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Cardiorespiratory and tissue adenosine responses to hypoxia and reoxygenation in the short-horned sculpin *Myoxocephalus scorpius*

T. J. MacCormack and W. R. Driedzic*

Ocean Sciences Centre, Memorial University of Newfoundland, St John's, NL, Canada A1C 5S7

*Author for correspondence (e-mail: wdriedzic@mun.ca)

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Summary

Adenosine is a product of adenylate phosphate breakdown that can exert protective effects on tissues during energy limitation. Accumulation of cardiac adenosine under hypoxia is well documented in mammals but has not been shown in fish. Adenosine content was measured in heart and brain tissue from short-horned sculpin *Myoxocephalus scorpius* L. exposed to acute hypoxia and to graded hypoxia and reoxygenation at 8°C. Cardiorespiratory parameters were recorded along with plasma lactate, K^+, Ca^{2+} and Na^+ levels and their relationship to adenosine levels investigated. Sculpin exhibited a large bradycardia during hypoxia, with a concomitant drop in cardiac output that recovers fully with reoxygenation. Ventilation rate also declined with hypoxia, suggesting a depression of activity. Plasma lactate concentration was significantly elevated after 4 h at 2.0 mg l^{-1} dissolved oxygen while K^+ levels increased during acute hypoxia. Adenosine levels were maintained in heart under acute and graded hypoxia. Brain levels fluctuated under hypoxia and showed no change with reoxygenation. It is concluded that a depression of cardiac activity in conjunction with an adequate anaerobic metabolism allow sculpin to avoid excessive adenosine accumulation under conditions of moderate hypoxia. Cardiac adenosine levels decreased and plasma K^+ levels and heart rate increased significantly at reoxygenation.

Key words: adenosine, fish heart, hypoxia, reoxygenation, short-horned sculpin, *Myoxocephalus scorpius.*

Introduction

Adenosine is a purine nucleoside that plays an important, well documented role in cardiac and neural function in mammals and amphibians (Mubagwa and Flameng, 2001; Lutz and Reiners, 1997). Adenosine accumulates in turtle neural tissue during anoxia and its neuroprotective effects are well documented, including defense of ion homeostasis (Buck and Bickler, 1998; Bickler and Buck, 1998) and suppression of excitotoxic neurotransmitter build-up (Milton et al., 2002). Adenosine also accumulates in mammalian cardiac muscle during hypoxia/ischemia when energy production is impaired and there is a net breakdown of phosphorylated adenylates. Under these conditions, adenosine exerts cardioprotective effects similar to those observed for neural tissue, and it has been intimately linked with the phenomenon of preconditioning (Sommerschild and Kirkebøen, 2000). Additional effects of adenosine include vasodilation (Berne, 1963) and stimulation of cardiac glucose uptake (Angello et al., 1993).

Adenosine has also been implicated in the control of cardiovascular and neural function in fish. In brain of the anoxia-tolerant shark *Hemiscyllium ocellatum*, adenosine levels increased almost fourfold during anoxia and data supported a neuroprotective role for adenosine in this species (Renshaw et al., 2002). However, to our knowledge, cardiac adenosine concentrations have not been reported in fish, despite the considerable physiological importance attributed to this compound (Colin et al., 1979; Bernier et al., 1996a,b; Sundin et al., 1999; Aho and Vornanen, 2002). Exogenously applied adenosine is widely used in investigations of cardiovascular physiology in fish (reviewed by Nilsson and Holmgren, 1992) but its effects are often highly variable and concentration-dependent. This observation is particularly relevant to investigations of blood flow, where distinct differences are noted both between species (Farrell and Davie, 1991a,b), and between coronary, systemic and branchial vessels (Mustafa and Agnisola, 1998; Small and Farrell, 1990). The prominence of concentration-dependent effects underlines the need to characterize adenosine levels in fish under a variety of physiological conditions.

The aim of the present study was to determine cardiac and brain adenosine levels throughout hypoxia and reoxygenation in a marine teleost, the short-horned sculpin *Myoxocephalus scorpius* L., to provide a framework for the study of adenosine action in fish. The short-horned sculpin is a benthic, north-temperate species that exhibits considerable hypoxia-tolerance. Cardiac and brain adenosine levels were measured during hypoxia and reoxygenation in conjunction with plasma ion and lactate levels. Plasma parameters were used to gain insight into the relationship between known hypoxia-inducible changes

and tissue adenosine levels. Cardiorespiratory parameters were also recorded to investigate the interplay between whole animal responses to hypoxia and the characteristics of adenosine accumulation.

Materials and methods

Short-horned sculpin *Myoxocephalus scorpius* L. of either sex (body mass 643±26 g, *N*=94) were captured locally, held in aerated seawater maintained at 8±3°C for 2 weeks before experiments and fed regularly with frozen herring.

Hypoxia/reoxygenation system

The experimental system consisted of a blackened, temperature-controlled (8±0.4°C) tank containing 35 l of water, aerated by an air pump combined with 100% O_2. Dissolved oxygen (DO_2) was monitored by pumping water from the tank to a DO_2 electrode (model 4000, VWR, West Chester, PA, USA) housed in a Plexiglas chamber, and back to the tank at 60 ml min^{-1}. Dissolved oxygen was reduced to desired values in less than 5 min by gassing with 100% N_2, and maintained within approximately 4% of the desired level. When necessary, DO_2 levels were adjusted by gassing with 100% O_2. While in the experimental system, instrumented fish were restrained in an adjustable, perforated plastic box to avoid entanglement of electrode leads.

Experiment 1: cardiorespiratory response to hypoxia/reoxygenation

Ventilation rate (f_V) was measured using customised brass electrodes and an impedance converter (model 2991EF, UFI, Morro Bay, CA, USA) interfaced to a PowerLab data acquisition system (ADInstruments, Castle Hill, NSW, Australia) as described previously (MacCormack et al., 2003).

Cardiac output was determined by fitting the ventral aorta with an ultrasonic flow probe (Transonic, Ithaca, NY, USA). Short-horned sculpin (*N*=6) were anaesthetized using 80 mg l^{-1} clove oil, positioned on their right side on a surgery table, iced and ventilated using cold 40 mg l^{-1} clove oil solution. The operculum was held open and an incision made ventral to the base of the third gill arch to expose the tip of the bulbus arteriosus and ventral aorta. A flow probe was secured around the ventral aorta immediately after the bulbus arteriosus and positioned for unimpeded blood flow. The lead was routed and secured inside the opercular cavity and anterior to the dorsal fin using silk sutures. Flow was monitored using a Transonic T106 flow meter interfaced to the data acquisition system described above. Following surgery fish were allowed to recover for approximately 24 h in a 200 l tank supplied with aerated, flow-through seawater before being transferred to the experimental system for an additional 18 h before trials were carried out.

Blood flow parameters were recorded overnight on undisturbed animals to attain the best approximation of resting values for subsequent comparisons. Oxygen was then reduced to 5.6 mg l^{-1} (60% saturation) and 3.8 mg l^{-1} (40% saturation), with animals held for 30 min at each level before DO_2 was reduced to 2.0 mg l^{-1} (20% saturation) and held for 6 h. In preliminary experiments, it was found that sculpin exposed to deeper levels of hypoxia exhibited frequent struggling and loss of equilibrium in a relatively short period. The final level of DO_2 was chosen to facilitate the examination of the hypoxia response over a sufficient time frame while still permitting the animal to survive reoxygenation. Following 6 h at 2.0 mg l^{-1}, the system was reoxygenated to approximately 100% saturation (9.4 mg l^{-1}) within 10 min and animals were held for an additional 1 h. Cardiorespiratory parameters, temperature and DO_2 content were continuously monitored and recordings collected just prior to each change in DO_2 content and at 1 h intervals during the period that the animals were at 2.0 mg l^{-1} DO_2. Preliminary work found that sculpin exposed to this protocol survived for the observation period of 7 days when returned to oxygenated holding tanks.

Experiment 2: metabolic response to hypoxia/reoxygenation

For biochemical and plasma ion determinations, fish were exposed to hypoxia and reoxygenation in a similar manner to that described in experiment 1. For hypoxia trails, fish were placed in the chamber under normoxia and held for 1 h before DO_2 levels were reduced to 2.0 mg l^{-1} through the same step-down procedure used in experiment 1. The sampling protocol for biochemical determinations is presented in Fig. 1. Fish were terminally sampled following the DO_2 step-down (0.5 h at 5.6 and 3.8 mg l^{-1}), and following 1, 2, 4 or 6 h at 2.0 mg l^{-1} DO_2 saturation (*N*=8 for all groups). Following 6 h at 2.0 mg l^{-1}, some animals were subjected to reoxygenation for 0.5 or 1 h. An additional set of animals was subjected to 10 h at 2.0 mg l^{-1} DO_2 saturation before sampling.

Fish were also exposed to acute, severe hypoxia. The experimental system was continuously gassed with 100% N_2 until a stable DO_2 saturation was reached (0.6 mg l^{-1}). Animals were then transferred directly from normoxic water to the system and sampled following loss of equilibrium (13±3.6 min).

For normoxia trials, animals were held under identical conditions to those described above except that DO_2 content in the chamber was maintained at approximately 100% saturation. Animals were placed in the chamber 1 h prior to the experiment and observations were made on DO_2 content and temperature every hour for a total of 8 h (*N*=8). An additional group of fish was taken directly from the holding tank to control for possible effects of confinement in the experimental system (*N*=8).

At the desired treatment time, fish were quickly removed from the system and a blood sample was collected from the caudal vein. Fish were then rendered unconscious by a blow to the head, killed by severing the spinal cord and the heart rapidly excised and frozen using tongs pre-cooled in liquid nitrogen (N_2). The brain was then quickly removed, placed in a cryovial, and submerged in liquid N_2. Blood samples were subsequently centrifuged and plasma was collected and frozen in liquid N_2.

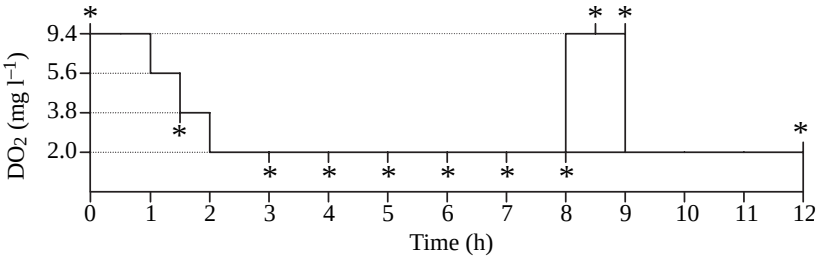

Fig. 1. Sampling protocol for biochemical and plasma ion determinations in short-horned sculpin *Myoxocephalus scorpius* exposed to hypoxia and reoxygenation. DO_2, dissolved oxygen saturation. Asterisks indicate the time at which each group of fish was sampled (*N*=8 for each group).

Analytical procedures

For high performance liquid chromatography (HPLC) analysis, tissue samples were homogenized in ice-cold perchloric acid (6%), centrifuged at 9°C for 5 min at 16000 ***g***, the supernatant removed and neutralized with 5 mol l^{-1} K_2CO_3. The neutralized sample was centrifuged as above and the supernatant removed and frozen in liquid N_2. For lactate determinations, plasma samples were deproteinated by addition of two volumes perchloric acid, centrifuged as described above and the supernatant removed. All extracts were stored at –80°C until use.

Adenosine levels were determined in tissue extracts by HPLC coupled to UV detection by a method modified from Hagberg et al. (1987). Tissue samples of either type from unknown treatments were assessed in random order to eliminate the possibility of experimenter bias. Samples (50 μl) were passed through a 0.45 μm filter and injected *via* a Waters (Milford, MA, USA) 717plus autosampler and eluted (1 ml min^{-1}) using 5.7/1 10 mmol l^{-1} $NH_4H_2PO_4/CH_3OH$ (pH 5.5 at room temperature). Separation was achieved with a C_{18} column (Waters, Symmetry 5 μm, 4.6 mm×250 mm) held at 35°C using a column heater compartment. Peaks were detected at 260 nm using a Waters 2487 dual channel absorbance detector and levels were quantified by comparing peak areas to known standards using Breeze 3.30 software (Waters). Adenosine peaks were identified by retention time comparisons and by spiking tissue samples with known quantities of adenosine. Adenosine levels in tissue extracts were found to be stable for at least 24 h at room temperature. Lactate was measured spectrophotometrically (model DU 640, Beckman Coulter, Inc, Fullerton, CA, USA) by following the reduction of NAD^+ to NADH (340 nm) using a kit obtained from Sigma diagnostics.

Plasma Na^+, K^+ and Ca^{2+} levels were measured using flame photometry (model PFP7, Jenway, Dunmow, Essex, England) at 589, 766 and 620 nm wavelengths, respectively. Plasma samples were diluted (25-fold for Ca^{2+}, 200-fold for Na^+ and K^+) with deionized water and ion concentrations were determined through comparison with a 5-point calibration curve prepared using standard solutions of NaCl, KCl and $CaCl_2$. The contribution of the anti-coagulant sodium-heparin to plasma Na^+ levels was assessed and found to comprise less than 5% of plasma concentrations, in agreement with previous estimates (Fletcher, 1977).

Statistics

Data are expressed as means ± S.E.M. throughout. Cardiorespiratory parameters were compared using a repeated-measures analysis of variance (RM-ANOVA) while biochemical data were compared using a general linear model (SPSS). *P* values <0.05 were considered significant in both cases. Biochemical and ion composition data from animals sampled directly from holding tanks, and those held under normoxia in the experimental system were pooled for statistical analysis since no differences were observed in any parameters between these groups.

Results

Experiment 1: cardiorespiratory parameters

Cardiorespiratory data for sculpin exposed to hypoxia and reoxygenation are presented in Fig. 2. Viability during hypoxia was excellent with the exception of one operated animal that expired following 3 h at 2.0 mg l^{-1} DO_2. Data from this fish are included in the analysis since no differences were observed in the animal's cardiorespiratory variables relative to other individuals prior to its death. *Post-mortem* examination revealed that the flow probe lead had been pulled through the suture holding it in position, causing it to cut off blood flow in the ventral aorta.

Ventilation rate under normoxia was approximately 24 breaths min^{-1}, which is within the range of values previously recorded for this species (Sundin et al., 2003). Ventilation rate increased slightly at 5.6 mg l^{-1} DO_2 but subsequently declined and remained significantly lower throughout hypoxia. No significant recovery was noted following reoxygenation.

Myoxocephalus scorpius exhibits a bimodal pattern of opercular movements. While resting under normoxia, water is pumped through a small flap in the dorsal region of the operculum, while under hypoxia movements of the operculum are much more exaggerated, with water exhaled along the entire length. Ventilatory characteristics in sculpin seem similar to the low-pressure, high-volume system described for the sea raven *Hemitripterus americanus* (Saunders and Sutterlin, 1971). In this regard impedance measurements accurately reflect changes in ventilation rate f_V between normoxia and hypoxia but do not allow accurate quantitation of relative changes in ventilation volume. Sculpins remain essentially motionless in the experimental system during extended periods in water at 2.0 mg l^{-1} DO_2. As little other obvious activity is noted in this situation, a decline in ventilation rate may reflect a decline in animal activity as a whole under hypoxia.

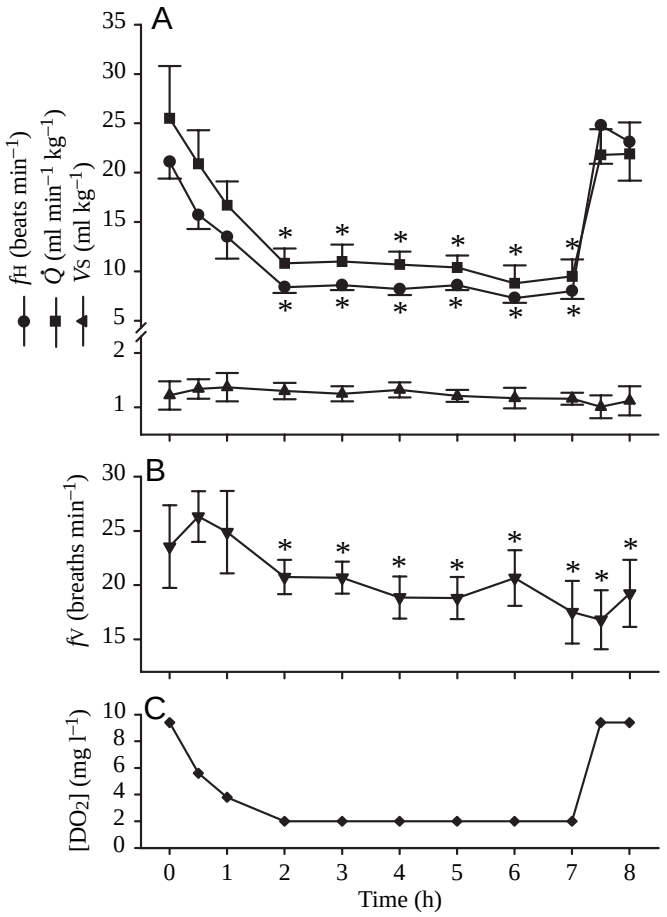

Fig. 2. Cardiovascular characteristics (A) and ventilation rate (B) of short-horned sculpin *Myoxocephalus scorpius* exposed to graded hypoxia and reoxygenation (8°C). (C) DO_2 was reduced to 5.6 and 3.8 mg l^{-1} for 0.5 h each, followed by 6 h at 2.0 mg l^{-1} and 1 h reoxygenation. *Significant difference (P<0.05) from normoxia. F_H, heart rate; $\dot{Q}$, cardiac output; V_S, stroke volume; F_V, ventilation rate.

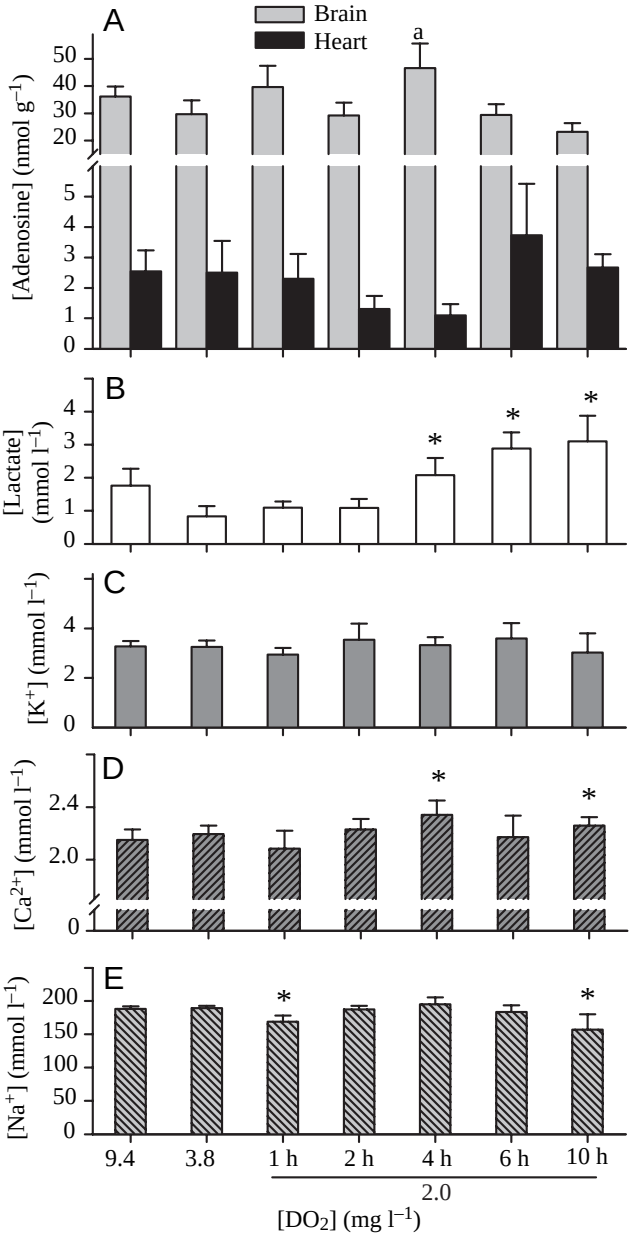

Fig. 3. Cardiac and brain adenosine levels (A) and plasma lactate (B), K^+ (C), Ca^{2+} (D) and Na^+ (E) levels from short-horned sculpin *Myoxocephalus scorpius* exposed to graded hypoxia (8°C). *Significant difference (P<0.05) from levels under normoxia; a indicates a significant difference from adjacent values (see text for details).

The onset of hypoxia resulted in a large, significant decrease in heart rate f_H, declining more than 60% from 20 beats min^{-1} under normoxia to less than 8 beats min^{-1} after 6 h at 2.0 mg l^{-1} DO_2. Normoxic f_H values measured in the present study were lower than published values that range from 30 beats min^{-1} to 50 beats min^{-1} at comparable temperatures (Fritsche, 1990; Sundin et al., 2003; Campbell et al., 2004). The presence of a hypoxic bradycardia in this species was also noted by Fritsche (1990), although of lesser magnitude, with f_H remaining at about 25 beats min^{-1} or above. The disparity in depth of bradycardia is likely to be related to differences in the duration of hypoxia and the method of induction. In the above-mentioned study, hypoxia of a similar magnitude was induced within 1 min and sustained for only 4 min. Heart rate increased at reoxygenation to levels not different from those observed while the animal was resting under normoxia. Synchrony between f_V and f_H was not obvious under the conditions tested.

Cardiac output for short-horned sculpin under normoxic conditions (25.5±5.3 ml min^{-1}) compared well with previous estimates obtained using the Fick Principle (27.75 ml min^{-1}; Goldstein et al., 1964). Hypoxic bradycardia was accompanied by a similar 65% decline in cardiac output, to a minimum of 8.8 ml min^{-1} following 5 h at 2.0 mg l^{-1} DO_2. Cardiac output recovered to resting normoxic levels following reoxygenation. Stroke volume V_S was high (1.23±0.06 ml kg^{-1}) compared to other species (Farrell and Jones, 1992) and was maintained under hypoxia and reoxygenation, further indicating that no attempt was made to defend normoxic blood flow at decreased f_H. High stroke volume in this instance is not a result of a large heart relative to body size, as the cardiosomatic index for short-horned sculpin is similar to, or lower than that for other north-

temperate species (C. E. Short and W. R. Driedzic, unpublished observations). Hypoxia does not influence ventral aortic blood pressure in this species (Fritsche, 1990). In light of the significant drop in cardiac output observed in the current study, together with the maintenance of ventral aortic blood pressure, peripheral circulation must be limited in this species during hypoxia.

Experiment 2: metabolic response to hypoxia/reoxygenation

The content of adenosine in brain ranged from 8.51 to 83.7 nmol g^{-1} with a mean concentration of 34.0±1.78 nmol g^{-1} (*N*=88). Results for biochemical measurements under graded hypoxia are presented in Fig. 3. There were no significant differences between adenosine levels under normoxia and those under hypoxia at any time point. Similar to the turtle brain, there seems to be a cyclic oscillation of adenosine content with time (Lutz and Kabler, 1997). A significant increase to about 46.6 nmol g^{-1} was observed between 2 and 4 h at 2.0 mg l^{-1} DO_2 that declined again at 6 h at 2.0 mg l^{-1}. Data for biochemical parameters following acute hypoxia exposure are presented in Fig. 4 and those for reoxygenation following graded hypoxia are shown in Fig. 5. Animals subjected to acute severe hypoxia or to reoxygenation following 6 h at 2.0 mg l^{-1} did not exhibit significant differences in brain adenosine levels compared to fish held under normoxia.

Cardiac adenosine contents ranged from undetectable to a maximum of 13.5 nmol g^{-1} with a mean concentration of 2.05±0.30 nmol g^{-1} (*N*=88). Although no significant increases were observed in cardiac adenosine levels under the conditions tested, a trend toward higher contents was noted between 4 and 6 h at 2.0 mg l^{-1} DO_2 (*P*=0.06), similar to that observed in brain (Fig. 3). Acute severe hypoxia did not effect cardiac adenosine levels (Fig. 4). The most substantial change noted in heart adenosine levels occurred following reoxygenation when, unlike brain, cardiac adenosine levels declined significantly following 0.5 h of reoxygenation after 6 h at 2.0 mg l^{-1} DO_2 (Fig. 5).

Plasma lactate concentrations increased significantly over normoxia levels following 4 h at 2.0 mg l^{-1} DO_2 and remained elevated through 0.5 h reoxygenation (Figs 3 and 5). Acute hypoxia did not significantly affect plasma lactate levels (Fig. 4). This is probably due the short exposure times required for the fish to lose equilibrium or to release lactate from the white muscle to the blood. Acute hypoxia (Fig. 4), as well as extended periods at 2.0 mg l^{-1} DO_2 (Fig. 3), resulted in a significant increase in plasma Ca^{2+} levels. Acute hypoxia significantly increased plasma K^+ levels (Fig. 4) but no increase was observed during graded hypoxia at any time point (Fig. 3). Reoxygenation resulted in a significant increase in plasma K^+ levels that did not return to prehypoxic values by the 1 h sampling point (Fig. 5). Increased plasma K^+ levels following reoxygenation may originate from the reperfusion of peripheral tissues that did not receive significant blood flow during hypoxia. No pattern was evident in plasma Na^+ levels, although significant and transient decreases were observed at 1 and 10 h at 2.0 mg l^{-1} DO_2 (Fig. 3). Plasma K^+ and Na^+ levels were in agreement with previous estimates for *M. scorpius* (Enevoldsen et al., 2003).

Discussion

Short-horned sculpin seem to recover fully from at least 10 h of exposure to 2.0 mg l^{-1} DO_2 without notable agitation. The ecological relevance of the sculpin's hypoxia tolerance is not readily apparent. These animals reside in cool, near-shore habitats (Scott and Scott, 1988) and, to our knowledge, are not regular visitors to tidepool environments that may be exposed to fluctuating oxygen levels. This animal's ability to survive hypoxia is more likely to be related to its sedentary nature, rather than to a specific environmental adaptation, although hypoxia tolerance is widespread amongst marine cottids (Martin, 1996).

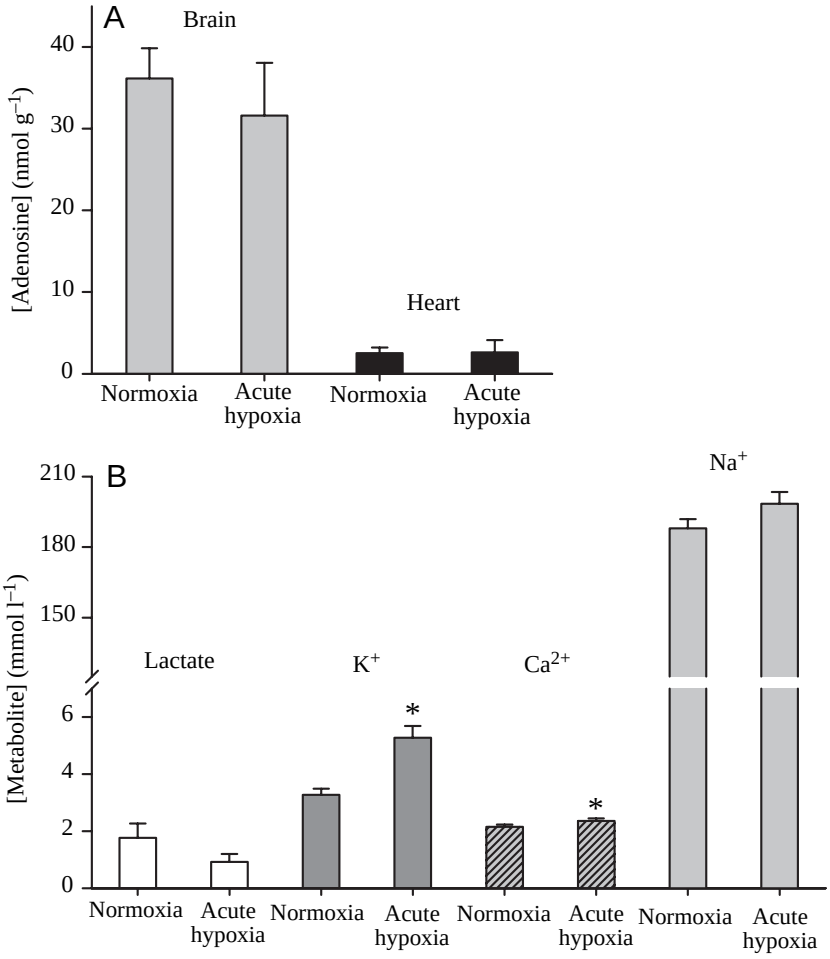

Fig. 4. Cardiac and brain adenosine levels (A) and plasma lactate, K^+, Ca^{2+} and Na^+ (B) levels from *Myoxocephalus scorpius* exposed to normoxia and acute hypoxia (8°C). *Significant difference (*P*<0.05) from levels under normoxia.

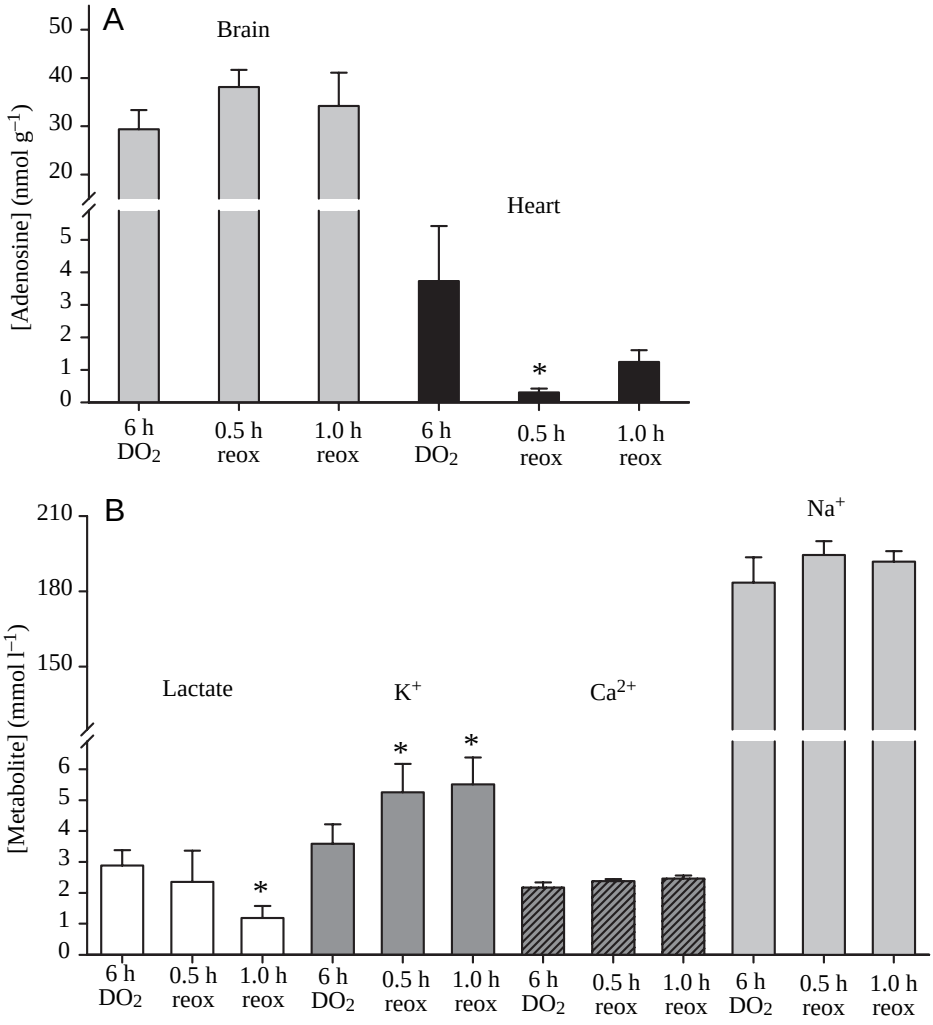

Fig. 5. Cardiac and brain adenosine levels (A) and plasma lactate, K^+, Ca^{2+} and Na^+ (B) levels from *Myoxocephalus scorpius* exposed to 2.0 mg l^{-1} DO_2 for 6 h followed by 1 h of reoxygenation (reox) (8°C). *Significant difference (P<0.05) from levels observed at 6 h at 2.0 mg l^{-1} DO_2.

The capacity to survive periods of oxygen limitation may also relate to the short-horned sculpin's nature as an opportunistic predator. Sculpins can be regularly observed preying on fish close, or equal to their own body size in the laboratory (T.J.McC., personal observations). During these events, prey can be held in the mouth for extended periods of time (several hours) before being fully consumed. This causes obvious changes in the ventilatory characteristics of the predator, as the buccal cavity is almost entirely blocked in some instances. Although arterial oxygen content is not known during these times, it seems reasonable that the inhibition of ventilation caused by the bolus of food could substantially reduce oxygen uptake resulting in systemic hypoxia. Regardless of when a high resistance to oxygen debt is critical to survival, short-horned sculpin are a useful model to help in understanding the underlying mechanisms.

This study provides the first information regarding cardiac adenosine levels in fish. The range of adenosine contents noted for sculpin cardiac muscle is very similar to those observed in reptiles and mammals. The lizard *Agama stellio stellio* had heart adenosine levels ranging from 1.8 to 13.5 nmol g^{-1} (Michaelidis et al., 2002) and in isolated perfused guinea pig hearts, levels were from 0.5 to 7 nmol g^{-1} (Rubio et al., 1974). This is remarkable considering the obvious differences in temperature and metabolism between these groups. Sculpin generally maintained cardiac adenosine levels throughout extended periods of hypoxia, showing only a trend towards accumulation following 6 h at 2.0 mg l^{-1} DO_2, which decreased after an additional 4 h of exposure. Preliminary experiments with more hypoxia-sensitive species, Atlantic cod *Gadus morhua* and rainbow smelt *Osmerus mordax*, yielded similar results. In contrast, cardiac adenosine levels can increase up to 55-fold following the onset of ischemia in mammals (Mullane and Bullough, 1995). Elevated plasma lactate concentrations indicate that fish were subjected to a level of hypoxia sufficient to impair aerobic metabolism. This suggests that the lack of adenosine accumulation cannot be ascribed to an adequate supply of oxygen.

The capacity of sculpin to avoid cardiac adenosine accumulation in the presence of hypoxia is probably due to the observed depression of cardiac activity. Cardiac output declined in concert with heart rate while stroke volume was maintained during hypoxia. In some teleosts stroke volume is increased during hypoxia and cardiac output is defended to some extent at lower heart rates (Farrell, 1982; Fritsche and Nilsson, 1989). Stroke volume in the sculpin is quite high relative to other teleosts, 1.23±0.06 ml kg^{-1} compared to about 0.5 ml kg^{-1} (Farrell and Jones, 1992), and may have a limited scope for further increase at low heart rates. Although this interpretation is speculative, it seems clear that short-horned sculpin do not attempt to maintain normal cardiac output under hypoxia. Oxygen consumption in short-horned sculpin correlates well with changes in heart rate (Campbell et al., 2004). A large drop in cardiac activity should therefore substantially reduce the oxygen requirements of the animal and help to preserve high-energy phosphate levels throughout hypoxia. Given that plasma lactate levels were elevated while cardiac adenosine levels were maintained throughout hypoxia, it seems reasonable to assume that anaerobic metabolism was robust enough to defend ATP levels in the sculpin heart. An alternative explanation is that cardiac adenosine washout may

be quite rapid, obscuring increases in production under hypoxia. This seems unlikely considering blood flow parameters were reduced under hypoxia, recovered fully subsequent to reoxygenation, and no functional impairment was evident that may have indicated injuries related to ATP depletion.

Heart performance in fish is intimately linked to the availability of extracellular Ca^{2+} (Driedzic and Gesser, 1994). Hypoxia-induced elevations in plasma Ca^{2+} levels may help to offset the negative effects of any decrease in pH on the sculpin heart. Increasing the extracellular Ca^{2+} level potentiates force development in fish cardiac muscle under anoxia (Nielsen and Gesser, 1983, 1984), and the possibility should not be excluded that elevated Ca^{2+} levels play a role in maintaining stroke volume in the sculpin heart. Increasing exogenous K^+ from 2.5 to 10 mmol l^{-1} exerts depressive effects on force development and oxygen consumption in rainbow trout heart (Kalinin and Gesser, 2002). It is conceivable that the positive inotropic effects of elevated Ca^{2+} levels on the heart balance the depressive effects of increased K^+ availability during acute hypoxia in the sculpin.

The large decrease in cardiac adenosine content in sculpin following reoxygenation may be associated with a reactivation of metabolic pathways for salvaging adenosine into the nucleotide pool. Adenosine kinase, which catalyses the phosphorylation of adenosine to AMP under normoxia, is inhibited by as much as 94% under hypoxia by increases in the concentration of inorganic phosphate (Gorman et al., 1997). In contrast, 5′-nucleotidase activity, which is responsible for catalyzing adenosine formation from AMP, is increased under hypoxia (Schrader, 1983). The net effect is an increase in the production and release of adenosine in the heart under hypoxia. At reoxygenation, the tenfold decrease in cardiac adenosine content in the sculpin is probably due to the activation of adenosine kinase and the reestablishment of inhibition on 5′-nucleotidase activity. This will result in decreased adenosine production and promote the phosphorylation of adenosine back to AMP.

Increases in plasma K^+ levels and decreases in cardiac adenosine content following reoxygenation may influence cardiovascular function in the sculpin. Elevations in plasma K^+ levels at reoxygenation probably reflect the reperfusion of peripheral tissues that experienced limited blood flow during hypoxia. This phenomenon is well documented and is supported by evidence that *M. scorpius* display a significant decline in heart rate and cardiac output while maintaining ventral aortic blood pressure under hypoxia (Fritsche, 1990). As discussed above, increased K^+ levels exert negative inotropic effects on the fish heart and this may influence cardiac function in sculpin during reoxygenation. Adenosine exerts inhibitory effects on the firing rate of the sino-atrial node and on atrial contractility, resulting in a slowing of heart rate and depressed atrial function in mammals (Collis, 1991). Adenosine also antagonizes the stimulatory effects of circulating catecholamines (Collis, 1991), which often increase in fish during hypoxia (Bonga, 1997). The decline in cardiac adenosine levels observed in the sculpin at reoxygenation may contribute to the recovery of heart function following hypoxia by releasing these inhibitory influences. This interpretation must be viewed with caution, however, since the response of the teleost heart to adenosine may be different from that of the mammalian heart. Decreased cardiac adenosine content may also counteract the depressive effects of the increased plasma K^+ concentrations observed in the sculpin at reoxygenation.

Brain adenosine levels increased significantly after 4 h at 2.0 mg l^{-1} DO_2 and were higher than cardiac levels as a whole, possibly reflecting an inability of the brain to maintain ATP levels under hypoxia. Adenosine contents remarkably similar to that of sculpin after 4 h at 2.0 mg l^{-1} DO_2, were observed in anoxic epaulette shark *Hemiscyllium ocellatum*, with levels reaching 46.4 nmol g^{-1} (Renshaw et al., 2002). Levels were also comparable to those observed in the active lizard *Agama stellio stellio* (about 30 nmol g^{-1}; Michaelidis et al., 2002). In the vast majority of vertebrates, brain ATP production is almost entirely aerobic (Bickler and Donohoe, 2002), and it is therefore not surprising that sculpin appear to exhibit hypoxic adenosine build up. It is interesting to note that after 10 h of hypoxia brain adenosine declined to levels not different from normoxic concentrations. In the freshwater turtle, *Trachemys scripta*, extracellular adenosine levels fluctuate by several-fold over roughly a 150 min period during long term anoxia (Lutz and Kabler, 1997) but the frequency of these cycles varies considerably between individual turtles. It is quite possible that sculpin exhibit similar fluctuations in neural adenosine under long-term hypoxia but that the terminal sampling methods employed here do not provide the necessary resolution to elucidate this phenomenon.

In summary, the short-horned sculpin possesses considerable tolerance to hypoxia and is able to avoid cardiac adenosine accumulation through a depression of cardiac activity and an activation of anaerobic metabolism. However, important changes in cardiac adenosine content were observed at reoxygenation that may facilitate the recovery of heart function under these conditions. The lack of hypoxic adenosine accumulation emphasizes the need to use caution in interpreting data on the effects of exogenously added adenosine on the fish heart. For instance, adenosine decreased contractility in atrial and ventricular preparations from rainbow trout *Salmo gairdneri* (Meghji and Burnstock, 1984) but had the opposite effect on the flounder *Platichthys flesus* heart (Lennard and Huddart, 1989). Fluctuations in brain adenosine content observed in the short-horned sculpin under hypoxia appear similar to those observed in the brains of anoxia-tolerant turtles and may serve a protective function during extended periods of hypoxia. Although the short-horned sculpin is able to withstand moderate hypoxia, it appears to be quite sensitive to more severe hypoxia. Information regarding adenosine metabolism in more hypoxia-tolerant species would be valuable to the overall understanding of the regulatory actions of purines in the fish cardiovascular system. Preliminary studies on more hypoxia-sensitive species suggest that the lack of cardiac adenosine accumulation under hypoxia may not be unique to short-horned sculpin.

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