RECENT ADVANCES TOWARD A NEW UNDERSTANDING

OF PISCINE HEART FUNCTION

J.B. Graham
CMBB and MBRD, Scripps Institution of Oceanography
UCSD, La Jolla CA 92093-0204,
Tel: (619) 534-8044, Fax: (619) 534-1305, email: jgraham@ucsd.edu

R. Shabetai, N.C. Lai, and V. Bhargava
VAMC, Cardiology Section and
Department of Medicine, UCSD

Discussion

Two paradigms for piscine heart function are:

i) that elasmobranch heart filling is dependent upon a strongly negative pericardial pressure and,

ii) in all fishes, atrial systole is the exclusive determinant of ventricular end-diastolic volume (Farrell and Jones, 1992).

Both of these principles are now being challenged by studies with a diversity of species, varied experimental approaches, and modern instrumentation.

That "normal cardiac function depends upon strongly negative pericardial pressure" has been a cornerstone of elasmobranch physiology for over a century. However, chronic pressure measurements in horn sharks (*Heterodontus*) reveal that although strongly negative at the time of surgery, pericardial pressure rises steadily and is near ambient in post-operated, resting sharks. Studies also show that sudden movements and activity bursts by a shark can displace fluid from the pericardium (via the pericardioperitoneal canal, ppc, which discharges into the peritoneum), thus lowering pericardial pressure and elevating cardiac stroke volume (Abel et al., 1987). Post-op leopard sharks (*Triakis*) also have a nearly ambient pericardial pressure. However, muscular tensions developed during sustained swimming compress (i.e., raise the pressure) the pericardium and

eject pericardial fluid. The volume of pericardial fluid that is displaced approximates the increase in cardiac stroke volume (Lai et al., 1989).

These findings indicate that a strongly negative pericardial pressure is not typical of resting sharks and that a negative pericardial pressure is not critical for cardiac performance during sustained swimming. Therefore, early findings of strongly negative pericardial pressures may reflect an unnatural physiological state which, because of ppc fluid ejection in the course of handling, indicated a functional link between strongly negative pressure and resting cardiac output. Finally, the newer data also suggest that, because elasmobranchs lack sympathetic heart innervation, the capacity to instantly increase in stroke volume by ejecting fluid from the pericardial space may represent a primitive biomechanical method for rapidly elevating cardiac output and increasing aerobic scope.

Another long-held paradigm of piscine heart function, "exclusive filling of the ventricle by the atrium," has its basis in observations that the atrio-ventricular (a-v) pressure gradient prevented flow into the ventricle except during atrial systole and that ventricular pressure records indicated a monophasic flow. However, there are few actual data or publications documenting this and many of the early conclusions about the a-v pressure gradient were based on composite depictions of separately obtained cardiac chamber pressures.

Doppler echocardiography, digital angiography, and simultaneous on-line measurements of the a-v pressure gradient reveal a bimodal ventricular-filling pattern in elasmobranchs (Lai et al., 1990, 1996). Doppler analyses also confirm bimodal filling in three teleost genera (*Paralabrax*, *Monopterus*, and *Channa*) having vastly different cardiac morphologies [the latter two genera lack a sinoatrial (sa) valve which means that the ventricle cannot be exclusively filled by atrial systole] (Lai et al., 1998). In both elasmobranchs and teleosts the early filling phase occurs during ventricular diastole, the late filling phase follows atrial systole (Figure 1). In *Paralabrax*, a strong similarity exists between the Doppler velocity profile and the diastolic a-v pressure gradient (Figure 2).

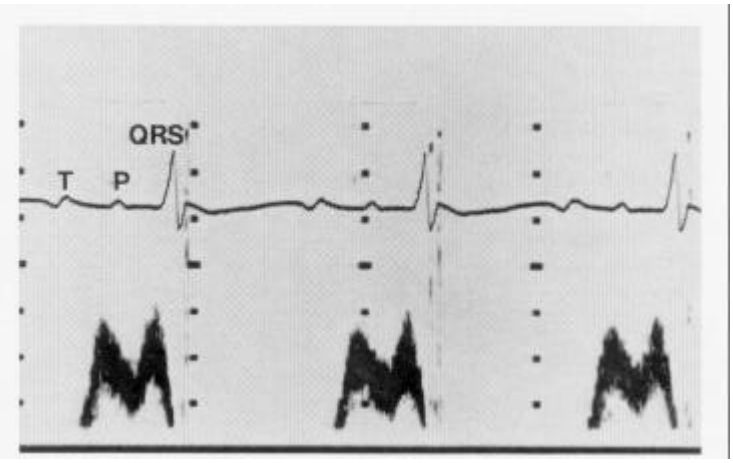


Figure 1. ECG and Doppler signal of blood flow velocity into ventricle through atrioventricular orifice showing an early filling phase and a late filling phase after P wave. Tick marks, 1 s.

Increased knowledge of specialized cardiac morphology, such as the absence of a sa valve, and of other structures affecting cardiac function (the ppc) requires new hypotheses about piscine heart function that extend beyond existing paradigms. There can be little doubt that a strongly negative pericardial pressure is not critical for normal elasmobranch heart function. It is also apparent that in elasmobranchs and teleosts the ventricle begins to fill with the onset of diastole and that the atrium's contribution to end-diastolic ventricular volume is not as large as was once thought, which is similar to most other vertebrates.

Acknowledgements

Supported by NSF IBN 93-16621.

References

Abel, D.C., Lowell, W.R., Graham, J.B., and Shabetai, R. 1987. Elasmobranch pericardial function II. The influence of pericardial pressure on cardiac stroke volume in horn sharks and blue sharks. Fish Physiol. and Biochem. 4:5-15

Farrell, A.P. and Jones, D.R. 1992. The heart. In: Hoar, W.S., Randall, D.J., and Farrell, A.P. eds., *Fish Physiology* Vol XII, Part A, pp. 1-88. Academic Press, New York

Lai, N.C., Graham, J.B., Bhargava, V., and Shabetai, R. 1996. Mechanisms of venous return and ventricular filling in elasmobranch fishes. Amer. J. Physiol. 270: H1776-1771

Lai, N.C., Graham, J.B., Dalton, N., Shabetai, R., and Bhargava, V. 1998. Echocardiographic and hemodynamic determinations of the ventricular filling pattern in some teleost fishes. Physiol. Zool. 71:157-167

Lai, N.C., Graham, J.B., Lowell, W.R., and Shabetai, R. 1989. Elevated pericardial pressure and cardiac output in the leopard shark, *Triakis semifasciata*, during exercise: the role of the pericardioperitoneal canal. J. Expt. Biol. 23:155-162

Lai, N.C., Shabetai, R., Graham, J.B., Hoit, B.D., Sunnerhagen, K.S., and Bhargava, V. 1990. Cardiac function of the leopard shark, *Triakis semifasciata*. J. Comp. Physiol. B 160:259-268

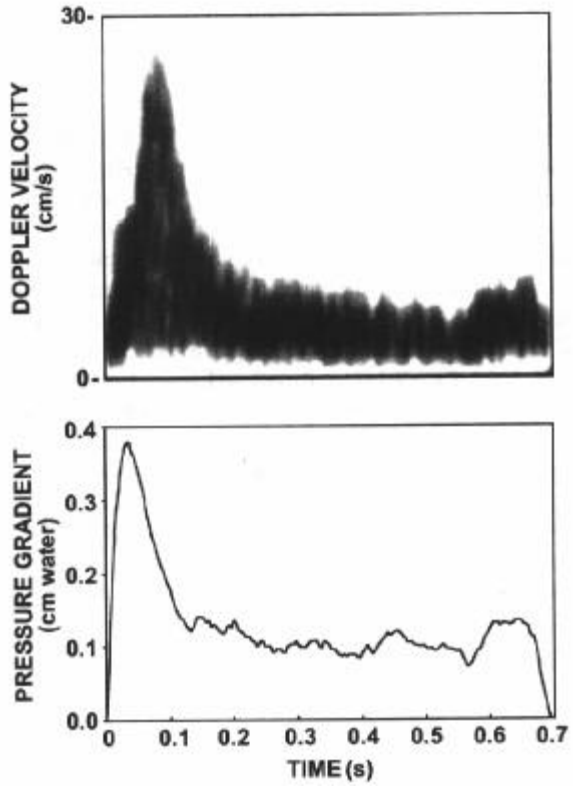


Figure 2. Superimposed tracings showing the similarity between the velocity profile (indicated by Doppler-flow wave form) and the atrioventricular pressure differential (the diastolic pressure gradient). Records were acquired within 1 min of each other. Note that the time scale is displayed for the pressure gradient profile only.

EFFECTS OF PERICARDIAL PRESSURE ON CARDIAC OUTPUT IN FREE-SWIMMING SPINY DOGFISH (*SQUALUS ACANTHIAS*)

Douglas A. Syme
University of Calgary
Department of Biological Sciences
2500 University Drive NW
Calgary, Alberta T2N 1N4, Canada
403-220-5281, FAX 289-9311, syme@acs.ucalgary.ca

David R. Jones
University of British Columbia
Department of Zoology
6270 Wesbrook Mall
Vancouver, British Columbia V6N 1Z4, Canada
604-822-4228, FAX 822-2416, jones@zoology.ubc.ca

Introduction

It is the prevailing view that pericardial pressures in elasmobranch fish are subambient, promoting a suction mode of cardiac filling; the negative pressure being transmitted to the atrium which aspirates blood from the central veins (see Lai *et al*. 1996 for an overview). However it has recently been shown that pericardial pressures are not always negative (Abel *et al.* 1986; Lai *et al.* 1989). We wanted to look at the role of subambient pericardial pressures in maintaining venous return to the heart in awake, prone, submerged animals with an intact pericardium.

Methods

Spiny dogfish (*Squalus acanthias*) were obtained from local suppliers and held at U.B.C. Anesthesia for surgery was achieved by alternately irrigating the gills with fresh and 0.01g/l MS222 aerated, sea water. The abdomen was opened by a ventral incision and 2000 IU heparin injected into the sinus venosus *via* an hepatic vein. A 22 gauge angiocath was inserted into the left cardinal sinus *via* the dorsolateral musculature. Medical vinyl tubing (#4Bolab) was advanced

through the pericardioperitoneal canal (PPC) into the pericardial cavity; the tip was blunted and vented with side ports. The PPC was tied securely around the catheter so fluid could not leak out of the pericardial cavity. The ventral aorta (VA) was exposed by a mid-line ventral incision and cannulated (#5 Bolab) *via* an anterior branchial artery, and a Doppler flow cuff (Iowa Bioengineering 545C-4) was attached just distal to the pericardium. The catheters and leads were secured to fascia, skin and the anterior dorsal fin. All incisions were then closed. The animals were allowed to recover for approximately 30 hours in a tank containing circulating, aerated, sea water. Catheters were connected to saline-filled syringes and pressure transducers (Deltran, Utah Medical Products Inc.) *via* 3-way stopcocks. Transducers were placed at the level of the water surface. Baseline recordings were made and then saline was injected or withdrawn through the PPC catheter.

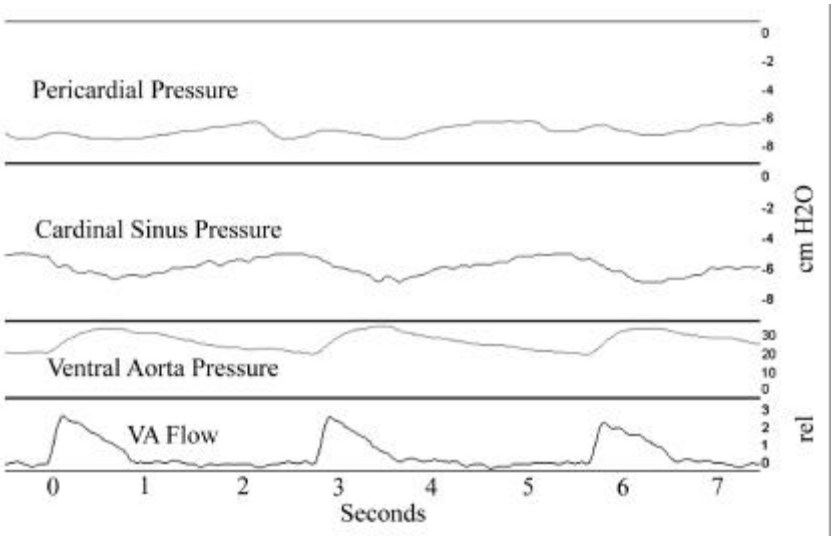


Figure 1. Pressures and flow at rest after recovery. Units of flow are relative volts.

Results and Discussion

Records from a resting fish, before any manipulations were made, are shown in Figure 1. Pressure in the cardinal sinus ranged from just super- to subambient; small fluctuations corresponding to systole/diastole were usually noted. Subambient venous pressures would necessitate subambient atrial pressures to elicit atrial filling.

Pericardial pressures were always notably subambient, falling even further during ventricular systole (presumably due to ejection of blood from the ventricle) and recovering during diastole. That pericardial pressure was negative after 30 hours or more of recovery suggests it is negative in fish with an unoccluded PPC; occluding the PPC should bias the pressures toward positive values. This also indicates that the negative pressures can be generated by fluid absorption from the pericardial cavity, independent of the patency of the PPC (see Shabetai *et al*. 1985 and references).

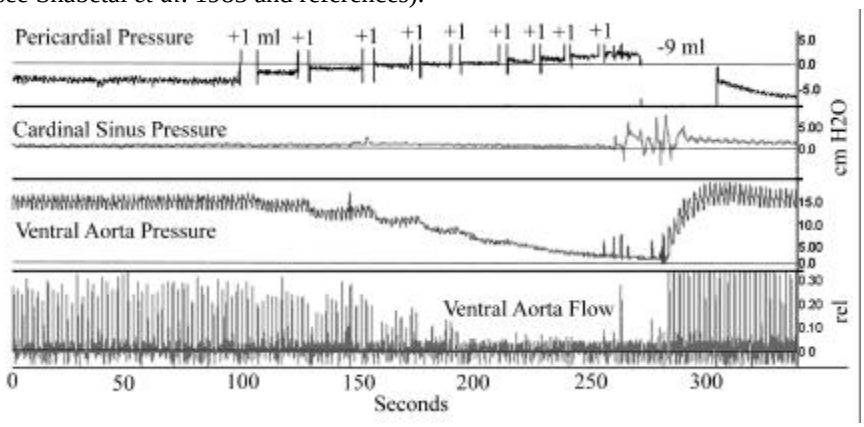


Figure 2. Pressures and flow during manipulation of pericardial pressure. Units for ventral aorta flow are relative volts. +1ml and -9ml markers on pericardial pressure record indicate injection or withdrawal of saline into/from the pericardial cavity

When saline was infused into the pericardial cavity (Fig. 2) there was a rise (less negative) in pericardial pressure toward ambient, a fall in flow and pressure in the VA, and a small rise in central venous pressure. Further infusions of saline resulted in progressive increases in pericardial pressure and falls in flow and pressure in the VA, until flow and pressure approached zero when pericardial pressure exceeded ambient. In all cases, after several seconds of severely reduced VA flow the fish contracted its respiratory muscles forcefully. It is suggested these contractions are attempts to expel fluid from the pericardial cavity *via* the PPC (Shabetai *et al*. 1985). Fish never displayed these contractions when pericardial pressures were subambient. Removal of the saline from the pericardial cavity resulted in a return of subambient pressures and restoration of VA flow and pressure.

This is the first study in which the PPC has been occluded while recording the effects of injection or withdrawal of fluid on cardiac function in free-swimming fish. This affords a precision in regulation of pericardial pressure which has not been possible in past experiments. In our preparations, it was obvious that negative pericardial pressures were necessary to maintain normal venous return. However some blood flow occurred even at ambient pressures. Only at positive pericardial pressures was flow arrested to the degree that the animal reacted to reduce pericardial pressures by active contraction of respiratory muscles.

Acknowledgements

We gratefully acknowledge the support of NSERC of Canada to both authors.

References

Abel, D.C., J.B. Graham, W.R. Lowell and R. Shabetai. 1986. Elasmobranch pericardial function. 1. pericardial pressures are not always negative. Fish Physiol. Biochem. 1:75-83.

Lai, N.C., J.B. Graham, W.R. Lowell and R. Shabetai. 1989. Elevated pericardial pressure and cardiac output in the leopard shark *Triakis semifasciata* during exercise: the role of the pericardioperitoneal canal. J. exp. Biol. 147:263-277.

Lai, N.C., J.B. Graham, V. Bhargava and R. Shabetai. 1996. Mechanisms of venous return and ventricular filling in elasmobranch fish. Am. J. Physiol. 270:H1766-1771.

Shabetai, R., D.C. Abel, J.B. Graham, V. Bhargava, R.S. Keyes and K. Witztum. 1985. Function of the pericardium and pericardioperitoneal canal in elasmobranch fishes. Am.J.Physiol. 248:H198-207.

FUNCTIONAL MORPHOLOGY

OF THE BULBUS ARTERIOSUS

Marvin H. Braun
Dept. of Zoology, University of British Columbia
6270 University Blvd., Vancouver, BC, Canada, V6T 1Z4
ph. (604) 822-4228, e-mail: braun@zoology.ubc.ca

R.W. Brill[1]., J. M. Gosline[2]., D. R. Jones[2].
1.Southwest Fisheries Center Honolulu Laboratory and
2.Dept. of Zoology, University of British Columbia

EXTENDED ABSTRACT ONLY DO NOT CITE

Introduction

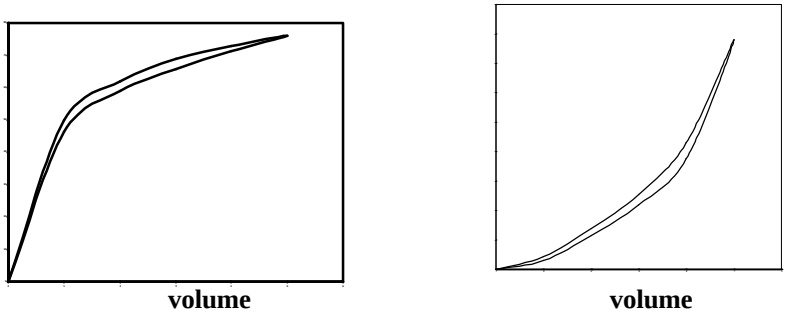
The bulbus arteriosus is a large chamber of the teleost heart and rests within the pericardial cavity between the ventral aorta and the ventricle. It is generally thought to function as a Windkessel, smoothing pulsatile flow (Santer, 1985), however, exactly how this is accomplished is unclear. The pressure-volume loops of bulbi (Fig. 1A.) are obviously different from the J-shaped pressure-volume curves of arteries (Fig. 1B) (Bushnell *et al*., 1992); despite arteries and bulbi being composed of the same three materials: elastin, collagen and smooth muscle.

We attempted to explain what possible design features of the bulbus generate the odd pressure-volume curves and to relate them to its function within the fish.

Methods and Results

The material properties of the bulbar wall were examined using uniaxial extensions on rings cut from bulbi. From the recordings of the extensions and the resulting forces generated, we were able to calculate stress and strain. Stress is **force/cross-sectional area** while strain is **change in length/initial length**. Pressure and volume are roughly analogous to stress and strain, respectively and

arteries have J-shaped stress-strain curves to match their J-shaped pressure-volume curves; perhaps the wall of the bulbus would have stress-strain behaviour reminiscent of its pressure-volume curves.



A. **B.**

Fig. 1. Pressure-volume loops from the (A) bulbus arteriosus and (B) ventral aorta of of a yellowfin tuna (*Thunnes albacares*).

The bulbar stress-strain curves were special in unexpected ways. The curves are typical J-shapes (Fig. 2) but what makes them unique are the values of stress, strain and modulus (stiffness). When comparing the modulus values of the bulbus with those of a ventral aorta, the ventral aorta is nearly ten times as stiff.

The extensibility of the material is also extraordinary. Bulbar rings are able to stretch nearly twice as much as most arteries.

While these properties of the wall material shed some light on the bulbus, the most unusual aspect of the inflations, the initial steep rise in pressure, was unexplained. Therefore, we decided to examine the architecture of the bulbus as a whole and perhaps glean some insights which would help us find an answer.

This answer appears to lie in the Law of Laplace:

Tension = Pressure X Radius

Conclusions

This special design greatly enhances the ability of the bulbus to smooth pressures and maintain flows. The key to these functions in arteries is the ability to act as a large reservoir and to this end, most air-breathing vertebrates have a relatively long arterial tree separating the heart from the vascular beds. The added length allows even relatively inelastic tubes to have a large overall compliance.

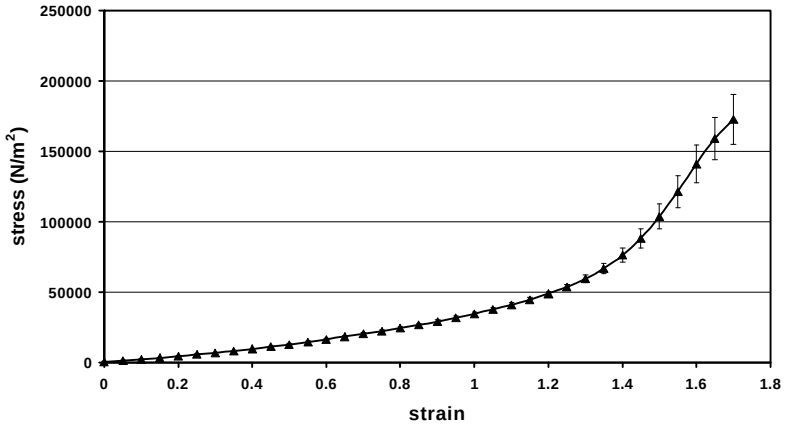


Fig. 2. Stress-strain data calculated from uniaxial extensions of rings cut from the bulbus arteriosus of yellowfin tuna (*Thunnus albacares*).

However, with only a short segment of ventral aorta separating the heart from the gills, the bulbus requires its exceptional compliance to smooth flows and pressures. The initial sharp rise of the curve allows the bulbus to reach physiological pressures quickly and to maintain those pressures over a large change in volume. With its special elastic properties, the bulbus is able to make flows only a few centimeters away from the heart remarkably smooth and to maintain diastolic perfusion of the gills.

Acknowledgements

This work was supported by equipment and operating grants awarded to D.R. Jones and J.M. Gosline from NSERCC.

References

Bushnell, P.G., Jones, D.R., and Farrell, A.P. (1992). The arterial system. In *Fish Physiology*, vol 12A (ed. W.S. Hoar, D.J. Randall and A.P.Farrell), pp. 89-139. New York: Academic Press.

Santer, R. M. (1985). Morphology and innervation of the fish heart. *Adv. Anat. Embryol. Cell Biol*. **89**, 1-102.

THE SIGNIFICANCE OF INCREASED HEART MASS IN FISH

Helgi Thorarensen
Holar Agricultural College
551 Saudarkrokur
Iceland
phone: 354 453 6300 fax: 354 453 6301
email: hthor@krokur.is

Discussion

There is considerable plasticity in the size of teleost hearts. For example, the relative heart mass (RVM = heart mass / body mass) of some species changes seasonally, being larger in summer than in winter. There are also indications that the level of activity of the fish affects the RVM. Heart mass may increase during periods of intensive training (Farrell et al. 1990) and wild fish tend to have larger hearts than their farmed conspecifics (Graham and Farrell 1992). During the reproductive season, the more active mature male salmonids have larger hearts than either females or immature males (Franklin and Davie 1992; Graham and Farrell 1992; Thorarensen et al. 1996). There also appears to be a correlation between the routine metabolic rate of fish and RVM (Higgins 1985; Goolish and Adelman 1987).

The significance of these changes in heart mass is not entirely clear. Experiments performed on *in situ* heart preparations show that larger hearts can generate both higher cardiac output and a greater maximum power output per unit body-mass than smaller hearts (Franklin and Davie 1992). However, only few studies have compared the performance of large and small hearts *in vivo.*

Some exercise training regimes increase the RVM of salmonids. When chinook salmon were exposed to a high intensity training regime (HI) and swum to U_{crit} on alternate days while otherwise maintaining a swimming velocity of 0.5 $bl \cdot s^{-1}$, the RVM increased by 10% compared with control fish that were exposed to a low intensity training regime swimming continuously at 0.5 $bl \cdot s^{-1}$. Following the training period the fish were cannulated in the dorsal aorta and a flow probe was placed on the ventral aorta. Swimming performance, oxygen consumption, cardiac output, blood pressure and blood gasses were measured in a swim-tunnel respirometer. The maximum oxygen consumption of the HI fish was 50% higher

than that of the control fish. This higher oxygen consumption was supported by a better extraction of oxygen from the blood in the tissues of the HI fish (90%) than in the control fish (62%). However, the maximum cardiac output was not significantly different, being 65.1 and 65.5 $ml \cdot min^{-1} \cdot kg^{-1}$ respectively. Thus, the increased heart mass of the HI trained fish did not result in a higher cardiac output.

The heart of male, but not female, rainbow trout increases in size during maturation and *in situ* measurements of cardiac performance, suggest that the cardiac output and power output of the male hearts is increased (Franklin and Davie 1992). The cardiac performance of mature male and female rainbow trout was measured while the fish swam to U_{crit}. The RVM of the mature males was 74% higher than that of females. The maximum cardiac output of the mature males (50.7 ± 5.2 $ml \cdot min^{-1} \cdot kg^{-1}$) was not significantly different from that of the females (45.1 ± 5.2 $ml \cdot min^{-1} \cdot kg^{-1}$).

This suggests that the significance of the greater heart mass of the males is not to increase maximum cardiac output. However, the maximum blood pressure was 30% higher and the estimated maximum power output per kg body-mass was 41% higher in the males than in the females. The increased blood pressure may have been caused by the higher haematocrit in the males (38%) than in the females (29%). The elevated haematocrit in the males will likely increase oxygen delivery through the cardiovascular system in the males and thus their maximum oxygen consumption. When haematocrit is acutely increased to this level in immature fish with smaller RVM, maximum cardiac output is reduced (Gallaugher et al. 1995). It is suggested that the increased heart mass allowed the mature males to increase their haematocrit without compromising maximum cardiac output.

The difference between the work load on the hearts of mature male and female rainbow trout was comparatively greater at rest than at maximum swimming speed. Resting cardiac output of the mature males was 46% higher and the estimated resting power output was 72% higher than that of the females. These results and the finding that RVM is correlated with routine metabolic rate (Higgins 1985; Goolish and Adelman 1987) suggest that the significance of the increased heart mass is to support higher routine or sustainable work load on the heart.

References

Farrell, A.P, J.A. Johansen, J.F. Steffensen, C.D. Moyes, T.G. West, and R.K. Suares 1990. Effects of exercise training and coronary ablation on swimming performance, heart size and cardiac enzymes in rainbow trout *Oncorhynchus mykiss*. Can. J. Zool. 68:1174-1179.

Franklin, C.E. and P.S.Davie 1992. Sexual maturity can double heart mass and cardiac power output in male rainbow trout. J. Exp. Biol. 171:139-148.

Gallaugher, P., H. Thorarensen and A.P. Farrell 1995. Haematocrit in oxygen transport and swimming performance of rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 171:301-314.

Goolish, E.M. and I.A. Adelman 1987. Tissue specific cytochrome oxydase activity in largemouth bass: The metabolic cost of feeding and growth. Physiol. Zool. 60:454-464.

Graham, M.S. and A.P. Farrell 1992. Environmental influences on cardiovascular variables in rainbow trout, *Oncorhynchus mykiss*. J. Fish Biol. 41:851-858.

Higgins, P.J. 1985. Metabolic differences between Atlantic salmon (*Salmo salar*) parr and smolts. Aquaculture 45:33-45.

Thorarensen, H., G. Young and P.S. Davie 1996. 11-ketotestosterone stimulates growth of heart and red muscle in rainbow trout. Can. J. Zool. 74:912-917.

PERSPECTIVES ON VENTRICULAR HYPERTROPHY

IN SEXUALLY-MATURE MALE RAINBOW TROUT

Kenneth. J. Rodnick
Department of Biological Sciences
Idaho State University
Pocatello, ID 83209 USA
phone: (208) 236-3790/fax: (208) 236-4570/e-mail: rodnkenn@isu.edu

Raymond J. Clark
Department of Biological Sciences
Idaho State University
Pocatello, ID 83209 USA
e-mail: clarraym@isu.edu

Introduction

The vertebrate heart displays a remarkable capacity for morphological and biochemical adaptation. One illustration of this adaptability is cardiac growth in adult animals, which functions to accommodate changes in work demand on the heart. Ventricular enlargement has been demonstrated in male rainbow trout in response to sexual maturation (Franklin and Davie, 1992; Graham and Farrell, 1992), however, no one has addressed the effects of hypertrophy on growth or cellular biochemistry of the layers of the trout ventricle, which consists of a compact, well-oxygenated epicardium and a trabecular, poorly-oxygenated endocardium. In addition, it is not known whether cardiac growth occurs through enlargement of individual cardiomyocytes (myocyte hypertrophy), proliferation of cardiomyocytes (myocyte hyperplasia), or both. Such knowledge would offer comparative insights concerning the relationship between cardiac development, functional capabilities, and plasticity in vertebrates.

Methods

Male rainbow trout, 18-36 months old (1533 ± 798 g) were reared at a commercial hatchery. Sexually-immature and mature animals were anesthetized with tricaine methanesulfonate and fork length and body weight was recorded. Ventricles were isolated and weighed, and myocardial layers were separated by blunt dissection. A portion of each layer was frozen in liquid N_2 for analysis of enzymes. An additional portion of each layer was rinsed in sodium phosphate buffer, fixed in a mixture of paraformaldehyde-gluteraldehyde, post-fixed in osmium tetroxide, dehydrated, and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and inspected at 4400x using a Zeiss EM 900 electron microscope. To determine morphometry (transverse cross-sectional area (CSA), circumference, and maximum diameter) of cardiac myocytes, we took micrographs in near-perfect cross section. Outlines of myocytes were traced from negatives using a photographic enlarger, then digitized subsequently to measure myocyte morphometrics. To facilitate measurements of myocyte length, we isolated intact ventricular myocytes from both layers using collagenase digestion, followed by filtration and centrifugation. Cell lengths were recorded at 400x from wet mounts using phase-contrast and a calibrated eyepiece micrometer.

To assess cellular biochemistry we measured maximal activities of aerobically and anaerobically-poised metabolic enzymes in whole homogenates of frozen tissue. These enzymes provided indices of sustainable glucose utilization (hexokinase), anaerobic metabolism (pyruvate kinase, lactate dehydrogenase), maximal aerobic metabolism (citrate synthase), and β-oxidation of fatty acids (β-hydroxyacyl CoA dehydrogenase). All enzyme activities were measured spectrophotometrically at 15^{o}C under saturating substrate concentrations. To examine the relationships between morphometric and biochemical variables, we calculated correlation coefficients, and conducted regressions analysis and analyses of variance. Statistical significance was established at $P < 0.05$.

Results and Discussion

Relative ventricle mass (ventricle weight ÷ body weight x 100) increased up to 2.4-fold during sexual maturation and this resulted in an increased proportion of epicardium relative to endocardium ($P<0.001$). Ventricular enlargement was associated with increased length (+31%) and transverse CSA (+83%) of cardiomyocytes, which resulted in up to a 2.2-fold expansion of mean myocyte

volume (from 1233 μm^3 to 2751 μm^3). Our observations indicate that sexual maturation induces ventricular enlargement through myocyte hypertrophy. Cell length and CSA were similar in both myocardial layers, and myocytes were elliptical rather than circular in transverse cross section. Ventricular hypertrophy did not alter transverse cell shape, perhaps reflecting the maintenance of short diffusion distances for small molecules as cells hypertrophy.

Measurements of maximal activities of metabolic enzymes demonstrated that ventricular hypertrophy was associated with (1) higher epicardial but not endocardial activities of citrate synthase (up to 23%) and β-hydroxyacyl CoA dehydrogenase (up to 20%); (2) lower activities of hexokinase (down to 50%) in both layers; and (3) no change in lactate dehydrogenase or pyruvate kinase, which were also similar between layers. These results suggest that the energetic needs of the hypertrophied trout ventricle may be met through increased reliance on fatty acid oxidation, particularly by the endocardium, but decreased reliance on glucose as a metabolic fuel in both layers.

References

Franklin, C. E. and Davie, P. S. (1992). Sexual maturity can double heart mass and cardiac power output in male rainbow trout. *J. exp. Biol.* **171**, 139-148.

Graham, M. S. and Farrell, A. P. (1992). Environmental influences on cardiovascular variables in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol*. **41**, 851-858.

PRESSURE AND VOLUME OVERLOADS

ARE ASSOCIATED WITH

VENTRICULAR HYPERTROPHY

IN MALE RAINBOW TROUT

Raymond J. Clark
Department of Biological Sciences
Idaho State University
Pocatello, ID 83209 USA
phone: (208) 236-3790/fax: (208) 236-4570/email: clarraym@isu.edu

Kenneth. J. Rodnick
Department of Biological Sciences
Idaho State University
Pocatello, ID 83209 USA
rodnkenn@isu.edu

Introduction

Cardiac hypertrophy and its underlying etiology has been studied intensively in mammals, but until recently has received little attention in fish. Several recent investigations have established that ventricular hypertrophy is induced during sexual maturation in male rainbow trout (*Oncorhynchus mykiss*) (Clark and Rodnick, 1998; Franklin and Davie, 1992). These studies show that the relative mass of the ventricle (RVM; ventricle weight ÷ body weight × 100) is 2-3 fold greater in mature males when compared with females or immature males. Studies of cardiac hypertrophy in mammals demonstrate repeatedly that the most important etiologic factor associated with development of hypertrophy is an elevated hemodynamic workload imposed on the heart (Scheuer and Buttrick, 1987). In this study, we demonstrate increased hemodynamic loads, specifically elevated blood pressure and blood volume, are associated with ventricular hypertrophy in sexually-mature male rainbow trout.

Materials and Methods

We measured blood pressure and blood volume in unanesthetized male rainbow trout (n = 15; 747 ± 116 g) at various stages of sexual maturation. Animals were fitted surgically with a ventral aorta (VA) cannula (Gamperl *et al*., 1994) and housed individually in plastic tubes during recovery (> 20 hrs) and experiments. Aortic pressure tracings were recorded using a pressure transducer connected to a chart recorder. We analyzed the tracings subsequently to determine the following hemodynamic variables: systolic blood pressure (SP), diastolic pressure (DP), mean pressure (MP), pulse pressure (PP), heart rate (HR), the positive and negative first derivatives of arterial pressure development (+/-dP/dt, respectively), and the absolute and relative duration of arterial systole.

Following blood pressure measurements, we injected Evans blue dye (7 mg/kg body weight) into the VA through the cannula, and collected blood samples (0.5 ml each) every 15 min for 1.5 hours to determine blood volume (BV) using indicator-dilution methodology (Kitzman *et al*., 1990). A separate group of male trout (n = 12; 1583 ± 619 g) were used to measure compliance and distensibility of the bulbus arteriosus. Briefly, the bulbus was excised, connected to a pressure transducer and saline-filled syringe pump, and taken through two inflation-deflation cycles which were recorded on video. Subsequent analysis of the video recording yielded pressure-volume relationships for each bulbus, from which compliance ($\Delta V/\Delta P$) and distensibility (percent $\Delta V/\Delta P$) were determined. At the conclusion of these procedures, we recorded body weight, fork length, and individually the weights of the ventricle, bulbus, and testes for each animal. Relationships between hemodynamic variables and RVM were determined using correlational analysis. Stastical significance was assumed at $P < 0.01$.

Results and Discussion

Our study demonstrates for the first time that hemodynamic workload is elevated in trout exhibiting ventricular hypertrophy. Maturation resulted in ventricular hypertrophy, as demonstrated by up to a 2.7 fold increase in RVM. Mature trout exhibited elevated SP (54.5 *versus* 37.5 mmHg; Fig. 1), PP (21.4 *versus* 3.0 mmHg; Fig. 1), and BV (63 *versus* 37 ml/kg body weight; Fig. 2), indicating that the enlarged ventricles of mature trout experience greater pressure and volume workloads than do ventricles of immature animals. The elevated PP observed in mature animals is particularly insightful, as recent clinical studies in humans demonstrate that PP predicts the degree of cardiac

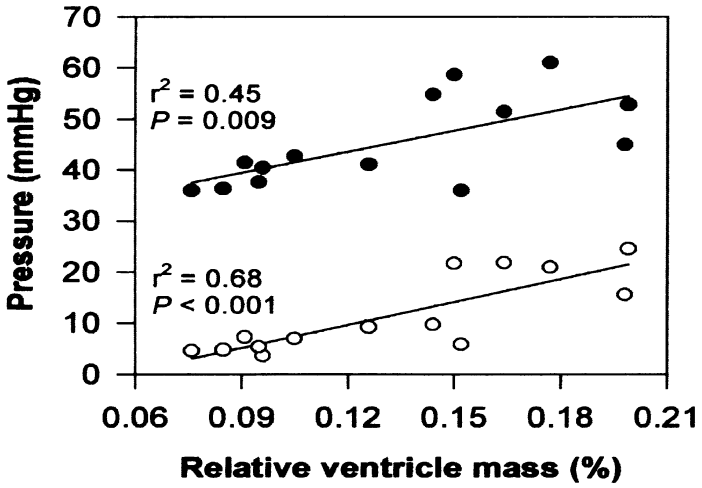


Figure 1. Increases in systolic pressure (●) and pulse pressure (○) with ventricular hypertrophy in trout.

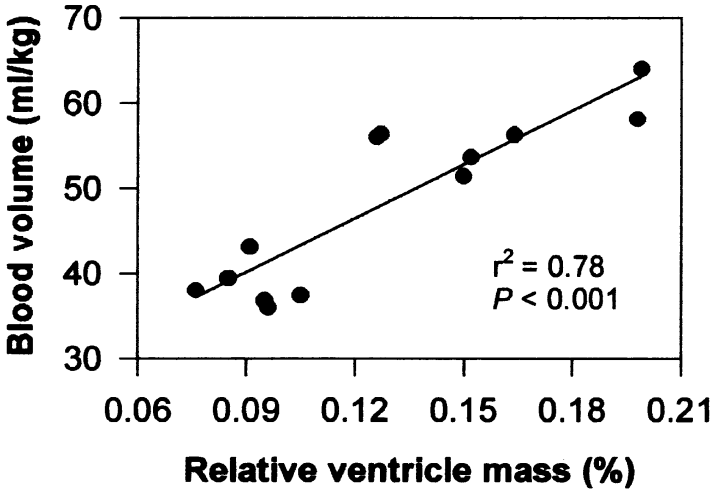


Figure 2. Increased blood volume with ventricular hypertrophy in trout.

All of our observations are consistent with either arterial stiffening (i.e., decreased compliance in the bulbus) or increased stroke volume. Arterial stiffening is unlikely based on two observations: (A) ventricle growth was associated with unaltered distensibility but increased compliance of the bulbus; (B) although arterial stiffening causes a simultaneous increase in SBP and decrease in DBP in mammals (Darne *et al*., 1989), we detected no change in DBP with increasing RVM. In the absence of arterial stiffening, an augmented stroke volume by itself can explain many of the cardiovascular changes we observed. This conjecture is supported by the observation that mature male trout have increased maximum stroke volumes (Franklin and Davie, 1992), a condition that can be facilitated by the increased BV and decreased HR of mature trout. Based on our observations, we conclude that ventricular hypertrophy with sexual maturation is associated with elevated preload and afterload, and this adaptive growth appears to be physiological rather than pathological.

Literature Cited

Clark, R. J. and Rodnick, K. J. (1998). Morphometric and biochemical characteristics of ventricular hypertrophy in male rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **201,** 1541-1552.

Darne, B., Girerd, X., Safar, M., Cambien, F. and Guize, L. (1989). Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. *Hypertension* **13,** 392-400.

Franklin, C. E. and Davie, P. S. (1992). Sexual maturity can double heart mass and cardiac power output in male rainbow trout. *Journal of Experimental Biology* **171,** 139-148.

Gamperl, A. K., Pinder, A. W. and Grant, R. R. (1994). Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater rainbow trout (*Oncorhynhchus mykiss*). *Journal of Experimental Biology* **193,** 209-232.

Kitzman, J. V., Martin, J. F., Holley, J. H. and Huber, W. G. (1990). Determinations of plasma volume, extracellular fluid volume, and total body water in channel catfish. *The Progressive Fish-Culturist* **52,** 261-265.

Scheuer, J. and Buttrick, P. (1987). The cardiac hypertrophic responses to pathologic and physiologic loads. *Circulation* **75,** I63-I68.

A COARSE SUBSTITUTE FOR THE FICK EQUATION

TO ESTIMATE THE METABOLIC RATE

OF EUROPEAN SEA BASS IN THE FIELD

Carole MERCIER
CREMA, CNRS-IFREMER, BP 5, L'Houmeau, France, 17137
phone: +33 05 46 50 06 18, fax: +33 05 46 50 06 00,
Email: cmercier@ifremer.fr

G. Claireaux[1] and D.M. Webber[2]
1: CREMA CNRS-IFREMER, 2: Dalhousie University, Halifax, Canada.

Introduction

The cardio-vascular system plays a key role in the processes which allow a fish to adapt to its environment. To date, heart rate telemetry is still the most effective technique available to evaluate the metabolic expenditure of free swimming fish (Priede, 1977, 1983; Lucas, 1991). However, heart rate (HR) is not an univocal correlate to metabolic rate (Thorarensen, 1996) as cardiac stroke volume (SV) and arterio-venous difference in oxygen content (Co_2^{a-v}) are also involved in setting the level of oxygen consumption (MO_2) of the organism. Unfortunately, in the field SV and Co_2^{a-v} are still out of reach.

To improve the estimation of metabolic dissipation in unrestrained fish, we tested a telemetry-compatible substitute to the Fick equation (*i.e.* $MO_2 = HR*SV*Co_2^{a-v}$). Cardiac stroke volume was approximated by the measure of the pressure in the pericardial cavity (PP) and ambient oxygen level (C_wO_2) was substituted for Co_2^{a-v}. The relationship between the product $HR*PP*C_wO_2$ and MO_2 was established in European sea bass (*Dicentrarchus labrax*) at three experimental temperatures: 11, 16 and 22°C.

Materials and Methods

Three groups of eight sea bass weighting approximately 400g were acclimated to water temperature of 11, 16 and 22°C.

Fish were anaesthetized with 2-phenoxyethanol 0.2‰. A surgical catheter (1mm diameter) filled up with NaCl solution (9‰) and coated with Benjoin dye was introduced ventral face above the heart in the pericardial cavity. The catheter was connected to a pressures sensor (Honeywell series 26PC, sensitivity 10 mV/psi, range 5.0 psi). Additionally two ECG electrodes were introduced on both sides of pericardial cavity. The same suture held in place the catheter and both ECG electrodes. Fish were then placed in a respirometer chamber (3.6 l).

Each fish was tested in wide range of experimental conditions : normoxia, hypoxia, chasing and all three parameters (*i.e.* ECG, PP and C_wO_2) were recorded simultaneous. In each situation, fish oxygen consumption (MO_2 in mg $O_2.kg^{-1}.h^{-1}$) was repeatedly calculated.

Results and Discussion

Figure 1 shows the relationship between the changes in pericardial pressure (PP) and the different waves of the ECG. Each decrease in PP is synchronised with the occurrence of a QRS complex which triggers myocardial contraction. The correspondence between both events indicates the good positioning of the surgical catheter in the pericardial cavity.

Classically, HR has been used as a mean to estimate metabolic expenditure in free ranging fish. If this approach was found satisfactory in some species (*e.g.* Armstrong, 1998), it turned out to be inappropriate in sea bass (Sureau and Lagardère, 1991). As shown in Figure 2, our own data confirmed this inadequacy, showing that there was no simple relationship between HR and MO_2 in this species. On the contrary, at all three temperature tested, experimental points tended to fill the entire area below the curve Active Metabolic Rate *vs* HR (solid lines in Figure 2).

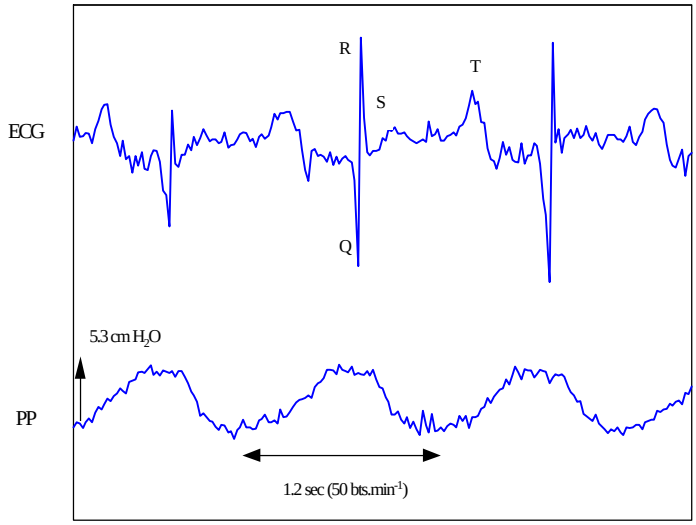


Figure 1: Correspondence between ECG waves (QRST) and changes in pericardial pressure (PP) at 22°C.

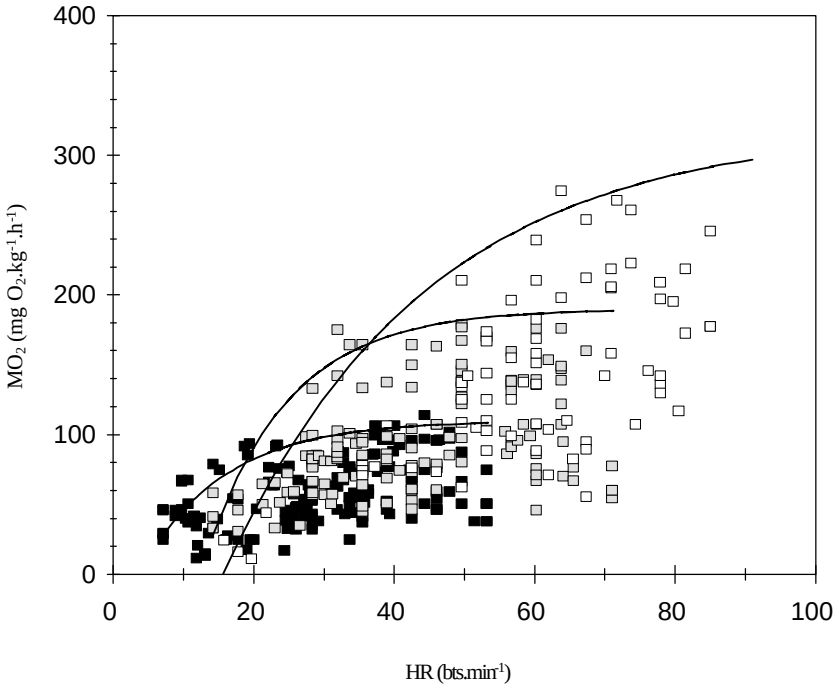


Figure 2: Relationship between oxygen consumption (MO_2) and heart rate (HR). Black symbols: 11°C, gray symbols: 16°C, opened symbols: 22°C. Solid lines: tentative relationships giving the maximum MO_2 (Active Metabolic Rate) for any given HR. At each temperature, experimental points distribute over the whole surface below the curve AMR=f(HR).

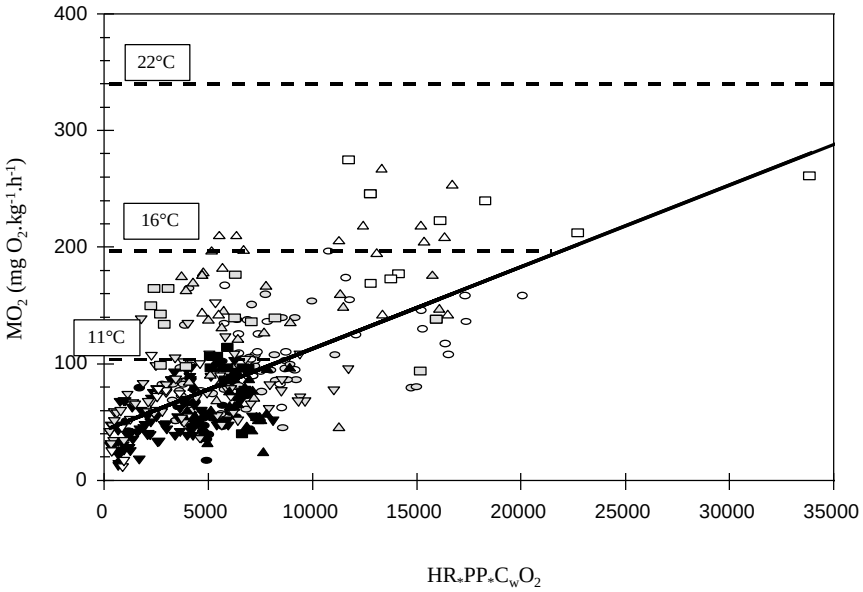


Figure 3: Relationship between oxygen consumption (MO_2) and the product $HR*PP*O_2$ where HR is the heart rate, PP the pericardial pressure and O_2 the water oxygen concentration. ❍: normoxia, ∇: hypoxia, Δ: recovery from hypoxia, ❑: recovery from stress; black symbols: 11°C, symbols: 16°C, opened symbols: 22°C. Solid line: $MO_2=0.007*(HR*PP*C_wO_2)+43.09$ ($r^2=0.39$). Dotted lines: Active Metabolic Rate at the temperature considered.

Taking into account PP and C_wO_2 greatly improved the accuracy of the evaluation of metabolic expenditure in sea bass (Figure 3). Our data set shows that routine MO_2 is linearly related to the product $HR*PP*C_wO_2$ and that this relationship is temperature independent. However some problems do remain. In situations of high metabolic demand (recovery from stress or from hypoxia) data points were mostly found above the regression line. It can be hypothesized that in such situations, C_wO_2 is not an adequate indicator of the conditions under which oxygen distribution throughout the organism takes place (Co_{2a-v}).

References

Armstrong, J.D., Relationships between heart rate and metabolic rate of pike: integration of existing data. J. Fish Biol. 52: 223-442.

Lucas, M.C., Priede, I.G., Armstrong, J.D., Gindy, A.N.Z. and De Vera, L. 1991. Direct measurements of metabolic activity and feeding behaviour of pike, *Esox lucius* L., in the wild, by the use of heart rate telemetry. J. Fish Biol. 39:325-342.

Priede, I.G. 1983. Heart rate telemetry from fish in the natural environment. Comp. Biochem. Physiol. 76: 515-524.

Priede, I.G. and Young, A.H. 1977. The ultrasonic telemetry of cardiac rhythms of wild brown trout (*Salmo trutta* L.) as indicator of bioenergetics and behaviour. J. Fish Biol. 10: 299-313.

Sureau, D. and Lagardère, J.P. 1991. Coupling of heart rate and locomotor activity in sole *Solea solea* (L.), and bass, *Dicentrarchus labrax* (L.), in their natural environment by using ultrasonic telemetry. J.Fish Biol. 38: 399-405.

Thorarensen, H., Gallaugher, P.E. and Farrell, A.P. 1996. The limitations of heart rate as a predictor of metabolic rate in fish. J. Fish Biol. 49: 226-236.

PROTEIN SYNTHESIS

BY THE MITOCHONDRIAL GENOME

OF RAINBOW TROUT *(ONCHORHYNCHUS MYKISS)*

HEART

W. R. Driedzic
Department of Biology, Mount Allison University,
Sackville, N.B. Canada E4L 1E4.
Tel – 506-364-2622. Fax – 506-364-2505.
wdriedzic@mta.ca

J.L. West, and J. R. Bailey

EXTENDED ABSTRACT ONLY DO NOT CITE

Introduction

Heart mass increases in male rainbow trout during sexual maturity and in both sexes when exposed to low temperature. Both conditions offer a valuable model system to study mechanisms of protein biosynthesis at the cellular and sub-cellular level. Heart growth is associated with an increase in mitochondrial population. Most mitochondrial proteins are encoded for by DNA in the nucleus, synthesised on ribosomes in the cytoplasm, and subsequently transported into the mitochondria. However, 4 polypeptides essential for synthesis of 4 inner-mitochondrial membrane proteins are encoded for by DNA in the mitochondria and synthesised on mitochondrial RNA. The trout heart is being exploited to study how the synthesis of mitochondrial encoded protein is controlled.

Methods

Mitochondria were isolated from trout heart and incubated with ^{3}H phenylalanine along with a complete mixture of amino acids, and malate and

pyruvate to serve as metabolic fuels. Following incubation total protein was isolated and level of label into the protein pool was tracked. Incorporation was linear over a three hour time period at 5°C, 15°C, and 25°C. Chloramphenicol which is a well established inhibitor of mitochondrial protein synthesis was included as a control in all studies. Isolated mitochondria were well coupled as evidenced by P:O ratios typically greater than 2 and RCR values typically about 8.

Results

Hearts from sexually mature male trout were relatively larger than hearts from sexually mature female trout [heart somatic index 0.104 ± 0.004 (n = 10) vs 0.087 ± 0.002 (n = 11)]. Mitochondria isolated from hearts of males had significantly higher rates of protein synthesis than mitochondria isolated from hearts of females [0.219 ± 0.021 (n = 10) vs 0.110 ± 0.022 (n = 11) picomoles phenylalanine incorporated/mg protein · min]. The mitochondria from male hearts are therefore predisposed to synthesize protein at a greater rate than mitochondria from female hearts. This is consistent with the elevated heart mass in males. Isolated mitochondria from male hearts consumed oxygen at a greater rate than mitochondria from female hearts [ADP activated rates - 0.124 ± 0.012 (n = 9) vs 0.096 ± 0.009 (n = 8) μmol O_2/mg protein · min]. However, within gender there was no correlation between rates of protein synthesis and any of the respiratory parameters measured (S3, S4, P:O, RCR).

Mitochondria were isolated from hearts of sexually immature fish which were acclimated to 13/C. There was no difference in the rate of protein synthesis at incubation temperatures of 25°C (0.456 ± 0.075 pmol phe/mg protein · min; n = 7) and 15°C (0.455 ± 0.027 pmol phe/mg protein · min; n = 8). However, at a test temperature of 5°C, the rate of protein synthesis decreased substantially (0.125 ± 0.020 pmol phe/mg protein · min; n = 6). Acclimation to 5°C (> 4 weeks) did not increase the rate of mitochondrial protein synthesis (0.071 ± 0.023 pmol phe/mg protein · min; n = 4) nor did a period of 7 - 12 days at 5°C reveal a transient increase in protein synthesis (0.088 ± 0.016 pmol phe/mg protein · min; n = 5). Therefore, low temperature alone has a substantial negative impact on protein synthesis. How mitochondria proliferate at low temperature remains to be determined. In contrast to protein synthesis, rates of oxygen consumption were temperature dependent across the full range of test temperatures. For fish acclimated to 13°C, rates of ADP activated oxygen consumption were 0.210 ± 0.012 μmol O_2/mg protein · min at 25°C, 0.150 ± 0.012 μmol O_2/mg protein · min at 15°C, and 0.100 ± 0.023 μmol O_2/mg

protein · min at 5°C. Therefore, metabolic rate is not a primary determinant of mitochondrial protein synthesis.

Acknowledgements

Support by N.B. Heart and Stroke Foundation and N.S.E.R.C. of Canada.

ISOLATED ATRIAL MUSCLE FROM YELLOWFIN TUNA UTILIZES CALCIUM RELEASED FROM THE SARCOPLASMIC RETICULUM DURING FORCE DEVELOPMENT

H.A. Shiels
Simon Fraser University, Department of Biological Sciences,
Burnaby BC, V5A 1S6
TEL: (604) 291-3977 FAX: (604) 291-3496
hollys@sfu.ca

E.V. Freund and B.A. Block
Hopkins Marine Station, Stanford University, Pacific Grove CA

A.P. Farrell
Simon Fraser University, Burnaby BC

Introduction

The routine heart rate of tunas (60-80 bpm) are not exceptional when compared with other fish. What is exceptional, is their maximum attainable heart rate. Maximal heart rates of 130-140 bpm and 154-230 bpm have been measured in yellowfin tuna and skipjack tuna, respectively (Brill, 1987). These heart rates slightly exceed the suggested upper limit to contraction frequency of 120 bpm observed for most fish (Farrell *et al*., 1991). This suggests differences between cardiac excitation-contraction coupling in tuna and other fish. One possibility for the apparent dichotomy between maximum heart rate in tuna and other fish, involves the cellular cycling of calcium during excitation-contraction coupling. Most fish cycle extracellular calcium back and forth across the cell membrane (sarcolemma; SL), with each beat. Mammals, on the other hand, cycle calcium between the intracellular calcium stores of the sarcoplasmic reticulum (SR) and the myofilaments. Therefore, the mode of E-C coupling in fish may result in longer diffusional distances for calcium movement, which may compromise maximum contraction frequencies. Skipjack tuna, which have relatively higher maximum contraction frequencies than other fish, utilize both intra- and extracellular calcium cycling to satisfy the calcium requirements of the

myofilaments (at 25°C, Keen *et al*., 1992) and thus appear intermediate between traditional "fish" and "mammal" excitation-contraction coupling models.

Although most other fish do not use SR calcium during contraction, studies with rainbow trout have suggested that warm temperatures can allow increased SR utilization. The temperature-dependency of SR utilization in fish may stem from the temperature-dependency of the SR-calcium-release channel itself. In mammals, cold temperatures can render the SR ineffective in sequestering and releasing calcium during contraction (Sitsapesan *et al*., 1991). Thus, the lack of SR involvement during excitation-contraction coupling in most fish hearts may reflect the fact that most studies are conducted at 5-15°C where there may be cold-induced opening of the SR-calcium-release channel (Tibbits *et al*., 1992). Conversely, the involvement of SR calcium during force development in skipjack tuna may simply reflect the 25°C test temperature. However, the effect of temperature on SR calcium release during E-C coupling has not been examined in tuna heart. Thus, the purpose of this research was to investigate how acute, physiological temperature change affects SR calcium contribution during force development in the atria of yellowfin tuna (*Thunnus albacares*) under physiological levels of adrenergic stimulation. Since calcium cycling has been implicated in contributing to the elevated maximal heart rates in tuna, the experiments were conducted over a range of pacing frequencies from sub- (0.2 Hz) to super-physiological (3.5 Hz).

Methods and Materials

Assessment of SR involvement is achieved using ryanodine, a specific and irreversible ligand for the calcium-release channel of the SR. When ryanodine is applied to muscle in the micromolar range, as in this study (10 μM), it locks the SR-calcium release channel closed rendering it ineffective in contributing calcium to force production. As such, tissue sensitivity to ryanodine is considered to reflect the dependence of contractility on calcium released from intracellular stores. Experiments were conducted at 15°C, 18°C or 25°C to test the temperature-dependency of the ryanodine response.

Trabecular muscle was dissected from the atrial lumen and hung in the isometric muscle apparatus (for detailed description of methods see Shiels and Farrell, 1997). Each experiment consisted of three force-frequency trials (1) low adrenaline (1 nM), (2) low adrenaline and ryanodine (10 μM), and (3) high adrenaline (1 μM) and ryanodine, performed sequentially on the same

trabeculae. Measurements of force, time to peak force, time to 50% relaxation, and rates (df/dt) of contraction and relaxation were calculated at each frequency. Significant increases after adrenaline stimulation, and significant decreases after ryanodine incubation were assessed using one-way student's t-tests ($P \leq 0.05$).

Conclusions

This is the first study to examine the relative importance of SR calcium cycling during force development in the yellowfin tuna. Our results indicate that SR calcium contribution is both frequency- and temperature-dependent. However, at maximal contraction frequencies, the SR can contribute up to 60% of the activator calcium during force development, independent of temperature. This is the greatest level of SR calcium involvement in force production reported for a teleost and is the first evidence directly linking the high heart rates of tunas with increased dependence on intracellular calcium cycling. Rates of contraction and relaxation were slowed after ryanodine treatment, further suggesting an active role for SR calcium involvement during excitation-contraction coupling. This active role for intracellular calcium cycling is in contrast to other fish species studied to date who rely primarily on extracellular calcium cycling.

In conclusion, the results of this study suggest that under normal conditions of temperature and heart rate, the SR plays an active role in contributing calcium to force development in the atrium of yellowfin tuna. Because % SR utilization increases in response to increased contraction frequency, we suggest that the ability to utilize SR calcium may be related to the high maximum attainable heart rates of yellowfin tuna.

References

Brill, R.W. 1987. On the standard metabolic rates of tropical tunas, including the effect of body size and acute temperature change. Fishery Bull. Fish Wldl. Serv. U.S. 85: 25-35.

Farrell, A.P. 1991. From hagfish to tuna-a perspective on cardiac function. Physiol. Zool. 64**:** 1137-1164.

Keen, J.E. Farrell, A.P., Tibbits, G.F., and Brill, R.W. 1992. Cardiac Physiology in Tunas. II. Effect of ryanodine calcium and adrenaline on force-frequency relationships in atrial strips from skipjack tuna, Katsuwonus pelamis. Can. J. Zool. 70: 1211-1217.

Shiels, H.A. and Farrell, A.P. 1997. The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing calcium to force development in isolated ventricular trabeculae from rainbow trout. J. exp. Biol. 200: 16.07-1621.

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

Tibbits, G.F., Moye, C.D., and Hove-Madsen, L. 1992. "Excitation-Contraction Coupling in the Teleost Heart". In, Fish Physiology (eds. Hoar, W.S., Randall, D.J. and Farrell, A.P.) Academic Press. pp. 267-296.

OXYGEN DIFFUSION LIMITATIONS

IN MYOCARDIALSLICES

OF RAINBOW TROUT

Jordi Altimiras
Center for Respiratory Adaptation. Dept.of Zoophysiology,
Univ. of Aarhus,Universitetsparken, Building 131,
DK-8000 Aarhus C, DENMARK
Phone +45 8942 2591 Fax +45 8619 4186
email: Jordi.Altimiras@biology.aau.dk

EXTENDED ABSTRACT ONLY DO NOT CITE

Introduction

The low oxygen capacitance of ringer salines is the major hurdle in its usage, although this problem is generally believed to be solved by full oxygenation of the saline, thereby increasing the partial pressure of oxygen above 93 kPa (700 torr). In these conditions, diffusion limitations in perfused organs or tissues have been neglected but, to our knowledge, not tested in an experimental set-up. We decided to evaluate how oxygen uptake (VO_2) of the trout myocardium was influenced by the oxygen tension and the oxygen capacitance of the perfusate.

Methods

Oxygen uptake was measured in 1 mm thick myocardial slices of rainbow trout Oncorhynchus mykiss in a closed chamber. In experiment I, VO_2 was measured in resting conditions and after poisoning the tissue with the uncoupler dinitrophenol (DNP 0.1 mM) at different PO_2. In some trials, glycolysis was blocked with IodoAcetate (1mM). In experiment II, the oxygen capacitance of a lactate ringer was increased by approximately 5 times by adding a perfluorinated oxygen carrier (perfluorodecalin 11% v/v) and VO_2 compared with and without the PFC.

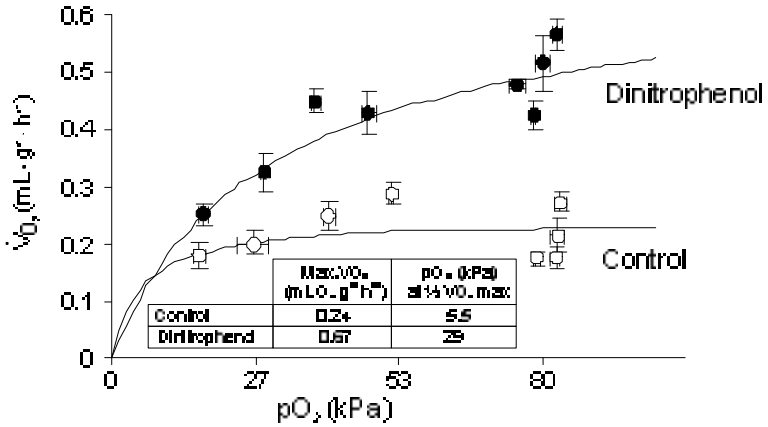


Figure 1. VO_2 as related to PO_2 of the ringer and "Michaelis-Menten-like" analysis to determine the threshold for O_2 limiting conditions.

Results

Resting oxygen consumption averaged 0.23 mL $O_2 \cdot g\text{-}1 \cdot h\text{-}1$ and was maintained at the lowest PO_2 tested (13 kPa, 100 torr). DNP increased oxygen consumption by 2 times at 80 kPa but a clear decrease was observed at 13 kPa, 26 kPa and 39 kPa although not at 52 kPa (Figure 1). The interruption of glycolysis did not alter oxygen consumption but dampened the effects of DNP, that only raised oxygen consumption by 50%. The increased oxygen content with PFC showed no difference versus the control ringer although VO_2 decreased linearly in the PO_2 range between 6-22 kPa (Figure 2).

Conclusions

At this stage our conclusions are:

a) the threshold PO_2 at which diffusion limitations occur is well below 20 kPa in resting non-working tissue but rise to 52 kPa in maximally stimulated tissue

b) a rise in oxygen capacitance has no effect in buffering the diffusion limitations at low PO_2

c) a revaluation of the problem in a perfused heart setting is required to understand how this applies to the in vivo oxygenation of the heart, with comparatively higher VO_2 (0.46 1mL O_2·g-1·h-1 extrapolating the results of Graham and Farrell (1990) to 0 mW power output).

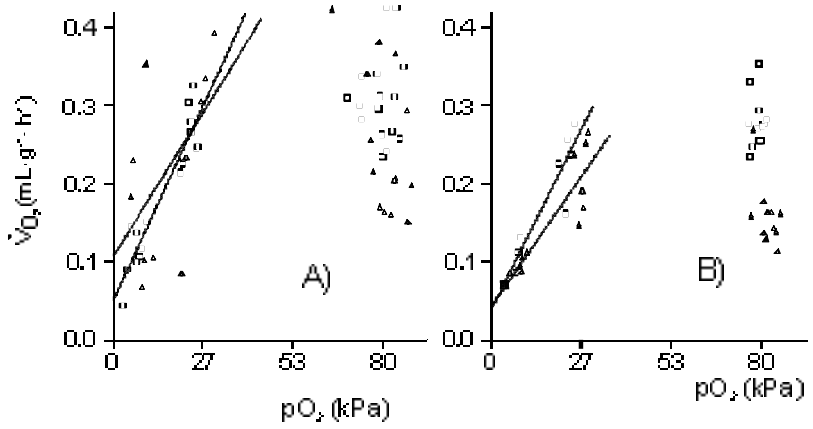


Figure 2. VO2 as related to PO2 of the ringerwith and without the presence of enriched oxygen solutions.
A) PFC solution with Pluronic as the emulsifying agent.
B) PFC solution with Egg-Yolk lecithin as emulsifying agent.

References

Graham & Farrell (1990). Physiol.Zool. 63:536

VASOACTIVE AGENTS AND THE BLOOD SYSTEM

OF HAGFISHES

Malcolm E. Forster
Department of Zoology, University of Canterbury, Private Bag 4800, Christchurch, New Zealand. Ph. ++64 3 364 2019. Fax ++64 3 364 2024. E-mail m.forster@zool.canterbury.ac.nz

It is because of their phylogenetic position that the cardiovascular system of hagfishes attracts attention. These ""phyletically ancient" animals are believed to have separated off from the vertebrate lineage about 375 to 550 mya (Mallat, 1985) and a study of living forms may cast light on properties of the blood system of the earliest vertebrates. Given their taxonomic position it is not surprising that people have described certain features of the cardiovascular system as primitive (Table 1).

Table 1. Features of the cardiovascular system of hagfish that have been described as "primitive".

1. Possession of large venous sinuses.
2. A high blood volume.
3. Low blood pressures.
4. The heart:
   a) long action potential.
   b) sparse myofibrils.
   c) hypoxia tolerance.
   d) cardiac control mechanisms - functionally aneural.
5. Lack of a functional renin-angiotensin system.

The cardiovascular system of hagfishes can be categorised as a high volume, moderate output system, with the lowest arterial blood pressures of any vertebrate animals (Forster, 1998). An extensive venous sinus system may hold 30% or more of the total blood volume. We do not understand the functional significance of the sinus system of hagfishes. Blood from the subcutaneous sinus is returned to the central circulation through the action of caudal and cranial "hearts". Blood from the pericardial sinus returns to the heart *via* the anterior cardinal anastomosis. The relationship of the sinuses and the central veins and the influence of mean circulatory filling pressures on cardiac function needs investigation.

Hagfishes use gill pouches for gas exchange, and their method of ventilation using a velum is very different from that in other fishes. The performance of isolated, perfused gill pouches indicates that a rise in pressure in the afferent branchial blood vessels is mirrored by a rise in the water ducts and *vice versa*. The relative lack of muscularity of the velum might require that the heart pumping blood to the gills be equally low-powered. The sparsity of myofibrils and the long conduction times of the cardiac action potential could ensure that pressures generated by the ventricle are not unsuitably high. The tolerance of the heart to hypoxia is almost certainly correlated with this low pressure development.

Branchial gas exchange is highly efficient in normoxic water and the PO_2 of venous blood can be very low, which indicates an efficient extraction of O_2 by the tissues. A number of agents, including catecholamines and natriuretic peptides, have possible effects on the pattern of blood flow through the gills, with implications for both gas exchange and ion transfer. The role of the subendocardial stores of catecholamines is not well understood. Perry *et al*. (1993) failed to demonstrate an effect downstream at the gills, but there is evidence implicating catecholamines in the regulation of branchial vascular resistance. The administration of the β-adrenergic blocking drug propranolol increased resistance markedly, and mimicked the action of hypoxia (Forster *et al*., 1992).

Of the agents tested to date , mammalian natriuretic peptides are the only ones which direct the outflow from branchial pouches to the venous sinus system of the gills (Fig. 1). By doing so they may reduce the volume of blood within the central circulation, which would be analogous to their function in other vertebrates.

In addition to catecholamines and natriuretic peptides a number of other pharmacological agents affect the circumferential tension in the walls of the branchial arteries. Carbachol, serotonin, cholecystokinin, arginine vasotocin and endothelin constrict afferent and efferent arteries. Prostaglandins and adenosine exhibit vasoactivity. We do not know how these agents control the circulation *in vivo*, if at all, but the responses reported suggest a sophisticated control of arterial vascular resistance that is at variance with the "primitive" label.

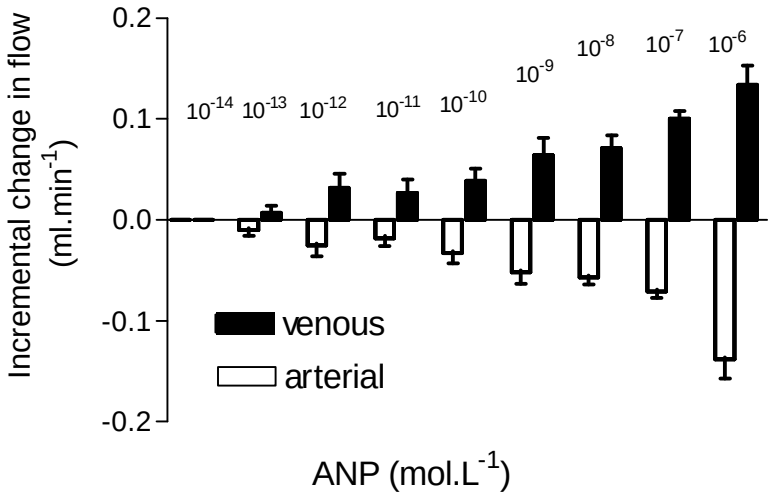


Fig. 1. The effect of rANP on the distribution of the fluid leaving perfused hagfish gill pouches. (C.W. Simpson and M.E. Forster, unpublished)

The lack of any cardiac response to acetylcholine and the fact that the rate and force of the branchial heart are not modulated by nerves may represent a derived condition. As with other "fishes" venous return is probably the major influence on cardiac output in hagfishes. The presence of a pressure sensitive sinus venosus pacemaker and endogenous catecholamines may help to make extrinsic control of the heart redundant.

References

Forster, M.E. (1998) Cardiovascular function in hagfishes. In *The Biology of Hagfishes*, Eds. J.M. Jørgensen, J.P. Lomholt, R.E. Weber and H. Malte, Chapman and Hall, London, pp.237-258.

Forster, M.E., Davison, W., Axelsson, M. and Farrell, A.P. (1992) Cardiovascular responses to hypoxia in the hagfish, *Eptatretus cirrhatus*. Respiratn. Physiol. 88:273-286.

Mallatt, J. (1985) Reconstructing the life cycle and the feeding of ancestral vertebrates. In Evolutionary Biology of Primitive Fishes, Eds. R.E. Foreman, A. Gorbman, J.M. Dodd and R. Olsson, Plenum, New York, pp. 59-68.

Perry, S.F., Fritsche, R. and Thomas, S. (1993) Storage and release of catecholamines from the chromaffin tissue of the Atlantic hagfish, *Myxine glutinosa*. J. Expt. Biol. 183:165-184.

CARDIAC STRETCH, VENOUS VASOACTIVITY AND BLOOD VOLUME IN FISH

A. P. Farrell
Biological Sciences, Simon Fraser University,
Burnaby, BC V5A 1S6, Canada
(604) 291-3647; Farrell@SFU.ca

K. R. Olson
Indiana University School of Medicine, University of Notre Dame,
Notre Dame, Indiana 46556, USA

Introduction

At this time we have a challenge to provide a more integrated view of the piscine cardiovascular system. This paper attempts to integrate three avenues of research that have proceeded somewhat independently over the years: the Starling response of the heart, the release of ANF from the heart, and the responsiveness of veins to vasoactive agents.

Atrial stretch and the increase in stroke volume

Vertebrate cardiac muscle responds intrinsically to stretch by increasing contraction strength, i.e., the Starling response of the heart. Consequently, hearts eject a greater stroke volume when atrial filling pressure is increased. Fish hearts are distinguished from mammalian hearts by being much more sensitive to stretch, as illustrated by Starling curves for the heart (a plot of stroke volume as a function of filling pressure). Indeed, stroke volume can triple in certain fish species with only a 0.2-0.3 kPa increase in filling pressure.

With cardiac stroke volume being so responsive to filling pressure, it is perhaps not surprising that most fish species studied thus far increase cardiac output more so by increasing stroke volume rather than increasing heart rate. While the sensitivity of the fish heart to atrial filling pressure is undoubtedly beneficial in

the regulation of cardiac output, another cardiac stretch-sensitive mechanism could be compromised.

Atrial stretch and the release of atrial natriuretic factors (ANFs)

ANF release from atrial myocytes in response to stretch may be as universal as the Starling response. ANFs are family of peptide hormones that are released from mammalian atrial myocytes in response to stretch. In trout, ANF release also occurs primarily from atrial tissue and is very sensitive to atrial filling pressure (Cousins and Farrell 1996).

A concern in fish, however, is that because the Starling response is much larger than in mammals, the appreciable increase in volume output from the heart could significantly dilute the ANF that is released. In perfused trout hearts, a step increase in filling pressure (from -0.08 to 0.36 kPa) caused immediate and large increases in both cardiac output and ANF release (Cousins and Farrell 1996); cardiac output increased 4.0-fold (from 17 to 69 mL/min/kg) and the ANF release rate increased 7.7-fold (from 30 to 230 pg ANF/min/g heart mass). Thus, even though some dilution occurs, the atrial sensitivity for ANF release more than matched the Starling response and the concentration of ANF leaving the heart increased by 3.7-fold.

The involvement of ANFs in blood volume regulation

ANFs are involved in blood volume regulation in mammals through their vasoactive, natiuretic, diuretic effects. In fish, however, the involvement of ANF in blood volume regulation is largely a matter of speculation, although central venous blood pressure and cardiac filling will be intimately involved.

Fish gills are the predominant binding site for ANF and recirculation of ANF is limited because as much as 60% of ANF is removed from blood within 5 min (Duff and Olson 1992; Sakaguchi et al. 1996). Any ANF returning to the heart is unlikely to directly affect its mechanical performance (Olson et al. 1997), even though ANF receptors are located there (Cerra et al 1996). ANF, however, is a potent vasodilator for large arteries and veins in trout (Olson et al. 1997), as well as having direct diuretic effects and ionoregulatory effects in other fish (Duff and Olson 1986).

Venous vasodilatation is particularly interesting because an ANF-mediated increase in venous compliance could provide a negative feedback inhibition of ANF release by reducing venous return to the heart and decreasing filling pressure. The possibility that ANF provides its own negative feedback takes on added importance when one considers how stable and prolonged ANF release can be in the absence of a negative feedback signal in a perfused trout heart preparation; ANF release rate was elevated 7-fold for 2.5 h, provided an elevated cardiac filling pressure was maintained (Cousins and Farrell 1996).

The precarious nature of blood volume homeostasis in teleost fish is evident when they exercise. Trout swimming in freshwater can gain (or lose in sea water) much as 10% body water. If, as we suspect, ANF is involved in piscine blood volume regulation, marine fish might differ with freshwater fish. Indeed, ANF binding sites tend to be downregulated in sea water compared with freshwater eels (Sakaguchi et al. 1996). However, the sensitivity of ANF release to filling pressure is no different in trout acclimated to marine and freshwater (Cousins et al. 1997). Thus, the ANF release mechanism is apparently unchanged despite the different environmental challenges in terms of volume regulation. Thus, important differences may lie with the actions of ANF.

Swimming presents a problem with the proposed negative feedback mechanism for ANF release. Fish need to sustain an increase in stroke volume during exercise, but if this is produced by an increase in cardiac filling pressure, the simultaneous increase in ANF release would increase venous compliance, reduce venous filling pressure and curtail ANF release. Unfortunately, stroke volume would also decrease. Obviously, other interactive mechanisms must be involved.

It is possible that an adrenergic controls could play an important interactive role in exercising fish. Adrenergic venoconstriction could offset the vasoactive effects of ANF on venous compliance. In addition, adrenergic stimulation of the myocardium could enhance the Starling response without altering the sensitivity for ANF release. Alternatively, adrenergic stimulation might directly inhibit ANF release from myocytes. Thus, several mechanisms can be postulated whereby stroke volume can be elevated during exercise either with or without elevated plasma ANF concentrations. Such possibilities need to be tested experimentally.

References

Cerra, M. C. M. Canonaco, Y. Takei and B. Tota. 1996. Characterization of natriuretic peptide binding sites in the heart of the eel, *Anguilla anguilla*. J. Exp. Zool. 275:27-35.

Cousins, K. L. and A. P. Farrell. 1996. Stretch-induced release of atrial natriuretic factor from the heart of rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool. 74:380-387.

Cousins, K. L., A. P. Farrell, R. M. Sweeting, D. L. Vesely and J. E. Keen. 1997. Release of atrial natriuretic factor prohormone peptides 1-30, 31-67 and 99-126 from freshwater- and seawater-acclimated perfused rainbow trout (*Oncorhynchus mykiss*) hearts. J. Exp. Biol. 200:1351-1362.

Duff, D. W. and K. R. Olson. 1986. Trout vascular and renal responses to atrial natriuretic factor and heart extracts. Am. J. Physiol. 251:R639-R642.

Duff, D. W. and K. R. Olson. 1992. Atrial natriuretic peptide clearance receptors in trout: Effects of receptor inhibition in vivo. J. Exp. Zool. 262:343-346.

Olson K. R., D. J. Conklin, A. P. Farrell, J. E. Keen, Y. Takei, L. Weaver, M. P. Smith and Y. Zhang. 1997. Effects of natriuretic peptides and nitroprusside on venous function in trout. Am. J. Physiol. 273:R527-R539.

Sakaguchi, H. H. Suzuki, H. Hagiwara, H. Kaiya, Y. Takei, M. Ito, S. Shibabe and S. Hirose. 1996. Whole body autoradiography and microautoradiography in eels after intra-arterial administration of 125I-labeled eel ANP. Am. J. Physiol. 271:R926-R935.

Acknowledgements

This work was funded by the National Science and Engineering Research Council of Canada.

HOW WATER TEMPERATURE REALLY LIMITS

THE VERTICAL MOVEMENTS OF TUNAS AND BILLFISHES –

IT'S THE HEART STUPID

Richard W. Brill,
Pelagic Fisheries Research Program, JIMAR, SOEST, University of Hawaii
Mailing address: NMFS, 2570 Dole St., Honolulu, HI 96822-2396, U.S.A.
Phone: 808-592-8304, FAX: 808-592-8301, email:
rbrill@honlab.nmfs.hawaii.edu

Timothy E. Lowe, and Katherine L. Cousins
Pelagic Fisheries Research Program, JIMAR, SOEST, University of Hawaii

Introduction

Accurate population assessments, especially for highly mobile pelagic species such as tunas (family *Scombridae*, tribe *Thunnini*) and billfishes (family *Istiophoridae*), require the ability to differentiate changes in abundance from changes in vulnerability to capture resulting from the natural variability in oceanographic conditions. Numerous studies have attempted to delineate the temperatures and oxygen levels that tunas and billfishes prefer, can withstand, or will avoid by employing catch statistics and oceanographic data averaged over time and space. Unfortunately, averaging catch statistics and environmental data can sometimes obscure, rather than elucidate, the relationships between species density and environment conditions. This occurs because fisheries and oceanographic data are often gathered separately in time and space, and because the inherent variability in both averages is usually too broad to clarify exact meaningful relationships (Sharp, 1978). More important, correlations of environmental data and catch rates do not prove causation and perpetuate a sort of circular logic. For instance, if tunas are rarely or never caught under a particular set of environmental conditions we assume the conditions are unsuitable. How is it known that they are unsuitable? -- because tunas are rarely or never caught when and where they occur. Entrapping circular arguments, missing data, limitations of catch per unit effort (CPUE) indexes, and the enormous difficulty of producing integrative models are just some of the obstacles fisheries biologists and fisheries managers face when attempting to

resolve pelagic fish population assessment issues and resource allotment questions with some confidence. The immediate objective of our research is, therefore, to combine laboratory and telemetry studies to investigate the interactions between environmental conditions and pelagic fish movements, distribution, and vulnerability to capture by specific fishing gears. Our overall objective is, however, ultimately to improve current tuna and billfish stock assessment methods.

Water Temperature Limits the Vertical Movements of Tunas and Billfishes

Ultrasonic depth telemetry studies of yellowfin tuna (*Thunnus albacares)*, skipjack tuna (*Katsuwonus pelamis*), blue marlin (*Makaira nigricans*), and striped marlin (*Tetrapturus audax*) all suggest that water temperature 8^{o}C colder than surface water temperature limit vertical movements in spite of wide differences in body mass and surface water temperatures (Brill et al., 1993, 1998). Recent studies of the behavior of adult (estimated body mass 60-90 kg) yellowfin tuna the near the Hawaiian Islands revealed that these fish spend the majority of their time in upper uniform temperature layer (i.e., shallower than 120 m). Moreover, their depth distribution was found to be essentially identical to that of the juvenile yellowfin tuna (body mass approximately 2-5 kg) followed in the same area some years earlier (Holland et al.1990). These observations, however, contradict much of what was thought to be understood about the thermal physiology of tunas.

Tunas have vascular counter-current heat exchangers which decouple heat production in the muscle and heat loss at the gills. These unique structures thus allow tunas to keep their muscles significantly warmer than the surrounding water. Vascular counter-current heat exchangers also slow the rate at which the tunas' muscle temperatures change when going from the warm surface layer to deeper, colder waters. Neill et al. (1976) were the first to propose that this enhanced "thermal inertia" should allow tunas to spend more time in deeper, colder water and to exploit more effectively whatever food resources are found there. Large yellowfin tuna should, therefore, have greater vertical mobility (i.e., ability to spend more time in deeper and colder water) then juvenile fish because their greater body mass affords even slower rates of muscle temperature change following abrupt decreases in ambient temperature. Yet, as stated, direct observations of adult and juvenile yellowfin tuna carrying depth sensitive ultrasonic transmitters showed identical vertical movement patterns in spite of the body mass of the adult fish being approximately 10-20 times larger than that

of the juvenile fish. In summary, neither differences in body mass, nor the presence of vascular heat exchanges in tunas and their absence in billfishes, appears to influence the limiting effect of water temperature on the vertical movements of these pelagic fishes.

A fresh perspective (or maybe we've been looking at the wrong end of the horse)

The basic premise underlying the idea that larger tunas should be able to spend more time in deeper, colder water is that body (i.e., swimming muscle) temperature is the most important factor limiting vertical movements. Our experiments, however, imply that it is the temperature of the heart that really limits the vertical movements of tunas and (by implication) billfishes. The heart is on the "water" side of tunas' vascular counter-current heat exchangers. This means its temperature will follow directly changes in water temperature regardless of the presence or absence of vascular counter-current heat exchangers or the size of the fish. The relatively simple recognition that the temperature of the heart is a limiting factor in behavior is a novel idea, but can it really explain the observed vertical movements of tunas and billfishes in the open ocean?

A nearly completed series of experiments on the effects of rapid ambient temperature change on the cardio-respiratory function of tunas revealed that reductions in water temperature result in an immediate and parallel decrease in heart rate. Figure 1 shows the response of a yellowfin tuna

exposed to an abrupt 25 to 15^{o}C change in water temperature (skipjack tuna respond in essentially the same manner). Note that heart rate follows the change in water temperature not the change in muscle temperature, which lags significantly behind. Because of tunas' limited ability to increase stroke volume (i.e., the volume of blood pumped per heart beat), cardiac output falls with heart rate (Farrell et al., 1992). Reductions in water temperature, therefore, directly and immediately impact the cardiac output of tunas (and by implication billfishes), thereby limiting swimming performance.

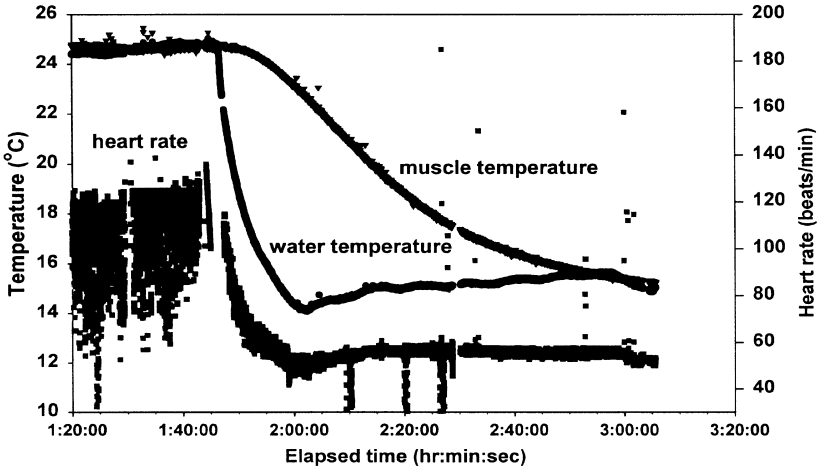


Figure 1. Effect of an abrupt change in water temperature (25 to 15°C) on heart rate in a yellowfin tuna. Note that heart rate follows the change in water temperature, not muscle temperature. Cardiac output (data not shown) follows heart rate because of tunas' limited ability to increase stroke volume.

A second key observation is that at 15°C, tunas have no ability to increase heart rate. Unlike most other teleosts, tunas increase heart rate rather than stroke volume during periods requiring elevated cardiac output (Farrell et al. 1992). In tunas, as in other vertebrates, the vagus nerve (i.e., the 10th cranial nerve) acts as a regulatory "break" on heart rate and increases in heart rate result from reductions in vagal nerve activity. The actions of the vagus nerve can be blocked pharmacologically with atropine. When tunas are given atropine at 25°C, heart rate approximately doubles. Yet at 15°C, atropine has no effect (Fig. 2). In other words, at 15°C, tunas have no ability to increase heart rate or cardiac output and, therefore, little or no ability to meet any increase in oxygen demanded by the swimming muscles while chasing prey, escaping a predator, or metabolizing lactate (i.e., recovering from exhaustive exercise). Hence, the effect of temperature on heart rate and cardiac output appears to explain the

limiting effects of water temperature on the vertical movements of tunas and (by implication) billfishes.

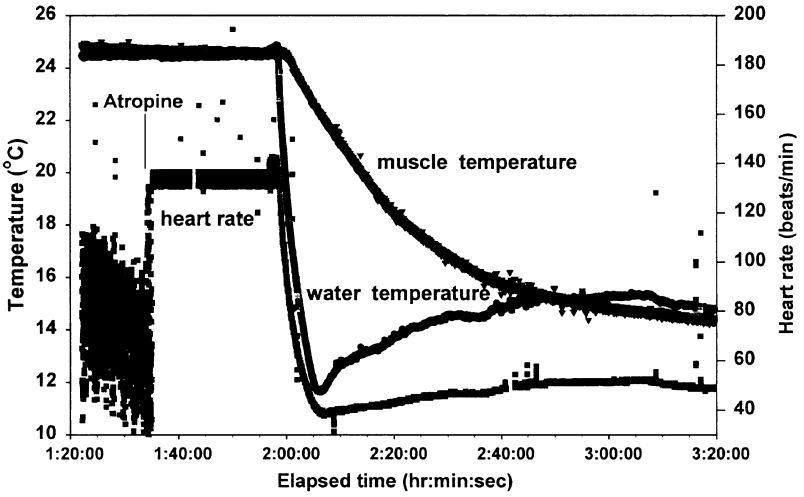


Figure 2. Effect of an abrupt change in water temperature (25 to 15°C) on heart rate in yellowfin tuna treated with atropine. Note that the heart rate is the same at 15°C is as in a yellowfin tuna not treated with atropine (Fig. 1). These data show that yellowfin tuna at 15°C have no ability to increase their heart rate or cardiac output.

Acknowledgments

This project was funded by Cooperative Agreements NA37RJ0199 and NA67RJ0154 from the National Oceanic and Atmospheric Administration with the Joint Institute for Marine and Atmospheric Research (JIMAR), University of Hawaii; and the National Marine Fisheries Service (Honolulu Laboratory, Southwest Fisheries Science Center). The views expressed herein are, however, those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies.

References

Brill R.W., D.B. Holts, R.K.C. Chang, S. Sullivan, H. Dewar, and F.G. Carey. 1993. Vertical and horizontal movements of striped marlin (*Tetrapturus audax*) near the main Hawaiian Islands, determined by ultrasonic telemetry, with simultaneous measurement of oceanic currents. Mar. Biol. 117: 567-574.

Brill, R., B. Block, C. Boggs, K. Bigelow, E. Freund, and D. Marcinek. 1998. Horizontal movements, depth distribution, and the physical environment of large adult yellowfin tuna (*Thunnus albacares*) near the Hawaiian Islands recorded using ultrasonic telemetry, with implications for their physiological ecology. Submitted to Marine Biology.

Farrell, A.P., P.S. Davie, C.E. Franklin, J.A. Johansen, and R.W. Brill. 1992. Cardiac physiology in tunas: I. In vitro perfused heart preparations from yellowfin and skipjack tunas. Cand. J. Zool. 70: 1200-1210.

Holland, K.N., R.W. Brill, and R.K.C. Chang. 1990. Horizontal and vertical movements of tunas (*Thunnus spp.*) associated with fish aggregating devices. Fish. Bull., U.S. 88: 493-507.

Neill, W.H., R.K.C. Chang, and A.E. Dizon. 1976. Magnitude and ecological implications of thermal inertia in skipjack tuna, *Katsuwonus pelamis* (Linnaeus). Environ. Biol. Fish. 1:61-80.

Sharp, G.D. 1978. Behavioral and physiological properties of tuna and their effects on vulnerability to fishing gear. In: G. D. Sharp and A. E. Dizon (eds), The Physiological Ecology of Tunas, Academic Press, New York, pp 397-441.

CONTROL OF THE HEART IN ELASMOBRANCH FISH:

EFFECTS OF AMBIENT TEMPERATURE AND OXYGEN LEVELS

E.W. Taylor
School of Biological Sciences, University of Birmingham, Edgbaston,
Birmingham B15 2TT, UK. 44-121-414-5472 (fax 5925)
E.W.Taylor@bham.ac.uk

C. Agnisola, University of Naples
M.J. Young and P.J. Butler. University of Birmingham

Discussion

Elasmobranch fish are unique amongst vertebrates in having no functional sympathetic innervation of the heart. Nervous control of heart rate is solely by varying levels of inhibitory, parasympathetic tone exerted via the cardiac branches of the Xth cranial nerve, the vagus (Taylor et al, 1977). This makes them the animal of choice when studying the essential features of vagal control of the vertebrate heart.

Their utility as a basic model for the study of vagal control of the heart is somewhat prejudiced by the fact that there are two pairs of cardiac vagi, the branchial and visceral branches (Taylor, 1992). However, the effects of progressive transection and electrical stimulation of these branches indicate that the branchial branches exert the majority of efferent inhibitory control, while the visceral branches are primarily sensory (Taylor et al, 1977; Short et al, 1977).

Study of vagal control of the elasmobranch heart is further complicated by the fact that *in vivo* cardiac function is influenced by an adrenergic tonus exerted by relatively high and varying levels of circulating catecholamines. In order to study their influence on the effectiveness of vagal control we have developed a preparation of the decerebrate dogfish which enables measurement of changes in cardiac performance following stimulation of the cardiac vagi. The *in situ* heart pumps saline through an extracorporeal circulation against a fixed resistance with controlled preload pressure (Agnisola and Taylor, 1998). This has enabled us to study the effects of adrenergic agonists and antagonists on cardiac

responses to peripheral stimulation of the branchial cardiac vagus. The degree of inhibition invoked by peripheral stimulation of a branchial cardiac branch varied with levels of noradrenaline added to the extracorporeal circulation. Levels that resembled those measured in resting fish augmented the degree of inhibition; whilst increasing the level tenfold, to that measured in disturbed or hypoxic fish, resulted in a reduced degree of inhibition, suggesting that increased levels of noradrenaline antagonise the effects of vagal stimulation on the heart, as would be predicted. These data are intriguing both for their physiological and for their mechanistic implications.

Vagal tone on the heart of dogfish varies directly with temperature and with oxygen level. Abolition of a normoxic vagal tone, either by cardiac vagotomy or injection of atropine, a muscarinic cholinoceptor antagonist, causes a larger increase in heart rate at higher temperatures (Taylor et al, 1977). Progressive hypoxia causes a reflex bradycardia, the onset and intensity of which varies with ambient temperature (Butler and Taylor, 1975).These data imply that the threshold for chemoreceptor induced changes in heart rate changes with temperature. The reflex bradycardia serves to improve the effectiveness of respiratory gas exchange (Taylor, 1992). This improvement is not associated with any change in cardiac output as the hypoxic bradycardia is accompanied by a proportional increase in cardiac stroke volume, following the Frank-Starling relationship (Taylor et al, 1977; Short et al, 1977), suggesting that the effect is subtle, possibly a result of increased pulsatility of flow or pressure, as suggested by Farrell (cited in Taylor, 1992).

In addition the bradycardia may serve to protect the heart from hypoxic damage (Farrell, pers. comm.). A proportion of the bradycardia developed in deep hypoxia in dogfish at 17°C persisted after injection of atropine (Taylor et al, 1977), indicating that it resulted from direct effects on the myocardium. This implies that performance of the heart was prejudiced under these conditions. We have noted in a current series of experiments on cod, that injection of atropine into an hypoxic animal only partially abolished a bradycardia and on occasions killed the fish, possibly due to heart failure.

Pulsed stimuli delivered to the peripheral cut end of a branchial cardiac branch entrained the heart, even when it was simultaneously being inhibited by continuous stimulation of the same nerve. Cardiac entrainment occurred over a wide range of pulse frequencies, with higher frequencies causing the heart to beat on alternate pulses. At some frequencies the heart was driven at a rate higher than that of the unstimulated preparation, so that stimulation of an

inhibitory pathway with pulsed stimuli was effectively increasing heart rate. These pulses mimic the respiration-related bursting activity recorded from the central cut ends of branchial cardiac branches (Taylor and Butler, 1982; Barrett and Taylor, 1985,a) and suggest a nervous mechanism for the establishment of cardiorespiratory synchrony.

Central recordings have revealed that the respiration-related bursting activity originates from cardiac vagal motoneurons (CVM) located together with respiratory vagal motoneurons (RVM), in the dorsal motonucleus of the vagus (DVN) (Barrett and Taylor, 1985,b and c; Taylor, 1992). CVM located outside the DVN do not show respiration-related activity and seem responsible for reflex changes in heart rate, such as that arising from stimulation of branchial mechanoreceptors or chemoreceptors. These latter responses can be invoked by central stimulation of branchial branches of the vagus.

Thus there are at least two separate populations of CVM in the dogfish, having different locations in the CNS and different functional properties. More recent physiological studies on mammals have accumulated evidence for a similar heterogeneity in the populations of CVM with some being affected by stimulation of lung stretch receptors and showing respiration related activity while others do not. The probable locations for these separate populations are the nA, where they will occurr together with respiratory vagal motoneurons, and the DVN.

References

Agnisola, C. and Taylor, E.W. (1998) Am. J. Physiol. (submitted)

Barrett, D.J. and Taylor, E.W. (1885, a,b,c) *J. Exp. Biol.* 117, 433-470 (3 papers)

Short,S. Butler, P.J. and Taylor, E.W.(1977) *J. Exp. Biol*. 70, 77-92.

Taylor, E.W. (1992) In: *Fish Physiology* Vol XXII B. 343-387 (ed.W.S Hoar, D.J. Randall and A. P. Farrell) Academic Press, NY.

Taylor, E.W. and Butler, P.J.(1982) *J. Appl. Physiol*. 53, 1330-1335.

Taylor, E.W. Short, S. And Butler, P.J. (1977) J. Exp. Biol. 70, 57-

CARDIOVASCULAR CONTROL

IN ANTARCTIC FISH

William Davison,
Department of Zoology, University of Canterbury,
Private Bag 4800, Christchurch, New Zealand.
ph +6433642029; FAX +6433642024;
e-mail bill@zool.canterbury.ac.nz

EXTENDED ABSTRACT ONLY DO NOT CITE

Antarctica is a region of both extremes and stability. It shows extremes of solar radiation, from 24h of daylight during summer to 24h of darkness in winter. This has flow on effects in terms of phytoplankton availability and thus along the food chain to food available for fish. Terrestrial temperatures show seasonal extremes. Seawater temperature, however, is at one extreme in that it is close to the freezing point of the liquid, yet is particularly stable, altering by no more than a few degrees throughout the year.

Although the physiology of Antarctic fish is poorly known, there are presumed to be fundamental differences compared with fish living in temperate and tropical waters, some of these differences being connected with the high viscosity of blood at low temperatures. The term "Antarctic fish" is itself somewhat misleading, as it refers almost exclusively to a single suborder (Notothenioidei). These are a dominant fish in Antarctica and do possess some interesting features. They are extremely stenothemal, have a high blood osmotic pressure which appears to be related to temperature and possess plasma antifreeze proteins. The notothenioids themselves are split into two obvious groups, those possessing red blood cells and those that do not. This paper presents some recent cardiovascular work carried out on red blooded fish in the family Nototheniidae.

Morphologically, the heart and gills of nototheniids are not greatly different to other teleosts, though total vascular resistances are much lower (Davison *et al*. 1997). Intrinsic heart rates of around 25 beats min^{-1} are not unduly different to those seen in temperate water fish indicating temperature compensation. However, these intrinsic rates are close to maximum and resting heart rates are much lower. Evidence indicates that the resting rate is maintained by cholinergic

inhibition, and that increased heart rate is achieved by reduction of this inhibition, rather than by adrenergic stimulation. Other evidence also points to a greater reliance on cholinergic neural control of the heart, gills and spleen. Nilsson *et al*. (1996) showed that release of red cells from the spleen during stress is due to cholinergic stimulation.

To date it has been reported that circulating catecholamines are low in nototheniid fish and that they do not change following a variety of stressors, lending weight to the concept of control via cholinergic mechanisms (Egginton, 1997). However, a recent study has shown that under some circumstances catecholamines are released. Forster *et al*. (1998) stressed two species of Antarctic nototheniids by placing them into water at 10^0C. This led to the release of extremely large amounts of adrenaline and noradrenaline into the blood stream, particularly in the active pelagic *Pagothenia borchgrevinki* (Fig 1). The catecholamines were shown to cause red cell swelling via a Na^+/H^+ antiporter. Interestingly, despite these high circulating levels of catecholamines, ventral aortic blood pressure and heart rate were kept constant in *P. borchgrevinki*. In the benthic *Trematomus bernacchii* blood pressure was maintained, while heart rate rose. It has been shown previously that in this species, control of heart rate is lost above 3^0C.

Some recent work has shown that control of blood flow through the gills of Antarctic nototheniids,is complex. Acetylcholine produced vasoconstriction in isolated perfused gill arches but was without effect on afferent or efferent arteries. Adrenaline showed both - and - responses throughout the gill vasculature, with - adrenergic constriction predominating, especially in efferent vessels. Interestingly, serotonin (5-HT) had a vasoconstrictory effect on afferent, efferent and lamellar blood vessels. Indeed, serotonin caused the greatest constriction of all drugs tested and the data indicate that antarctic fish tissues are much more sensitive to this drug than are temperate water teleosts.

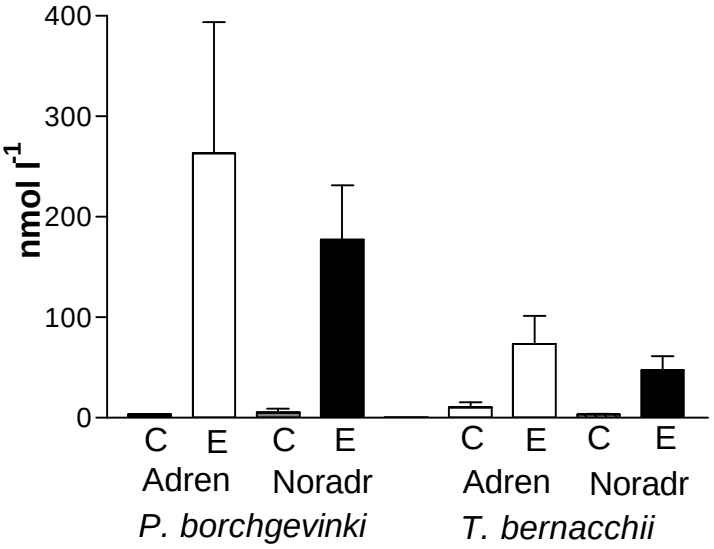


Fig. 1 Levels of adrenaline and noradrenaline in two species of antarctic fish (*Pagothenia borchgrevinki* and *Trematomus bernacchii*) at rest (C) and following exposure to water at 10^0C (E).

References

Davison, W., M. Axelsson, S. Nilsson and M.E.Forster. 1997. Cardiovascular function in Antarctic Notothenioid fishes. Comp. Biochem. Physiol. 118A: 1001-1008.

Egginton, S. 1997. The physiological reponse of Antarctic fishes to environmental and experimental stress. Cybium 21: 415-421.

Forster, M.E., W. Davison, M. Axelsson, L. Sundin, C.E. Franklin and S. Gieseg. 1998. Catecholamine release in heat stressed Antarctic fish causes proton extrusion by the red cells. J. Comp. Physiol. B. (In press).

Nilsson, S., M.E. Forster, W. Davison and M. Axelsson. 1996. Nervous control of the spleen in the red-blooded Antarctic fish *Pagothenia borchgrevinki*. Am. J. Physiol. 270: R599-R604.

IMPACT OF TEMPERATURE ON HEART FUNCTION

AND CALCIUM MANAGEMENT

IN EURYTHERMAL TROPICAL FISH

Ana Lúcia Kalinin
Department of Physiological Sciences, Federal University of São Carlos,
Rodovia Washington Luiz, km 235, 13565-905 - São Carlos, SP, Brazil
phone: ++55 16 2608314/fax: ++55 16 2608328/e-mail:
akalinin@power.ufscar.br

Monica J. Costa [1], F. Tadeu Rantin [1], Hans Gesser [2] &
William R. Driedzic [3]
1. Department of Physiological Sciences,
Federal University of São Carlos - SP, Brazil
2. Department of Zoophysiology, Aarhus University - Aarhus, Denmark
3. Biology Department, Mount Allison University - Sackville, Canada

Introduction

The ability of cardiac muscle to maintain pump performance under different physiological conditions is one of the most important characteristics that allow vertebrates to survive in extreme conditions. It is well known that changes in environmental temperature have an immediate and substantial effect upon cardiac performance in north-temperate teleosts. By contrast, there little information is available concerning the impact of temperature on tropical species. The present study report on two tropical species: the tide pool fish *Bathygobius soporator* (Gobiidae), normally exposed to acute temperature shifts from about 25 ^{o}C to greater than 40 ^{o}C during the summer in South America Atlantic coast, and the freshwater cichlid *Oreochromis niloticus*, tolerant to a wide range of temperatures (from ~ 10 to 35 ^{o}C) in different Brazilian regions. Both species were acclimated to 25 ^{o}C.

Methods

Control values of the i*n vivo* heart rate (f_H - bpm) were obtained by electrocardiography at 25 ^oC. The temperature was then increased to 30, 35 and 40 ^oC. For the *in vitro* preparations, pairs of strips (~1 mm diameter) were excised from the ventricle, placed in the bathing medium and connected to a isometric force transducer and to a stimulator. Muscle preparations were subjected to temperature transitions from 25 to 40 ^oC, maintained at 40 ^oC for 30 min and decreased back to 25 ^oC. To examine the dependence of the cardiac muscle responses on the excitation-contraction coupling, an experimental series was performed at a physiological level of 1.25 mM extracellular Ca^{2+} and at an elevated level of 9.25 mM Ca^{2+}. The maximal capacity of hearts to develop force was assessed trough the addition of Ca^{2+} or adrenaline to the bathing medium. Preparations were also subjected to an increase in imposed contraction frequency. The force development upon the first stimulation, following a rest period of 5 min, was determined with and without ryanodine.

Results

The f_H in whole animals is presented in Table 1. *O. niloticus* presented f_H values similar to those of north-temperate teleost fish, in which resting f_H are typically under 100 beats $\cdot$ min^{-1} while *B. soporator* presented f_H mean values higher than other species adapted to high temperatures. The controlled decrease in f_H of *B. soporator* at 40 ^oC may have a protective effect in maintaining low levels of intracellular Ca^{2+}.

In both species, at extracellular Ca^{2+} concentrations of 1.25 and 9.25 mM, a transition from 25 to 40 ^oC resulted in a decrease in twitch force. During the subsequent restoration to 25 ^oC, twitch force increased in both Ca^{2+} concentrations but did not achieve the initial values in *O. niloticus*, indicating a possible irreversible damage in the myocardial function after reaching the highest temperature. The mechanisms for the biphasic response of heart muscle of both species are unclear and might be multiple since many aspects of contractility and excitation-contraction coupling are influenced by temperature. However, the relationship between temperature and intracellular pH seems a likely candidate. At 25 ^oC, increments in extracellular Ca^{2+} from 1.25 mM to 7.25 mM in *B. soporator* and 9.25 mM in *O. niloticus* resulted in increases in twitch force development. However, at 40 ^oC, the twitch force of *O. niloticus*

increased progressively to reach maximum values at 9.25 mM, indicating a large dependence on extracellular Ca^{2+}. *B. soporator* presented increases only in the resting tension, probably due to increases in intracellular Ca^{2+}. In *B. soporator*, twitch force at 25 ^{o}C declined as frequency was increased above 30 contractions.min^{-1} and became irregular above 120 contractions.min^{-1}. In *O. niloticus* twitch force became irregular above 72 contractions.min^{-1}. At 40 ^{o}C twitch force development remained constant at frequencies up to about 150 contractions.min^{-1} in *B. soporator*, becoming irregular above 240 contractions.min^{-1}, similar to resting heart rates encountered *in vivo* in small mammals. In *O. niloticus*, twitch force development remained constant at frequencies up to about 120 contractions.min^{-1} and became irregular above 180 contractions.min^{-1}. In both species the post rest potentiation was not influenced by ryanodine at either temperature indicating that heart performance does not involve a functional sarcoplasmic reticulum to sequester Ca^{2+} during relaxation.

Temperature (^{o}C)	**f_H (bpm)**	
	B. soporator	***O. niloticus***
25	141,8 ± 6,1	33,8 ± 5,8
30	177,0 ± 6,3	66,0 ± 10,1
35	229,6 ± 13,6	87,2 ± 12,4
40	161,7 ± 10,3	97,2 ± 6,5

Table 1. Effect of a change in temperature from 25 to 40 ^{o}C on heart rate (f_H) of *O. niloticus* and *B. soporator*. Data are means ± S.E.

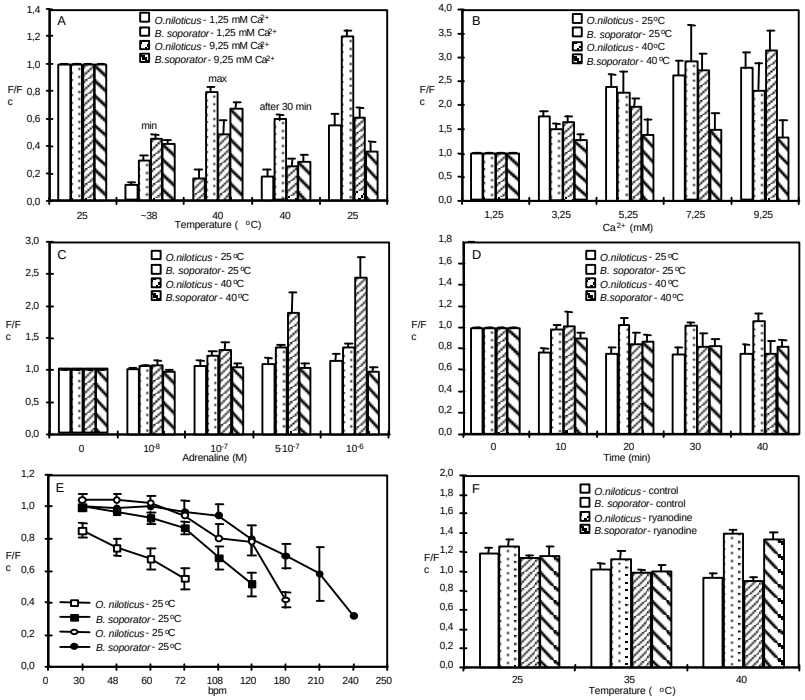


Figure 1. Effect of different treatments on twitch force of cardiac muscle of *O. niloticus* and *B. soporator* (mean ± S.E.): A. Changes in temperature from 25 to 40 ^{o}C and back to 25 ^{o}C; B. Extracellular Ca^{2+}; C. Adrenaline; D. Time exposure to different temperatures; E. Stimulation frequency; F. Twitch force of the first contraction following a rest period of 5 min.

TEMPERATURE SENSITIVITY OF CONTRACTILITY

IN THE SALMONID HEART;

THE ROLE OF TROPONIN C

Todd E. Gillis
School of Kinesiology, Simon Fraser University,
Burnaby, British Columbia, V5A 1S6
phone: (604) 291-4974; email: tegillis@sfu.ca

X Xue[†], Chris D. Moyes[‡], Thor Borgford[†], Glen F. Tibbits[†]
Simon Fraser University[†]; Queens University[‡], Kingston, Ontario, Canada

Discussion

As environmental temperatures decrease the maximal contractile force of cardiomyocytes and the sensitivity of these cells to Ca^{2+} is reduced (Brandt and Hibberd, 1976; Harrison and Bers, 1989; Churcott et al. 1994). Cytosolic Ca^{2+} concentration is used by cardiomyocytes to initiate contraction and regulate contractility. A reduction in these control mechanisms can disrupt or inhibit cardiac function. Teleost fish living where environmental temperatures seasonally range from 0^oC to 20^oC are exposed to conditions which are potentially cardioplegic. However, hearts isolated from rainbow trout, *Oncorhynchus mykiss*, acclimated to 11^oC were more sensitive to Ca^{2+} than rat hearts over the range 7^oC to 21^oC (Churcott et al., 1994). At 7^oC the $K_{0.5}$ value for teleost hearts was 5.8 while in rat heart it was 5.1. This higher sensitivity of teleost cardiomyocytes to Ca^{2+} suggests that the contractile element of these cells are adapted to function at lower temperatures. High Ca^{2+} sensitivity would help to offset the debilitating effects of low temperature by allowing regulatory control of contractility (Churcott et al., 1994).

One possible explanation for the differences in Ca^{2+} sensitivity between mammals and teleosts is the contractile protein troponin C (TnC). The replacement of native cardiac TnC with skeletal TnC in mammalian cardiomyocytes relieves the negative effect of lowered temperature on Ca^{2+} sensitivity (Harrison and Bers, 1990). TnC along with troponin I (TnI), troponin

T (TnT), actin, and tropomyosin constitute the functional unit of a thin filament from cardiomyocytes. The binding of Ca^{2+} to TnC triggers a cascade of events through the thin filament leading to the force generating conditions of muscle contraction.

The cardiac isoform of TnC has three functional Ca^{2+} binding sites, the first of these, called the regulatory site, is a low-affinity site which is unoccupied in diastole. The other two functional sites have a high affinity for Ca^{2+} and remain bound under all physiological Ca^{2+} concentrations. With an increase in intracellular Ca^{2+} concentration at the onset of systole the regulatory site of TnC becomes bound causing a change in the conformation of the protein. It has been suggested that this change in conformation exposes a hydrophobic region of TnC which then interacts with a hydrophobic region on TnI forming a strong bond (Herzberg et al. 1986). In diastole, TnI interacts strongly with actin, inhibiting the actin-myosin force generating reaction. The increased interaction between TnC and TnI decreases the inhibitory effect of TnI allowing crossbridge cycling between actin and myosin resulting in force generation and myocyte contraction. The binding kinetics of the regulatory site on TnC as well as the specific interactions between the individual protein components of the thin filament determine the characteristics of the contractile reaction (Solaro and Van Eyk, 1996). The secondary and tertiary structure of these proteins are, therefore, critical to how or if the thin filament will react when triggered by Ca^{2+}.

The higher Ca^{2+} sensitivity in teleost cardiac myocytes may be the result of differences in the primary sequence of TnC between mammals and teleosts. Such sequence variation could translate into differences in how the protein binds Ca^{2+}, reacts when bound by Ca^{2+}, or interacts with TnI in the contractile reaction. To investigate this possibility our research group has cloned and sequenced the salmonid isoform of TnC (Moyes et al., 1996). The primary sequence of this protein is very similar to that of the mammalian isoform. At the amino acid level only 13 of the 161 amino acids differ in sequence to the mammalian isoform. Remarkably, there is complete sequence identity between the two isoforms at the regulatory binding site. It is primarily in the region which constitutes the first high affinity Ca^{2+} domain where the amino acid sequence differs. This section of the protein is one of the regions which interacts with TnI. Differences in the amino acid sequence here could alter the overall conformation of the protein effecting the Ca^{2+} binding kinetics of the regulatory site, and/or effect how TnC would interact with TnI during the

contractile event. Either of these two possibilities could have an effect on the Ca^{2+} sensitivity of the teleost myocyte.

To determine if the differences in protein sequence effect Ca^{2+} sensitivity our laboratory is currently undertaking 2 different sets of experiments. The first of these is an invitro examination the Ca^{2+} binding kinetics (K_A, K_{on}, K_{off}) of teleost and bovine TnC isoforms at different temperatures. These will determine if there are differences in how TnC alone binds Ca^{2+} and if there are differences in how the binding kinetics of these different isoforms are effected by lowered environmental temperature. The second set of experiments are using recombinant DNA techniques to manipulate the primary structure of the teleost TnC isoform. Mutants of the teleost isoform where sections of the protein are replaced with the corresponding section from the bovine sequence will be engineered and then used to replace native TnC in functional teleost cardiac myocytes. Measurement of how these mutants effect myocyte contractility should determine if or what specific amino acid residues of the teleost TnC are responsible for high Ca^{2+} sensitivity. Taken together these two sets of experiments should establish if the high Ca^{2+} sensitivity demonstrated in teleost cardiac myocytes are the result of differences in the primary structure of TnC and then if so, if the TnC effect is the result of differences in Ca^{2+} binding kinetics or in how it interacts with TnI during contraction.

Acknowledgements

This work was supported through a Heart and Stroke Foundation of Canada Research Fellowship to T.E. Gillis, a Medical Research Council of Canada Fellowship to C.D. Moyes and a NSERC operating grant to G.F. Tibbits.

References

Brandt, P.W. and M.G. Hibberd. 1976. Effect of temperature on pCa-tension relation of skinned ventricular muscle of the cat. J. Physical. Loon. 258:76P.

Churcott, CS, C.D. Moyes, B.H. Bressler, K.M. Baldwin, and G.F. Tibbits, 1994, Temperature and pH effects on Ca^{2+} sensitivity of cardiac myofibrils: a comparison of trout with mammals. Am. J. Physical. 36:R62-R70.

Harrison, S.M., and D.M. Bers. 1989. The influence of temperature on the calcium sensitivity of the myofiliments of skinned ventricular muscle from the rabbit. J. Gen Physical. 93:411-427

Harrison, S.M., and D.M. Bers. 1990. Modification of temperature dependence of myofilament Ca^{2+} sensitivity by troponin C replacement. Am. J. Physical. 258:C282-C288.

Herzberg, O., J. Moult, and M.N.G. James. 1986. A model for the Ca^{2+} induced conformational transition of troponin C: a trigger for muscle contraction. Biol. Chem. 261:2638-2644.

Moyes, C.D., T. Borgford, L. LeBlanc, and G.F. Tibbits. 1996. Cloning and expression of salmon cardiac troponin C: titration of the low-affinity Ca^{2+}-binding site using a tryptophan mutant. Biochemistry. 35:11756-11762.

Solaro, R.J., and J. Van Eyk, 1996. Altered interactions among thin filament proteins modulate cardiac function. J. Mol. Cell. Cardiol. 28:217-230.

ATRIOVENTRICULAR CONTRACTILE DIFFERENCES IN THE HEART OF THERMALLY ACCLIMATED TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM)

Eija Aho
Department of Biology, University of Joensuu,
P.O.Box 111, 80101 Joensuu Finland
Phone: 358-13-2513569; Fax: 358-13-2513590;
E-mail: eija.aho@joensuu.fi

Matti Vornanen
Department of Biology, University of Joensuu,
P.O.Box 111, 80101 Joensuu Finland

Introduction

In mammalian heart atrial contraction is much faster than ventricular one. The cellular and molecular basis for this difference is complicated involving action potential duration, kinetics of intracellular Ca^{2+}-transient and properties of myofibrillar proteins. Although the importance of atrial contraction for the function of the heart is much greater in fish than in warm-blooded animals (Johansen and Burggren 1980), atrioventricular contractile properties have not been directly compared in teleost species. In the present study basic contractile characteristics and their cellular basis were delineated in thermally acclimated rainbow trout.

Material and methods

Trout were acclimated for more than three weeks at either 4°C or 18°C. Isometric contractile properties of excised atrial and ventricular tissues were determined at the acclimation temperatures of the animals. Thapsigargin-sensitive Ca^{2+}-uptake of sarcoplasmic reticulum (SR) was determined fluorometrically from crude cardiac homogenates using Fura-2 (Aho and Vornanen 1998). Ca^{2+}-Mg^{2+}-ATPase activity of purified atrial and ventricular

myofibrils was measured at three different temperatures (5, 10 and 15°C) . Electrophysiological experiments were conducted in enzymatically isolated myocytes using whole-cell patch clamp techniques (Vornanen 1998).

Results and Conclusions

Contractile properties.

The rate of isometric contraction was much faster in atrial than ventricular preparations. Time-to-peak force in atrial and ventricular tissue of WA fish at 18°C were 174 ±8 and 384 ±11 ms, respectively; corresponding values for CA fish at 4°C were 274 ±8 and 588 ±21 ms. Due to the shorter action potential and twitch duration the frequency-dependence of contractile force of atrial tissue differed from that of ventricular muscle. In preparations paced to contract at physiological rates full restitution of contractile force was achieved at much shorter diastolic intervals in atrial than ventricular muscle.

Cessation of steady-state stimulation for longer diastolic periods caused variable strengthening of post-rest contraction. Rest-potentiation was somewhat stronger in ventricular (157 and 204% for WA and CA fish, respectively) than atrial tissue (129 and 148% for WA and CA fish, respectively). In ventricular preparations rest-potentiation was associated with marked prolongation of twitch duration and it was not affected by ryanodine (10 μM, 60 min). In contrast to ventricular tissue, atrial rest-potentiation was completely abolished by ryanodine (10 μM, 60 min) in CA fish (Fig. 1).

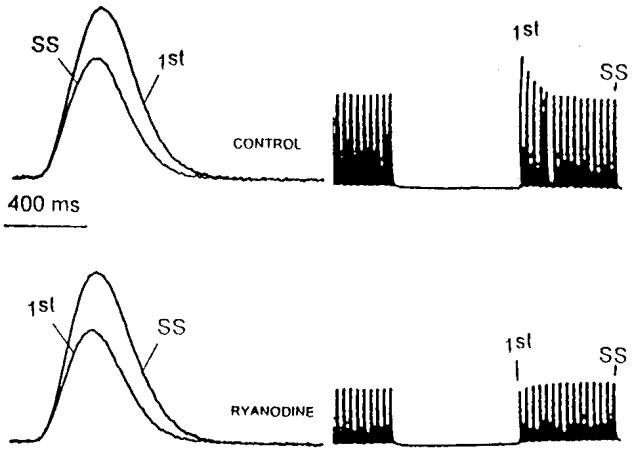


Figure 1. Effect of ryanodine on atrial rest-potentiation in CA rainbow trout at 4°C. SS = steady state contraction; 1st = first contraction after rest.

It is notable that the blocking effect of ryanodine occurred at the acclimation temperature of the fish (4°C), indicating that SR is capable for retaining its Ca^{2+}-load and that the Ca^{2+}-release channels of the fish cardiac SR are not leaking at low ambient temperature. The effect of ryanodine on rest-potentiation of atrial tissue in WA fish was weak. At physiological pacing rates the effect of ryanodine (10 μM, 60 min) on contraction force was negligible in the preparations of WA fish, while in atrial and ventricular muscle of CA trout a slight negative inotropic effect was noted. In contrast to ryanodine, nifedipine (10 μM) effectively inhibited force generation in all preparations.

Myofibrillar Ca $^{2+}$-Mg^{2+}-ATPase.

One explanation for the fast contraction of atrial tissue is the high Ca^{2+}-Mg^{2+}-ATPase activity of atrial myofibrils (Fig. 2). At the acclimation temperature of the fish ATPase activity was significantly higher in atrial than ventricular preparations. Furthermore, there was clear difference in temperature dependence between atrial and ventricular tissue, Q_{10}-values being much higher for atrial than ventricular preparations. On the other hand, Q_{10}-values of CA fish were lower than those of WA fish.

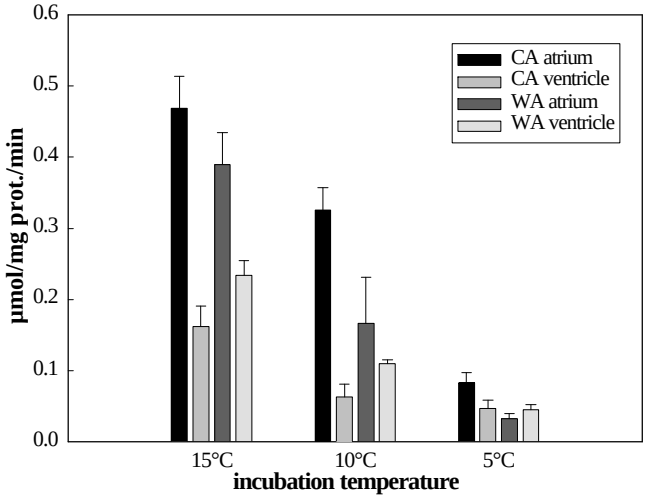


Figure 2. Myofibrillar Ca^{2+}-Mg^{2+}-Atpase activity of trout heart preparations.

SR Ca^{2+}-uptake.

The fast contraction kinetics of the atrial tissue can be partly attributed to fast Ca^{2+}-uptake of atrial SR. Ca^{2+}-uptake rate (µM/mg protein/min) of atrial preparations (0.391 ±0.052 and 0.299 ±0.028 for CA and WA atrium, respectively) was significantly higher than that of ventricular preparations (were 0.293 ±0.049, and 0.168 ±0.022, respectively) in both acclimation groups. Acclimation to cold enhanced Ca^{2+}-uptake rate of both atrial and ventricular

myocardium.

The present results show prominent differences in contractile properties between atrial and ventricular myocardium of the trout heart. The faster contraction kinetics of atrial tissue can be attributed to shorter action potential duration, higher myofibrillar ATPase activity and faster Ca^{2+}-uptake rate of SR in atrium when compared to ventricle. Contribution of intracellular Ca^{2+}-stores to contractile activation seems to be negligible in ventricular muscle but may be involved in atrial contraction especially in CA fish. Acclimation to cold causes positive compensation in SR Ca^{2+}-uptake rate.

Acknowledgements

This study was supported by the Academy of Finland (project #7641).

References

Johansen and Burggren Cardiovascular functioning lower vertebrates. In: *Hearts and heart-like organs*, edited by Bourne, G.H. New York: Academic Press, 1980, p. 61-117.

Aho, E. and Vornanen, M. 1998. Ca-ATPase activity and Ca-uptake by sarcoplasmic reticulum in fish heart: effects of thermal acclimation. J.Exp.Biol. 201: 525-532.

Vornanen, M. 1998. L-type Ca current in fish cardiac myocytes: effects of thermal acclimation and -adrenergic stimulation. J.Exp.Biol. 201: 533-547.

INFLUENCES OF AMBIENT OXYGENATION AND TEMPERATURE ON THE METABOLIC AND HEART RATES OF FREE SWIMMING EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

Christel Lefrançois
CREMA, CNRS-IFREMER, BP 5, L'Houmeau, France, 17137.
phone: +33 05 46 50 94 40, fax: +33 05 46 50 06 00, e-mail: clefranc@ifremer.fr

G. Claireaux.
CREMA, CNRS-IFREMER

Introduction

Many species of fish live in environments where abiotic parameters continuously fluctuate and so alter the physiological capacities of the animal. Priede (1983) reported that survival "is based on the continuous interplay between the demands of the environment and the physiological capacities of the animal represented by its metabolic scope". In the present study, our objective was to show how water conditions, by setting physiological limits, play a key role in the bioenergetic interactions between a fish and its environment.

In order to do so, we analysed and paralleled the influence of water temperature and oxygenation conditions on sea bass metabolic capacity and scope for increasing heart rate.

Materials and Methods

Four groups of three sea bass (*Dicentrarchus labrax*) were successively acclimated at 15, 20 and 25°C. At each temperature, groups were submitted to the following protocol. Two individuals were directly introduced into a 240 l respirometer, while the third one was equipped with an acoustic heart rate

transmitter (Vemco V16) according to the method described by Claireaux and Lefrançois (1998). Oxygen consumption (MO_2) and cardiac frequency (HR) were recorded in normoxic conditions and during stepped decrease in ambient oxygenation from normoxia to 20% air saturation. In some instances, in normoxia, fish metabolic rate was risen through feeding (specific dynamic action) or stress (chasing) in the respirometer.
At each temperature, the relationships between water oxygen saturation and maximum sustainable MO_2 (MO_2max) or maximum sustainable HR (HRmax) was established using the same general equation:

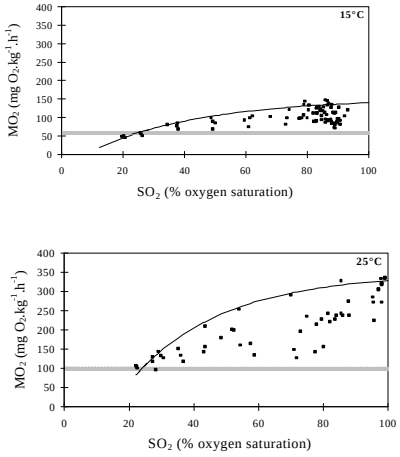
$$Y = Y_{as} (1 - e^{(\alpha_1 So_2 + \beta_1)}). \quad (1)$$

where Y represents MO_2max or HRmax, Y_{as} the curve asymptote at air saturation, So_2 the oxygen saturation (%), α and β two constants.

Results and Discussion

During hypoxia, the limiting effect of ambient oxygenation reduced the maximum sustainable MO_2 (figure 1) indicating that, for any given metabolic rate, there is a limiting oxygen concentration (LOC) below which the associated MO_2 can no longer be maintained. This notion of LOC developed by Fry (1971) and Neill and Bryan (1991) is illustrated on Figure 1 by the LOC-MO_2 curves (solid line). At 100% air saturation, the maximal MO_2 or active metabolic rate is strongly temperature-dependent and attained 152, 360 and 345 $mgO_2.kg^{-1}.h^{-1}$ at 15, 20 and 25°C respectively.

In a similar way, during hypoxia, the limiting effect of ambient oxygenation induced a marked decrease in the maximum sustainable HR (LOC-HR curve on Figure 2). Again these curves differed with temperature as maximal heart beat frequency at 100% saturation rose linearly, attaining 65, 87 and 103 $beats.min^{-1}$ at 15, 20 and 25°C respectively (Q_{10}=1.79). This Q_{10} is in accordance with the range of values from 1.3 to 3.0 found in others fish species (Farrell, 1997). Furthermore, the heart rate of sea bass at 25°C approached the upper limit of 120 $beats.min^{-1}$ admitted for fish (Farrell, 1997).



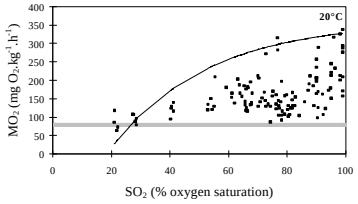
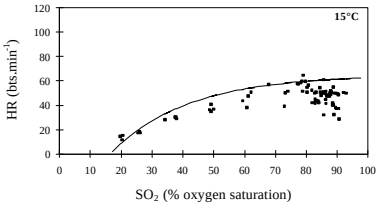
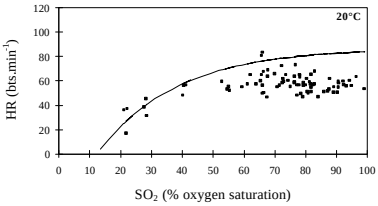


Figure 1. Influence of ambient oxygen saturation (SO_2) on the metabolic performance of the sea bass. Solid line: LOC-MO_2 curve (equation 1). Shaded area: standard metabolic rate (Claireaux and Lagardère, 1998).





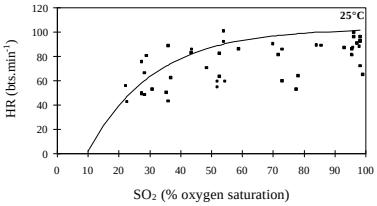


Figure 2. Influence of ambient oxygen saturation (SO_2) on the maximal heart rate of the sea bass. Solid line: LOC-HR curve (equation 1).

Figure 3 sums up the main results obtained. It shows how temperature and ambient oxygenation, by setting MO_2max and HRmax, delimit the framework within which sea bass must regulate its metabolic and heart rates. The size of this framework rises with temperature, but diminishes with oxygen saturation. During hypoxia, the limiting effect of low oxygen availability was quickly recognizable and was the strongest below 40% (Figure 3). This particular

threshold was also noticed by Schurmann et al. (1998) who showed that free swimming sea bass absolutely avoided oxygen saturation inferior to 40%.

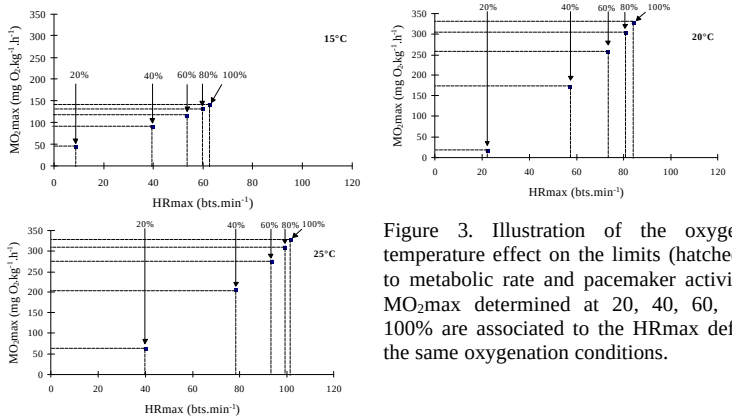


Figure 3. Illustration of the oxygen and temperature effect on the limits (hatched lines) to metabolic rate and pacemaker activity. The MO_2max determined at 20, 40, 60, 80 and 100% are associated to the HRmax defined in the same oxygenation conditions.

Conclusion

In terms of energy, the most important problem facing an animal trying to survive is to attain the power output necessary to live in its selected niche while operating well below its maximum power rating i.e., metabolic capacity (Priede, 1983). Measuring metabolic demand and heart rates in free swimming fish is a way to get an insight into the constraint associated with life in a given set of environmental conditions. However, the present study shows that in order to fully understand the bioenergetic processes which underlie the interaction between a fish and its environment, one must not only measure metabolic or heart rates, but also compare these data with the physiological capacities of the animal as they are imposed by the environment considered.

References

Claireaux, G. and C. Lefrançois. 1998. A method for the external attachment of acoustic tags on roundfish. Hydrobiologia: in press.

Farrell A.P. 1997. Effects of temperature on cardiovascular performance. *In* Global warming: implications for freshwater and marine fish. Series for

experimental biology seminar series 61. *Edited by* Wood C.M. and D.G. Mc Donald. Cambridge University Press, pp.135-158.

Fry F.E.J. 1971. The effect of environmental factors on the physiology of fish. *In* Fish Physiology, Vol. VI. *Edited by* W.S. Hoar and D.J. Randall. Academec press, New-York.

Neill, W.H. and J.D. Bryan. 1991. Responses of fish to temperature and oxygen, and response integration through metabolic scope. *In* Aquaculture and water quality, advances in world aquaculture. *Edited by* Brune D.E. and J.R. Tomasso. The world aquaculture society Baton Rouge, pp.30-57.

Priede I.G. 1983. Heart rate telemetry from fish in the natural environment. Comp. Biochem. Physiol. 76: 515-524.

Schurmann H., G. Claireaux and H. Chartois. 1998. Change in vertical distribution of sea bass (*Dicentrarchus labrax* L.) during hypoxic episode. Hydrobiologia: in press.

ATRIOVENTRICULAR CONTRACTILE DIFFERENCES IN THE HEART OF THERMALLY ACCLIMATED CRUCIAN CARP

Virpi Tiitu
Department of Biology, University of Joensuu,
P.O. Box 111, 80101 Joensuu Finland
Phone: 358-13-2514075; Fax: 358-13-2513590;
Email: Tiitu@joensuu.fi

Heikki Ståhlberg and Matti Vornanen
Department of Biology, University of Joensuu,
P.O. Box 111, 80101 Joensuu Finland

Introduction

The function of atrial and ventricular tissue in the pumping action of the heart is quite different. Ventricular contraction generates pressure for the ejection of blood, while atrial contraction regulates end-diastolic volume of the heart and thereby indirectly modifies cardiac performance. Due to the functional differences the contractile properties and molecular composition of atrial and ventricular muscle are markedly different. In mammalian heart atrial contraction and relaxation are much faster than those of ventricular tissue. The faster kinetics are contributed to shorter action potential duration, better developed sarcoplasmic reticulum and faster myosins in atrial myocytes when compared with ventricular cells (for references see Minajeva et al. 1997). In the fish heart atrial contraction is the principal mechanism for ventricular filling and therefore atrial muscle plays a far greater role in cardiac function in fishes than in mammals (Johansen and Burggren 1980). However, contractile properties of atrial and ventricular muscle have not been directly compared in fish.

Therefore, the aim of the present study was to clarify the basic characteristics of atrial and ventricular contraction in the crucian carp heart. Because our previous studies have shown that thermal acclimation modifies the contractile properties of the ventricular tissue in this fish species (Matikainen and Vornanen 1992;

Vornanen 1994; Vornanen 1996), the effects of thermal acclimation on atrial and ventricular contraction were studied as well.

Materials and Methods

Crucian carp were acclimated at either 4°C or 23°C for a minimum of three weeks. Contractile experiments were conducted at the acclimation temperature of the animal or at an intermediate temperature of 13°C. Pacing frequencies were selected to correspond the physiological heart rates. Contractile parameters were recorded on computer and were analyzed off-line using PClamp6-software.

Results and conclusions

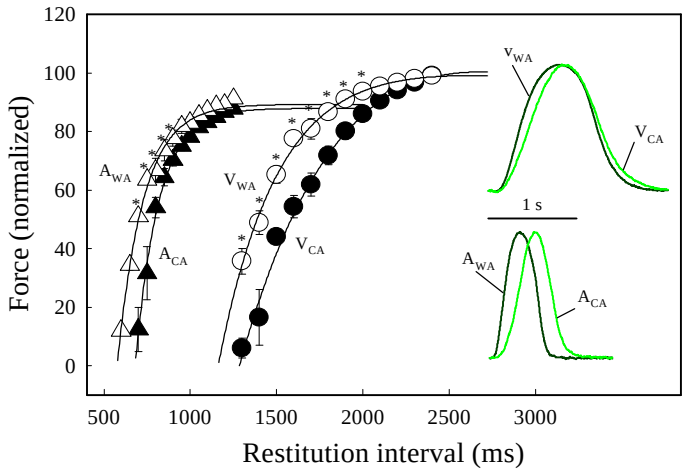


Figure 1. Force restitution in atrial and ventricular muscle of thermally acclimated crucian carp heart at 13°C. Time courses of ventricular (top) and atrial (bottom) contractions are shown at right.

Force restitution and rest-potentiation were measured to clarify frequency-dependent differences in force development between atrial and ventricular tissues. Contraction and action potential durations were much shorter in atrial than ventricular tissue. As a consequence, the restitution curve of the atrial tissue

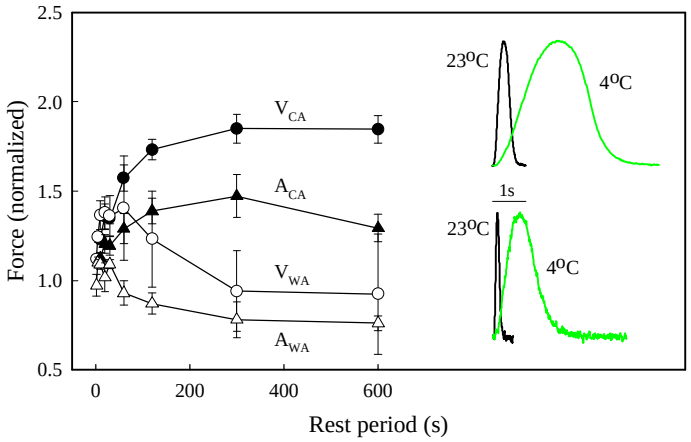


Figure 2. Rest-potentiation in atrial and ventricular muscle of thermally acclimated crucian carp heart at the acclimation temperatures of the fish (4° and 23°C). Time-courses of ventricular (top) and atrial (bottom) contractions are shown at right.

was sifted to the left in regard to ventricular one (Fig. 1). Experiments at 130C revealed acclimation-dependent changes in atrial and ventricular contraction; restitution curves of warm-acclimated (WA) fish were sifted to shorter diastolic intervals in comparison to those of cold-acclimated (CA) fish (Fig. 1). Furthermore, both ventricular and atrial contractions were slightly longer in duration and the rates of contraction and relaxation were slower in CA than in WA fish. Cyclopiazonic acid (20 M) and ryanodine (10 M), blockers of SR function, did not have any effect on the restitution of either atrial or ventricular tissue. Instead, the force of contraction was effectively inhibited by nifedipine (20 M), a Ca^{2+}-channel blocker.

Prolonged diastolic rest period caused variable potentiation of post-rest contraction. In the ventricle of CA fish the maximum potentiation was achieved at the rest period of 5 min and was 185% of the control force (0.16 Hz, 40C); this level of potentiation was maintained at longer rest periods. In the ventricle of WA fish the maximum potentiation was only 140% of the control force (0.8 Hz, 230C) (Fig. 2). This was achieved at the rest period of 2 min, whereas at longer diastolic intervals a prominent decay of contraction force occurred. The difference in rest-potentiation between acclimation groups was also evident at the common experimental temperature of 130C (0.5 Hz). Rest-potentiation of atrial muscle was much weaker than in the ventricular tissue. In CA fish atrial rest-potentiation was maximally 147% (5 min) of the steady-state twitch. In the atrium of WA fish a weak rest-potentiation (~ 110%) was present at short rest intervals (5-30 s), while longer diastolic intervals were associated with clear rest-decay (Fig. 2). Rest-potentiation was not modified by 10 TM ryanodine (60 min) in either atrial or ventricular preparations. Instead, rest-potentiation was always associated with marked prolongation of the twitch. The positive correlation between contraction force and twitch duration is explained by frequency-dependency of action potential duration, recorded in enzymatically isolated cells.

The present experiments show that the atrial contraction is much faster than the ventricular one. As a consequence, the frequency-dependent properties of force production differ significantly between atrial and ventricular tissues. Furthermore, acclimation to cold slows contraction kinetics and reduces the ability of ventricular and atrial tissues of the crucian carp heart to produce force at high contraction frequencies.

Acknowledgments.

This study was supported by the Academy of Finland (project #7641).

References

Minajeva, A., Kaasik, A., Paju, K., Seppet, E. K., Lompre, A. M., Veksler, V. and Ventura-Clapier, R. 1997. Sarcoplasmic reticulum function in determining atrioventricular contractile differences in rat heart. Am.J.Physiol. 273: H2498-H2507

Johansen, K. and Burggren, W.W. Cardiovascular function in lower vertebrates. In: *Hearts and heart-like organs*, edited by Bourne, G.H. New York: Academic Press, 1980, p. 61-117.

Matikainen, N. and Vornanen, M. 1992. Effect of season and temperature acclimation on the function of crucian carp (*Carassius carassius*) heart. J.Exp.Biol. 167: 203-220.

Vornanen, M. 1994. Seasonal and temperature-induced changes in myosin heavy chain composition of the crucian carp hearts. Am.J.Physiol. 267: R1567-R1573

Vornanen, M. 1996. Effects of extracellular calcium on the contractility of warm- and cold-acclimated crucian carp heart. J.Comp.Physiol.B 166: 1-11.

ELECTROCARDIOGRAPHIC CHARACTERIZATION AND MYOCARDIAL FUNCTION OF *PIARACTUS MESOPOTAMICUS* (TELEOSTEI, SERRASALMIDAE) EXPOSED TO GRADED HYPOXIA

Francisco Tadeu Rantin
Federal University of São Carlos, Department of Physiological Sciences,
Laboratory of Zoophysiology and Comparative Biochemistry.
Via Washington Luiz, km 235 - P.O. Box 676
13565-905 - São Carlos, SP - Brazil
Phone: ++55 16 260 8314, Fax: ++55 16 260 8328,
e-mail: ftrantin@power.ufscar.br

Cecilia Del Rosario Guerra, Ana L. Kalinin, Roberto
M.M. Verzola & Sergio E.A. Perez
Federal University of São Carlos, Department of Physiological Sciences
Via Washington Luiz, km 235 - P.O. Box 676
13565-905 - São Carlos, SP - Brazil

Abstract

We have developed a method that allows a detailed analysis of the electrical phenomena during the cardiac cycle of fish, including the 3 bipolar and monopolar leads of standard electrocardiography. Fish were fitted with 3 ECG electrodes, to configure the classic Einthoven triangle. Recordings were obtained under normoxia, graded hypoxia, and during recovery to normoxia. Normoxic fish presented f_H of 67 ± 4.5 bpm, P wave duration of 50 ms, PR intervals of 124 ms, and QRS complexes of 55 ms. In the frontal plane the SÂP was -120°, SÂQRS was 30°, and SÂT was -60°. The maximum amplitudes of P and T waves, and QRS complexes were in aVL (0.08 mV), D_{III} (-0.13 mV) and D_I (0.088 mV), respectively. SÂP and SÂQRS remained constant in all O_2 tensions. The SÂT remained nearly constant (60 to 90°) down to 30 mmHg,

shifted abruptly to 30^{o} at 10 mmHg, and shifted back to left (0^{o}) after 10 min of recovery to normoxia. After 30 min of recovery, this orientation shifted further to the left (-60^{o}). At 10 mmHg the T wave assumed an "ischaemic" pattern, with the highest amplitude (0.49 mV) at D_{III}. The changes in the morphology of the T waves and S$\hat{A}$T indicate that myocardial impairment is due to a "global ischaemia" under severe hypoxia and these changes are reversible when the O_2 supply returns to normal.

Introduction

Electrocardiography is a simple and usual method to examine the physiological state of the heart, and has been used in the past to monitor the changes in heart rate of fish under some specific conditions (Ueno et al., 1986; Mitsuda et al., 1988; Rantin et al., 1995). The use of several leads in fish is difficult for many reasons, such as the anatomic diversity of these animals and lack of information on the correct sites of the electrodes in relation to the heart (Gehrke & Fielder, 1988). In this respect, the ECG patterns depends on body shape and should be established for each species (Guerra, 1992; Rantin et al., 1993; Delavechia, 1994). The main purpose of the present study was to develop of a method for a detailed analysis of the electrical phenomena during the cardiac cycle in a serrasalmid fish, *Piaractus mesopotamicus*, recording simultaneously from 6 ECG leads from minimally disturbed animals under normoxic and hypoxic conditions. This species was chosen due to its cardiac responses to normoxia and graded hypoxia. The characterization of its myocardial function under such conditions were previously documented for the D_I ECG lead (Guerra, 1992; Rantin et al., 1995).

Materials and Methods

Prior to experiments, specimens of pacu, *Piaractus mesopotamicus* (n = 50; Wt = 195 to 571g), were maintained in holding tanks with an aerated flow-through water supply at constant temperature (25 ^{o}C) and kept at a photoperiod of 12:12 h. Commercial food pellets were provided *ad libitum*.

To obtain the ECG signals, fish were fitted with three electrodes placed in the frontal plane: the first electrode (RA) was inserted and sutured near to the base of the right pectoral fin, the second (LA) near to the base of the left pectoral fin, and the third (LF) near to the base of the left pelvic fin. This strong attachment

prevented relative displacements of the electrodes and improved the signal quality (Altimiras, 1995). The electrode positions configured the classic Einthoven triangle (Figure 1). All preparations were completed within 15 min while a light benzocaine anesthesia was applied. The level of anesthesia was sufficient but allowed for spontaneous breathing. After surgery the fish was placed into the experimental chamber to recover for at least 12 h in normoxia (140 mmHg) and a fourth electrode was immersed in the water to be used as reference. Meanwhile all disturbances were kept at a minimum to obtain resting control values. The electrodes were connected to a six channels ECG amplifier and recorder (Mikromed Type ER 661).

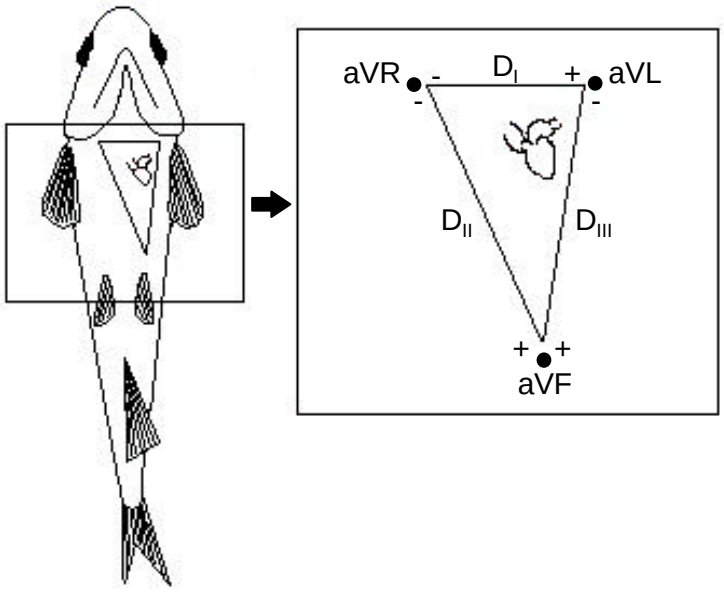


Figure 1. Schematic representation of the ECG electrode positions in relation to the heart of *Piaractus mesopotamicus*, configuring the Einthoven triangle with the 3 bipolar (D_I, D_{II} and D_{III}) and the 3 monopolar "limb" leads (aVR, aVL and aVF).

Simultaneous recordings of heart rate and standard electrocardiography, including the 3 bipolar (D_I, D_{II} and D_{III}) and the 3 monopolar "limb" leads (aVR, aVL and aVF), were obtained from fish in normoxia (140 mmHg), graded hypoxia (100, 70, 50, 30 20 and 10 mmHg) and during the subsequent recovery

to normoxia. The spatial orientation of the P (SÂP) and T (SÂT) waves, and QRS complexes (SÂQRS) axis were determined in all experimental tensions.

Results

Figure 2 presents the heart rate of *P. mesopotamicus* in normoxia, graded hypoxia, and during the subsequent recovery to normoxia.

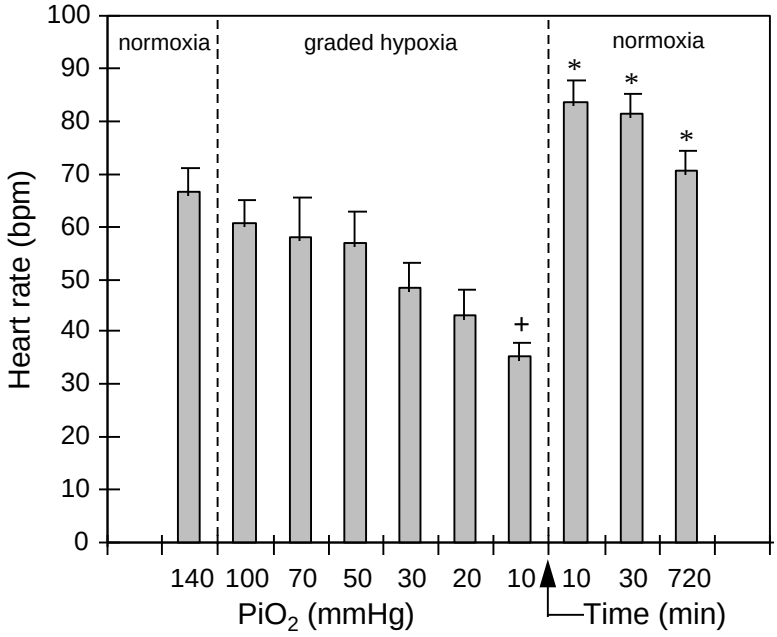


Figure 2. Heart rate (f_H - bpm) of *Piaractus mesopotamicus* (n = 12) in normoxia, graded hypoxia and during the subsequent recovery to normoxia (mean ± S.E.). + - indicates statistical differences (Friedman's test) in relation to control values ($P < 0.05$). ✻ - indicates statistical differences in relation to the more hypoxic tension of 10 mmHg ($P < 0.05$).

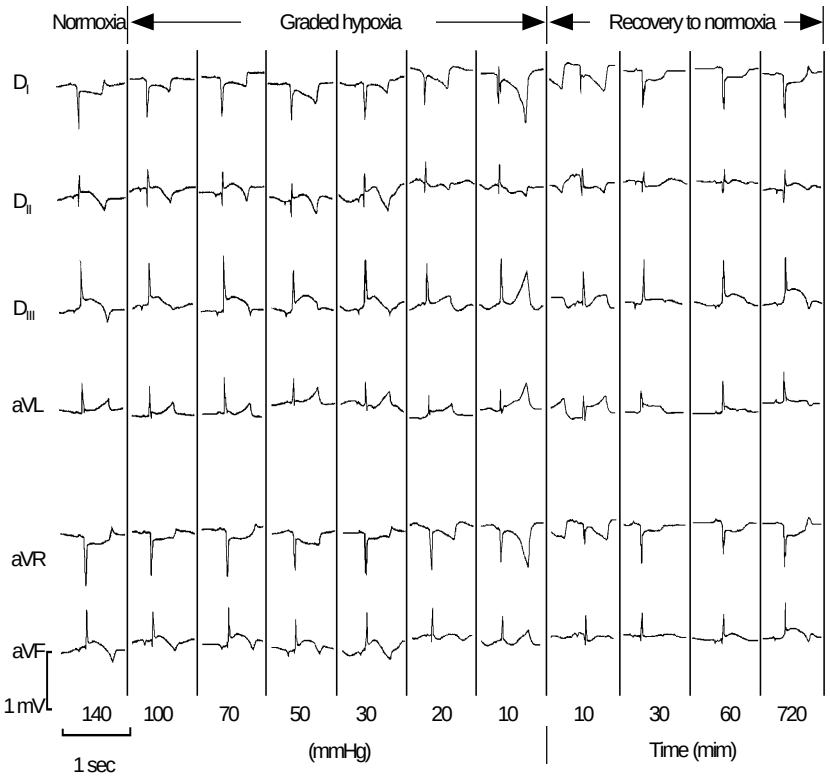


Figure 3. Representative ECGs recorded for *P. mesopotamicus* in the experimental O_2 tensions of inspired water (PiO_2 - mmHg) and in the different bipolar and monopolar leads.

Figure 3 depicts the typical ECG recordings in the bipolar (D_I, D_{II}, and D_{III}) and monopolar (aVR, aVL and aVF) leads from *P. mesopotamicus* in normoxia, exposed to graded hypoxia and during subsequent recovery to hypoxia.

The amplitude mean values (mV) of P wave, QRS complex, and T wave of the ECGs obtained from 12 specimens of *P. mesopotamicus* in normoxia, exposed to graded hypoxia and during the recovery to normoxia are presented in Table I. No significant differences were observed in the amplitudes of the P waves and QRS complexes during all experimental O_2 tensions. However, significant

differences occurred in the T wave amplitudes at 20 and 10 mmHg in all the six leads recorded.

Table I. Mean values (± S.E.) of the amplitudes (mV) of P wave, QRS complex and T wave of *Piaractus mesopotamicus* (n = 12) in normoxia, graded hypoxia and during the subsequent recovery to normoxia (0, 10, 30 and 720 min at 140 mmHg). ☐ - indicates statistical differences ($P < 0.05$) in relation to the initial control values. ▒ - indicates statistical differences ($P < 0.05$) in relation to the more hypoxic values (10 mmHg).

	P_IO_2 (mmHg)	D_I (Mean ± S.E.)	D_{II} (Mean ± S.E.)	D_{III} (Mean ± S.E.)	aVR (Mean ± S.E.)	aVL (Mean ± S.E.)	aVF (Mean ± S.E.)
P	140	-0.028 ± 0.032	-0.117 ± 0.016	-0.095 ± 0.012	0.062 ± 0.020	0.064 ± 0.021	-0.095 ± 0.014
	100	-0.013 ± 0.029	-0.115 ± 0.011	-0.086 ± 0.009	0.061 ± 0.018	0.071 ± 0.010	-0.099 ± 0.011
	70	-0.033 ± 0.033	-0.110 ± 0.012	-0.075 ± 0.008	0.050 ± 0.014	0.080 ± 0.012	-0.086 ± 0.013
	50	0.000 ± 0.000	-0.100 ± 0.012	-0.066 ± 0.008	0.061 ± 0.018	0.071 ± 0.010	-0.072± 0.014
	30	-0.027 ± 0.029	-0.027 ± 0.029	-0.066 ± 0.008	0.050 ± 0.016	0.078 ± 0.010	-0.050 ± 0.026
	20	-0.007 ± 0.033	-0.075 ± 0.027	-0.069 ± 0.009	0.056 ± 0.017	0.067 ± 0.010	-0.040 ± 0.025
	10	-0.013 ± 0.024	-0.076 ± 0.027	-0.075 ± 0.009	0.063 ± 0.018	0.080 ± 0.012	-0.039 ± 0.025
	140-0’	-0.028 ± 0.033	-0.117 ± 0.017	-0.095 ± 0.013	0.063 ± 0.021	0.064 ± 0.021	-0.095 ± 0.014
	140-10’	-0.017 ± 0.040	-0.017 ± 0.039	-0.095 ± 0.009	0.050 ± 0.020	0.092 ± 0.024	-0.090 ± 0.007
	140-30’	-0.022 ± 0.031	-0.127 ± 0.020	-0.114 ± 0.021	0.078 ± 0.022	0.092 ± 0.024	-0.086 ± 0.014
	140-720’	-0.017 ± 0.030	-0.105 ± 0.017	-0.083 ± 0.012	0.067 ± 0.022	0.044 ± 0.022	-0.091 ± 0.013
QRS	140	1.073 ± 0.133	1.050 ± 0.130	-0.320 ± 0.078	-1.014 ± 0.148	0.677 ± 0.111	0.368 ± 0.130
	100	1.155 ± 0.127	1.125 ± 0.138	-0.330 ± 0.054	-1.166 ± 0.184	0.718 ± 0.103	0.432 ± 0.125
	70	1.045 ± 0.134	1.085 ± 0.130	-0.30 ± 0.080	-0.991 ± 0.142	0.684 ± 0.098	0.423 ± 0.135
	50	0.968 ± 0.127	1.000 ± 0.134	-0.282 ± 0.079	-0.918 ± 0.146	0.646 ± 0.101	0.400 ± 0.123
	30	1.068 ± 0.126	1.125 ± 0.125	-0.385 ± 0.063	-1.025 ± 0.149	0.650 ± 0.102	0.455 ± 0.136
	20	0.982 ± 0.131	0.965 ± 0.200	-0.305 ± 0.075	-0.932 ± 0.138	0.641 ± 0.102	0.436 ± 0.134
	10	0.882 ± 0.108	0.825 ± 0.196	-0.200 ± 0.085	-0.755 ± 0.145	0.586 ± 0.095	0.390 ± 0.153
	140-0’	1.073 ± 0.133	1.050 ± 0.130	-0.320 ± 0.138	-1.014 ± 0.148	0.677 ± 0.111	0.368 ± 0.130
	140-10’	0.927 ± 0.143	0.700 ± 0.214	-0.205 ± 0.187	-0.782± 0.151	0.618 ± 0.167	0.386 ± 0.130
	140-30’	0.915 ± 0.156	0.823 ± 0.222	-0.200 ± 0.191	-0.891 ± 0.182	0.623 ± 0.099	0.436 ± 0.145
	140-720’	1.118 ± 0.1462	0.932 ± 0.240	-0.300 ± 0.154	-1.086 ± 0.168	0.705 ± 0.104	0.405 ± 0.176
T	140	-0.041 ± 0.063	-0.045 ± 0.061	0.010 ± 0.038	0.045 ± 0.056	-0.005 ± 0.046	-0.015 ± 0.043
	100	0.045 ± 0.071	0.021 ± 0.060	0.022 ± 0.048	0.017 ± 0.077	0.014 ± 0.045	0.025 ± 0.056
	70	0.105 ± 0.095	0.050 ± 0.079	-0.022 ± 0.048	-0.028 ± 0.086	0.027 ± 0.062	0.050 ± 0.071
	50	0.105 ± 0.096	0.044 ± 0.104	-0.030 ± 0.061	-0.091 ± 0.078	0.072 ± 0.068	0.0167 ± 0.076
	30	0.255 ± 0.118	0.165 ± 0.153	-0.018 ± 0.064	-0.159 ± 0.132	0.133 ± 0.083	0.094 ± 0.105
	20	0.164 ± 0.127	0.240 ± 0.142	-0.045 ± 0.057	-0.300 ± 0.122	0.159 ± 0.059	0.170 ± 0.076
	10	0.395 ± 0.163	0.490 ± 0.135	-0.130 ± 0.059	-0.427 ± 0.113	0.291 ± 0.076	0.215 ± 0.085
	140-0’	-0.041 ± 0.062	-0.045 ± 0.061	0.010 ± 0.037	0.045 ± 0.055	-0.005 ± 0.047	-0.015 ± 0.043
	140-10’	0.100 ± 0.065	0.044 ± 0.053	-0.095 ± 0.040	-0.063 ± 0.069	0.080 ± 0.046	-0.081 ± 0.038
	140-30’	0.018 ± 0.049	-0.031 ± 0.048	-0.025 ± 0.037	0.029 ± 0.049	0.022 ± 0.028	-0.025 ± 0.027
	140-720’	-0.036 ± 0.043	-0.010 ± 0.049	-0.033 ± 0.046	-0.427 ± 0.045	0.028 ± 0.030	-0.044 ± 0.052

The duration mean values (ms) of the P wave, QRS complex, and T wave of the ECGs obtained from 12 specimens of *P. mesopotamicus* in normoxia, exposed to graded hypoxia and during the recovery to normoxia are presented in Table II. No significant differences were observed in the duration of the P waves and QRS complexes during all experimental O_2 tensions. However, the T wave duration was significantly higher in the leads D_I, D_{II}, aVL and aVF at 20 mmHg

and in the six leads recorded at 10 mmHg. During the recovery to normoxia, the duration of P and T waves and QRS complex returned to values similar to those recorded during the initial normoxic level.

The duration mean values (ms) of the PR, ST, and QT intervals, calculated from the ECGs of 12 specimens of *P. mesopotamicus* in normoxia, exposed to graded hypoxia and during the recovery to normoxia are presented in Table III. No significant differences were observed in the duration of the PR and ST intervals in all experimental O_2 tensions. However, the duration of the QT intervals was significantly higher in the six leads recorded at 10 mmHg. During the recovery to normoxia, the duration of PR and ST and QT intervals approached the values recorded during the initial normoxic level.

Discussion

This study was motivated by the lack of data available to characterize of electrical events in the fish heart. Therefore, a methodology was developed to obtain electrocardiographic signals in the teleost *Piaractus mesopotamicus* under conditions that approach those of the natural environment.

Table II. Mean values (± S.E.) of the duration (ms) of P wave, QRS complex and T wave of *Piaractus mesopotamicus* (n = 12) in normoxia, graded hypoxia and during the subsequent recovery to normoxia (0, 10, 30 and 720 min at 140 mmHg). ☐ - indicates statistical differences ($P < 0.05$) in relation to the initial control values. ☐ - indicates statistical differences ($P < 0.05$) in relation to the more hypoxic values (10 mmHg).

	PIO_2 (mmHg)	D_I (mean ± S.E.)	D_{II} (mean ± S.E.)	D_{III} (mean ± S.E.)	aVR (mean ± S.E.)	aVL (mean ± S.E.)	aVF (mean ± S.E.)
P	140	38,0 ± 1,5	42,0 ± 1,5	43,0 ± 1,3	37,0 ± 1,5	40,0 ± 2,2	40,9 ± 0,9
	100	38,0 ± 1,7	42,0 ± 1,5	43,0 ± 1,3	37,0 ± 1,3	38,6 ± 2,6	40,9 ± 0,9
	70	39,0 ± 1,7	42,0 ± 1,5	43,0 ± 1,3	37,0 ± 1,0	38,6 ± 2,6	40,9 ± 0,9
	50	42,2 ± 3,6	42,0 ± 3,3	43,0 ± 3,3	39,0 ± 3,0	41,3 ± 4,4	40,9 ± 0,9
	30	44,4 ± 3,4	43,0 ± 35,8	43,3 ± 4,0	40,0 ± 3,3	42,5 ± 4,1	40,9 ± 0,9
	20	43,8 ± 3,8	43,3 ± 3,3	43,3 ± 3,3	40,0 ± 3,3	42,9 ± 4,7	40,9 ± 0,9
	10	42,2 ± 3,6	42,0 ± 3,3	42,2 ± 3,6	38,0 ± 3,3	43,3 ± 5,6	40,9 ± 0,9
	140-0’	40,0 ± 1,7	39,0 ± 1,5	38,0 ± 1,3	38,0 ± 1,5	38,0 ± 2,2	38,0 ± 0,9
	‘140-10’	42,0 ± 1,7	41,0 ± 0,3	39,0 ± 3,2	40,0 ± 3,2	39,0 ± 4,7	39,0 ± 2,8
	140-30’	40,0 ± 0,9	38,0 ± 1,3	39,0 ± 1,3	39,0 ± 1,3	39,0 ± 2,6	39,0 ± 2,8
	140-720’	40,0 ± 1,7	39,0 ± 1,3	39,0 ± 1,0	38,0 ± 1,5	38,0 ± 2,0	38,0 ± 2,8
QRS	140	42,0 ± 0,9	40,0 ± 1,0	39,0 ± 1,0	45,0 ± 0,9	45,0 ± 0,9	44,0 ± 0,9
	100	43,0 ± 0,9	40,0 ± 1,0	38,0 ± 0,9	46,0 ± 0,9	44,0 ± 0,9	44,0 ± 0,9
	70	43,0 ± 0,9	40,0 ± 1,0	39,0 ± 0,9	45,0 ± 0,9	44,0 ± 0,9	45,0 ± 0,9
	50	46,0 ± 0,9	40,0 ± 1,0	37,0 ± 0,9	47,0 ± 0,9	46,0 ± 0,9	44,0 ± 0,9
	30	48,0 ± 0,9	40,0 ± 1,0	40,0 ± 1,0	48,0 ± 0,9	46,0 ± 0,9	45,0 ± 0,9
	20	47,0 ± 0,9	40,0 ± 1,0	40,0 ± 0,9	48,0 ± 0,9	46,0 ± 0,9	46,0 ± 0,9
	10	46,0 ± 0,9	40,0 ± 1,0	40,0 ± 0,9	49,0 ± 2,8	48,0 ± 2,8	44,0 ± 2,8
	140-0’	44,0 ± 0,9	43,0 ± 1,0	44,0 ± 1,0	41,0 ± 0,9	42,0 ± 0,9	41,0 ± 0,9
	‘140-10’	46,0 ± 2,8	45,0 ± 3,0	46,0 ± 3,0	45,0 ± 2,7	44,0 ± 2,7	43,0 ± 2,8
	140-30’	44,0 ± 2,8	46,0 ± 1,0	46,0 ± 3,0	45,0 ± 2,7	44,0 ± 2,8	43,0 ± 2,8
	140-720’	43,0 ± 2,8	45,0 ± 0,9	45,0 ± 3,0	44,0 ± 2,7	45,0 ± 2,8	43,0 ± 2,8
T	140	116,4 ± 23,2	100,0 ± 8,9	84,0 ± 15,1	88,0 ± 10,0	88,0 ± 10,0	64,0 ± 6,5
	100	90,9 ± 12,2	92,0 ± 10,4	92,0 ± 13,4	93,3 ± 6,7	87,3 ± 11,8	75,6 ± 8,0
	70	98,2 ± 15,6	108,0 ± 29,8	116,0 ± 24,2	120,0 ± 32,0	120,0 ± 27,5	108,9 ± 32,2
	50	138,2 ± 27,2	164,4 ± 36,2	136,0 ± 18,1	127,3 ± 22,1	128,0 ± 20,5	92,0 ± 8,5
	30	138,2 ± 26,1	180,0 ± 32,8	123,6 ± 19,0	130,9 ± 25,9	127,3 ± 14,1	96,0 ± 20,8
	20	185,5 ± 28,7	168,0 ± 41,2	105,5 ± 20,4	128,0 ± 30,3	138,2 ± 22,5	140,0 ± 31,1
	10	210,9 ± 35,4	180,0 ± 30,0	152,7 ± 33,2	174,5 ± 25,7	178,2 ± 24,3	149,1 ± 25,4
	140-0’	116,4 ± 23,2	100,0 ± 9,0	84,0 ± 3,3	88,0 ± 9,9	88,0 ± 9,9	64,0 ± 18,0
	‘140-10’	112,7 ± 21,0	108,0 ± 2,1	96,0 ± 1,5	92,0 ± 18,0	80,0 ± 9,3	85,0 ± 12,0
	140-30’	76,3 ± 8,4	84,0 ± 2,6	88,0 ± 1,3	80,0 ± 18,0	64,0 ± 6,5	93,0 ± 10,0
	140-720’	73,0 ± 7,3	72,0 ± 5,3	80,0 ± 14,0	83,0 ± 10,0	84,0 ± 13,9	72,0 ± 8,0

Table III. Mean values (± S.E.) of the duration (ms) of the PR, ST, and QT intervals calculated from the ECG recordings of *Piaractus mesopotamicus* (n = 12) in normoxia, graded hypoxia and during the subsequent recovery to normoxia (0, 10, 30 and 720 min at 140 mmHg). ☐ - indicates statistical differences ($P < 0.05$) in relation to the initial control values. ▒ - indicates statistical differences ($P < 0.05$) in relation to the more hypoxic values (10 mmHg).

	PIO_2 (mmHg)	D_I (mean ± S.E.)	D_{II} (mean ± S.E.)	D_{III} (mean ± S.E.)	aVR (mean ± S.E.)	aVL (mean ± S.E.)	aVF (mean ± S.E.)
PR	140	121,8 ± 4,2	124,0 ± 4,0	121,8 ± 4,2	121,8 ± 4,2	120,8 ± 4,2	121,0 ± 4,2
	100	121,8 ± 4,2	124,0 ± 4,0	121,8 ± 4,2	121,8 ± 4,2	120,8 ± 4,2	121,0 ± 4,2
	70	121,8 ± 4,2	124,0 ± 4,0	121,8 ± 4,2	121,8 ± 4,2	120,8 ± 4,2	121,0 ± 4,2
	50	118,2 ± 1,8	120,0 ± 0,0	118,2 ± 1,8	118,2 ± 1,8	121,5 ± 4,7	118,2 ± 1,8
	30	112,7 ± 4,1	114,0 ± 4,3	112,7 ± 4,1	112,7 ± 4,1	110,9 ± 4,1	112,7 ± 3,1
	20	105,5 ± 5,5	106,0 ± 6,0	105,5 ± 5,5	105,5 ± 3,5	104,5 ± 3,5	104,8 ± 3,5
	10	109,1± 4,9	110,0 ± 5,4	109,1 ± 4,9	109,1 ± 3,9	108,1 ± 3,9	108,6 ± 2,9
	140-0’	121,8 ±4,2	124,0 ± 4,0	121,8 ± 4,2	122,8 ± 4,2	121,8 ± 4,2	120,8 ± 4,2
	140-10’	130,9 ± 5,6	130,9 ± 5,6	130,4 ± 5,6	131,9 ± 5,6	130,9 ± 5,6	130,4 ± 5,6
	140-30’	127,3 ± 4,9	127,3 ± 4,9	127,0 ± 4,9	128,3 ± 4,9	127,3 ± 4,9	126,3 ± 4,9
	140-720’	123,8 ± 3,6	123,6 ± 3,6	123,4 ± 3,6	123,6 ± 3,6	123,0 ± 3,6	120,0 ± 0,0
ST	140	225,5 ± 23,7	256,0 ± 19,0	272,0 ± 11,6	268,0 ± 18,9	264,0 ± 16,0	272,0 ± 14,4
	100	265,5 ± 17,3	272,0 ± 19,6	260,0 ± 14,9	248,9 ± 23,8	258,2 ± 15,6	288,9 ± 14,6
	70	250,9 ± 20,3	272,0 ± 19,6	272,0 ± 18,7	253,3 ± 29,1	269,1 ± 18,0	288,9 ± 19,8
	50	243,6 ± 20,5	226,7 ± 32,0	252,0 ± 23,1	240,0 ± 25,9	232,0 ± 23,7	283,6 ± 14,7
	30	221,8 ± 28,2	200,0 ± 33,7	265,5 ± 16,4	232,7 ± 27,9	243,6 ± 20,5	268,0 ± 19,8
	20	210,9 ± 26,5	204,0 ± 44,0	276,4 ± 23,2	240,0 ± 29,8	243,6 ± 21,2	248,0 ± 29,7
	10	207,3 ± 26,3	252,0 ± 32,1	269,1 ± 25,9	254,5 ± 27,7	236,4 ± 28,8	272,7 ± 26,9
	140-0’	225,5 ± 23,7	256,0 ± 19,0	272,0 ± 11,6	268,0 ± 18,9	264,0 ± 16,0	272,0 ± 14,4
	140-10’	229,1 ± 20,3	244,0 ± 24,9	228,0 ± 19,8	236,0 ± 19,3	247,3 ± 16,9	231,1 ± 25,6
	140-30’	247,3 ± 13,0	231,1 ± 21,9	271,1 ± 11,1	236,0 ± 20,2	252,0 ± 12,0	245,0 ± 22,0
	140-720’	261,8 ± 14,6	256,0 ± 17,1	266,7 ± 9,4	243,6 ± 15,7	256,0 ± 16,0	268,0 ± 10,4
QT	140	341,8 ± 12,5	356,0 ± 15,1	360,0 ± 14,6	356,0 ± 15,1	352,0 ± 16,7	332,0 ± 14,7
	100	349,1 ± 12,2	364,0 ± 15,1	360,0 ± 10,3	351,1 ± 16,0	341,8 ± 9,9	364,4 ± 12,4
	70	363,6 ± 11,4	380,0 ± 21,7	388,0 ± 22,4	377,8 ± 15,1	385,5 ± 16,4	393,3 ± 25,4
	50	381,8 ± 19,7	382,2 ± 20,1	380,0 ± 12,3	367,3 ± 15,1	360,0 ± 11,9	368,0 ± 14,4
	30	363,6 ± 13,7	380,0 ± 14,9	381,8 ± 9,9	367,3 ± 11,8	370,9 ± 10,9	364,0 ± 11,1
	20	396,4 ± 17,4	380,0 ± 19,1	381,8 ± 12,5	364,0 ± 16,3	381,8 ± 13,6	388,0 ± 20,7
	10	418,2 ± 19,7	432,0 ± 25,2	421,8 ± 22,5	429,1 ± 22,9	414,5 ± 18,1	425,5 ± 15,5
	140-0’	341,8 ± 12,5	356,0 ± 15,1	360,0 ± 14,6	356,0 ± 15,1	352,0 ± 16,7	332,0 ± 14,7
	140-10’	345,5 ± 22,4	352,0 ± 11,6	324,0 ± 13,9	336,0 ± 16,0	330,9 ± 18,0	324,4 ± 15,6
	140-30’	320,0 ± 9,3	320,0 ± 9,4	364,4 ± 23,5	336,0 ± 13,6	316,0 ± 11,1	330,0 ± 16,5
	140-720’	341,8 ± 15,6	328,0 ± 14,4	337,8 ± 9,7	327,3 ± 10,5	272,0 ± 29,9	304,0 ± 31,1

In mammals, a normal P wave has an amplitude below 0.25 mV and a duration of less than 110 ms. In *P. mesopotamicus*, a P wave of 40 ± 1.5 ms and a maximum amplitude of -0.117 ± 0.017 mV were observed in the D_{II} bipolar lead, where the waves were better identified, and the projection of the P wave onto the Einthoven triangle was approximately -110°. This suggests that the SÂP vector is projected in the fourth quadrant, above and to the right, different from the situation in mammals in which this vector is oriented towards the bottom, to the left, and slightly towards the front or back. The change of the site of the pacemaker from the upper part to the middle of the atrial sinus node may result in the change of the polarity of the P wave in the aVL monopolar limb lead from negative to positive (Eifler et al., 1980).

The duration of the QRS complex in mammals in normally less than 0.10 sec, and the difference in the amplitudes in D_I and D_{III}, when projected onto the Einthoven triangle, results in an average electric axis of the heart located between 0° and 90° (approximately 60°). *P. mesopotamicus* presented average duration of 40.91 ± 0.9 ms, with the maximum projection observed in the D_I bipolar lead (1.073 ± 0.133 mV), at approximately 40°, and the minimum in D_{III} (-0.320 ± 0.132 mV). These results suggest that the average depolarization vector of the heart of *P. mesopotamicus* is projected in the second quadrant, *i.e.*, below and to the left, like in mammals.

The P wave and the QRS complex represent the depolarizations of the atrium and ventricle, respectively, and the excitation wave in the heart is conducted from the atrium to the ventricle (Saito, 1969; Namba et al., 1973; Mitsuda et al., 1988). The results obtained for *P. mesopotamicus* show that both of the axes may point in opposite directions without presenting a pattern of agreement or disagreement between them.

The conduction of the ventricular depolarization may occur by means of a specialized tissue which would facilitate the synchronism necessary during the cardiac systole. Although a rapid conduction path has not yet been discovered, physiological evidence found in the teleosts suggests its existence (Satchell, 1991). The fact that *P. mesopotamicus* does not present alterations in the amplitude and duration of the QRS and SÂQRS complexes is evidence in support of this hypothesis.

In mammals, the T wave is generally oriented in the same direction as the main QRS vector. In *P. mesopotamicus*, different directions were measured for T vector of repolarization, located in the triangle at approximately -150°, and the QRS vector of depolarization at 40°. This is because in mammals the intraventricular septum and other areas of the ventricular muscle depolarize first. It would seem logical that these areas would also be the first to repolarize. However, this does not occur in *P. mesopotamicus*, probably due to the fact that fish do not have an intraventricular septum. Furthermore, the contraction of the endocardiac area may be more prolonged than for the majority of the external surface of the heart. Therefore, the part of the ventricular muscle which depolarizes first would be that which forms all of the external surface of the ventricle, and especially that closest to the apex of the heart (Bayés de Luna, 1995) and the endocardiac areas would be the last to repolarize.

The reason for an abnormal repolarization sequence may also be related to the elevated pressure which exists inside the ventriculum during contraction, which would greatly decrease the blood irrigation of the endocardium, possibly delaying the repolarization process in the endocardiac areas. However, hystomorphological studies of the heart tissue of *P. mesopotamicus* must be done in order to elucidate this question.

Future studies on the conditions of the myocardium of different teleost species under conditions similar to those found in the environment should be done using the techniques developed in this study, in order to elucidate fundamental questions about these physiological processes.

In conclusion, there is no pattern of agreement between the SÂT, SÂP, and SÂQRS vectors in this species during normoxia, which may be due to various factors such as their anatomic variability, the position of the electrodes, and the sex and physiological conditions of the animals. The average vector of depolarization of the heart of *P. mesopotamicus* is projected in the second quadrant, that is below and towards the left, similar to that observed in mammals, and may indicate a verticalization of the heart. The conduction of the ventricular depolarization may occur through a specialized tissue which would facilitate the synchronism necessary during cardiac contraction. The fact that *P. mesopotamicus* does not present alterations in the amplitude and duration of the QRS complexes nor in the SÂQRS supports this hypothesis.

Acknowledgements

This project was supported by FAPESP and CNPq. Fellowships were provided to C.D.R. Guerra by CAPES and ASO (American States Organization - Proc. 22808-1-PN). Specimens of pacu were kindly provided by CEPTA/IBAMA, Pirassununga, SP, Brasil.

References

Altimiras, J. 1995. Heart rate variability: Its significance in lower vertebrates. PhD thesis, Barcelona University, Spain.

Bayés de Luna, A. 1995. Electrocardiografia clínica. Ed. Mosby-Doyma Libros, Barcelona, 294 p.

Delavechia, M.L. 1994. Respostas cardio-respiratórias de Tilápia-do-Nilo, *Oreochromis niloticus* (Schwartz 1983) às variações de oxigênio dissolvido e durante a recuperação subsequente. PhD thesis, Federal University of São Carlos, SP, Brazil.

Eifler, W.J.; E. Macchi, H.J. Ritsema, B.M. Horacek and P.M. Rautaharju. 1980. Mechanims of generation of body surface eletrocardiographic P-waves in normal, midle, and lower sinus rhythms. Circ. Res.,48:168-182.

Gehrke, P.C. and D.R. Fielder. 1988. Effect of temperature and dissolved oxygen on heart rate, ventilation rate and oxygen consumption of spangled perch, *Leiopotherapon unicolor* (Günther 1859) (Percoidei, Teraponidae). J. Comp. Physiol., 157:771-782.

Guerra, C.D.R. 1992. Respostas cárdio-respiratórias de pacu, *Piaractus mesopotamicus* (Holmberg, 1887), às variações de oxigênio no ambiente e durante recuperação subsequente. Master thesis, Federal Univerity of São Carlos, SP, Brazil.

Mitsuda H, Y. Ishida, H. Yoshikawa and S. Ueno. 1988. Effects of high concentration of CO_2 on electrocardiograms in carp, *Cyprinus carpio*. Comp. Biochem. Physiol., 91A: 749-757.

Namba, K., S. Murachi, S. Kawamoto and Y. Nakano. 1973. Studies on electrocardiograms of fishes: 1. Test of method to detect the ECG from fish. J. Fac. Fish. and Animal Husb., Hiroshima University, 12:147-154.

Rantin, F.T., A.L. Kalinin, C.D.R. Guerra, M. Maricondi-Massari & R.M.M. Verzola. 1995. Electrocardiographic characterization of myocardial function in normoxic and hypoxic teleosts. Braz. J. Med. Biol. Res., 28:1277-1289.

Rantin, F.T., M.L. Glass, A.L. Kalinin, R.M.M. Verzola and M.N. Fernandes M.N. 1993. Cardio-respiratory responses in two ecologically distinct erythrinids (*Hoplias malabaricus* and *Hoplias lacerdae*) exposed to graded environmental hypoxia. Env. Biol. Fish., 36:93-97.

Saito, T. 1969. Electrophysiological studies on the pace-maker of several fish heart. Zool. Mag., 78:291-296.

Satchell G.H. 1991. Physiology and form of fish circulation. Cambridge Univ. Press. Cambridge pp. 10-40.

Ueno S, H. Yoshikawa, Y. Ishida and H. Mitsuda. 1986. Electrocardiograms recorded from the body surface of the carp, *Cyprinus carpio*. Comp. Biochem. Physiol., 85A: 129-133.

CARDIORESPIRATORY RESPONSES

TO EMERSION, HYPOXIA, AND SUBMERSION

IN THE MUDSKIPPER, *PERIOPHTHALMODON SCHLOSSERI*

Aguilar, N.M.
Scripps Institution of Oceanography, U.C. San Diego, La Jolla, CA 92093-0204, USA; Tel (619-534-8044); Fax (619-534-1305);
e-mail: nmaguilar@ucsd.edu

K. Ogawa, and A. Ishimatsu, Marine Research Station, Nagasaki University, Nomo 714, Nagasaki 851-05, Japan

Methods

Physiological responses to emersion, hypoxia, and submersion were studied in *Periophthalmodon schlosseri*, one of the largest amphibious mudskippers. *P. schlosseri* were chronically cannulated at the coeliac artery and exposed to one of three experimental conditions:

1) complete emersion

2) partial submersion in hypoxic water ($Po_2 \leq 0.9$ kPa), but with free access to air and

3) complete submersion in normoxic water.

The control condition for each fish was partial submersion in normoxic water with free access to air.

We took blood samples at t=0, 1, 3, 5, 12 hr, and 2 hr recovery and measured blood Po_2, O_2 content (Co_2), hematocrit, pH, and total CO_2 (Tco_2). In addition, we measured total blood O_2 capacity and hemoglobin concentration at the end of each experiment in order to calculate percent saturation and produce *in vivo* oxygen dissociation curves. Some of these parameters could not be measured

for every experiment. All experiments were run at 30°C with 50% seawater. All fish were starved at least 24 hrs prior to an experiment.

In separate experiments, changes in aerial ventilatory tidal volume and ventilatory frequency in response to hypoxia, hyperoxia, and hypercapnia, were determined using a pneumotach and video camera. In each experiment, the fish was partially submerged in a sealed chamber. The experiments were:

1) altered aerial Po_2 (20.0, 10.0, 5.07, and 40.0 kPa),

2) altered aquatic Po_2 (20.0, 2.0, 40.0 kPa), and

3) altered aerial Pco2 (0.0267 and 2.05 kPa).

Results

During complete emersion, Po_2 did not change significantly relative to the control condition (Table 1). There was as a significant increase in pH and slight decreases in Pco_2 and Tco_2. (Pco_2 was calculated from Tco_2 and pH using the Henderson-Hasselbalch equation). This is unlike most fish, which show a respiratory acidosis upon emersion (*e.g.*, *Channa argus*, Ishimatsu and Itazawa, 1983). However, other marine amphibious fishes have been shown to maintain similar rates CO_2 release in both air and water (Steeger and Bridges, 1995).

	EMERSION	**Emersion** recovery	**Submersion**	**Submersion** recovery	**Hypoxia**	**Hypoxia** recovery
PO_2	--	--	⋁	⋁	--	--
Co_2	nm	nm	⋁	--	--	⋁
Total CO_2	--	--	⋁	--	⋁	⋁
pH	⋀	⋀	--	⋀	--	--
Hct	⋁	⋁	--	⋁	--	⋁
Heart rate	nm	nm	⋁	⋀	⋀	⋀
Blood pressure	nm	nm	⋁	⋁	--	--

Table 1. Summary of results from cardiovascular experiments. ⋀ = significant increase relative to control; ⋁ = significant decrease relative to control; -- = no change; nm = not measured.

Aquatic hypoxia did not significantly affect Po_2, Co_2, pH, or Pco_2. Tco_2 decreased at 5 hr and recovery, but without corresponding changes in pH or Pco_2. This result may be attributable to a few individuals which had significantly decreased hematocrits as a result of repeated sampling, although some of the sampled blood was returned to the fish. Heart rate and blood pressure were highly variable and probably reflect minor disturbances during blood sampling.

Complete submersion of *Pn. schlosseri* resulted in a precipitous drop in Po_2 (4.5 kPa to less than 2 kPa), and corresponding decreases in Co_2 (3.5 to 1.5 mmol O_2 l^{-1}) and percent saturation (70% to 30%). Tco_2 and Pco_2 decreased significantly relative to the control values, but there was no corresponding change in pH. However, pH increased significantly during recovery. Both heart rate and blood pressure decreased significantly within one hour of submersion. As soon as the fish was returned to control conditions, heart rate increased significantly.

Under control conditions, *Pn. schlosseri* uses intermittent aquatic ventilation, however, when completely submerged, the fish ventilated continuously. This combined with the higher solubility coefficient for CO_2 in water relative to air accounts for the decreased Tco_2 and Pco_2. The relatively constant pH suggests a very high blood buffering capacity. The pronounced bradycardia upon submersion is similar to that reported for the congener *Pn. freycineti* (Garey, 1966) and confirms diving bradycardia for this genus of mudskipper.

In the aerial ventilation experiments, mild aerial hypoxia (10.0 kPa) produced a significant increase in ventilation frequency (opercular beats/min) and minute tidal volume (ml/100g/min). Figure 1 shows the results expressed as minute tidal volume (ventilation volume * ventilation frequency). Changes in aquatic Po_2 did not affect tidal volume or frequency. Aerial hypercapnia resulted in a significant increase in ventilation frequency and minute tidal volume.

This study demonstrates that for *Pn. schlosseri*, aerial oxygen tension is a much stronger modulator of ventilation than aquatic oxygen tension. Even under normoxia, the respiratory system is not able to take up as much oxygen from water as it does from air. This fish is clearly capable of maintaining acid-base balance during extended periods of aerial exposure, but the exact mechanism is still unknown.

Acknowledgements

(Funding from NSF Summer in Japan Fellowship and Fogerty Travel Fellowship, Monbusho Science Ministry (Japan), NSF 96004699)

References

Garey, W.F. (1966) *Biol. Bull.* **122**: 362-368.

Ishimatsu, A. and Y. Itazawa (1983) *Comp. Biochem. Physiol.* **74A**: 787-793.

Steeger, H.-U. and C.R. Bridges (1995) *J Fish Biol.* **47**: 308-320.

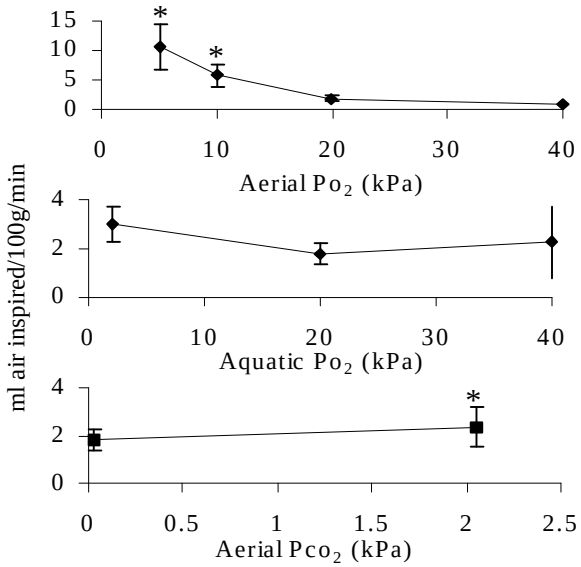


Figure 1. Minute ventilation as a function of aerial Pco_2 and Po_2, and aquatic Po_2. * = significantly different from control; ± 1

EFFECT OF ACUTE ANOXIA ON THE FUNCTION OF CRUCIAN CARP HEART: SIGNIFICANCE OF CHOLINERGIC AND PURINERGIC CONTROL

Matti Vornanen
University of Joensuu, Department of Biology,
P.O. Box 111, 80101 Joensuu, Finland
Phone: 358-13-2513578; Fax: 358-13-2523590;
Email: Matti.Vornanen@joensuu.fi

Introduction

Crucian carp inhabit small shallow ponds where prolonged anoxia is a regular seasonal phenomenon. The extraordinary anoxia-tolerance of this fish species is based on huge glycogen stores, specialized anaerobic metabolism and reduced locomotor activity (Vornanen 1994; Johnston and Bernard 1983; Nilsson et al. 1993). The seasonal anoxia tolerance is improved by low ambient temperature which effectively suppresses physiological functions. In the absence of positive thermal compensation the contractility of cardiac muscle is strongly inhibited by low ambient temperature; heart rate is depressed and the ability to produce force at high pacing frequencies is compromised.

The cellular mechanisms underlying these functional changes include temperature-induced reductions in myofibrillar ATPase activity and Ca-uptake rate by sarcoplasmic reticulum, as well as anoxia-induced depression of sarcolemmal Na,K-ATPase (Vornanen 1994; Aho and Vornanen 1997; Aho and Vornanen 1997). These functional changes appear as a consequence of long-term thermal acclimation in the laboratory or during seasonal acclimatization in the wild and improve the survival of the fish by reducing energy demands during anoxic season. It is not, however, clear how the function of the crucian carp heart will change when subjected to acute anoxia without prior acclimatization period. The acute anoxia response should be particularly

important during summer months when the fish are likely to encounter hypoxic or anoxic water on the bottom of the pond during feeding bouts.

Therefore, the aim of the present study was to characterize the response of crucian carp heart to acute anoxia in summer-acclimatized fish. Especially, the significance of cholinergic and purinergic systems in the acute anoxic reaction was the target of this investigation.

Materials and Methods

Warm-acclimated (22°C) summer fish were used throughout. The experiments were conducted at room temperature (22 ± 1°C). *In vivo* heart rate was recorded by small needle electrodes placed on both sides of the pericardial cavity. Contractile activity of excised heart was recorded by force-transducers and the results were analyzed off-line with a computer using PClamp6-software. Electrophysiological experiments were conducted on enzymatically isolated atrial and ventricular myocytes using whole-cell patch clamp techniques.

Results and Conclusions

Anoxia caused immediate bradycardic reflex that was completely abolished by intraperitoneal injection of atropine (2 mg/kg). Aminophylline (50 mg/kg), a blocker of purine receptors, was without effect on anoxic bradycardia. Thus, the anoxic bradycardia is exclusively mediated by muscarinic cholinergic receptors. Carbamylcholine (Cch), a muscarinic cholinergic agonist, effectively depressed spontaneous beating rate of excised hearts (Fig. 1) as well as force generation of paced atrial preparations *in vitro*. In contrast, ventricular muscle was completely unresponsive to Cch. The cholinergic depression of atrial contractility was associated with shortening of action potential and twitch durations. These effects are explained by Cch-induced increase in inwardly rectifying potassium current ($I_{K,Ach}$) (Fig. 2 b, d). Cch did not modify ventricular action potential (Fig. 2a).

Adenosine (Ado), a purinergic agonist, had a slight negative chronotropic effect on excised crucian carp heart *in vitro* (Fig. 1). In paced atrial preparations Ado caused a slight negative inotropic effect, while in ventricular tissue a weak positive inotropic effect was noted. All these responses required high (mM) concentrations of Ado. Ado (100 μM) did not modify electrical activity of atrial or ventricular myocytes.

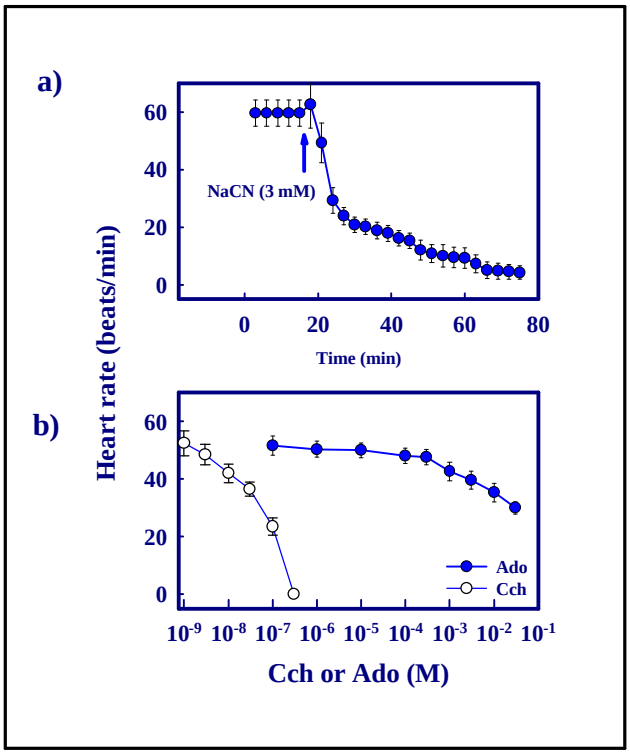


Figure 1. Effect of aerobic block (3 mM NaCN (**a**), carbamylcholine (Cch) and adenosine (Ado) (**b**) on the spontaneous beating rate of crucian carp heart *in vitro*.

Blockade of aerobic metabolism with 3 mM NaCN caused rapid decline of spontaneous beating rate (Fig. 1) and force generation of crucian carp hearts *in vitro*. In the continuous presence of cyanide partial recovery of force generation was noted, possibly due to the strong reduction of heart rate. In atrial and ventricular preparations that were forced to contract at the rate of 1.0 Hz, cyanide caused rapid deterioration of contractile function. In ventricular tissue

the negative inotropic effect was associated with marked prolongation of twitch, while in atrial tissue only the final phase of relaxation was slowed down. In single atrial and ventricular cells cyanide (0.1 mM) caused strong and rapid depolarization of resting membrane potential. In ventricular cells also the duration of action potential was increased. The depolarization was due to a cyanide-induced inward current. The voltage-dependence of the cyanide-induce current component was nonlinear with a maximum at -60 mV and crossing zero potential at about -120 and -20 mV.

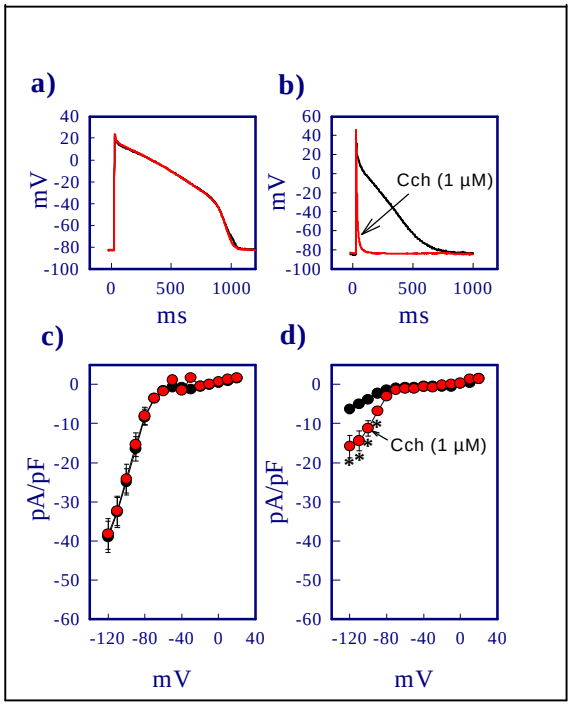


Figure 2. Effect of carbamylcholine (1 µM) on action potential duration and inwardly rectifying potassium current in ventricular (**a, c**) and atrial (**b, d**) myocytes of crucian carp heart.

The present experiments show that the contractility of the crucian carp heart is sensitive to oxygen shortage. Upon exposure to acute anoxia heart rate and atrial contraction are rapidly depressed by activation muscarinic cholinergic receptors through the opening of inwardly rectifying potassium channels ($I_{K,Ach}$). If the duration of anoxia is sufficiently long, the depletion of tissue oxygen content will directly depress heart rate as well as the force of atrial and ventricular contraction. Instead, purinergic system (Ado) does not seem to be involved in anoxic response of the crucian carp heart.

Acknowledgments.

This study was supported by the Academy of Finland (project #7641).

References

Vornanen, M. 1994. Seasonal adaptation of crucian carp (*Carassius carassius* L.) heart: glycogen stores and lactate dehydrogenase activity. Can.J.Zool. 72: 433-442

Johnston, I. A. and Bernard, L. M. 1983. Utilization of the ethanol pathway in carp following exposure to anoxia. J.Exp.Biol. 104: 73-78

Nilsson, G. E., Rosen, P. and Johansson, D. 1993. Anoxic depression of spontaneous locomotor activity in crucian carp quantified by a computerized imaging technique. J.Exp.Biol. 180: 153-162

Vornanen, M. 1994. Seasonal and temperature-induced changes in myosin heavy chain composition of the crucian carp hearts. Am.J.Physiol. 267: R1567-R1573

Aho, E. and Vornanen, M. 1997. Seasonality of ATPase activities in crucian carp (*Carassius carassius* L.) heart. Fish Physiol.Biochem. 16: 355-364

Aho, E. and Vornanen, M. 1998. Ca-ATPase activity and Ca-uptake by sarcoplasmic reticulum in fish heart: effects of thermal acclimation. J.Exp.Biol. 201: 525-532.

ANOXIC TROUT HEARTS:

PRECONDITIONING IN A NON-MAMMALIAN MODEL

A. Kurt Gamperl
Dept. of Biological Sciences, Simon Fraser University,
8888 University Drive, Burnaby, B.C., Canada. V5A 1S6.
604-291-3977, (FAX) 604-291-3496,
e-mail gamperl@sfu.ca

A.E. Todgham[*] W.S. Parkhouse[‡], R. Dill[‡] and A.P. Farrell[*].
Depts. of Biological Sciences ([*]), and Kinesiology ([‡]),
Simon Fraser University, Burnaby, B.C., Canada. V5A 1S6.

Introduction

Brief periods of ischaemia can greatly reduce the degree of myocardial necrosis associated with a subsequent prolonged ischaemic insult (Murray *et al*., 1986). This phenomenon, termed preconditioning, has been studied in many mammalian and avian models, and has been shown to be initiated by factors other than brief ischaemia (e.g. hypoxia, rapid pacing, myocardial stretch, etc.). Although the hearts of some fishes (e.g. crucian carp; Gesser, 1977) appear to be resistant to short-term anoxia (ie. myocardial function quickly returns to pre-anoxic levels following reoxygenation), no studies have examined whether mechanisms of myocardial protection can be induced in hypoxia/anoxia-intolerant species.

This study measured the reduction in trout (*Oncorhynchus mykiss*) *in situ* cardiac performance caused by 15 minutes of anoxia, and examined whether prior anoxic exposure (5 min) can diminish or eliminate this anoxia-induced myocardial dysfunction.

Methods

An *in situ* heart preparation was obtained from anaesthetized trout (400-700 g) as detailed in Farrell *et al*. (1986). After completion of surgery, the anterior part

of the fish was immersed in a temperature-controlled saline bath at 10^{o} C, and the heart was connected to a perfusate reservoir filled with O_2 equilibrated saline. Output pressure (P_{out}) was set at 50 cm H_2O, and filling pressure (P_{in}) was adjusted to provide a physiological cardiac output (Q = of 16 ml kg^{-1} min^{-1}). The heart was allowed to recover from surgery for 25 min, thereafter maximum cardiac output (Q_{max1}) was measured in all hearts by increasing P_{in} in steps to 4.5 cm H_2O. With Q reset to 16 ml kg^{-1} min^{-1} the trout hearts were then exposed to one of four treatments (N = 7-8).

(1) Anoxia - Low P_{out}; 15 min of anoxia (saline PO_2 < 5 mm Hg) at a P_{out} of 10 cm H_2O, 40 min after Q_{max1}.

(2) Anoxia - High P_{out} ; 10 min of anoxia at a P_{out} of 10 cm H_2O, followed by 5 min of anoxia at P_{out} = 50 cm H_2O, 40 min after Q_{max1}.

(3) Preconditioned; 5 min of anoxia at P_{out} = 10 cm H_2O, 15 min after Q_{max1}, but 20 min prior to the anoxic exposure protocol described for the Anoxia - High P_{out} group.

(4) (4) Control; the hearts of this group were only exposed to oxygenated saline, and changes in P_{out} were identical to those for Group 3.

After all treatments, the hearts were perfused with oxygenated saline for 30 minutes with Q at 16 ml kg^{-1} min^{-1}. Maximum cardiac output was then remeasured (Q_{max2}).

Perfusate samples were taken 15 and 30 minutes after the 15 min anoxic period to determine myocardial lactate efflux. Hearts were sampled 2 min after Q_{max2} for the analysis of myocardial lactate, glycogen, ATP and PCR.

Results

There was a minor, but non-significant, deterioration of maximum cardiac performance in control hearts. Fifteen minutes of anoxia at low P_{out} significantly decreased maximum stroke volume and cardiac output (Fig. 1). Increasing P_{out} during the anoxic period caused a further reduction in both of these parameters. Maximum stroke volume and cardiac output in the Anoxia-High P_{out} group were 23% and 38% lower, respectively, following anoxic exposure. Prior exposure to 5 min. of anoxia completely ameliorated the reductions in cardiac function that

were observed in the Anoxia-Low P_{out} and Anoxia-High P_{out} groups. This effect was not due to a diminished level of cardiac work by the preconditioned hearts during the 15 min anoxic period. For example, cardiac power output measured at the end of the 15 min anoxic exposure was 0.22, 0.33 and 0.39 mW g^{-1} ventricle in the Anoxia-Low P_{out}, Anoxia-High P_{out}, and preconditioned hearts, respectively (results not shown).

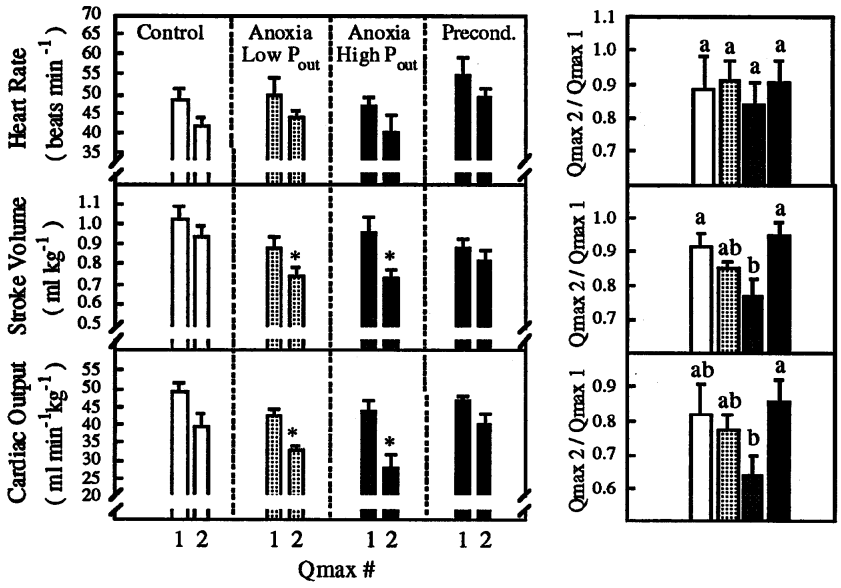


Figure 1. Performance of *in situ* rainbow trout hearts measured at Q_{max}, before and after exposure to anoxia. (*) Indicates a significant decrease ($P < 0.05$) between $Qmax_1$ and Q_{max2} as determined by paired t-tests. Dissimilar letters indicate $Qmax_1/Qmax_2$ values which were significantly different from each other. Significant differences between groups were identified using ANOVA and Fisher's LSD tests.

Myocardial concentrations of lactate, glycogen, PCR and ATP at the end of the experiment were similar in all experimental groups (Table 1). However, lactate efflux was significantly elevated in the preconditioned hearts (by approx. 7 fold) at 30 min post-anoxia as compared with the other three groups (Table 1).

Table 1. Myocardial biochemistry, and lactate efflux, in trout hearts exposed to anoxia or normoxia. Hearts were sampled for biochemical analysis 2 minutes after the second Q_{max} test. Dissimilar letters indicate group values which were significantly different from each other ($P < 0.05$) as determined by ANOVA and Fisher's LSD tests. SEM values are enclosed by brackets.

	Lactate Efflux ($nM\ g^{-1}\ min^{-1}$)		**Myocardial Concentrations ($uM\ g^{-1}$)**			
	15 min	**30 min**	**L actate**	**Glycogen**	**ATP**	**PCR**
Control	24.5 (5.0)	13.7 (2.7)[a]	45.1 (0.7)	51.5 (3.7)	0.95 (0.13)	2.3 (0.3)
Anoxia Low P_{out}	37.4 (21.9)	4.3(2.0)	36.4 (5.1)	55.3 (7.6)	0.79 (0.09)	2.3 (0.4)
Anoxia High P_{out}	42.2 (10.5)	12.8 (3.6)[a]	38.9 (3.7)	58.0 (5.1)	0.81 (0.18)	3.0 (0.6)
Precond.	72.9 (24.7)	80.0 (31.9)[b]	40.3 (7.1)	46.9 (3.6)	0.70 (0.28)	2.1 (0.4)

Discussion and Conclusions

In the hypoxia-intolerant rainbow trout, it is clear that a short period of anoxia can eliminate the reduction in maximum cardiac performance that is concommitant with a more prolonged anoxic exposure. Therefore, our results strongly suggest that a "pre-conditioning like" phenomenon also exists in fishes, although we were not able to measure the extent of myocardial necrosis (cell death) in these experiments. The mechanisms mediating this phenomenon are unknown, but are likely to include adenosine, protein kinase C and ATP-sensitive K^+ channels (Dekker, 1988; Yellon et al., 1998). An interesting finding was that the "preconditioned" hearts released more lactate following 15 minutes of anoxic exposure. The significance of this result is unclear, but may indicate that anoxic trout hearts were better able to support myocardial energy requirements through anaerobic pathways as a result of preconditioning.

Acknowledgements

This research was supported by an NSERC operating grant to A.P. Farrell.

References

Dekker, L.R.C. 1998. Toward the heart of ischemic preconditioning. Cardiovasc. Res. 37: 14-20.

Farrell, A.P., K.R. MacLeod and B. Chancey. 1986. Intrinsic mechanical properties of the perfused rainbow trout heart and the effects of catecholamines and extracellular calcium under control and acidotic conditions. J. exp. Biol. 125: 319 - 345.

Gesser, H. 1977. The effect of hypoxia and reoxygenation on development of force in the myocardium of carp and rainbow trout: protective effects of CO_2/HCO_3. J. exp. Biol. 69: 199-206.

Murray, C.E., R.B. Jennings and K.A. Reimer. 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 74: 1124-1136.

Yellon, D.M., G.F. Baxter, D. Garcia-Dorado, G.Heusch and M.S. Sumeray. 1998. Ischaemic preconditioning: present position and future directions. Cardiovasc. Res. 37: 21-33.

CELLULAR ASPECTS OF MYOCARDIAL OXYGEN LACK

IN FISH AND OTHER ECTOTHERMS

Hans Gesser
Department of Zoophysiology, Institute of Biological Sciences,
University of Aarhus Universitetsparken, Building 131,
DK-8000 Aarhus C, Denmark
Phone: +45 8942 2588, fax: +45 8619 4186,
e-mail: hans.gesser@biology.aau.dk

EXTENDED ABSTRACT ONLY DO NOT CITE

Discussion

Ambient oxygen content is more variable for water breathers than for air breathers. This in combination with differences in swimming capabilities may result in bouts as well as prolonged periods with cellular activities constrained by hypoxia. The tolerance to hypoxia varies largely among species, being high in species like goldfish, flounder and hagfish and low in cod and salmonid fishes. The heart attracts particular interest because of the ultimate importance of its continuous function in vertebrates, and of its commonly strong dependence on aerobic energy liberation. The mechanical performance of isolated cardiac muscle subjected to hypoxia varies largely among vertebrate species and tends to correlate with the hypoxic tolerance of the whole animal.

A lowered pO_2 at the cell surface will decrease the diffusion of oxygen to the mitochondria. This effect should be counteracted by myoglobin, which should increase the diffusion rate for oxygen at a given gradient (Meyer et al., 1984). In accordance with this, a comparison of myoglobin-rich with myoglobin-poor fish hearts indicated a positive effect of myoglobin on hypoxic performance (Driedzic and Gesser, 1994).

Oxygen lack at the mitochondrial level depresses aerobic energy liberation, with a consequent imbalance between demand and liberation of energy. In turn, contractility and ATP hydrolysis will be depressed and glycolytic ATP

production stimulated, whereby a new balance and a new cellular energy state ($\Delta G=\Delta G_0$-ln(ATP/ADP*1/P_i)) is approached. The contractility and energy state approached vary among species due to variations in rates of hydrolysis and resynthesis of ATP. For example, a study of heart tissue from marine fish species demonstrated a positive correlation between the mechanical performance during hypoxia and the anaerobic relative to the aerobic energy liberating capacity estimated by the maximal activity of pyruvate kinase and cytochrome oxidase respectively (Driedzic and Gesser, 1994).

Seemingly unlike mammals, anoxia does not immediately elicit the full anaerobic potential in cardiac muscle of ectotherms. Thus, adrenaline or increases in extracellular Ca^{2+} increase twitch force by a similar factor under anaerobic and aerobic conditions (Driedzic and Gesser, 1994).The relationship between force loss during hypoxia expressed in percentage of the prehypoxic twitch force and the cellular energy state is remarkably constant as it did not differ substantially among four ectothermic species (Hartmund and Gesser, 1996). Neither was it substantially influenced by extracellular Ca or temperature in rainbow trout myocardium (Driedzic and Gesser, 1994).

Creatine kinase may act as a temporal as well as a spatial buffer to the energy state in muscle cells (Meyer et al., 1984). A comparison of vertebrate species showed that the cardiac activity of creatine kinase relative to the capacity for energy liberation estimated by the cytochrome oxidase activity, was extremely high in freshwater turtle, goldfish and Atlantic hagfish, all of which show a high cardiac tolerance to severe hypoxia. The high creatine kinase activity may attenuate a removal of the creatine kinase reaction from equilibrium at PCr levels lowered by hypoxia (Christensen et al., 1994).

The loss of contractility during hypoxia is probably less related to the lowered energy state itself than to the changes in the concentrations of the substances defining it. Hence, in skinned cardiac muscle of rabbit, contractility is depressed by the increases in P_i. This effect of P_i was to some extent offset by an elevated ADP (Godt and Nosek, 1989). In contrast to the likely possibility that the negative inotropic effect of P_i in a hypoxia tolerant heart is small, it was found to be very prominent in skinned cardiac muscle from turtle, an effect which, however, could be completely off-set by an elevation of ADP (submitted).

To an extent depending on species, a decrease in the cellular energy state may entail a decrease in phosphorylated adenylates, possibly by a transformation of ADP to AMP, which is further processed. This degradation may be important, as

it may counteract a lowering of the energy state as well as release substances e.g. adenosine which act on purinergic receptors and modify cardiac activity (Hartmund and Gesser, 1996). This relates to the interesting question to what extent different activities are modified in the face of a decreased energy liberation.

At last a point of caution should be made relating to the existence of some controversy regarding what preparation (isolated tissue, heart *in situ* etc) to employ in studying different aspect of cardiac hypoxia.

References

Christensen M., T. Hartmund and H. Gesser.1994. Creatine kinase, energy-rich phosphates and energy metabolism in heart muscle of different vertebrates. J. Comp. Physiol. B. Biochem. Syst. Environ. Physiol. 164:118-123

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

Godt R. E. and T. M.. Nosek. 1989. Changes of intracellular milieu with fatigue or hypoxia depress contractility of rabbit skeletal and cardiac muscle. J. Physiol. Lond. 412:155-180

Hartmund T. and H. Gesser. 1996. Cardiac force and high-energy phosphates under metabolic inhibition in four ectothermic. vertebrates Am. J. Physiol. 271 (Regulatory, Integrative Comp. Physiol. 40): R946-R954

Meyer, R.A., L.H. Sweeney and L.H. Kushmerick. 1984. A simple analysis of the phosphocreatine shuttle. Am. J. Physiol. 246 (Cell Physiol 15): C365-C377

CARDIORESPIRATORY RESPONSES OF TUNA

TO ACUTE HYPOXIA

Peter G. Bushnell
Indiana University South Bend
Department of Biological Sciences, 1700 Mishawaka Avenue,
P.O. Box 7111, South Bend, Indiana 46634-7111
phone: (219) 237-4888; fax: (219) 237-6589;
email: pbushnell@iusb.edu

EXTENDED ABSTRACT ONLY DO NOT CITE

Anatomical, physiological, and biochemical adaptations enable tunas to consume oxygen at rates that are unmatched by other teleosts. As a consequence of their high metabolic demand, the heterogeneous distribution of tuna species has been attributed, in part, to the availability of oxygen in the water column. By using multiple instrumentation techniques on free-swimming and restrained yellowfin (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*) tuna, cardiovascular and respiratory variables were monitored in fish acutely exposed to hypoxia ranging from 130 mmHg to 50 mmHg. The results indicated that both species of tuna respond physiologically to moderate hypoxia (130-100 mmHg) and can not maintain oxygen delivery when PO_2 falls below 90 mmHg.

In the first set of experiments (Bushnell and Brill, 1991), free-swimming yellowfin and skipjack tuna were instrumented with heart rate transmitters and cannulae for measuring PO_2 of water entering and leaving the gills. These cannulae were also used to deliver dye to the inhalant water stream and collect it from the exhalant water stream in order to estimate ventilation volume (V_g) by dye dilution. When the PO_2 of the tuna's aquarium was lowered from 150 mmHg to 70 mmHg over a 15 minute period (Fig. 1), both yellowfin and skipjack tuna increased their swimming speed despite a concomitant reduction in heart rate (HR). These responses were first noted at an ambient PO_2 of ~130 mmHg. Normoxic ventilation volume was ~3-5 $L^{-1} \cdot min^{-1} \cdot kg^{-1}$ with an oxygen

utilization (U) of 45-55%. An increase in swimming speed and/or mouth gape in response to hypoxia increased V_g by 45% resulting in a 35% reduction in U.

In a second set of experiments, a detailed study of the tuna's oxygen delivery system in normoxic and hypoxic conditions was conducted using multiply-instrumented, restrained fish (Bushnell *et al.,* 1990; Bushnell and Brill, 1992). Yellowfin and skipjack tuna were equipped with dorsal and ventral aortic cannulae, a pulsed Doppler flow probe, and inhalant and exhalant water sampling cannulae. Tuna were prevented from swimming with a spinal

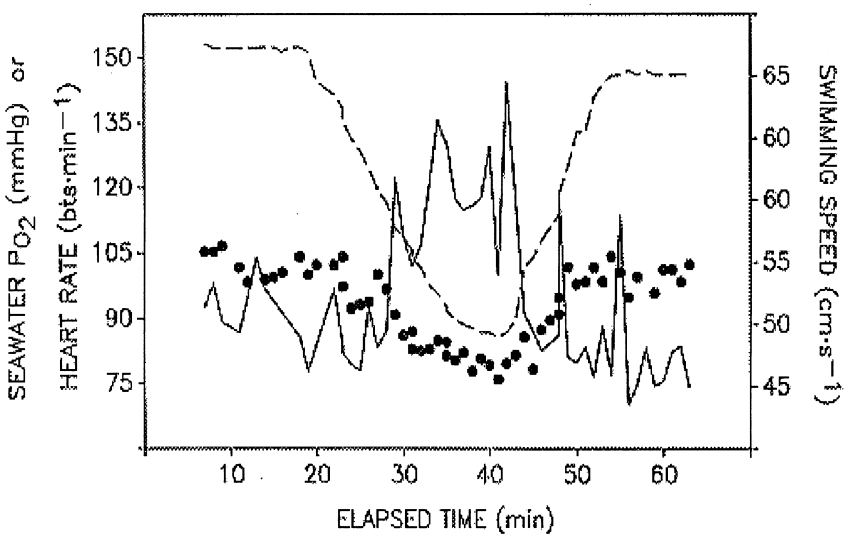


Fig.1. The effect of ambient oxygen levels (dashed line) on heart rate (solid circles) and swimming speed (solid line) in a free-swimming yellowfin tuna.

injection of lidocaine HCl, placed in a tank, and situated in front of a seawater delivery pipe to provide ram ventilation of the gills. Although they could not swim, the tuna had full control of their respiratory apparatus which allowed them to set their own Vg by adjusting their mouth gape and opercular aperture. Heart rate, dorsal and ventral aortic blood pressures, and cardiac output were continuously monitored during normoxia and three levels of hypoxia (PO_2 ~

120, 90, 50 mmHg). Water and blood samples were taken for O_2, CO_2 and pH measurements in fluids afferent and efferent to the gills and used to calculate measures of oxygen transfer effectiveness.

Cardiorespiratory variables monitored in normoxic and hypoxic restrained tuna closely matched those measured in their free-swimming counterparts. When exposed to hypoxia, the cardiorespiratory responses in the restrained tuna were similar to other teleosts, differing only in magnitude and sensitivity to inspired oxygen levels. In general, it appeared that skipjack tuna were less hypoxia tolerant than yellowfin. As was noted in the free-swimming studies, hypoxic tuna increased V_g and decreased HR. The hypoxic bradycardia, however, was not accompanied by a compensatory increase in stroke volume, resulting in a progressive decline in cardiac output. Therefore, in order to maintain oxygen consumption in the face of declining oxygen levels, both yellowfin and skipjack tuna were forced to draw on substantial venous oxygen reserves. The failure to maintain oxygen delivery to the tissues occurred at a higher inhalant water PO_2 in skipjack tuna (130-90 mmHg) than in yellowfin tuna (90-50 mmHg).

The results of these studies lends a physiological basis for using oxygen levels as an important factor in modeling tuna distribution. Since skipjack tuna appear to be less hypoxia tolerant, one would predict that they would tend to be restricted to the oxygen-rich mixed layers. Yellowfin tuna, on the other hand, should be able to tolerate moderate hypoxia for extended periods of time and would be capable of penetrating the upper layers of the oxycline common in many areas of the Pacific. Ultrasonic tagging studies (Cayré and Marsac, 1993) have indicated that yellowfin tuna behavior is, indeed, strongly affected by the depth of the oxycline. Although the depth distribution of yellowfin tuna indicates that they tend to stay above the oxycline, yellowfin tuna will occasionally penetrate it, presumably in search of prey. Interestingly, the lower limit of yellowfin tuna distribution appears to be shaped by relatively moderate levels of hypoxia (120-100 mmHg); oxygen levels which are significantly above their lower lethal limit but similar to levels where physiological responses were first noted in our laboratory experiments.

References

Bushnell, P.G. and R.W. Brill. 1992. Oxygen transport and cardiovascular responses in skipjack (*Katsuwonus pelamis*) and yellowfin tuna

(*Thunnus albacares*) exposed to acute hypoxia. J. Comp. Physiol. 162:131-143

Bushnell, P.G. and R.W. Brill. 1991. Responses of swimming skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas to acute hypoxia, and a model of their cardiorespiratory function. Physiol. Zool. 64(3):787-811

Bushnell, P.G., R.W. Brill, and R.E. Bourke. 1990. Cardiorespiratory responses of skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*Thunnus obesus*) to acute reductions in ambient oxygen. Can J. Zool. 68(9):1857-1865

Cayré, P and F. Marsac. 1993. Modeling the yellowfin tuna (*Thunnus albacares*) vertical distribution using sonic tagging results and local environmental parameters. Aquat. Living Resour. 6:1-14

THE EFFECTS OF HYPOXIA

ON FISH VASCULATURE

Kenneth R. Olson and Malcolm E. Forster
Indiana University School of Medicine,
South Bend Center for Medical Education, University of Notre Dame,
Notre Dame, IN 46556, USA
Ph: (219) 361-7560, Fax: (219) 361-7821, e-mail: olson.1@nd.edu and
Department of Zoology, University of Canterbury,
4800 Private Bag, Christchurch,
New Zealand

Discussion

Perhaps the most primal function of the cardiovascular system is delivery of oxygen to tissues. To ensure this end, vertebrates are endowed with a hierarchy of control systems that affect the convective process. These systems can be viewed as operating on two (or more) levels. The remote system operates for the "good" of the whole organism. It consists of a variety of feedback mechanisms that adjust the primary effectors, the heart and blood vessels, in order to maintain an optimal perfusion (arterial) head-pressure. It is well known in mammals that if oxygen availability becomes limited, i.e., hypoxia, the remote system may allocate flow distribution to more oxygen-sensitive tissues, or to tissues whose continued function is most crucial to survival, such as the brain and heart. The local system is found within the tissues, primarily at the arteriolar and pre-capillary level and it matches convective delivery with the metabolic needs of the cells. In hypoxic situations the intent of the remote and local systems may be at odds.

For many fish, the threat of an acute or chronic reduction in ambient oxygen is both real and reoccurring. How the remote and local systems respond to these crises, and how the two systems are integrated is largely unknown. This is further complicated by an incomplete understanding of the criticality of oxygen delivery to individual organs, as there are numerous reports of the extreme (compared to mammals) anaerobic capacity of some organs including the heart (see this symposium) and brain.

As the cardiovascular system of most fish seems to respond to hypoxia in some way, it can be assumed that virtually all these vertebrates have some receptive mechanism for detecting ambient or internal oxygen, or both. Oxygen sensitive sensors in the central nervous system, gills and probably elsewhere serve these functions in elasmobranchs and teleost, and perhaps the cyclostomes as well (3). However, the cardiovascular responses to hypoxia are quite variable and there does not appear to be a uniform, clear-cut response.

In the hagfish, *Eptatretus cirrhatus*, hypoxia increases cardiac output (CO) and gill vascular resistance Rg, but it does not affect systemic resistance Rs (1). Heart rate (HR) is unaffected and the increased CO is achieved solely through an increased stroke volume (SV). This is strongly suggestive of an increased venous return, which can be accomplished through venoconstriction without an apparent increase in Rs. The increased Rg may be due in part to catecholamines (1), however local responses (see below) can not be excluded.

In both elasmobranchs and teleosts, hypoxia usually invokes bradycardia, while CO is maintained through an increased SV (3). In many fish, there is a concomitant increase in ventral aortic blood pressure while dorsal aortic pressure may increase or decrease depending on the change in Rg relative to Rs. Generally, however, in *in vivo* studies changes in Rg have been found to be minimal or non-existent compared to changes in Rs. Gill sensors are responsible for the bradycardia in the cod, whereas it has been proposed that O_2 receptors located elsewhere in the body produce the rapid (2 min) increase in Rs (2). It has also been suggested that the efferent loop producing the increased Rs is mediated by the sympathetic nervous system (2). While reflex control of the vasculature is probably involved in these resistance changes, the contribution of local control must also be considered.

In spite of the somewhat equivocal effects of hypoxia on Rg *in vivo*, especially in the cod, hypoxic vasoconstriction has been well documented in perfused gills and in anesthetized intact preparations. The elegant studies by Sundin and coworkers showed in the cod that hypoxia decreased gill arteriovenous (AV) resistance and produced a -mediated dilation of the arterioarteriolar (AA) pathway. A concomitant constriction of the AA pathway, of unknown origin negated the dilation in fish without blockade, suggesting additional regulatory mechanisms. In the trout, hypoxic constriction of the AA pathway was mediated by cholinergic mechanisms, although an unidentified component also remained (4).

It has been clearly shown that local mechanisms are vitally important in the responses of mammalian vessels to tissue hypoxia. Typically, systemic vessels dilate, whereas pulmonary vessels constrict. A myriad of factors have been shown to contribute to these responses, including paracrine signals of endothelial origin as well as direct responses of vascular smooth muscle. Relatively little is known about the activity, or even existence of similar mechanisms in fish at the level of individual vessels. The remaining portion of this presentation will focus on this topic.

In isolated postbranchial arteries of trout (see Smith, et al., this symposium for additional details) and tuna, hypoxia decreases vascular tone through mechanisms independent of neural or endocrine control. Upon return to normoxia, there is a further decrease in tone prior to return to resting tension. In pre-branchial arteries of the ghost shark and Pacific hagfish hypoxia elicits complex responses, some of which may be mediated by endothelium-dependent systems. The interactions of endothelium-dependent, and direct smooth muscle responses to hypoxia in these fish will be discussed.

References

Forster, M.E., Davison, W., Axelsson, M., and Farrell, A.P. 1992 Cardiovascular responses to hypoxia in the hagfish, *Eptatretus cirrhatus.* Respiration Physiol. **88:** 373-386.

Fritsche, R., and Nilsson, S. 1993 Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. Exp. Biol. **48:** 153-160

Fritsche, R., and Nilsson, S. 1993 Cardiovascular and ventilatory control during hypoxia. In: *Fish Ecophysiology.* Rankin, J.C., and Jensen, F.B., eds. Chapman & Hall, London. pp 180-206

Sundin, L. 1995 Responses of the branchial circulation to hypoxia in the Atlantic cod, *Gadus morhua*. Am. J. Physiol. **268:** R771-R778.

Sundin, L., and Nilsson, G. 1997 Neurochemical mechanisms behind gill microcirculatory responses to hypoxia in trout: an in-vivo microscopy study. Am. J. Physiol.**272:** R576-R585.

CARDIOVASCULAR EFFECTS

OF ENVIRONMENTAL HYPERCAPNIA

IN THE RAINBOW TROUT *IN VIVO*

Todd M. Hoagland
University of Notre Dame, Department of Biological Sciences
and South Bend Center for Medical Education
B 19 Haggar Hall, University of Notre Dame, Notre Dame IN 46556
Work (219)631-8293 Fax (219)631-7821 Home (219)283-0708

Kenneth R. Olson and Steve F. Perry
University of Ottawa, Department of Biology
30 Marie Curie, Ottawa, Ontario, K1N 6N5, CANADA
Work (219)631-7560 , Work (613)562-5800x6005

Introduction

The systemic vascular resistance in teleosts is a primary determinant of blood pressure and has indirect affects on gas transfer at the gill. During hypoxia, the systemic circulation is influenced by neural, humoral and potentially local mechanisms (Fritsche and Nilsson, 1990). Most teleosts show an increase in pre- and post-branchial blood pressures in response to hypoxia (Holeton and Randall, 1967; Fritsche and Nilsson, 1990). In the Atlantic cod, these increases in pressures are attributed to the significant rise in the systemic vascular resistance. Elevated levels of circulating catecholamines combined with increased adrenergic nervous activity are responsible for this increase in resistance (Fritsche and Nilsson, 1990).

Environmental hypercapnia elicits a respiratory acidosis in fish and produces similar responses as hypoxia including, hyperventilation (Janssen and Randall, 1975) and catecholamine release (Perry et al. 1989). The increased ventilation most likely enhances branchial oxygen transfer and blood oxygen transport to offset the hemoglobin-oxygen binding problems of the hypercapnic acidosis. Although respiratory perturbations have been examined regarding hypercapnia, cardiovascular changes have not. Hypercapnic catecholamine release will most

likely have significant cardiovascular implications and the present study will elucidate (arterial CO2, PaCO2; arterial oxygen, PaO2; arterial pH, pHa; ventilation amplitude, V_{amp}; ventral aortic, P_{VA} and dorsal aortic pressure, P_{DA}; central venous pressure, P_{VEN}; cardiac output, Vb and heart rate, Hf) cardiovascular and concomitant respiratory changes in response to environmental hypercapnia.

Methods

Rainbow trout (0.3-0.8 kg) of either sex were used. Trout were initially anesthetized in ethyl aminobenzoate (benzocaine, 1:12,000 wt:vol) and during surgery the gills were continuously irrigated with 10°C aerated water containing 1:24,000 benzocaine. Pressure readings were taken from the dorsal aorta, ventral aorta, and venous sinus via Hewlett-Packard 7853A patient monitors and digitized signals collected and stored in a 486 IBM-compatible computer. Vb was determined using Transonic flow probes (Transonic, Ithaca, NY). PE-160 was implanted into the buccal cavity to facilitate measurement of inspired water PCO2 (PwCO2) and PO2 (PwO2). Small (1cm2) brass plates were stitched to the external surface of each operculum to allow the measurement of ventilation amplitude using an impedance converter. A caudal artery and vein were cannulated allowing an extracorporeal circuit to be initiated in which the arterial blood flowed continuously over a series of electrodes (pH, PO2, PCO2) by means of a peristaltic pump.

Upon establishment of a stable baseline, experiments commenced with a period of normocapnia followed by periods of hypercapnia and recovery. During normocapnia, the inflowing water was provided from a gas equilibration column being gassed vigorously with air. Hypercapnia was initiated by gassing the equilibration column, that provided the fish boxes with water, with CO2 in air mixed by a GF-3/MP gas-mixing flowmeter(Cameron Instrument Company, Port Arkansas, TX). Fish were divided into three groups. The first was treated with (α2-adrenoreceptor antagonist) yohimbine 2 h before the experiment. The second served as a control with saline injected 2 h prior and the third group was not pretreated. All groups were subjected to normocapnia, immediately followed by a dose-response to hypercapnia and then back to normocapnia while cardiovascular variables were recorded concomitantly with PaO2, PaCO2, pHa, PwCO2 and PwO2. All data are presented as means ± 1SE.

Results

CO2 dose response curves

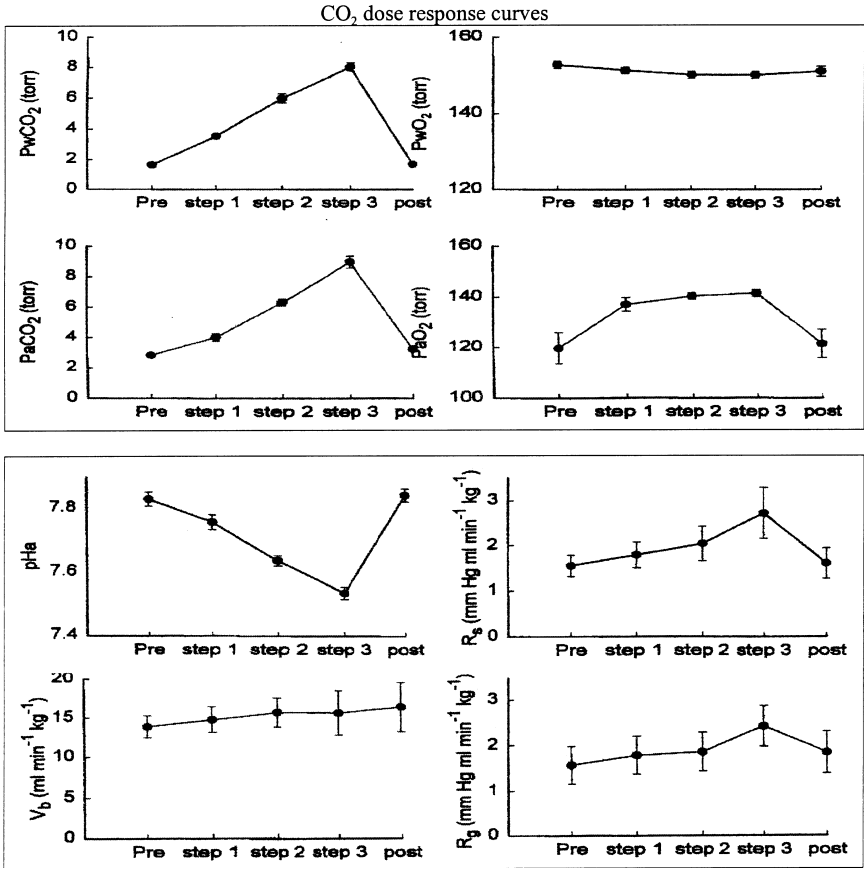


Figure 1. Effects of incremental increasing hypercapnia on chosen cardio-respiratory variables.

Trout responded to hypercapnia via an increase in PDA, PVA and Vamp. The Vb and Hf showed no significant differences from normocapnia and PVEN

increased at step 3 of hypercapnia (the highest PwCO2). Branchial and systemic vascular resistances increased markedly. The PaO2 increased with hypercapnia while PwO2 showed no significant differences.

Conclusions

Exposure of fish to hypercapnic acidosis caused marked cardio-respiratory changes including: hyperventilation (as indicated by increased opercular displacement), increased P_{VA}, P_{DA} and no significant changes in Vb. The increased P_{VA} and P_{DA} most likely resulted from the increased sys-temic and branchial resistances. The hypercapnia-mediated increase in P_{DA} was abolished by pre-treating fish with the 2-adrenoreceptor antagonist, yohimbine. This lends evidence to circulating catecholamines being an important mediator of the hypercapnic response. Hypoxia was not the driving force in these cardio-respiratory changes in that PaO2 actually increased during the steps of hypercapnia most likely from the increased Vamp causing hyperventilation. Although further elucidation of the cardiovascular mechanisms of action are needed, it appears clear that CO2/H+ are important modulators of cardiovascular and respiratory function in fishes.

Acknowledgements

This work was supported by the National Science Foundation Grant IBN-9723306.

References

Fritsche, R. & Nilsson, S. (1990). Autonomic nervous control of blood pressure and heart rate during hypoxia in the cod, Gadus morhua. J. Comp. Physiol. B 160, 287-292.

Fritsche, R. & Nilsson, S. (1993). Circulatory and ventilatory responses to hypoxia. In: Fish Ecophysiology (F. Jensen & C. Rankin, eds.), London: Chapman and Hall, pp 180-206.

Holeton, G.F. & Randall, D.J. (1967). Changes in blood pressure in the rainbow trout during hypoxia. J. Exp. Biol. 46, 297-305.

Janssen, R.G. & Randall, D.J. (1975). The effects of changes in pH and PCO2 in blood and water on breathing in rainbow trout, Salmo gairdneri. Respir. Physiol. 25, 235-245.

Perry, S.F., Kinkead, R., Gallaugher, P. & Randall, D.J. (1989). Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (Salmo gairdneri). Respir. Physiol. 77, 351-364.

REGULATION OF VASCULAR SMOOTH MUSCLE TONE

IN THE TROUT:

LOCAL EFFECTS OF HYPOXIA, HYPERCAPNIA AND PH

Michael P. Smith
University of Notre Dame, Department of Biological Sciences
and South Bend Center for Medical Education
B19 Haggar Hall, University of Notre Dame, Notre Dame IN 46556
Work 219-631-8293 Fax 219-631-7821 Home 616-668-5312

Jeffrey T. Wincko[†] and Kenneth R. Olson[‡]
University of Notre Dame, Department of Biological Sciences[†]
and South Bend Center for Medical Education[‡]
B19 Haggar Hall, University of Notre Dame, Notre Dame IN 46556

Introduction

With blood flow related to the fourth power of vessel radius and radius governed by medial vascular smooth muscle (VSM), modulation of VSM tone is critical in matching regional flow to metabolic demand at the tissue level. VSM tone reflects the degree of activation of the actin-myosin contractile apparatus by intracellular calcium $[Ca^{2+}]_i$ so it follows that regulation of flow ultimately rests upon modulation of $[Ca^{2+}]_i$. Local metabolic signals such as P_{O2}, P_{CO2} and $[H^+]$ are ideally positioned to influence VSM directly or in conjunction with adenosine (ADO) and K^+-channels. Hypoxia generally relaxes systemic vessels in an attempt to restore flow to deprived tissues while in the pulmonary circulation hypoxia elicits constriction (HPV) to assure ventilation-perfusion matching (Wadsworth, 1994).

The effects of hypercapnia parallel those of hypoxia and are often inseparable from the attendant acidosis (Nagi and Ward, 1997). In all cases, vasodilation is associated with reductions in $[Ca^{2+}]_i$ (Kozlowski, 1995). Although well characterized in mammalian VSM, little is known about metabolic coupling of VSM tonus in fish. Hypoxia has been shown to cause branchial constriction

with accompanying elevations in gill resistance in fish (Holeton and Randall, 1967) which is not adenosinergic in origin (Sundin and Nilsson, 1997) . Recent *in vivo* studies from this lab demonstrate that hypercapnic effects resemble those of hypoxia in the gill, suggesting a vasoregulatory role for P_{CO2} in fish. These experiments characterize the effects of changes in P_{O2}, P_{CO2} and $[H^+]$ on isolated vascular segments and gills of Rainbow trout (*Oncorhynchus mykiss*).

Methods

Vessels (efferent branchial arteries; EBAs) were harvested from wild Steelhead trout or captive Rainbow trout and suspended in tissue baths in PBS with glucose (pH 7.8, 12^{o}C), set to nominal tension (500 mg) and bubbled with room air. EBAs were pre-contracted with arginine vasotocin (AVT; 1 nM), endothelin (ET-1; 1 nM) or potassium chloride (KCl; 50 mM).Changes in isometric tension (mg) in un-stimulated or pre-contracted EBAs were recorded by polygraph following gassing to equilibrium with N_2, O_2 and CO_2 custom mixes. Final bath dissolved oxygen (DO) and pH were measured following exposures up to 1 hour long. Effects of pH were assessed in HEPES at various $[H^+]$. Gill arches were isolated from captive trout and perfused with filtered PBS via afferent branchial artery at constant flow by peristaltic pump while suspended in an aerated bath (10 mOsm, 12^{o}C). Bath or perfusate gas partial pressures or pH were manipulated and input pressure recorded during 10-20 minute exposures. Differences between treatments were considered significant if $p<0.05$ by ANOVA and Student-Newman-Keul's test.

Results

Isolated Vessels.

Preliminary studies in ET-1 contracted EBAs of captive trout showed acidity- and N_2-induced relaxation. N_2/CO_2 elicited a bi-phasic contraction/relaxation profile in ET-1 contracted vessels which was absent in KCl-contracted vessels (Figure 1a). O_2/CO_2 produced a robust contraction that diminished within 15 minutes in untreated vessels. Further studies in wild trout vessels confirmed tone-independent relaxation due to declining pH_o and contraction upon alkalinization. AVT pre-contracted EBAs responded to both N_2/CO_2 and N_2 with bi-phasic profiles which were altered by KCl contraction. Extreme hypoxia/hypercapnia (CO_2) produced a bi-phasic contraction/relaxation in both

AVT- and KCl-contracted EBAs, suppressed contractions due to AVT or KCl until air was returned and was unique among all gas treatments in eliciting any effect (i.e. robust contractions) in EBAs without pre-existing contractile tone (Figure 1b). Interestingly, CO_2 caused only mild transient contractions which gave way to relaxations in both un-stimulated and contracted EBAs of captive trout. CO_2 effects were reversible with return to air, repeatable and therefore not due to a pathological rigor. The degree of contractile agonist rebound after CO_2 relaxation was contingent on the duration of exposure.

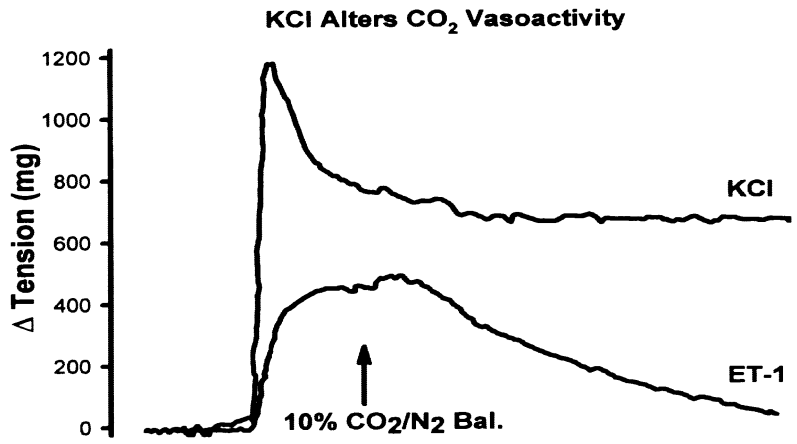


Figure 1a

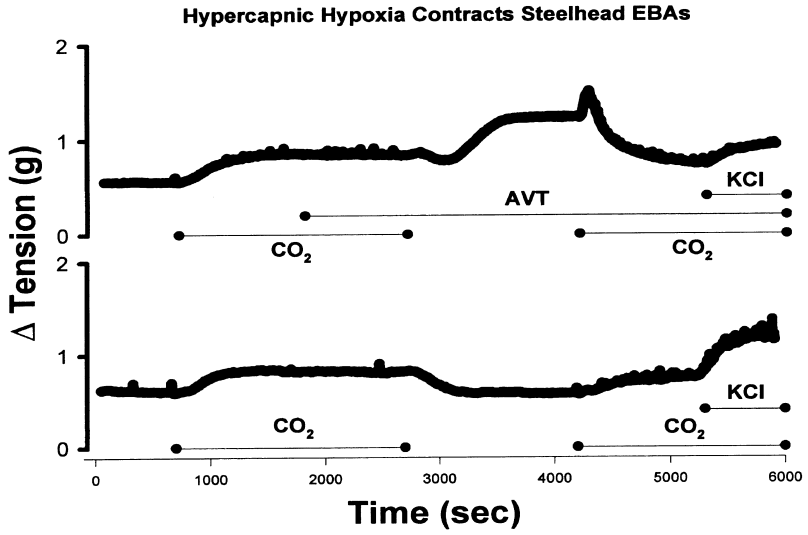


Figure 1b

Isolated Gills.

Elevations in bath P_{CO2} reduced gill resistance (R_{GILL}) independent of DO content. Reductions in perfusate pH dose-dependently and reversibly diminished R_{GILL}, effects which did not vary with re-exposure. Perfusate aciditity (<pH 7.8) reduced while alkalinity elevated R_{GILL}. Hypoxic perfusate (N_2, <10% DO) had no effect but both hypoxic/hypercapnic (95% N_2/5% CO_2, <10% DO) and hyperoxic/hypercapnic (95% O_2/5% CO_2) perfusates elevate R_{GILL}, a profile invariant upon re-exposure (Figure 2a). Extreme hypoxic hypercapnia (CO_2, pH 5.6) rapidly and robustly elevates input pressure and elicits a unique vasomotion (Figure 2b) which is reversible and repeatable while acidified hypoxic perfusate (N_2, pH 5.6) reduces gill resistance.

Clinical Data.

Captive trout were determined to be hypercapnic (P_{CO2} = 34 mm Hg with metabolic compensation ($[HCO_3^-]_p$ = 15 mmol/L). Wild trout, though not tested, were assumed to be normocapnic due to raceway oxygenation facilities.

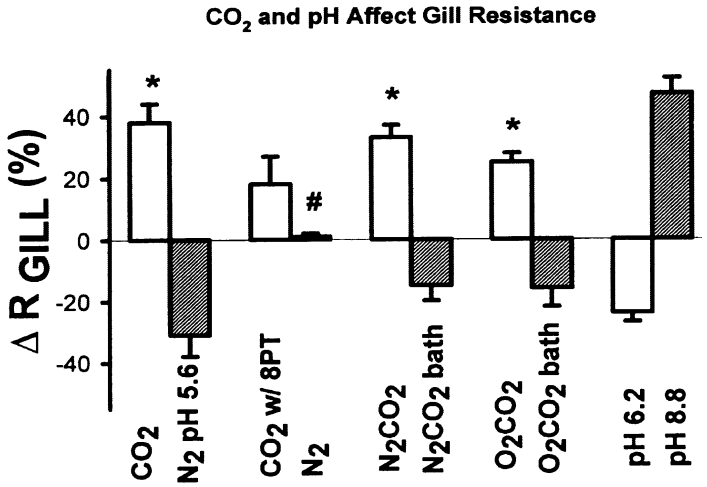


Figure 2a

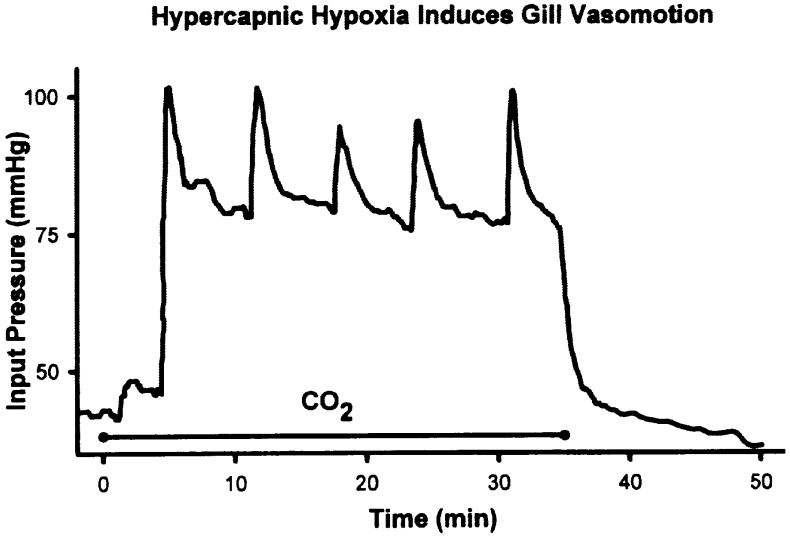


Figure 2b

Conclusions

Alterations in gas partial pressures elicit changes in VSM tone *in vitro* which may be related to membrane potential via K^+ channel activity. CO_2 elicits contractile responses not related to pH_o but relaxations due to CO_2 may be attributable to concomitant acidosis. Differences in CO_2 responsiveness between captive and wild trout vessels may reflect an adaptation of the branchial vasculature to chronic hypercapnia and acidosis. P_{CO2} is a determining factor in resistance in isolated trout gills and CO_2 effects are divorcible from pH_o effects in the gill. CO_2 induces a unique vasomotion in the isolated gill which cannot be duplicated by matching hypoxic and acidotic conditions.

Acknowledgements

This work was supported by the National Science Foundation Grant IBN-9723306. Gratitude to the Staff of the Richard Clay Bodine State Fish Hatchery, Mishawaka, IN for their generous gift of Steelhead tissues.

References

Holeton, G.F. and D.J. Randall. 1967. Changes in blood pressure in the rainbow trout during hypoxia. J. Exp. Biol. 46: 297-305.

Kozlowski, R.Z. 1995. Ion channels, oxygen sensation and signal transduction in pulmonary arterial smooth muscle. Cardio. Res. 30: 318-325.

Nagi, M.M. and M.E. Ward. 1997. Modulation of myogenic responsiveness by CO_2 in rat diaphragmatic arterioles: role of the endothelium. Am. J. Physiol. 272 (Heart Circ. Physiol. 41): H1419-H1425.

Sundin, L. and G.E. Nilsson. 1997. Neurochemical mechanisms behind gill microcirculatory responses to hypoxia in trout: an in-vivo microscopy study. Am. J. Physiol. 272 (2 Part 2): R576-R585.

Wadsworth, R.M. 1994. Vasoconstrictor and vasodilator effects of hypoxia. TIPS. 15: 47-53.

CARDIAC PERFORMANCE OF A TELEOST FISH

DURING HYPOXIC CONDITIONS:

ELECTROCARDIOGRAPHIC CONSIDERATIONS AND

NERVOUS CONTROL OF THE HEART RATE

Cecilia Del Rosario Guerra[1] and Franscisco Tadeu Rantin[2]
1 - Universidad Santa Maria La Antigua, Panama (pcguerrb@canaa.usma.ac.pa)
2 - Universidade Federal de Sao Carlos, SP, Brasil (ftrantin@power.ufscar.br)

EXTENDED ABSTRACT ONLY DO NOT CITE

Six lead electrocardigrams were recorded simultaneously in the teleost, *Piaractus mesopotamicus*, in normoxia (140 mmHg), during graded hypoxia (30 min at 100, 70, 50, 30, 20, and 10 mmHg), acute hypoxia (1 and 2 h at 30 mmHg), and during the subsequent recovery to normoxia. Antagonist drugs (atropine and propranolol) were used to elucidate the nervous control of the heart rate (f_H) during hypoxia. Simultaneous ECG recordings, including the 3 bipolar (D_I, D_{II} and D_{III}) and the 3 monopolar 'limb' leads (aVR, aVL and aVF) were obtained from minimally disturbed fish. The spatial orientation of the P (SAP) and T (SAT) waves, and QRS complexes (SAQRS) axis were determinated in all experimental conditions. In normoxia, sinus rhythm was characterized by a mean heart rate of 65 b.p.m. The P wave amplitude and QRS complexes remained constant in all experimental protocols.

No significant differences were observed in the durations of the ECG components (P, T waves, QRS complexes, ST segments, PR and QT intervals) in any condition. During normoxia, the SAP was -120°; SAQRS was 30°, and SAT was -150° in the frontal plane. The maximum amplitude of P and T waves were observed in D_{II} (-0.117 and -0.045 mV, respectively), and QRS was in D_I (1.073 mV). In severe hypoxia, the maximum amplitude of P, T waves, and QRS complexes were in aVL (0.08 mV), D_{III} (-0.13 mV) and D_I (0.088 mV) respectively. The SAP and SAQRS remained constant in all experimental O_2 tensions. The increase in the amplitude and area of the T wave and shift of the

SAT during hypoxia indicated alterations in the direction of ventricular repolarization suggesting myocardial “ischemia”. Atropine injections blocked the bradycardia recorded during graded hypoxia and propranolol injection provoked bradycardia during severe hypoxia. Drug injections did not provoke any alteration in the morphology of the ECG waves during normoxia. In severe hypoxia the alterations in the morphology of the T waves were observed in atropinized animals. The results suggest that the variation of the cholinergic tonus on the heart is a major factor in the regulation of the heart rate and that the adrenergic control of the heart is of particular importance in this species.

Acknowledgements

Financial support: CAPES, SENACYT, USMA and Ford-MacArthur foundation

CARDIAC ANGIOTENSIN II RECEPTORS

IN THE DOGFISH, *SCYLIORHINUS CANICULA*

M.C. Cerra
Departments of Biology and Pharmaco-Biology, University of Calabria,
Arcavacata di Rende, CS, Italy;
Tel. +984 492909; Fax +984 492911;
email: cerramc@pobox.unical.it

A.Gattuso
Biology, University of Calabria;

N. Hazon,
Gatty Marine Lab., University of St. Andrews;

Y. Takei,
Ocean Research Institute, University of Tokyo; and

B. Tota,
Biology, University of Calabria, and Zoological Station "A. Dohrn".

Introduction

The octapeptide angiotensin II (ANGII) is a potent homeostatic hormone not only in mammals but also in non-mammalian vertebrates, including Elasmobranch fish. The recently isolated Elasmobranch ANGII has a unique structure for the presence of a proline at position 3 and, as suggested by studies conducted in the hearts of *Raja erinacea*, *Squalus acanthias*, and *Triakis scyllia* (Hazon et al., 1995), it can play a role in the modulation of cardiac function (for review see Kobayashi and Takei, 1996). As in other non-mamalian species, also in Elasmobranchs, ANGII may elicits its functions through the binding to specific receptors that have been identified and partially characterized in osmoregulatory organs of*Triakis scyllia* (Tierney et al., 1997) and *Scyliorhinus canicula* (Hazon et al., 1997).

Materials and methods

In vitro quantitative autoradiography was associated to saturation and competitive binding studies in order to investigate the presence and type of ANGII receptors in the atrium, ventricle and conus arteriosus of the dogfish *Scyliorhinus canicula*. Cardiac ANGII receptors were identified and characterized using labeled dogfish ANGII (^{125}I-dfANGII), unlabeled dfANGII, and two specific antagonists for the ANGII receptors subtypes, AT1 and AT2 (CV11974 and CGP42112, respectively). For the saturation study, 16µm cardiac sections were incubated in the presence of ^{125}I dfANGII (10^{-12} - $5x10^{-9}$ M) and of 10^{-6} M of unlabeled dfANGII (N=3). For the competitive inhibition study cardiac sections were incubated in the presence of 100pM of ^{125}I-dfANGII and increasing concentrations (10^{-13}-10^{-6}M) of unlabeled dfANGII, CGP42112 and CV11974 (N=6).

Results and discussion

Specific ANGII binding sites were heterogeneously distributed among the dogfish cardiac regions with a prevalent localization of the labeling in the outer layer of the conus (i.e. tunica media plus adventitia). The results from saturation and competitive experiments indicated us that the different heart regions of the dogfish are characterized by two types of receptors. One type, mainly observed in the ventricular myocardium and in the inner and outer layers of the conus, showed low affinity values (398±83<Kd<4960±930 pM) and was better displaced by the AT2 antagonist, CGP42112 than by the AT1 antagonist, CV11974, with a ranking order comparable to that reported for the mammalian AT2 receptors (Timmermans et al., 1992). The second type, identified in all cardiac regions of the dogfish, except the inner conal layer, demonstrated high affinity binding values (16±9<Kd<106±3 pM). This receptor, according to the standard nomenclature (Timmermans et al., 1992), at present can be considered an atypical ANGII receptor because it showed a low discrimination capacity between the AT1 and AT2 antagonists used to displace the radioligand. These results indicated that the dogfish cardiac ANGII receptors differ from both the low affinity-low discriminative and the high affinity-AT2 selective receptors described in the osmoregulatory organs of *Triakis scyllia* (Tierney et al., 1997) and *Scyliorhinus canicula* (Hazon et al.,

1997). It remains to be explored if the atypical ANGII receptor identified in the dogfish heart is either a primitive type, evolved before the diversification into AT1 and AT2, or it represents the counterpart of one of the two major types of ANGII receptors.

References

Hazon, N., Tierney, M.L., Hamano, K., Ashida, K., and Y. Takei. 1995. The effect of endogenous angiotensins, angiotensin II competitive binding inhibitors and converting enzyme inhibitor in elasmobranch fish. Net. J. Zool. 45(1-2): 117-120.

Hazon, N., Cerra, M.C., Tierney, M.L., Tota, B., and Y. Takei. 1997. Elasmobranch renin angiotensin system and the elasmobranch receptor. In: Kawashima S. and Kikuyama S., eds, Advances in Comparative Endocrinology, Monduzzi Editore, Bologna, Italy, pp. 1307-1312.

Kobayashi, H., and Y. Takei. 1996. The renin-angiotensin system. Comparative aspects. In: Bradshaw S.D., Burggren W., Heller H.C., Ishii S., Langer H., Neuweiler G., Randall D.J. (eds.) Springer, New York.

Tierney M.L., Takei Y., and N. Hazon. 1997. The presence of Angiotensin II receptors in elasmobranchs. Gen. Comp. Endocrinol. 105: 9-17.

Timmermans, P.B.W.M., Benfield, P., Chiu, A.T., Herblin, W.F., Wong, P.C., and R. D. Smith 1992. Angiotensin II receptors and functional correlates. Am. J. Hypertens. 5:221S-235S.