E. An *asterisk* denotes significant difference from the lowest [AD] while a *dagger* denotes significant difference from the lowest pacing frequency. See text for details

increased PP. In combination, ventricular PP increased by 370% (Fig. 2b).

In *N. coriiceps*, increasing stimulation frequency caused a significant increase in PP of 175%, while the greatest increase was seen with an increase in [AD] which resulted in a ~400% increase in PP without an increase in pacing frequency (Fig. 3b).

Contraction kinetics

The time required for reaching PT (TPT) and THR are given in Table 1 and 2. In *C. aceratus* TPT and THR were significantly longer than in *N. coriiceps*. Increasing pacing frequency caused a significant decrease in TPT for both tissues in *C. aceratus*, but only in ventricular tissues was

Table 1 Time to peak tension (TPT) and half-relaxation (THR) in *C. aceratus* ventricular tissue at different pacing frequencies (F_{STIM}) and adrenaline concentrations [AD]

[AD]		F_{STIM}			
		0.1 Hz	0.2 Hz	0.3 Hz	0.4 Hz
1 nM	TPT	2.50 ± 0.10	2.27 ± 0.07	1.92 ± 0.05†	1.55 ± 0.05†
	THR	1.27 ± 0.07	1.01 ± 0.05†	0.81 ± 0.03†	0.60 ± 0.01†
	N	13	13	12	3
10 nM	TPT	2.63 ± 0.05	2.35 ± 0.04†	1.97 ± 0.02†	1.60 ± 0.00†
	THR	0.98 ± 0.05*	0.87 ± 0.03	0.75 ± 0.03†	0.64 ± 0.00†
	N	13	13	12	1
100 nM	TPT	2.54 ± 0.07	2.25 ± 0.06†	1.87 ± 0.04†	1.60 ± 0.03†
	THR	0.94 ± 0.05*	0.90 ± 0.04	0.73 ± 0.03†	0.57 ± 0.01†
	N	13	13	11	6
1,000 nM	TPT	2.34 ± 0.09	2.05 ± 0.07*†	1.75 ± 0.05*†	1.56 ± 0.04†
	THR	1.14 ± 0.07	1.08 ± 0.07	0.81 ± 0.03†	0.59 ± 0.02†
	N	13	13	11	5

An asterisk denotes significant differences from the lowest [AD] while a dagger denotes significant difference from the lowest pacing frequency

N number of functional tissues

Table 2 Time to peak tension (TPT) and half-relaxation (THR) in *N. coriiceps* ventricular tissue at different pacing frequencies (F_{STIM}) and adrenaline concentrations [AD]

[AD]		F_{STIM}			
		0.2 Hz	0.3 Hz	0.4 Hz	0.5 Hz
1 nM	TPT	1.35 ± 0.06	1.28 ± 0.06	1.13 ± 0.05	1.01 ± 0.09†
	THR	0.56 ± 0.03	0.55 ± 0.03	0.55 ± 0.01	0.57 ± 0.02
	N	7	7	4	2
10 nM	TPT	1.31 ± 0.04	1.24 ± 0.04	1.15 ± 0.04	1.06 ± 0.17†
	THR	0.53 ± 0.02	0.51 ± 0.02	0.51 ± 0.01	0.51 ± 0.01
	N	7	7	4	2
100 nM	TPT	1.35 ± 0.04	1.25 ± 0.03	1.25 ± 0.10	
	THR	0.53 ± 0.03	0.49 ± 0.01	0.49 ± 0.01	
	N	7	7	6	
1,000 nM	TPT	1.37 ± 0.03	1.33 ± 0.03		
	THR	0.60 ± 0.04	0.52 ± 0.02		
	N	7	5		

An asterisk denotes significant differences from the lowest [AD] while a dagger denotes significant difference from the lowest pacing frequency

N number of functional tissues

THR significantly affected. TPT in ventricular tissue was only significantly affected by [AD] at the highest concentration, while atrial tissues were unaffected (Table 1). In ventricular tissue from *N. coriiceps*, TPT was significantly reduced only at the highest pacing frequency, while no other effects were observed (Table 2).

In *C. aceratus* there was a significant increase in rates of contraction and relaxation of ventricular muscle with increasing [AD], while increases in pacing frequency had only moderate effects at the tonic adrenaline level (Fig. 2c, d). In *N. coriiceps* contraction and relaxation rates increased significantly with increasing [AD] at the lowest pacing frequencies (Fig. 3c, d).

Discussion

The range in which ventricular strips could be paced is in good agreement with published in vivo ranges previously reported for *N. coriiceps* (Campbell et al. 2008) and *C. aceratus* (Hemmingsen and Douglas 1972; Acierno et al. 1997). Heart rate in sea caged *N. coriiceps*, for example, ranged from 12 bpm (0.2 Hz) to 30 bpm (0.5 Hz) (Campbell et al. 2008).

Cardiac tissues under baseline conditions showed a more than twofold difference in PT between species. The explanation for this is likely to be found in the presence or absence of Hb/Mb and cardiac morphometry. Ventricular preparations from *N. coriiceps* contracted with significantly less force (1.14 mN mm^{-2}) than those from the haemoglobinless ice fish, *C. aceratus* (2.6 mN mm^{-2}). The force of contraction generated by *C. aceratus* at 0°C are similar to or greater than for several active species at warmer temperatures (Shiels and Farrell 1997, 2000, Rivaroli et al. 2006), while that of *N. coriiceps* was surprisingly small.

Our initial conjecture for the impressive tension generation by *C. aceratus*, was based on its large ventricular size. According to LaPlace's Law for a cylindrical vessel

[$T = P \times R$, where T is the tension required to generate pressure (P) in a ventricle with a given radius (R)] a doubling of the radius doubles the tension required to generate the same pressure. With RVMs up to three times greater than red-blooded notothenioids, greater wall tension must be a prerequisite for maintaining functionality of the large ventricle of *C. aceratus*. However, this is not consistent with earlier investigations on the function of the icefish heart, specifically that the icefish heart is considered to displace large volumes at and against relatively low pressures (Zummo et al. 1995; Tota et al. 1991; Tota and Gattuso 1996). In their study on the ventricular ultrastructure of *C. aceratus*, Johnston and Harrison (1987) found large mitochondrial volumes, compared to red-blooded fishes. While this reduces the diffusion distance for oxygen, it also reduces the volume available for contractile proteins. As such, myofibril content is much less than for red blooded Notothenioids which should mean that the icefish ventricle is capable of generating less work per unit mass than other fish (Johnston and Harrison 1987). The results from this study are contradictory to both these concepts, and warrants further investigations on the properties of icefish myofibrils. Assuming that the large force of contraction generated by the ventricular muscle in *C. aceratus* is not a physiological requirement for the initiation of contraction during diastole, it may be a morphological requirement to sufficiently shorten myofilaments to achieve systole in a large ventricle.

By comparison, the force of contraction at baseline levels for *N. coriiceps* was small. While red-blooded Notothenioids generally have larger ventricles than temperate fishes, the RVM in *N. coriiceps* is only ~0.10% (Johnston and Harrison 1987; Egginton 1997). Ventricular mass has been suggested as an indicator of stroke volume (Sänger et al. 2005), and as such the small force of contraction in *N. coriiceps* under baseline conditions is not surprising.

In both species the force of contraction (PT) of ventricular muscle increased in response to increasing adrenaline concentrations, although the degree of response varied. For *C. aceratus* at baseline stimulation frequency, a significant increase (~60%) was found in the force of contraction and power production at 100 and 1,000 nM AD. As with most, if not all teleost hearts, increasing stimulation frequency was associated with a negative force–frequency relationship, the severity of which was not influenced by adrenaline concentration. Thus, at a stimulation frequency of 0.4 Hz and 1,000 nM AD, force of contraction remained significantly greater than with 1 nM AD. While increases in stimulation frequency and increases in adrenaline concentrations were able to significantly increase power production independently (166 and 227%, respectively) the greatest increase in power production came from a combination of the two, yielding a 370% maximum increase.

The initial power production was greater for atrial than for ventricular tissues, and atrial power production at the highest attainable heart rates reached 135 mN mm^{-2} while ventricular power production was 51 mN mm^{-2}. Adrenergically mediated increases in atrial pumping at higher frequencies would increase ventricular filling yielding an increase in ventricular end-diastolic volume and an increase in stroke volume due to the length dependent activation of ventricular myofilaments (de Tombe 2003). However, an isometric experimental setup used in the present study cannot be used to quantify the increase in ventricular contraction force mediated through this mechanism. Rather, an oscillating muscle length preparation (see Shiels et al. 1998) would provide valuable insight into this mechanism. Tota et al. (1991) found that changing the preload from −0.07 to −0.04 kPa in the isolated heart from the icefish *Chaenocephalus hamatus*, caused a two- to threefold increase in stroke volume, demonstrating beyond doubt that this mechanism plays a significant role in ventricular performance.

Notothenia coriiceps showed by far the greatest inotropic response to adrenaline, with a fourfold increase in PT between 1 and 1,000 nM adrenaline at 0.2 Hz (Fig. 3a). By comparison, increasing stimulation frequency at low [AD] yielded a 75% increase in power production (Fig. 3b). As such, this infers that an acute increase on cardiac demand would be most effectively met by an adrenergically mediated increase in force of contraction. However, during routine activity changes in heart rate may be a more economical way to meet moderate changes on cardiac demand, particularly if adrenaline is a precious resource.

Interestingly, the large interspecific differences in developed tension at tonic AD levels were not sustained during maximal adrenergic stimulation, where *C. aceratus* and *N. coriiceps* contracted with similar force (3.82 ± 0.54 and 4.23 ± 0.63 mN mm^{-2} respectively). This would imply differences in the affinity of adrenergic receptors between species, and future work on the effects of AD might consider plotting saturation curves.

Ventricular tissue from *C. aceratus* took twice as long to contract and relax as *N. coriiceps*. We hypothesise that the explanation for this is found in the lack of oxygen binding proteins which has caused an up-regulation of slow myosin isoforms. This leads to a longer tension-time integral which serves as an energy conserving mechanism (Tiitu and Vornanen 2001). In addition the increase in retention time of venous blood in the cardiac lumen provides longer oxygen diffusion times from the plasma to the myocardium, no doubt a benefit for *C. aceratus* given the absence of a coronary blood supply.

In both species, increasing pacing frequency was associated with a significant reduction in TPT for all tissues. Particularly in *N. coriiceps* absolute rates of contraction and relaxation (Fig. 3c, d) follow the same trends as PT which suggests that this effect is a result of a loss in force of contraction. TPT in *C. aceratus* was significantly reduced by adrenaline at a concentration of 1,000 nM. Why this effect is only seen at the highest [AD] is unclear. Although in mammalian cardiac tissue, β-adrenergic stimulation reduces the time required to reach PT and half-relaxation (Winegrad 1984), this is not a typical response in teleost fishes, where adrenergic stimulation rarely affects TPT or THR. Graham and Farrell (1989) suggested that adrenergic stimulation may be more important for the inotropic properties of cardiac muscle rather than for chronotropic regulation. With the exception above, this statement appears to hold true in respect to the two species examined in the present study.

In summary, adrenergic stimulation in the absence of a cholinergic inhibition does not enable ventricular tissue to operate at higher pacing frequencies. However, it is clear that adrenaline has large positive inotropic effects on ventricular tissue. While it does increase absolute rates of contraction and relaxation, it does not decrease contraction times. The latter may be the limiting factor in achieving high contraction frequencies, if there is not time for mechanical restitution.

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